Getting-started with

Population Lifecourse Exposure to Health Effects Model

(PLETHEM)

PLETHEM v0.1.7

Revised February 11, 2019

ScitoVation
INNOVATIVE CELL BASED SCIENCE

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1 List of attached files

This guide includes reference to number of samples files. These are available for download from this Dropbox folder.

- UserDb.sqlite: User database file containing chemical data for running the workflows in this document
- Demo Project.Rdata: Prepopulated PLETHEM project file
- In-vivo time course data.csv: CSV file containing dummy in vivo blood concentration time course
- Exposure.xlsx: Excel file for importing exposures into PLETHEM. This file can be edited if the sheet names and column names in each sheet are kept the same

2 Introduction

Population Lifecourse Exposure to Health Effects Model (PLETHEM) is an open-source package for physiologically-based pharmacokinetic (PBPK) modeling written in R statistical language. The main aim of the package is to ease PBPK modeling for most applications by providing powerful modeling workflows wrapped in an easy-to-use and intuitive user interface. PLETHEM supports PBPK modeling, in vitro to in vivo extrapolation (IVIVE), and high-throughput IVIVE. PLETHEM consists of a master database of physiological parameters, lifecourse equations, QSAR models, and chemical information. Additional physiological parameter sets or chemicals can also be stored externally as a user database. Finally, PBPK modeling workflows in PLETHEM save all the modeling data in a project file that is platform agnostic and can be opened through any other PLETHEM installation.

R can be accessed from the command line or via a number of interactive development environments (IDEs). We recommend using RStudio[1] as the IDE when you are working in PLETHEM. Guidelines for installing Rstudio are included below. To test the capabilities of the package, we will be running a demo project that is already created and saved. We will also create a new project for simulating a dummy Coumarin dataset using PBPK models. Finally, we will run an HT-IVIVE workflow for two exemplar compounds: coumarin and all-trans retinoic acid (ATRA). The files needed to run the workflows are available here or can be obtained by emailing plethem@scitovation.com.

3 Scope and goal

The goal is to test the PBPK modeling and HT-IVIVE workflow within PLETHEM package by running through a number of sample scenarios. We will provide detailed step-wise instruction and all the corresponding data files needed to perform testing on the following topics:

1. Installing R and Rstudio.
2. Installing PLETHEM from the Comprehensive R Archive Network (CRAN).
3. Loading and running an existing PLETHEM project for PBPK modeling.
4. Creating, running, and saving a new PLETHEM project for PBPK modeling.
5. Importing exposure estimates for PBPK modeling.
6. Running HT-IVIVE for estimating equivalent dose from in vitro point of departure.

Problems, typos, or areas for improvement encountered by the testing team can be sent to plethem@scitovation.com with "PLETHEM Bug Report" as the subject of the email. We have already identified several bugs through internal testing of the package. These will be mentioned where appropriate in the document below to make it easier on the testers.

There are two planned updates to the PLETHEM package in the first quarter of 2019. This guide is written for PLETHEM version 0.1.7, the version on CRAN as of February 11th, 2019. The first update to PLETHEM focusses on a redesign of the exposure import user interface that makes it more user friendly. It also adds support for exposure estimation via ConsExpo. Additionally, most bugs that have been identified previously will be fixed in this update as well. The second update will include additional bugs identified through the beta testing process.

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[1] https://www.rstudio.com/

4 Installing prerequisites and getting started

4.1 Installing R[2]

PLETHEM requires R version 3.4.0 (released April 2017) or newer.

4.1.1 Installing on Windows

    a. In a web-browser, navigate to https://cran.r-project.org/
    b. Select "Download R for Windows" under "Download and Install R."
    c. Select "base."
    d. Select "Download R 3.(version) for Windows."
    e. Save and run the installer file.

4.1.2 Installing on Mac OS X

    a. In a web-browser, navigate to https://cran.r-project.org/
    b. Select "Download R for (Mac) OS X" under "Download and Install R"
    c. Select the "Latest release".[3]
    d. Click the link (e.g., "R-3.5.2.pkg") to download and run the installer file.

4.2 Installing Rstudio Desktop

    a. In a web browser, navigate to:

        https://www.rstudio.com/products/rstudio/download/#download

    b. Select from "Installers for Supported Platforms" an option that matches your operating system:
        i. For Windows: "RStudio 1.(ver) - Windows Vista/7/8/10"[4]
        ii. For Mac OS X: "RStudio 1.(ver) - Mac OS X 10.6+ (64-bit)"
    c. Save and Run the executable file.

4.3 Installing PLETHEM

The PLETHEM interface runs in R using some menu support from Rstudio. R is an interpreted command-line language. Installation of PLETHEM is easily achieved by downloading it from the Comprehensive R Archive Network (CRAN) repository directly within Rstudio.

    a. Open RStudio. The interface will look similar to Figure 1.
    b. Install PLETHEM by going to "Tools" > "Install Packages..."
    c. Enter "plethem" in the "Packages" field.

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[2] Depending on your system and institutional policy, installation may require assistance from your information technologies department.
[3] At the time of preparing this document, the latest release was R-3.5.2.
[4] At the time of preparing this document, the current version of RStudio Desktop was 1.1.463.

d. Ensure "Install dependencies" is checked.
e. If you are prompted to select a mirror, choose one near to your current geographic location.
f. Click "Install."
g. If this is your first time installing PLETHEM this can take a significant amount of time (i.e., minutes) as the package downloads and installs. Once PLETHEM is installed completely you will see the following message in the console window (Figure 1, purple arrow):

```
package 'plethem' successfully unpacked and MD5 sums checked
```

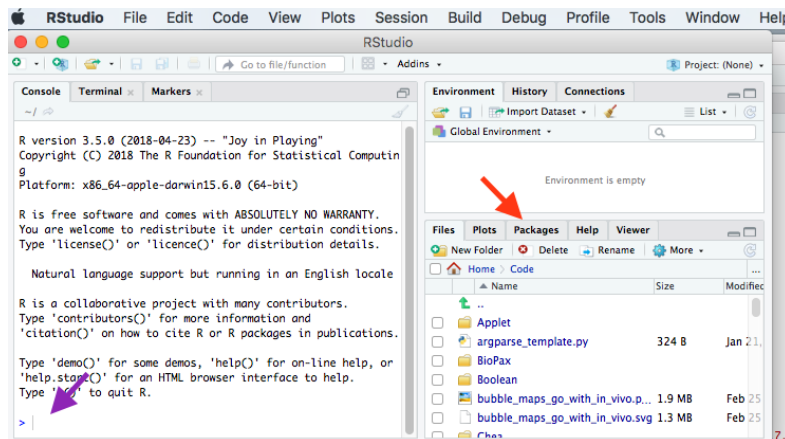PLETHEM is now installed and loaded.

Figure 1. The Rstudio environment. Rstudio is broken up into a series of windows that communicate different information. On the left is the console, where a user could enter R commands for execution. It provides feedback on the status of commands. Success in step 5.4.g will result in "package 'plethem' successfully unpacked and MD5 sums checked" appearing in the console window. On the right side of the screen is a series of tabbed windows, including "Packages" which will assist in the import of PLETHEM (red arrow).

4.4 Getting PLETHEM set up

4.4.1 Loading the PLETHEM package

a. Click the "Packages" tab on bottom-right panel (Figure 1, red arrow).
b. Find "plethem" in the list by scrolling through the installed packages or by searching, and tick the box next to it.
c. You should see a message in the console:

```
You are now using PLETHEM. If you have installed PLETHEM for the
first time or have just updated it, please run setUserDb() to select
an existing database or createUserDb() to create a new one.
```

Depending on the version of R you have installed, you may get an additional warning stating that PLETHEM was built under a different version of R. This warning will not affect the functioning of the package in any way.

4.4.2 Initializing a user database

PLETHEM uses portable sqlite databases to store information about chemical properties, kinetics, and physiological parameters. You can either create a new user database or set an existing database as the default one to use. We will be using the file UserDb.sqlite provided.

 a. Go to Tools -> Addins -> Browse Addins in Rstudio. This will open an addins dialog box.
 b. Search for the plethem addin labeled "Select a new user database".
 c. Select that addin and click "execute".
 d. This will open a Select file dialog box with the title "Select User Database". (note: this window may open behind you current Rstudio window)
 e. In that dialog box, navigate to where you have stored files outlined in Section 1 of this document provided with this document and open the file called "UserDb.sqlite"
 f. If the file loads successfully, you will see the following message on the RStudio Console:

```
[1] "Selected User Database has 3 chemicals"
```

5 Modeling using the rapidPBPK model

5.1 Loading and running an existing project

5.1.1 Loading an existing forward dosimetry project

 a. Go to Tools -> Addins -> Browse Addins in Rstudio. This will open the addins dialog box.
 b. Search and execute the "Load Plethem Project" addin. If you are not able to see the execute button on the addin dialog, select the addin and hit the enter key on your keyboard.
 c. This opens a new window (note: this window may open behind you current Rstudio window) called "Select PLETHEM Project". Navigate to the location where you have stored the materials for this and select "Demo Project.Rdata."
 d. Once the project loads, the forward dosimetry UI will launch in the systems default browser with all the datasets and simulations populated in the UI. We recommend using Google Chrome as the default browser.

5.1.2 Running a forward dosimetry simulation and looking at the results.

 a. Click on "Model Setup" on the top navigation bar. Navigate to the "Simulations" tab on the model setup page.
 b. Select "Simple Forward Dosimetry Simulation" from the dropdown menu and click on Run Simulation.
 c. Under the Model Outputs tab, select the concentration plots pane. Plot Arterial Plasma and Total Fat concentration. The resulting plot should look like Figure 2.

5.1.3 Running a Monte Carlo simulation and looking at results.

 a. Navigate back to the "Simulations" tab under the "Model Setup" UI.
 b. Select "Forward Dosimetry with Monte Carlo simulation" from the dropdown menu and click on run simulations.
 c. Under the Model Outputs tab, select the concentration plots pane. Plot Arterial Plasma and Fat total concentration. These would be box plots for Cmax of the Monte Carlo simulation.
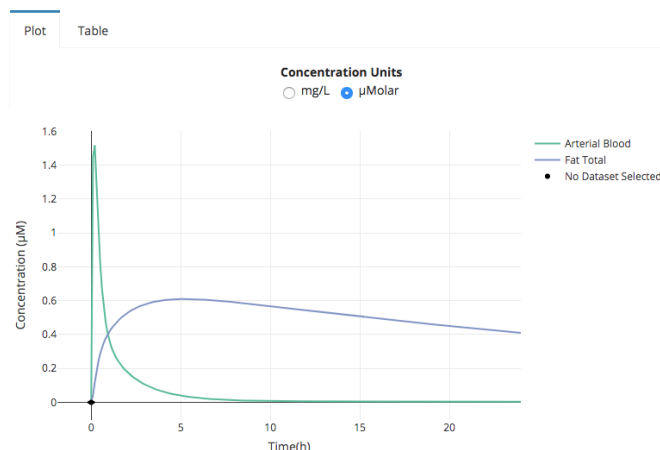
Figure 2. Time course for arterial plasma and fat concentration. Units for the plot can be changed to mg/L by using the radio buttons above the plot.

5.1.4   Exiting the modeling user interface.

    a. To exit the modeling interface at any point in time just click on the power button on the main navigation bar.

    b. This will open a dialog box asking if the user wants to exit this modeling session and save any changes made to the project. (Note: PLETHEM can only run one project at a time. To open a different project, the user must exit the existing modeling session as outlined here)

    c. Click the confirm button to exit the session. This saves the project information and the main window turns grey.

5.2   Creating a project for the model

5.2.1   Create a new forward dosimetry project

    a. Go to Tools -> Addins -> Browse Addins in Rstudio. This will open the addins dialog box.

    b. Search and execute the "Create PLETHEM Project" addin. If you are not able to see the execute button on the addin dialog, just double click the addin name to run.

    c. This opens new window asking for the name of the project and model. Give the project a name of your choosing and set the model to be "rapidPBPK".

    d. Clicking "Done" will open window where you can choose the folder where the project file will be saved when you exit the app (note: this window may open behind you current Rstudio window).

    e. Once a folder is selected, the forward dosimetry UI will launch in the systems default browser. We recommend Chrome, but the app should work in all modern browsers.

Once you have created a project, you can exit it anytime by clicking the power button symbol on the navbar at the top. This will create a dialog box stating that the project will be saved to the location selected above.

If you quit the project as outlined above, please reload it by following the instructions in the "Load existing project" section before proceeding.

5.3   Creating datasets for the simulation

This guide aims to take users through creating datasets and simulations. PLETHEM has built in defaults for a large set of parameters needed to fully parameterize PBPK models. The guide does not ask the users to set all these parameters individually and uses the defaults built into PLETHEM for inputs not directly specified by the users.

5.3.1   Creating an exposure dataset

    a.   Open the model setup user interface by clicking on "Model Setup" on the navbar at the top.

    b.   Exposure will be selected by default.

    c.   Enter a value of 10 for "Oral dose mg/kg/day", 1 for "Number of bolus doses."

    d.   Click the "Save As" button.

    e.   Give the dataset the name "Oral Exposure 1" and a description "Demo Oral exposure."

    f.   Click the "Add" button.

    g.   This creates an exposure set called "Oral Exposure 1" and the drop down menu in the exposure tab is auto updated to show "Oral Exposure 1."

5.3.2   Creating a chemical dataset

    a.   Click on the "Chemical" tab in the UI.

    b.   Click the import button. This opens window with a tab for the user database and the main database. If you have forgotten to set a user database as described in the steps above, instead of new window and the entire UI greys out. To solve this issue, reload the webpage and then close the app using the power button on the navbar.

    c.   From the user database, select Coumarin.

    d.   Enter name as "Default Coumarin Set" and description as "Data from User Database"

    e.   Set the "First order metabolism in liver" to 3500.

    f.   Click the "Calculate chemical params" button to calculate fraction dissolved in water

    g.   Click the "Save/Restore" button to open a dialog box that can save all the changes we just made to the chemical dataset.

    h.   Click "Save All" in the window that opens. If you want to save only a few changes or restore some of the changes made, you can click on the table shown to select the values you want to change or save.

    i.   Repeat steps above to import "ATRA" from the chemical database.

5.3.3   Creating physiological dataset.

    a.   Click on Physiological tab to open the physiological UI.

    b.   Click on "Calculate Parameters" button to use life course equations to calculate physiological parameters for an average male adult male.

    c.   Click on the "Calculate Partition" button to calculate partition coefficients for coumarin using built in QSAR models in PLETHEM.

    d.   Change the value of "Rate of absorption in Gut Lumen (per hour)" to 1.

    e.   Click on the "Save as" to save the changes we made as a new physiological parameter set. Name the dataset "Adult Physiology for Coumarin" and description as "Partitioning using default coumarin data". If the dataset does not appear in the dropdown menu right away, please refresh the page in the browser and check again.

5.3.4   Creating a parameter variability dataset.

a. Click on the "Uncertainty and Variability" tab to load that UI.
b. Click on Physiological on the sidebar. This brings up the UI to create variability sets for physiological parameters.
c. Click the "New" button. This opens a window where we can create a new variability set.
d. Call the set "Physiological Variability" and a description "Standard variability values."
e. From the dropdown menu select "Body weight" and "Cardiac Output". We will be defining a variability for these parameters.
f. Click on the "Update List" button to populate the table below.
g. Define a CV of 0.3 and a distribution type of "Log-normal" for both Body weight and cardiac output.
h. Click done to create the dataset.

5.4   Combining all the sets to make a simulation

5.4.1   Simulation Set- Creating a simple forward dosimetry simulation

a. Go to the simulations tab.
b. Click on the "Create new Simulation" button. This opens a dropdown panel for selecting datasets previously created.
c. Name the simulation "Simple Forward Dosimetry" with a description "Forward dosimetry simulation for coumarin with no variability."
d. Make sure for each set the "Select Variability" dropdown says "None."
e. Select "Use Chemical Vkm1" under the "Select Metabolism" dropdown.
f. Set the Simulation Duration to 24 hrs.
g. Uncheck the "Run Monte Carlo Simulation" checkbox.
h. Click the save button to save the simulation.
i. The UI indicates that the simulation is saved successfully and the dropdown menu on the simulation tab is populated with the name of the newly created simulation.

5.4.2   Simulation Set – Creating a simulation for performing Monte Carlo analysis

a. Click on the "Create new Simulation" button. This opens a dropdown panel for selecting datasets previously created.
b. Name this simulation "Forward Dosimetry with Variability" with a description "Forward dosimetry simulation for coumarin with variability in physiological parameters."
c. Make sure for exposure and chemical set the "Select Variability" dropdown says "None."
d. For the compartment set, choose "Physiological Variability" from the "Select Variability" dropdown.
e. Select "Use Chemical Vkm1" under the "Select Metabolism" dropdown.
f. Set the Simulation Duration to 24 hours.
g. Make sure that the "Run Monte Carlo Simulation" checkbox is checked.
h. Click the save button to save the simulation.
i. The UI indicates that the simulation is saved successfully. A simulation with the name "Forward Dosimetry with Variability" has been added to the dropdown menu on the simulation tab.

5.5 Running simulations and plotting results with the rapidPBPK model

5.5.1 Run Simulation

a. Select "Simple Forward Dosimetry" model from the simulation dropdown, then click "Run Simulation."
b. Once the simulation completes running, the UI switches to the model output tab.

5.5.2 Concentration Plots – Plotting concentration plots

a. Click on concentration plots to open the concentration plot pane.
b. Select different compartments from the model tab.
c. By default, the data is plotted in μM. Select the mg/L radio button and ensure that the model scale and graph window changes to reflect the change in units.
d. Click on the table tab. This should show the concentration data for the compartments selected as returned by the simulation.
e. Click the "Get Data" button. This opens a window that asks the user for a name and location to save the model results as a csv file.

5.5.3 Adding external PK data to project

a. External concentration data in mg/L can be added to the project and plotted against concentration simulation.
b. Click the "Add dataset" button at the top of the model output page to open the "Add dataset" dialog box.
c. Enter "Plasma concentration in vivo" for the name and "Data from in-vivo studies" for the description.
d. Click the "Browse" button and navigate to the location on your machine where all the testing data is stored. Select the file "In-vivo time course data.csv."
e. This should create a table displaying the data that will be imported.
f. Click the "Add dataset" button to add the data to the project.

5.5.4 Plotting PK data against simulation results

a. Make sure that the "Plot" tab is selected in the "Concentration Plot" pane.
b. Select the "Dataset" tab (next to the "Model" tab from where you selected compartments in the previous step).
c. Select the "Plasma concentration in vivo" from the "Select Datasets" dropdown.
d. This plots the data on the graph beside it. Make sure the units are selected to be "mg/L."

5.5.5 Plotting exposure

a. Exposure plots can be seen under the "Exposure Plots" pane.
b. Plot both instantaneous exposure values as well as total exposure across the simulation duration for the active route of exposure (oral) or for all exposures (oral, drinking water, inhalation and intravenous).
c. The data behind the plots can be seen in the "Table" tab.

5.5.6 Plotting amounts

    a. Amounts can be plotted from the "Amount Plots" pane.

    b. Currently we cannot plot amount data from external sources.

    c. Plot compartment values to view amounts of chemical in different compartments in the model.

5.5.7 Parameters

    a. Click the parameters tab at the top to switch over to the view the parameters table.

    b. You can see three tables, one each for exposure, physiological and chemical parameters. (**BUG – chemical parameters table heading says "All parameters"**)

5.5.8 Running Monte Carlo analysis

    a. Navigate back to the "Simulations" Tab on the "Model Setup" page.

    b. Select the "Forward Dosimetry with Variability" Simulation.

    c. Click the "Run simulation" button. Running Monte Carlo simulations take more time. Once the simulation is complete, the UI will switch to the Model Output tab.

    d. The concentration and amount plots change to box plots for Cmax values for each dose.

    e. (BUG – Axis labels not correct. Exposure data does not plot in Monte Carlo mode. Mass balance plots does not plot in Monte Carlo mode.)

5.6 Importing exposures using the batch import file

Instead of creating exposures, we will import them from the batch import file provided to you, "Exposures.xlsx". This is an excel file and can be edited to add new exposures as required. This allows users to create a set of exposures that will be used frequently or to import exposure estimates from tools that PLETHEM does not support natively.

We will continue using the project currently open in your browser. If you have closed that project, just refer to the instructions in "Loading an existing project" above to reload the model.

1. Importing the batch exposure file.

    a. On the exposure tab click on the "Import batch exposure file" button. This launches a dialog box with the title "Import exposure data."

    b. Load the provided batch exposure file by clicking the "Browse" button and navigating to the location where the file is stored. Once the file is read in, the progress bar below the browse button will show "Upload Complete". Imported exposure data can be viewed and selected for import by navigating the panes below the "Browse" button.

    c. Click on "Oral Exposure" to open that pane. Select any of the exposures from the table.

    d. Click on "Import Selected Exposures" button to import these exposures in the project.

    e. The imported exposures are added to the project and can be selected from the dropdown menu under the "Exposure" tab.

    f. Proceed to make a simulation with the new exposure as described in "Combining all datasets to create a simulation" section above and run the new simulation.

2. Exposure import UI will undergo a complete overhaul in the next update of PLETHEM (0.2.0). If you are using that version of PLETHEM (Will be displayed on the home page), the batch import UI can be accessed by clicking the "Import" button on the exposure tab.

6   Performing high-throughput IVIVE using PLETHEM

6.1   Starting HT-IVIVE UI and loading chemicals

6.1.1   Launching the HT-IVIVE UI

    a. Got to Tools -> Addins -> Browse Addins in Rstudio. This will open the addins dialog box.

    b. Search and execute the "HT-IVIVE using PLETHEM" addin. If you are not able to see the execute button on the addin dialog, select the addin and hit the enter key on your keyboard.

    c. This launches the HT-IVIVE UI in your systems default browser. We recommend using Google Chrome

6.1.2   Importing chemicals to the project

    a. Click on the "Import chemical" button at the top of the Setup page. This opens the import chemical dialog. This opens window with a tab for the user database and the main database. If you have forgotten to set a user database as described in the steps above, instead of new window and the entire UI greys out. To solve this issue, reload the webpage and then close the app using the power button on the navbar.

    b. From the user database, select Coumarin.

    c. Enter name as "Default Coumarin Set" and description as "Data from User Database"

    d. Click "Import" button.

    e. Repeat steps a-e above to import ATRA. Set the name of this dataset to be "Default ATRA dataset".

6.2   Parameterizing the HT-IVIVE model

    a. Click the "Add new row" button on the UI. This open a dialog box for parameterizing the HT-IVIVE model.

    b. Select "Default Coumarin Set" from the Select chemical dropdown.

    c. Navigate to "Invitro POD" tab. Enter 500 in the numeric box and select "µM" as the units.

    d. Navigate to "HT-IVIVE Type" tab and select "Oral Exposure non-volatile chemical."

    e. Navigate to "Hepatic Clearance."

    f. Select "Whole Hepatocyte Clearance". Enter 0.000413 for the numeric value and select units to be L/h/10^6 hepatocytes

    g. Navigate to "Renal Clearance" and click the checkbox that says "Include Renal Clearance"

    h. Click the "OK" button to complete parameterizing the model. The model is saved as a line item on the UI..

    i. Repeat the steps above for the ATRA dataset. Set the "Invitro POD" to 38 µM. Select "Subcellular Clearance" under the "Hepatic Clearance" tab and set the microsomal value and units to 0.00016 and µmol\min\mg Protein respectively.

6.3 Running the HT-IVIVE Model and saving results

6.3.1 Running the HT-IVIVE model

a. Once a line item is created for both the chemicals, we can run the HT-IVIVE by clicking the "Perform HT-IVIVE" button on the setup tab.

b. After the model is done running, the UI focusses on the "Results" page.

c. Here we see a line corresponding to each line in the setup tab. The results table also shows the steady state blood concentration (Css) and the equivalent applied dose that corresponds to the invitro POD.

d. The table can be exported as a csv file by clicking on the csv button above the table. Similar clicking the button "Copy", copies the table onto the clipboard and it can then be pasted in excel, word or similar programs.

e. To quit the HT-IVIVE app, click on the power icon on the top navigation bar. Unlike PBPK modeling, HT-IVIVE models are not stored as projects.