< Dallphin-AtoM ver. 0.8.5 Brief users' guide>

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 Dall-Phin.

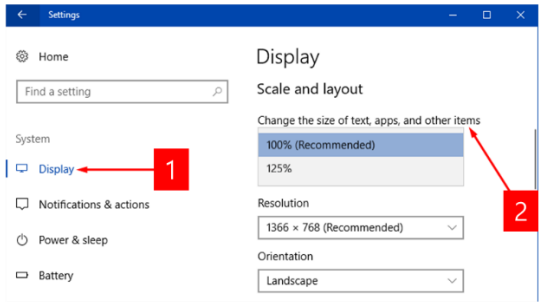
- Elements are too small or large compared to the rest of the desktop.
- Elements such as icons, toolbars, text, and dialog boxes appear to be fuzzy or disoriented.
- Blurry text appears in Dall-Phin interface.

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

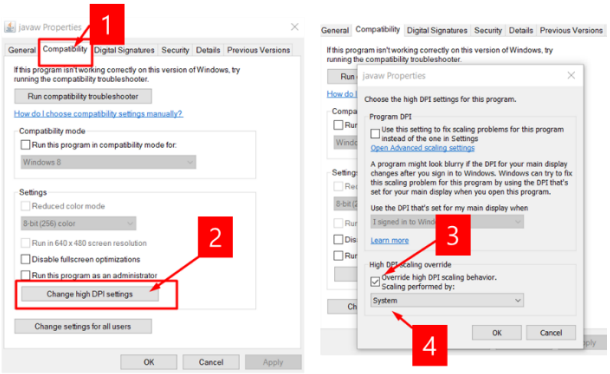
1. Change the size of apps: You can right-click on the Desktop (background image) > Display Setting > Change the size of apps, text, and other items on the screen. (100%) (Figure 1A)

2. Change the Java compatibility properties of Javaw.exe: You can find javaw.exe in C:₩DAll-Phin₩jre₩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



What is Dallphin-AtoM?

- Dallphin is the acronym of the phrase '**D**rugs with **ALL**ometry and **PH**ysiology **IN**side' and AtoM is '**A**nimal to hu**M**an'.
- It is a PBPK software that predicts human PK parameters and plasma drug concentrations based on many published methods using physicochemical properties, in vitro and animal PK data.

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.

Right to use Dallphin-AtoM

- Dallphin-AtoM (Dallphin, for simplicity) 0.8.5 is distributed to beta testers for test use only.
- Any suggestion, comments or bug reports via email (yimds@catholic.ac.kr) are welcomed.

Who developed Dallphin-AtoM?

- Dallphin 0.8.5 was developed by researchers of PIPET (Pharmacometrics Institute for Practical Education and Training) of College of Medicine, the Catholic University of Korea for the education of students and researchers.

- Dallphin 0.8.5 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).

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.

What this guide tells

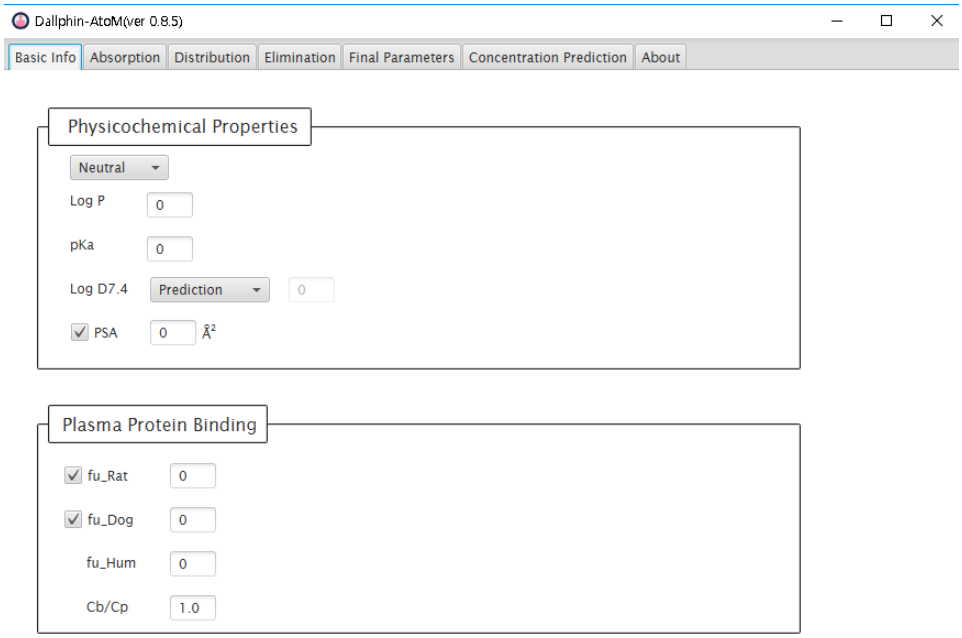
- To predict drug exposure in human, we need to know four major PK parameters - Ka, Vd (Vc, Vp and Q), CL and F if the drug follows first-order kinetics.
- This guide introduces the principles used in the prediction of each of the 4 PK parameters based on in vitro or animal PK data together with corresponding references. (Demonstrating how to use Dallphin is not the purpose of this guide: it will be open as a video file in the web.)
- You may find more detailed introduction to the 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 Introduction of the tabs

- When Dallphin is launched in your computer, you'll see six tabs. From the leftmost one, you

should enter your own 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" tappane.



Basic info Tab

- In the Basic info tabpane, physicochemical properties, blood-to-plasma concentration ratio and plasma protein binding are input.
- Log D may be directly input 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.

Absorption Tab

<Caco-2 permeability and Fa>

- Caco-2 permeability of the drug should be input as Papp. Those from reference standard drugs (atenolol and/or verapamil) are also recommended to input if available.
- 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 [2].
- Caco-2 cell based Fa (fraction absorbed, F = Fa x Fgut x F_H) was then calculated using equations on the relationship between Fa, Peff, small intestine surface area and its transit time [3].
- MDCK-II cell permeability can be input or predicted using Caco-2 Papp to estimate passive CL that may be used when microsomal method is chosen for the calculation of hepatic CL.
- The Fa calculated herein is given as Fa_Caco in the bottom of the pane.

<MDCK permeability>

MDCK-II cell permeability may be input here when available.

<first-order absorption rate constant: ka>

- We used an equation [4] to predict human ka that is calculated from Caco-2 permeability data, intestinal surface area and Vc (central compartment Vd).
- In a one compartment model, to predict the ka value, we assume the Vd as Vc. This may cause some discrepancy in ka prediction, we highly recommend you to use two compartment model.

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

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 Vd in Dallphin-AtoM. The user should opt for one in the two.

<Allometric method>

- Compartmental PK parameters on distribution (Vc, Vp and Q) obtained from i.v. PK study in more than two animal species (mouse, rat, dog, monkey) is a necessity to use the allometric method.
- The user may choose either one- or two-compartment models, and the number of compartments should be same for all of the animal species used. (The number of distribution compartments ≥3 is not acceptable.)
- In case of two compartment model:
    - Vc and Vp in each species are to be input by users and Vss is automatically filled up (Vss = Vp + Vc).
    - Dallphin calculates human Vss and Vc allometrically with the exponent to body weight fixed to 1.
    - Although there is no report on the allometric estimation of Vc, we assumed that both of Vc and Vss are proportional to body weights regardless of species.
- In case of one compartment model:
    - The user may also opt for "1-compartment" model instead of "2-compartment model". Occasionally, the i.v. PK data may be better fitted by a 1-compartment model according to the PK sampling scheme, infusion time, or else. Even when the i.v. PK profiles in animals seem to follow multi-compartment models, software for compartmental analysis may not be available to the user. Then, the user may input Vss obtained from non-compartmental analysis in the "1-compartment"model.
    - Vp or Q are not requested.

<PBPK method>

- If animal PK data is not availabile, Vss is predicted from the PBPK-based prediction method which uses drug lipophilicity, organ fat content and other tissue physiologic factors [5-7]. All of the physiological variables from the references are used in Dallphin AtoM as built-in

equations to calculate Vss. When using the PBPK method, only Vss is calculated (without presenting Vc and Vp separately) as if the drug follows a 1-compartment model.

Elimination tab

Hepatic CL

- For hepatic CL, the user should opt for either of microsome or hepatocyte based on the user's experimental data (done with microsome or human hepatocyte).
  - The well-stirred model equation which defines the relationship of hepatic blood flow, hepatic intrinsic CL, plasma unbound fraction and blood-to-plasma concentration ratio was used to calculate various hepatic CLs from microsomal or hepatocyte experimental data.

<microsome experiment data>

- The microsome protein concentration can be input considering the user's experimental condition.
- In the microsome experiment, when the user inputs microsome intrinsic CL (CL int_ini), it is corrected by nonspecific binding fraction predicted by 'logD' or 'logP' value [7] and scaled up by the amount of protein per gram liver and weight of liver to predict the human hepatic CL (CLh).
- As intrinsic CL can be over-predicted in microsome experiments, passive diffusion CL may be applied on intrinsic metabolic CL to reflect the diffusion rate of drug molecules across the hepatocyte cell membrane. The user can select whether the CLh is corrected by passive diffusion CL (to obtain CL_PS) or just use the CLh as is.
  - Passive diffusion CL was calculated using either the apparent passive permeability (Papp) of MDCK-II cell and surface area of one million human hepatocytes. However, in case the MDCK-II cell Papp is not available, the user may use Papp of Caco-2 cell permeability or logD to predict the MDCK-II cell Papp value. [8]. The corrected CLh obtained using MDCK-II or Caco-2 Papp is CLh_PS. When the CLh is corrected with logD instead of MDCK-II or Caco-2 permeability data, it is named CLh_PS(logD).
- Correction using rat in vitro-in vivo correlation (ivivc) data:

- When both of rat microsomal experiment 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.
- First, corrected CLint_ini of rat (CLint_ini') is back-calculated from observed CLh of rat (CLh_ratinvivo)
- Second, the ratio between the CLint_ini and its corrected value in rat is calculated.
- Third the ratio is used to correct the CLint_ini of human.
- Last, the human corrected CLint_ini is used to calculate CLh_fromrativivc using the well-stirred model equation.

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

<When using hepatocyte experiment data>

- The hepatocyte cell volume ratio can be input considering the user's experimental condition. Generally, it is considered to be 0.005 when 10^6 hepatocyte cells were used.
- In this case, when the user inputs intrinsic CL of hepatocyte, it is corrected by nonspecific binding fraction predicted by 'logP' or 'logP' value [7] and scaled up by the number of hepatocytes per gram liver and weight of liver to predict the intrinsic metabolic CL.
- The well-stirred model equation which defines the relationship of hepatic blood flow, hepatic intrinsic CL, plasma unbound fraction and blood-to-plasma concentration ratio was used to calculate the hepatic CL.
- The ivivc correction using rat data as in the case of microsomal experiment is not provided because in vitro metabolism study using rat hepatocytes is not frequently performed.

Renal CL

- Renal CL of human is predicted directly from the rat or dog renal CL corrected by fraction unbound in plasma and kidney blood flow of each animal. [9]
- If an assumed human renal CL value of the new molecule is available (from information on

the same category drugs or else), the user may directly input the value in the Final parameters tabpane.

- 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 human in the Final parameters tabpane..

Final Parameters tab

- The user may choose preferred hepatic CL, Fh, renal CL and Fa values for simulation among several differently calculated parameters in the previous tabpanes.
- Since Fh value is derived from hepatic CL, Fh and hepatic CL is paired and the Fh value will be automatically selected when the hepatic CL is selected.
- The user has four pairs of choices in hepatic CL and Fh when using microsome intrinsic CL, and the user has one pair of choice in hepatic CL and Fh when using hepatocyte intrinsic CL.
- The user may select one of the two renal CL (from rats or dogs) or enter the assumed renal CL value for simulation.

Concentration Prediction tab

- 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. Results and input parameters are also saved in the result folder ('Open Result Folder').

References

1. **Palm K, Stenberg P, Luthman K, Artursson P.** Polar molecular surface properties predict the intestinal absorption of drugs in humans. *Pharm Res*. 1997;14:568-571.
2. **Sun D, Lennernas H, Welage LS, Barnett JL, Landowski CP, Foster D, Fleisher D, Lee KD, Amidon GL.** 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;19:1400-1416.

3. **Krishna R, Yu L.** Biopharmaceutics applications in drug development. Springer Science & Business Media; *2007*. page 87
4. **Usansky HH, Sinko PJ.** 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;314:391-399.
5. **Berezhkovskiy LM.** Volume of distribution at steady state for a linear pharmacokinetic system with peripheral elimination. *J Pharm Sci*. 2004;93:1628-1640.
6. **Poulin P, Theil FP.** 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;98:4941-4961.
7. **Kilford PJ, Gertz M, Houston JB, Galetin A.** Hepatocellular binding of drugs: correction for unbound fraction in hepatocyte incubations using microsomal binding or drug lipophilicity data. *Drug Metab Dispos*. 2008;36:1194-1197.
8. **Li R, Bi YA, Lai Y, Sugano K, Steyn SJ, Trapa PE, Di L.** Permeability comparison between hepatocyte and low efflux MDCKII cell monolayer. *Aaps j*. 2014;16:802-809.
9. **Paine SW, Menochet K, Denton R, McGinnity DF, Riley RJ.** 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;39:1008-1013.