

httk: High-Throughput Toxicokinetics Virtual Training

Breakout Group Worksheet: Intermediate

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 \(r-project.org\)](http://httk.r-project.org)
 - Explore httk vignettes: <https://CRAN.R-project.org/package=httk>
 - Learn more about EPA's [Rapid Chemical Exposure and Dose Research](#)
 - Read more in:
 - Wambaugh et al. (2018): <https://doi.org/10.1093/toxsci/kfy020>
 - Pearce et al. (2017): [10.18637/jss.v079.i04](https://doi.org/10.18637/jss.v079.i04)
 - For questions, contact: NAM@epa.gov
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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 \geq 2.10) and a graphical user interface (RStudio recommended)
- R packages:
 - httk (version 2.2.2)
 - readxl
- Excel file of experimental data: IntermediateFiles_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_pbtck)” into the console and you will see the help page for the pbtck 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)Clear all previous calculations to begin the training with a clean environment.

```
# 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)

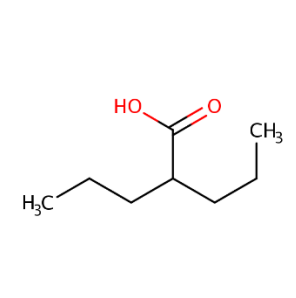
## Warning: package 'httk' was built under R version 4.1.3

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

Directions

A. Chemical of Interest

Valproic Acid



Valproic acid is a low-molecular weight branched-chain fatty acid which is widely used for treating several diseases, including epilepsy and bipolar disorder. Until the 1960s, it was used only as a solvent for pharmaceuticals and some industrial chemicals. In 1963, several

compounds were found to be positive for anticonvulsant activity when valproic acid was used as a solvent, which led the use of valproic acid for treating epilepsy in 1967 followed by the US FDA approval in 1978 for similar use. Valproic acid is a medication used for the treatment of seizures and migraines.

Preferred IUPAC Name: 2-propylpentanoic acid

Other names: Valporate

Name in httk: Valproic acid

CASRN: 99-66-1

DSSTox substance identifier (DTXSID): DTXSID6023733

Molecular Formula: C₈H₁₆O₂

SMILES: CCCC(CCC)C(=O)O

<https://comptox.epa.gov/dashboard/chemical/details/DTXSID6023733>

- 1) How do you read an excel file? Let's practice with the Valproic acid iv data dosed at 10 mg/kg (For the following questions, read in the IntermediateFiles_toxsci.xlsx excel file and the "valproic_acid_iv_oral" sheet using the following commands to read in individual sheets

- 2) What are the contents of this file and how can we load the time course data into R?
Hint: *You will need to select the Time and Value columns using the database name and the \$ operator. You can call the columns by their names or column number. Note the species of interest and the time frame for the experiment.*

- 3) How can the experimental data be compared to the httk simulations? Remember that you can choose between a simpler model (solve_3comp) and a more complex model (solve_pbtk). You can run the simulations for both and compare them in one plot. Make sure that you set input.units and output.units to the same units found in the excel file (Note that ug/mL = mg/L)
 - a. Solve the 3 compartmental model for rats at 10 mg/kg IV
 - b. Solve the pbtk model
 - c. Plot the experimental data (**Hint:** *Make sure to plot the output that corresponds to the tissue from the excel file, shown in the "Media" column.*)
 - d. Plot output for each solution (**Hint:** *You can plot the experimental data points from the excel file starting with the plot() function, then use lines() for each additional simulation line.*)

- 4) Why are the i.v. results between 3comp and pbtk so different?

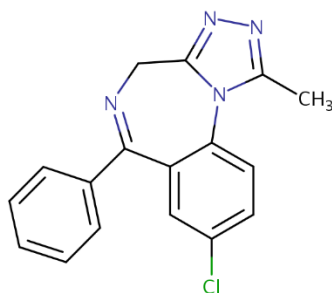
- 5) Note: To see the schematics for each model, go to the console and type `??solve_3comp` or `??solve_pbtok`. Let's assume you made a mistake in entering the initial dose and want to correct the error. Let's assume you entered the initial dose as 10 mg/kg and you would like to change it to 12 mg/kg.
 - a. Demonstrate how to change the listed dose inside the solve function using the pbtok model.
 - b. Plot the output and compare it to the output from the 10 mg/kg solution using lines
 - c. What happens to the results?

- 6) What if the parameter we need to change is not listed in the solve function? How do we change it from the default values? Note- we will use the pbtok model for the rest of the analysis.
 - a. We need to use the parameterize function for the corresponding pbpk model. Using the excel file, find the body weight for the rat in the study. Compare this to the default body weight ("BW") value you get from running `parameterize_pbtok(chem.name = chem.name, species = "Rat")` Are these values different? .
 - b. Run the simulations with the modified body weight (recall, this corresponds to 10 mg/kg IV dose for rats for 4 hours)
 - c. Plot both the solution from part 3d and new solution you just found.

- 7) Let us continue using the valproic acid example for rats but now consider changing clearance or other model parameters. How might some of the other parameters affect the model solutions? First list the pbtok parameters so we can choose which one we want to change.
 - a. Let's start with `Clmetabolismc`(hepatic clearance) for this specific example. *(Hint: Begin by finding the default value for hepatic clearance. Then either increase or decrease the value using `parameterize_pbtok()` and compare solutions you get when using the default value vs. a different value.)*

B. Chemical of Interest

Alprazolam



Alprazolam also known as Xanax, is a fast-acting, potent tranquilizer of moderate duration within the triazolobenzodiazepine group of chemicals called benzodiazepines. It is most commonly used in management of anxiety disorders. Alprazolam is used mainly orally.

Preferred IUPAC Name: 8-Chloro-1-methyl-6-phenyl-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine

Other names: Xanax

Name in htk: Alprazolam

CASRN: 28981-97-7

DSSTox substance identifier (DTXSID): DTXSID4022577

Molecular Formula: C₁₇H₁₃ClN₄

SMILES: CC1=NN=C2CN=C(C3=CC=CC=C3)C3=CC(Cl)=CC=C3N12

<https://comptox.epa.gov/dashboard/chemical/details/DTXSID4022577>

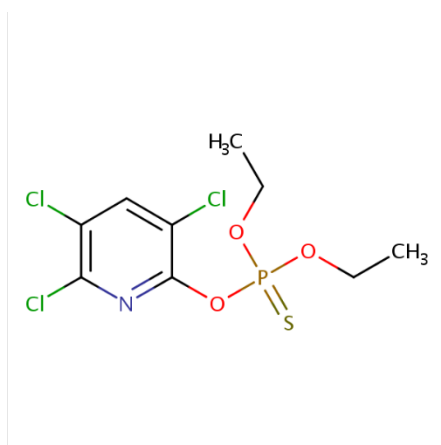
- 1) Now we will focus on another chemical, Alprazolam. Read the sheet titled “alprazolam_oral7mgperkg” from the IntermediateFiles_toxsci.xlsx excel file and find the details of the experiment as you did previously (i.e. find dose, route, species, time, etc.). Note- clear your environment!
 - a) Identify the default values for fraction unbound in rat plasma and pKa_Donor. Then
 - b) Compute solutions using both the 3 compartment and pbtk models, like we did in the first example. Plot both solutions and compare these to the experimental data.

- 2) What happens if we change the stomach absorption? In htk, the default value for the parameter for fraction absorbed, “Fgutabs” is 1. (fraction absorbed = “Fgutabs. For this question, use the 3 compartment model only.
 - a) First, find the experimental rat body weight, apply it to the parameters, and find the solution using the 3 compartment model using default values for all other parameters besides BW.

- b) We wish to see how changing F_{gutabs} can impact the fit. Reduce the fraction of chemical absorbed by assigning numerous values to F_{gutabs} that are less than 1 (i.e. try setting F_{gutabs} to 0.6, 0.3, 0.15) and find solutions for each case. Plot all of these solutions together.
 - c) How does reducing the fraction absorbed affect time curve concentrations for our chemical?
- 3) How do the oral dose and oral absorption affect the solution? (**Hint:** Use the excel file sheet titled "*alprazolam_oral12mgperkg*" (for alprazolam 12.5 mg/kg) in combination with the excel file already loaded for 7 mg/kg.)
- a) Read in the data for 12.5 mg/kg alprazolam. Find the body weight of the rat from this experiment and parameterize the 3 compartment model with that body weight. Also set F_{gutabs} to the value you found to give the best fit from the previous question. Solve the model.
 - b) Compare solutions for 12.5 mg/kg alprazolam with the ideal F_{gutabs} to the solution for 7 mg/kg alprazolam with the ideal F_{gutabs} you found above. You may plot data points and solutions to see how they compare.
 - c) You may wish to compare other F_{gutabs} values as well. Recall from the previous exercise that higher values of F_{gutabs} overpredicted the solution for 7 mg/kg of alprazolam. Does this hold true for the higher dose of 12.5 mg/kg alprazolam or does it appear that fraction absorbed changes as dose changes? That is, does experimental data indicate that a higher dose may raise or lower fraction absorbed or does it remain constant?

C. Chemical of Interest

Chlorpyrifos



Chlorpyrifos, also known as Chlorpyrifos ethyl, is an organophosphate pesticide that has been used on crops, animals, and buildings, and in other settings, to kill several pests, including insects and worms. It acts on the nervous systems of insects by inhibiting the acetylcholinesterase enzyme. Chlorpyrifos is considered moderately hazardous to humans (Class II) by the World Health Organization.

Preferred IUPAC Name: O,O-Diethyl O-(3,5,6-trichloropyridin-2-yl) phosphorothioate

Other names: Chlorpyrifos ethyl

Name in htkk: Chlorpyrifos

CASRN: 2921-88-2

DSSTox substance identifier (DTXSID): DTXSID4020458

Molecular Formula: C₉H₁₁Cl₃NO₃PS

SMILES: CCOP(=S)(OCC)OC1=NC(Cl)=C(Cl)C=C1Cl

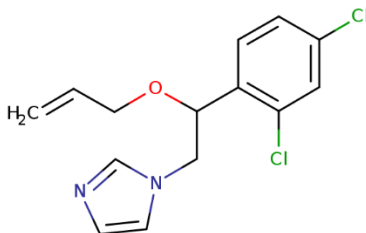
<https://comptox.epa.gov/dashboard/chemical/details/DTXSID4020458>

- 1) Let us now consider Chlorpyrifos. Please load the experimental chlorpyrifos data by using the excel sheet titled "chlorpyrophos_po_50mgperkg" (`read_excel("IntermediateFiles_toxsci.xlsx", "chlorpyrophos_po_50mgperkg")`) for these set of questions.
 - a) determine the following parameters using `get_physchem_param()`: logP, MW, logHenry . What might these parameters tell us about Chlorpyrifos? (**Hint:** *Glycerol (a compound with logHenry = -7.8) is a non-volatile chemical that is often considered to be a benchmark chemical for volatility; if a chemical's logHenry > -7.8, then that compound is more volatile than glycerol. Note that VOCs can be inhaled.*)
 - b) Find the specs of the experiment (dose, route, time, species, etc.), set `Fgutabs = 0.15`, and find solutions to correspond with the given experimental data using `solve_pbtok`.

- c) Optional: consider why we might initially use the pbtk model (over the 3comp model) for this chemical. Plot the experimental data vs the httk solution for plasma concentration. Does the solution fit the data well? Why or why not do you think this may be the case?
- 2) httk also include an inhalation model (solve_gas_pbtk). Due to the modular approach designed in httk, the gas model can simulate oral, iv, and inhalation exposures; it contains a lung compartment with exhalation. To set an oral route, use the following command:
iv.dose = FALSE
- a. Using solve_gas_pbtk(), find the solution for the oral dose of chlorpyrifos (use the same scenario as the previous question). (**Hint:** *gas_pbtk has slightly different inputs than the 3comp and pbtk models. Set exp.conc = 0 (because there is no inhalation/concentration), iv.dose = FALSE, and dose = 50. Make sure to set correct species, time, and input.units and output.units as always.*)
- b. Plot the results. What do you notice?

D. Chemical of Interest

Imazalil



Imazalil is a fungicide widely used in agriculture, particularly in the growing of citrus fruits. It is found on a wide variety of fruits and vegetables, but it is primarily used on tubers for storage. Trade names include Freshgard, Fungaflor, and Nuzone. It is also used in veterinary medicine as a topical antimycotic.

Preferred IUPAC Name: 1-[2-(2,4-Dichlorophenyl)-2-[(prop-2-en-1-yl)oxy]ethyl]-1H-imidazole

Other names: Enilconazole, chloramizole, 1-[2-(2,4-Dichlorophenyl)-2-(2-propenyloxy)ethyl]-1H-imidazole, (+/-)-Imazalil, 1-[2-(aliloxi)-2-(2,4-diclorofenil)etil]-1H-imidazol

Name in htkk: imazalil

CASRN: 35554-44-0

DSSTox substance identifier (DTXSID):

Molecular Formula: C₁₄H₁₄Cl₂N₂O

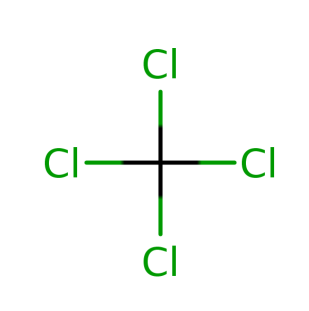
SMILES: ClC1=CC(Cl)=C(C=C1)C(CN1C=CN=C1)OCC=C

<https://comptox.epa.gov/dashboard/chemical/details/DTXSID8024151>

- 1) Consider Imazalil, a volatile chemical given by an IV dose. Read the sheet name corresponding to imazalil ("imazalil_iv_1mgperkg") into R using `read_excel("IntermediateFiles_toxsci.xlsx", "imazalil_iv_1mgperkg")`
 - a. Find the following parameters corresponding to imazalil: logP, MW, logHenry, pKa_Accept
 - b. Change `Fgutabs = 0.15`. Then using the experimental specs for this chemical, simulate plasma concentration solutions using `solve_gas_pbtok()`. (**Hint:** Use `iv.dose = TRUE`.)
- c) Run the gas pbtok model?
- d) Plot the plasma concentration /time for rat at 1 mg/kg IV

E. Chemical of Interest

Carbon tetrachloride



Carbon tetrachloride, is a non-flammable, colourless liquid with a "sweet" chloroform-like smell that can be detected at low levels. It was formerly widely used in fire extinguishers, as a precursor to refrigerants and as a cleaning agent but has since been phased out because of environmental and safety concerns. It is persistent in the environment and a model compound for hepatotoxicity. Exposure to high concentrations of carbon tetrachloride can affect the central nervous system and degenerate the liver and kidneys. Prolonged exposure can be fatal.

Preferred IUPAC Name: Tetrachloromethane

Other names: carbon tet, tetrachloromethane, methane tetrachloro-

Name in httk: carbon tetrachloride

CASRN: 56-23-5

DSSTox substance identifier (DTXSID): DTXSID8020250

Molecular Formula: CCl₄

SMILES: ClC(Cl)(Cl)Cl

<https://comptox.epa.gov/dashboard/chemical/details/DTXSID8020250>

- 1) In this example we will use the VOC carbon tetrachloride. Please use the excel file sheet titled 'ccl4_Rat_1000ppm' from the pre-training materials provided. Read in the data. Unlike the previous examples, we only have time and arterial blood concentration data. The details of this experiment are as follows: A rat (of unknown body weight, so assume it to be default) was given a constant 1000 ppmv inhaled dose of carbon tetrachloride for the duration 1.68 hours – 2 hours. The rat's arterial blood was measured in mg/L.
 - a. Use `solve_gas_pbtk()` to find the solution. (**Hint:** To set event dosing such as this, create a dosing matrix where the first column are the times corresponding to start of dose and end of dose duration and the second column corresponds to the dose given (i.e. 1000 at the start of the dose and 0 at the end of the dose). The column names of this matrix are `c("time", "forcing_values")`. Instead of `dose = "some dose"`, use `forcings = "dosing matrix"`.)

- i. Solve_gas_pbtk defaults to linear clearance unless "vmax" and "km" are input. Use $v_{max} = 0.25$ and $k_m = 0.25$.
- b. Plot the experimental data and htkk solutions.