httk: High-Throughput Toxicokinetics Virtual Training

Breakout Group Worksheet: Beginner

This worksheet was developed for the Breakout Group session of the httk Virtual Training, hosted by the U.S. Environmental Protection Agency's Center for Computational Toxicology and Exposure on November 8-9, 2023.

For more information about httk:

- Visit the httk package manual: httk: High-Throughput Toxicokinetics (r-project.org)
- Explore httk vignettes: https://CRAN.R-project.org/package=httk
- Learn more about EPA's Rapid Chemical Exposure and Dose Research | US EPA
- Read more in
  - Wambaugh et al. (2018): https://doi.org/10.1093/toxsci/kfy020
  - Pearce et al. (2017): 10.18637/jss.v079.i04
- For questions, contact: NAM@epa.gov

Goals

- Use R's package httk to explore toxicokinetics of a compound of interest.
- Identify physiological and compound-specific parameters.
- Simulate tissue concentrations of chemicals using different exposure scenarios.
- Extract experimental dosing and time course data from data provided in Wambaugh et al. (2018) and replicate the experiment in httk.

Tools

- R software environment (release ≥ 2.10) and a graphical user interface (RStudio recommended)
- R packages:
  - httk (version 2.2.2)
  - readxl
- Excel file of experimental data: BeginnerFiles_toxsci.xlsx. (This file contains subsets=from the Supplementary data provided in Wambaugh et al. (2018): https://doi.org/10.1093/toxsci/kfy020).

Hints

- Navigate to the "help" tab in the pane in the bottom right corner of your Rstudio screen. Here, you can search for httk functions. You can also type "help([function])" into the

consol. For example, type “help(solve_pbtk)” into the console and you will see the help page for the pbtk model.

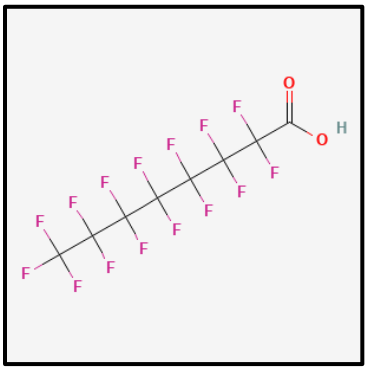
- Chemical-specific data and in vitro data for a compound can be found in the table “chem.physical_and_invitro.data”. One way to subset this table and see data for a specific chemical is the following command:
- subset(chem.physical_and_invitro.data, Compound==chem.name)

```
# Load packages
 # Make sure the following packages are installed:
 #readxl, httk, ggplot2, car, openxlsx

library(readxl)

## Warning: package 'readxl' was built under R version 4.1.3

library(httk)

## Warning: package 'httk' was built under R version 4.1.3

rm(list=ls()) #To start with a clean environment, clear previous calculations.
```

Directions

A. Chemical of Interest

Perfluorooctanoic acid



Perfluorooctanoic acid (PFOA) is a perfluorinated carboxylic acid, belonging to the per- and polyfluorinated substances (PFAS) group of chemicals. It is a lab-made compound that began being used commercially in the mid-1950s as an industrial surfactant. Other applications include textiles and floor wax. Low levels of PFOA can be found in drinking water, some foods, and household dust.

Preferred IUPAC Name: Pentadecafluorooctanoic acid

Other names: Perfluorooctanoic acid, PFOA, C8, Perfluorooctanoate, PFO, Perfluorocaprylic acid, C_8-PFCA, FC-143, F-*n*-octanoic acid
Name in httk: Perfluorooctanoic acid
CASRN: 335-67-1
DSSTox substance identifier (DTXSID): DTXSID8031865
Molecular Formula: $C_8HF_{15}O_2$
SMILES: OC(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F

1. Confirm the DTXSID and CAS number for the chemical Perfluorooctanoic acid.

2. Say we wish to simulate the concentration of Perfluorooctanoic acid acid in the liver.
   a. Which of the following models would be appropriate for this: 1comp, 3comp, pbtk? (**Hint:** *Search solve_'modelname' in the help file to see a diagram of the compartment models for each model.*)

   b. Which model could we use if we needed concentration in the kidney?

   c. What compartments does the pbtk model use? What compartments does 3comp use?

3. Find chemical and physical parameters within httk.

   a. Find logHenry and logP for this chemical (**Hint:** *Search chem.physical_and_invitro.data or get_physchem_param*)

   b. Find human and rat plasma fraction unbound. [**Hint:** *Use chem.physical_and_invitro.data*]

   c. Run parameterize_pbtk or parameterize_3comp to find the fraction unbound (funbound.plasma) in the plasma for rabbits. Were you able to find a value or did you run into an error?

   d. Say that you still want to generate parameters and simulate solutions for rabbits. Mitigate the issue from part (c) and find the funbound.plasma value (**Hint:** *Use the "default.to.human" input.*)

4. We are now going to simulate Perfluorooctanoic acid tissue concentrations to mirror the experimental data found in Wambaugh et al. (2018). The excel file BeginnerFiles_toxsci.xlsx contains multiple data points for multiple experimental chemicals, including Perfluorooctanoic acid.
    - Load the excel file.
        - Make sure the readxl package is installed [install.packages("readxl")] and loaded into R [library("readxl")]
        - Read the .xlsx file into R and name it: my_data = read_excel("path_to_file/ BeginnerFiles_toxsci.xlsx ")
    - Extract the data pertaining only to Pentadecafluorooctanoic acid. (**Hint:** *You can do this by creating a subset-- subset(my_data, Compound == chem.name*)

    a. Notice that there are multiple routes of exposure and subjects tested for our chemical. Also note that each subject corresponds to one longitudinal experiment in which the subject was given a single dose at the start of the experiment. Our first goal is to compare oral dose httk simulations to the oral dose experimental concentrations obtained from subject 81, so we start by extracting experiment specifications for this case.

        i. Find the oral dose and dose units for subject 81 (**Hint:** *Route="po" pertains to oral dosing.*)

        ii. What is the output media and output units? What species was studied?

        iii. How long did the experiment last?

    b. Using these specs, generate oral dose results using both the 3comp and pbtk models and plot them against the experimental data.
    Hints:
        - *Use the solve_3comp() and solve_pbtk() functions with appropriate inputs.*
        - *Output units: httk has units in days. use days = number-of-hours-in-experiment/24. When plotting, make sure to convert httk output units into hours.*
        - *Note that ug/mL = mg/L*
        - *Make sure to use iv.dose=FALSE*
        - *When plotting overlayed items, you can use the following commands (and make sure units of time are the same):*

```
plot(time.experimental, conc.experimental) # will plot points for experimental
data
lines(time.simulation1, conc.simulation1) # will plot a line for simulation 1
lines(time.simulation2, conc.simulation2) # will plot a line for simulation 2
```

Explain the results. Which model produced results simulations that seem to fit the data well? Consider why this might be the case.

c. Our second goal is to compare the IV dose experimental data pertaining to subject 85 with IV dose httk simulations. Extract the experiment specifications for IV.

   i. Find the IV dose and IV dose units for subject 85.

   ii. What is the output media and output units? What species was studied?

   iii. How long did the experiment last?

d. Using these specs, generate iv dose results using both the 3comp and pbtk models and plot them against the experimental data. (**Hint***: Make sure to use iv.dose=TRUE*)

e. Explain the results. Which model produced results simulations that seem to fit the data well? Consider why this might be the case.

i. Compare the oral dose and IV dose time curves. Do they make sense?

ii. Compare the AUC for both cases and explain similarities or differences.

5. Some chemical exposure occurs frequently. In the case of PFOS, a subject might be exposed to multiple doses over a period of time rather than just one single initial exposure. Say you wish to investigate how multiple exposures affect the concentration of the substance in the plasma by splitting the 1 mg/kg oral dose into 3 even oral bolus doses: 33% of the chemical was given at time 0, 33% was given at 24 hours, and 33% was given at 36 hours. The experiment lasts for 3 days total. (**Hint:** *Use a dosing matrix in the solve function and set dose = 0*)
    - Example of how to create a dosing matrix. Use the following example of a simple arbitrary dosing matrix to create one for the above scenario.
        - Example: At time = 0 hours a dose of 10 mg/kg was given and at time = 6 hours a dose of 20 mg/kg was given.
          dose.mat = matrix(c(0, 0.25, 10, 20), nrow=2)
          colnames(dose.mat) = c("time","dose")

    a. Use the model you think gave the best fit (3comp or pbtk). Plot the concentration in plasma over time. How does the solution differ from that in 4b?

6. Find conversion factor for chemical from ppmv to uM for PFOA. (**Hint:** *Use convert_units()*)

7. httk contains physiological/biochemical parameters for some or all of the following species: humans, rats, rabbits, and mice. For which species are there parameters for Perfluorooctanoic acid?

8. Find the following parameters using the pbtk model (**Hint:** *Use parameterize_pbtk()*):
    a. Liver to plasma partition coefficient for PFOA. (**Hint:** *Partition coefficients have the suffix "2pu".*)

b. Fractional volume of the kidney and fractional blood flow rate to kidney for an average human.