

httk: High-Throughput Toxicokinetics Virtual Training

Breakout Group Worksheet: Intermediate **ANSWERS**

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: [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](https://doi.org/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: 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_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:

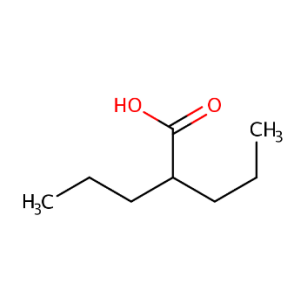
```
subset(chem.physical_and_invitro.data, Compound==chem.name)Clear all previous calculations to begin the training with a clean environment.
```

```
# 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 htk: 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 read_excel("IntermediateFiles_toxsci.xlsx", "valproic_acid_iv_oral").

```
setwd("set/path_to_working_directory") #Directory where all data and files are located.
valproic_acid_iv_10mgperkg <- read_excel("IntermediateFiles_toxsci.xlsx", "valproic_acid_iv_10mgperkg")
View(valproic_acid_iv_10mgperkg)
```

- 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.

```
colnames(valproic_acid_iv_10mgperkg)# Lists all the column names to help you select the ones you need.
## [1] "Compound" "DSSTox_Substance_Id" "CAS"
## [4] "Reference" "Species" "Species.Weight"
## [7] "Species.Weight.Units" "Dose" "Dose.Units.and.Type"
## [10] "Time" "Time.Units" "Media"
## [13] "Media.Units" "Value" "Units"
## [16] "Route"
time.valproic<-(valproic_acid_iv_10mgperkg$Time)/24 #httk uses days as default time unit.
conc.valproic<-valproic_acid_iv_10mgperkg$Value
```

- 3) How can the experimental data be compared to the htk 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.**)

```

out3<-solve_3comp(chem.name = "Valproic acid",
                  species="Rat",
                  iv.dose=TRUE,
                  dose=10,
                  input.units = "mg/kg",
                  output.units = "mg/L", days = 4/24)

## Warning in apply_clint_adjustment(Clint.point, Fu_hep = Fu_hep,
## suppress.messages = suppress.messages): Clint adjusted for in vitro partitioning
## (Kilford, 2008), see calc_hep_fu.

## Warning in apply_fup_adjustment(fup.point, fup.correction = fup.adjustment, :
## Fup adjusted for in vivo lipid partitioning (Pearce, 2017), see
## calc_fup_correction.

## Warning in available_rblood2plasma(chem.cas = chem.cas, species = species, : Rat
## in vivo measured Rblood2plasma used.

## None of the monitored components undergo unit conversions (i.e. conversion factor of 1).
##
## AUC is area under the plasma concentration curve in uM*days units with Rblood2plasma = 0.74.
## The model outputs are provided in the following units:
## mg: Asyscomp, Atubules, Ametabolized
## mg/l: Cliver, Csyscomp
## mg/l*days: AUC

    out.pbt<-solve_pbt(chem.name = "Valproic acid",
                      species="Rat",
                      iv.dose=TRUE,
                      dose=10,
                      input.units = "mg/kg",
                      output.units = "mg/L", days = 4/24)

## Warning in apply_clint_adjustment(Clint.point, Fu_hep = Fu_hep,
## suppress.messages = suppress.messages): Clint adjusted for in vitro partitioning
## (Kilford, 2008), see calc_hep_fu.

```

```

## Warning in apply_fup_adjustment(fup.point, fup.correction = fup.adjustment
, :
## Fup adjusted for in vivo lipid partitioning (Pearce, 2017), see
## calc_fup_correction.

## Warning in available_rblood2plasma(chem.cas = chem.cas, species = species,
: Rat
## in vivo measured Rblood2plasma used.

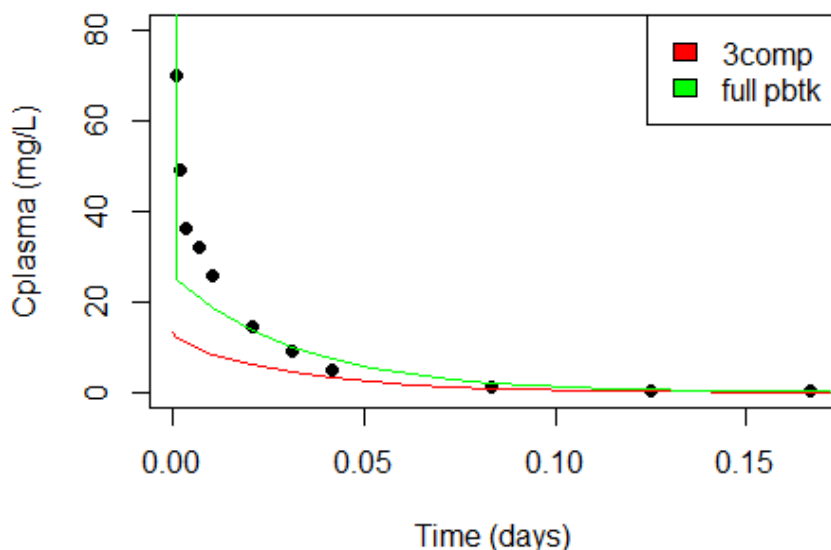
## None of the monitored components undergo unit conversions (i.e. conversio
n factor of 1).
##
## AUC is area under the plasma concentration curve in uM*days units with Rbl
ood2plasma = 0.74.
## The model outputs are provided in the following units:
## mg: Aven, Atubules, Ametabolized
## mg/l: Cgut, Cliver, Cven, Clung, Cart, Crest, Ckidney, Cplasma
## mg/l*days: AUC

#First plot the experimental data
plot(time.valproic, conc.valproic, ylim=c(0,80), xlab="Time (days)",
      ylab="Cplasma (mg/L)", main="i.v. Valproic Acid in Rat 10 mg/kg", pch=19
)
#Plot the output of each solution using lines. out3 contains the solutions fo
r solve_3comp. Similarly, out.pbtk contains the solutions for solve_pbtk.

lines(out3[, "time"], out3[, "Cplasma"], col="red")
lines(out.pbtk[, "time"], out.pbtk[, "Cplasma"], col="green")
legend(x="topright", legend=c("3comp", "full pbtk"), fill=c("red", "green"))

```

i.v. Valproic Acid in Rat 10 mg/kg



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

Answer: The difference between the iv models consists of where the injection is made and the volume of each compartment. For the 3comp model, the iv injection site is part of the rest of the body compartment. For the pbtk model, the iv has a separate venous compartment, which is much smaller than the rest of the body compartment. In essence the pbtk gives a much higher initial peak due to the difference in compartment sizes. The concentration will be divided by the volume of each compartment, making the pbtk result much higher.

- 5) Note: To see the schematics for each model, go to the console and type `??solve_3comp` or `??solve_pbtk`. 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.
- Demonstrate how to change the listed dose inside the solve function using the pbtk model.
 - Plot the output and compare it to the output from the 10 mg/kg solution using lines.
 - What happens to the results?

```
out2.pbtk<-solve_pbtk(chem.name = "Valproic acid",
                      species="Rat",
                      iv.dose=TRUE,
                      dose=12,    #Change from 10 to 12 mg/kg
                      input.units = "mg/kg",
                      output.units = "mg/L", days = 4/24)

## Warning in apply_clint_adjustment(Clint.point, Fu_hep = Fu_hep,
## suppress.messages = suppress.messages): Clint adjusted for in vitro partit
ioning
## (Kilford, 2008), see calc_hep_fu.

## Warning in apply_fup_adjustment(fup.point, fup.correction = fup.adjustment
, :
## Fup adjusted for in vivo lipid partitioning (Pearce, 2017), see
## calc_fup_correction.

## Warning in available_rblood2plasma(chem.cas = chem.cas, species = species,
: Rat
## in vivo measured Rblood2plasma used.

## None of the monitored components undergo unit conversions (i.e. conversio
n factor of 1).
##
## AUC is area under the plasma concentration curve in uM*days units with Rbl
ood2plasma = 0.74.
```

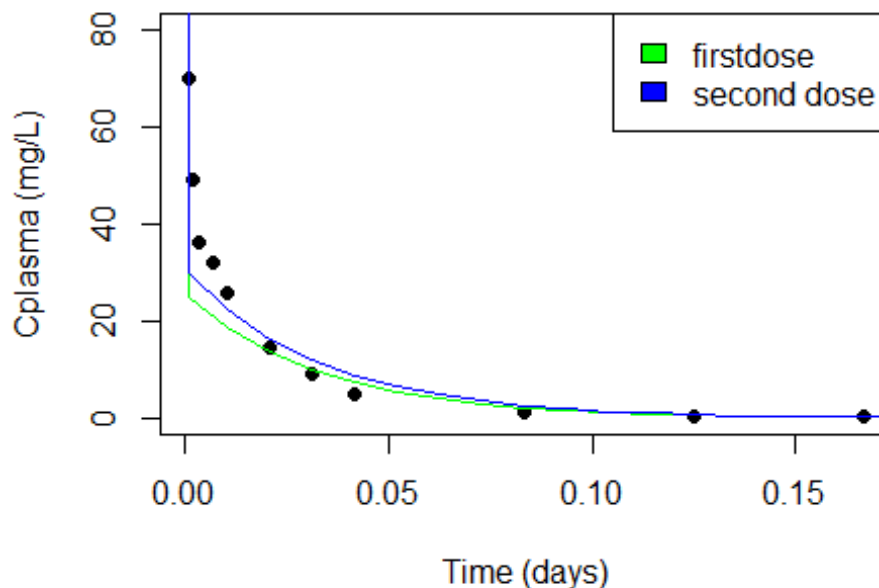
```

## The model outputs are provided in the following units:
## mg: Aven, Atubules, Ametabolized
## mg/l: Cgut, Cliver, Cven, Clung, Cart, Crest, Ckidney, Cplasma
## mg/l*days: AUC

plot(time.valproic, conc.valproic, ylim=c(0,80),xlab="Time (days)",
      ylab="Cplasma (mg/L)", main="i.v. Valproic Acid in Rat 10 mg/kg", pch=19
)
lines(out.pbtk[, "time"],out.pbtk[, "Cplasma"],col="green")
lines(out2.pbtk[, "time"],out2.pbtk[, "Cplasma"],col="blue")
legend(x="topright",legend=c("firstdose", "second dose"),fill=c("green", "blue
"))

```

i.v. Valproic Acid in Rat 10 mg/kg



Answer: The initial dose changes the simulations during the transition from the early peak to the clearance phase.

- 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 pbtk 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_pbtk(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.

```

parms<-parameterize_pbtck(chem.name = "valproic acid", species = "Rat")

## Warning in apply_clint_adjustment(Clint.point, Fu_hep = Fu_hep,
## suppress.messages = suppress.messages): Clint adjusted for in vitro partitioning
## (Kilford, 2008), see calc_hep_fu.

## Warning in apply_fup_adjustment(fup.point, fup.correction = fup.adjustment
, :
## Fup adjusted for in vivo lipid partitioning (Pearce, 2017), see
## calc_fup_correction.

## Warning in available_rblood2plasma(chem.cas = chem.cas, species = species,
: Rat
## in vivo measured Rblood2plasma used.

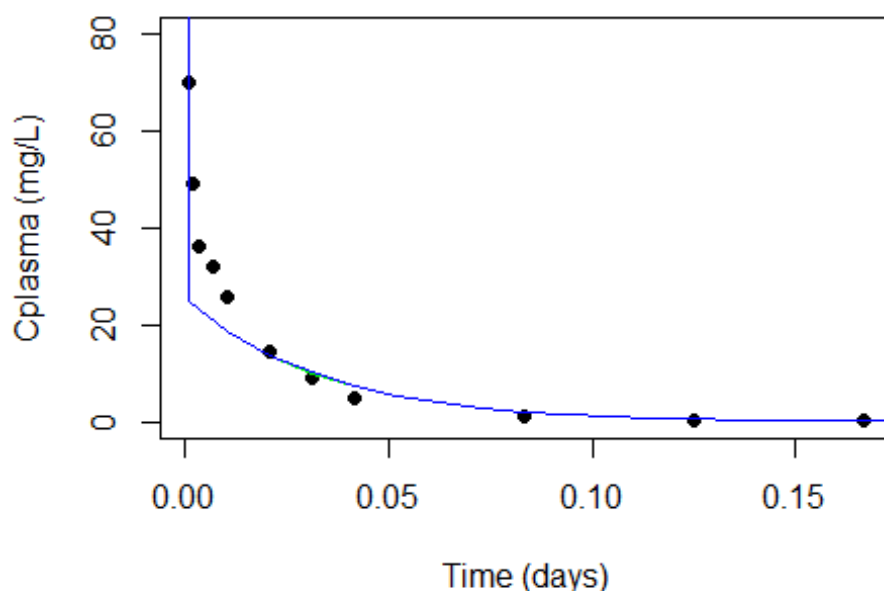
#The body weight listed in the excel file is different from the default. To change use the parms object created in the previous line.
parms$BW<-0.3
#Run the simulations with the new parameter list
out2.pbtck<-solve_pbtck(chem.name = "valproic acid",
                        parameters = parms,
                        species = "Rat",
                        iv.dose=TRUE,
                        dose=10,
                        input.units = "mg/kg",
                        output.units = "mg/L", days = 4/24)

## None of the monitored components undergo unit conversions (i.e. conversion factor of 1).
##
## AUC is area under the plasma concentration curve in uM*days units with Rblood2plasma = 0.74.
## The model outputs are provided in the following units:
## mg: Aven, Atubules, Ametabolized
## mg/l: Cgut, Cliver, Cven, Clung, Cart, Crest, Ckidney, Cplasma
## mg/l*days: AUC

#Now you can plot both the new and old line plots
plot(time.valproic, conc.valproic, ylim=c(0,80),xlab="Time (days)",
      ylab="Cplasma (mg/L)", main="i.v. Valproic Acid in Rat 10 mg/kg", pch=19
)
lines(out.pbtck[, "time"],out.pbtck[, "Cplasma"],col="green")
lines(out2.pbtck[, "time"],out2.pbtck[, "Cplasma"],col="blue")

```


i.v. Valproic Acid in Rat 10 mg/kg



Result: Not much difference observed between the two body weights.

- 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 pbtk 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_pbtk()` and compare solutions you get when using the default value vs. a different value.)

```
parms<-parameterize_pbtk(chem.name = "valproic acid", species = "Rat")
## Warning in apply_clint_adjustment(Clint.point, Fu_hep = Fu_hep,
## suppress.messages = suppress.messages): Clint adjusted for in vitro partitioning
## (Kilford, 2008), see calc_hep_fu.
## Warning in apply_fup_adjustment(fup.point, fup.correction = fup.adjustment
, :
## Fup adjusted for in vivo lipid partitioning (Pearce, 2017), see
## calc_fup_correction.
## Warning in available_rblood2plasma(chem.cas = chem.cas, species = species,
: Rat
## in vivo measured Rblood2plasma used.
```

parms *#The object created to store the parameters from parameterize*

```
## $BW
## [1] 0.25
##
## $Clint
## [1] 4.855
##
## $Clint.dist
## [1] NA
##
## $Clmetabolismc
## [1] 1.173
##
## $Fgutabs
## [1] 1
##
## $Fhep.assay.correction
## [1] 0.9474
##
## $Funbound.plasma
## [1] 0.3656
##
## $Funbound.plasma.adjustment
## [1] 0.988
##
## $Funbound.plasma.dist
## [1] NA
##
## $hematocrit
## [1] 0.46
##
## $Kgut2pu
## [1] 1.432
##
## $kgutabs
## [1] 2.18
##
## $Kkidney2pu
## [1] 3.904
##
## $Kliver2pu
## [1] 4.398
##
## $Klung2pu
## [1] 1.161
##
## $Krbc2pu
## [1] 0.5593
##
```

```
## $Krest2pu
## [1] 1.001
##
## $liver.density
## [1] 1.05
##
## $MA
## [1] 10.99
##
## $million.cells.per.gliver
## [1] 110
##
## $MW
## [1] 144.2
##
## $pKa_Accept
## pKa_Accept
## "None"
##
## $pKa_Donor
## pKa_Donor
## "5.25"
##
## $Pow
## [1] 562.3
##
## $Qcardiacc
## [1] 12.56
##
## $Qgfr
## [1] 0.2223
##
## $Qgutf
## [1] 0.1324
##
## $Qkidneyf
## [1] 0.1243
##
## $Qliverf
## [1] 0.0271
##
## $Qrestf
## [1] 0.6762
##
## $Rblood2plasma
## [1] 0.74
##
## $Vartc
## [1] 0.02889
##
```

```

## $Vgutc
## [1] 0.02585
##
## $Vkidneyc
## [1] 0.006952
##
## $Vliverc
## [1] 0.03486
##
## $Vlungc
## [1] 0.00476
##
## $Vrestc
## [1] 0.7312
##
## $Vvenc
## [1] 0.02889

parms$Clmetabolismc #Default value is 1.17 L/h/kg BW.
## [1] 1.173

out.cl<-solve_pbtk(chem.name = "Valproic acid",
                   species="Rat",
                   iv.dose=TRUE,
                   dose=10,
                   input.units = "mg/kg",
                   output.units = "mg/L", days = 4/24)

## Warning in apply_clint_adjustment(Clint.point, Fu_hep = Fu_hep,
## suppress.messages = suppress.messages): Clint adjusted for in vitro partitioning
## (Kilford, 2008), see calc_hep_fu.

## Warning in apply_fup_adjustment(fup.point, fup.correction = fup.adjustment
, :
## Fup adjusted for in vivo lipid partitioning (Pearce, 2017), see
## calc_fup_correction.

## Warning in available_rblood2plasma(chem.cas = chem.cas, species = species,
: Rat
## in vivo measured Rblood2plasma used.

## None of the monitored components undergo unit conversions (i.e. conversion factor of 1).
##
## AUC is area under the plasma concentration curve in uM*days units with Rblood2plasma = 0.74.
## The model outputs are provided in the following units:
## mg: Aven, Atubules, Ametabolized

```

```
## mg/l: Cgut, Cliver, Cven, Clung, Cart, Crest, Ckidney, Cplasma
## mg/l*days: AUC
```

```
parms$Clmetabolismc<-10
```

```
parms
```

```
## $BW
```

```
## [1] 0.25
```

```
##
```

```
## $Clint
```

```
## [1] 4.855
```

```
##
```

```
## $Clint.dist
```

```
## [1] NA
```

```
##
```

```
## $Clmetabolismc
```

```
## [1] 10
```

```
##
```

```
## $Fgutabs
```

```
## [1] 1
```

```
##
```

```
## $Fhep.assay.correction
```

```
## [1] 0.9474
```

```
##
```

```
## $Funbound.plasma
```

```
## [1] 0.3656
```

```
##
```

```
## $Funbound.plasma.adjustment
```

```
## [1] 0.988
```

```
##
```

```
## $Funbound.plasma.dist
```

```
## [1] NA
```

```
##
```

```
## $hematocrit
```

```
## [1] 0.46
```

```
##
```

```
## $Kgut2pu
```

```
## [1] 1.432
```

```
##
```

```
## $kgutabs
```

```
## [1] 2.18
```

```
##
```

```
## $Kkidney2pu
```

```
## [1] 3.904
```

```
##
```

```
## $Kliver2pu
```

```
## [1] 4.398
```

```
##
```

```
## $Klung2pu
```

```
## [1] 1.161
```

```
##  
## $Krbc2pu  
## [1] 0.5593  
##  
## $Krest2pu  
## [1] 1.001  
##  
## $liver.density  
## [1] 1.05  
##  
## $MA  
## [1] 10.99  
##  
## $million.cells.per.gliver  
## [1] 110  
##  
## $MW  
## [1] 144.2  
##  
## $pKa_Accept  
## pKa_Accept  
## "None"  
##  
## $pKa_Donor  
## pKa_Donor  
## "5.25"  
##  
## $Pow  
## [1] 562.3  
##  
## $Qcardiacc  
## [1] 12.56  
##  
## $Qgfrf  
## [1] 0.2223  
##  
## $Qgutf  
## [1] 0.1324  
##  
## $Qkidneyf  
## [1] 0.1243  
##  
## $Qliverf  
## [1] 0.0271  
##  
## $Qrestf  
## [1] 0.6762  
##  
## $Rblood2plasma  
## [1] 0.74
```

```

##
## $Vartc
## [1] 0.02889
##
## $Vgutc
## [1] 0.02585
##
## $Vkidneyc
## [1] 0.006952
##
## $Vliverc
## [1] 0.03486
##
## $Vlungc
## [1] 0.00476
##
## $Vrestc
## [1] 0.7312
##
## $Vvenc
## [1] 0.02889

out.cl.high<-solve_pbt(chem.name = "Valproic acid",

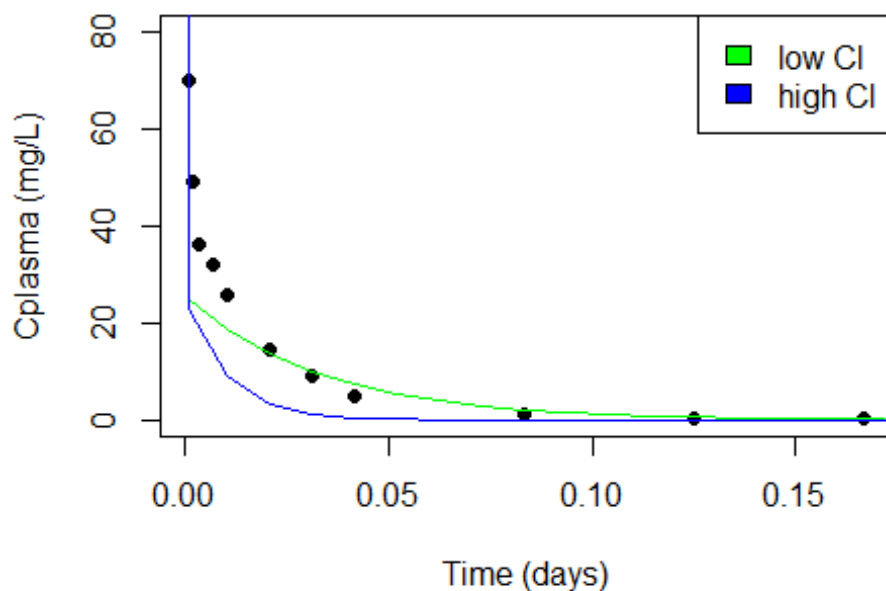
                        parameters=parms, #This makes the change
                        species=Rat,
                        iv.dose=TRUE,
                        dose=10,
                        input.units = "mg/kg",
                        output.units = "mg/L", days = 4/24)

## None of the monitored components undergo unit conversions (i.e. conversion factor of 1).
##
## AUC is area under the plasma concentration curve in uM*days units with Rblood2plasma = 0.74.
## The model outputs are provided in the following units:
## mg: Aven, Atubules, Ametabolized
## mg/l: Cgut, Cliver, Cven, Clung, Cart, Crest, Ckidney, Cplasma
## mg/l*days: AUC

plot(time.valproic, conc.valproic, ylim=c(0,80),xlab="Time (days)",
      ylab="Cplasma (mg/L)", main="i.v. Valproic Acid in Rat 10 mg/kg", pch=19
)
lines(out.cl[, "time"],out.cl[, "Cplasma"],col="green")
lines(out.cl.high[, "time"],out.cl.high[, "Cplasma"],col="blue")
legend(x="topright",legend=c("low Cl", "high Cl"),fill=c("green", "blue"))

```

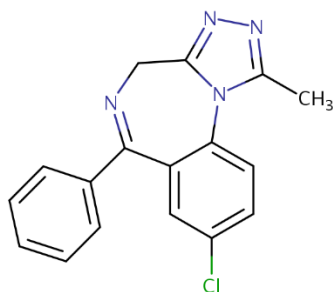
i.v. Valproic Acid in Rat 10 mg/kg



Result: The htk Clearance value provides a good fit for the data. There is no need for further adjustment.

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


```

## None of the monitored components undergo unit conversions (i.e. conversion factor of 1).
##
## AUC is area under the plasma concentration curve in Um*days units with Rblood2plasma = 0.78.
## The model outputs are provided in the following units:
## mg: Aintestine, Atubules, Ametabolized
## mg/l: Cliver, Csyscomp
## mg/l*days: AUC

p<-parameterize_pbtok(chem.name="alprazolam",species="Rat")

## Warning in apply_clint_adjustment(Clint.point, Fu_hep = Fu_hep,
## suppress.messages = suppress.messages): Clint adjusted for in vitro partitioning
## (Kilford, 2008), see calc_hep_fu.

## Warning in apply_fup_adjustment(fup.point, fup.correction = fup.adjustment, :
## Fup adjusted for in vivo lipid partitioning (Pearce, 2017), see
## calc_fup_correction.

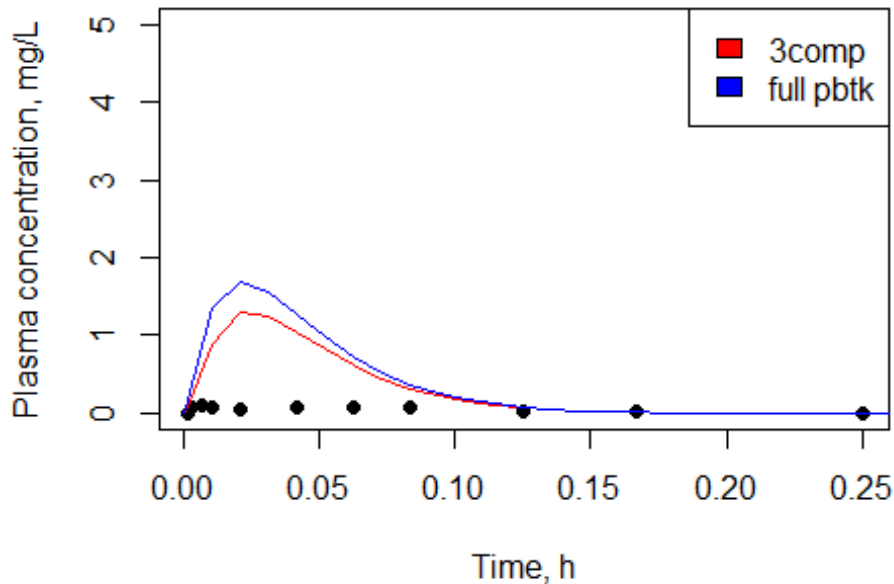
## Warning in available_rblood2plasma(chem.cas = chem.cas, species = species, :
## Human in vivo measured Rblood2plasma substituted.

out.alp_pbtok<-solve_pbtok(chem.name="alprazolam",
                           parameters=p,
                           species="Rat",
                           iv.dose= FALSE,
                           dose = 7,
                           input.units = "mg/kg",
                           output.units = "mg/L", days = 6.24)

## None of the monitored components undergo unit conversions (i.e. conversion factor of 1).
##
## AUC is area under the plasma concentration curve in Um*days units with Rblood2plasma = 0.78.
## The model outputs are provided in the following units:
## mg: Agutlumen, Atubules, Ametabolized
## mg/l: Cgut, Cliver, Cven, Clung, Cart, Crest, Ckidney, Cplasma
## mg/l*days: AUC

plot(alprazolam_oral7mgperkg$Time/24,alprazolam_oral7mgperkg$Value,xlab = "Time, h",ylim=c(0,5),ylab="Plasma concentration, mg/L",pch=19)
lines(out.alp[,"time"],out.alp[,"Cplasma"],col="red")
lines(out.alp_pbtok[,"time"],out.alp_pbtok[,,"Cplasma"],col="blue")
legend(x="topright",legend=c("3comp","full pbtok"),fill=c("red","blue"))

```



2) What happens if we change the stomach absorption? In htkk, 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 Fgutabs can impact the fit. Reduce the fraction of chemical absorbed by assigning numerous values to Fgutabs that are less than 1 (i.e. try setting Fgutabs 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?

```
param<-parameterize_3comp(chem.name="alprazolam",species="Rat")
## Warning in apply_clint_adjustment(Clint.point, Fu_hep = Fu_hep,
## suppress.messages = suppress.messages): Clint adjusted for in vitro partit
ioning
## (Kilford, 2008), see calc_hep_fu.
## Warning in apply_fup_adjustment(fup.point, fup.correction = fup.adjustment
, :
```

```

## Fup adjusted for in vivo lipid partitioning (Pearce, 2017), see
## calc_fup_correction.

## Warning in available_rblood2plasma(chem.cas = chem.cas, species = species,
:
## Human in vivo measured Rblood2plasma substituted.

#Read the Rat BW directly from the spreadsheet
BW<-unique(alprazolam_oral7mgperkg$Species.Weight)
param$BW<-BW
out.alp1<-solve_3comp(chem.name="alprazolam",
                      parameters=param,
                      species="Rat",
                      iv.dose= FALSE,
                      dose = 7,
                      input.units = "mg/kg",
                      output.units = "mg/L", days=6/24)

## None of the monitored components undergo unit conversions (i.e. conversion
n factor of 1).
##
## AUC is area under the plasma concentration curve in uM*days units with Rblood2plasma = 0.78.
## The model outputs are provided in the following units:
## mg: Aintestine, Atubules, Ametabolized
## mg/l: Cliver, Csyscomp
## mg/l*days: AUC

#Now try to reduce Fgutabs to less than 1. This is the absorption rate constant for solve_3comp.
param$Fgutabs<-0.6
out.alp3<-solve_3comp(chem.name="alprazolam",
                      parameters=param,
                      species="Rat",
                      iv.dose= FALSE,
                      dose = 7,
                      input.units = "mg/kg",
                      output.units = "mg/L", days=6/24)

## None of the monitored components undergo unit conversions (i.e. conversion
n factor of 1).
##
## AUC is area under the plasma concentration curve in uM*days units with Rblood2plasma = 0.78.
## The model outputs are provided in the following units:
## mg: Aintestine, Atubules, Ametabolized
## mg/l: Cliver, Csyscomp
## mg/l*days: AUC

```

```

param$Fgutabs<-0.3
out.alp4<-solve_3comp(chem.name="alprazolam",
                      parameters=param,
                      species="Rat",
                      iv.dose= FALSE,
                      dose = 7,
                      input.units = "mg/kg",
                      output.units = "mg/L", days=6/24)

## None of the monitored components undergo unit conversions (i.e. conversion factor of 1).
##
## AUC is area under the plasma concentration curve in uM*days units with Rblood2plasma = 0.78.
## The model outputs are provided in the following units:
## mg: Aintestine, Atubules, Ametabolized
## mg/l: Cliver, Csyscomp
## mg/l*days: AUC

param$Fgutabs<-0.15
out.alp5<-solve_3comp(chem.name="alprazolam",
                      parameters=param,
                      species="Rat",
                      iv.dose= FALSE,
                      dose = 7,
                      input.units = "mg/kg",
                      output.units = "mg/L", days=6/24)

## None of the monitored components undergo unit conversions (i.e. conversion factor of 1).
##
## AUC is area under the plasma concentration curve in uM*days units with Rblood2plasma = 0.78.
## The model outputs are provided in the following units:
## mg: Aintestine, Atubules, Ametabolized
## mg/l: Cliver, Csyscomp
## mg/l*days: AUC

plot(alprazolam_oral7mgperkg$Time/24,alprazolam_oral7mgperkg$Value,xlab = "Time, h",ylim=c(0,2),ylab="Plasma concentration, mg/L",pch=19)
lines(out.alp1[, "time"],out.alp1[, "Cplasma"],col="red")

lines(out.alp3[, "time"],out.alp3[, "Cplasma"],col="magenta")

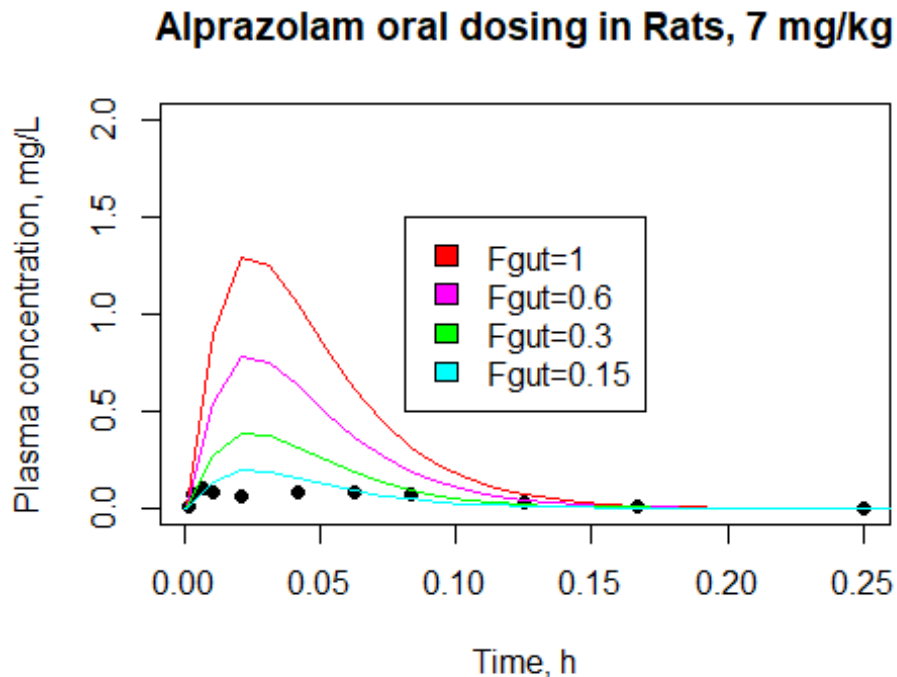
lines(out.alp4[, "time"],out.alp4[, "Cplasma"],col="green")

```

```

lines(out.alp5[, "time"], out.alp5[, "Cplasma"], col="cyan")
legend(x="center", legend=c("Fgut=1", "Fgut=0.6", "Fgut=0.3", "Fgut=0.15"), fill=c(
("red", "magenta", "green", "cyan"))
title(main="Alprazolam oral dosing in Rats, 7 mg/kg")

```



- 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 Fgutabs 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 Fgutabs to the solution for 7 mg/kg alprazolam with the ideal Fgutabs you found above. You may plot data points and solutions to see how they compare.
 - c) You may wish to compare other Fgutabs values as well. Recall from the previous exercise that higher values of Fgutabs 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?

```
alprazolam_oral12mgperkg <- read_excel("IntermediateFiles_toxsci.xlsx", "alprazolam_oral12mgperkg")

param<-parameterize_3comp(chem.name="alprazolam",species="Rat")

## Warning in apply_clint_adjustment(Clint.point, Fu_hep = Fu_hep,
## suppress.messages = suppress.messages): Clint adjusted for in vitro partitioning
## (Kilford, 2008), see calc_hep_fu.

## Warning in apply_fup_adjustment(fup.point, fup.correction = fup.adjustment, :
## Fup adjusted for in vivo lipid partitioning (Pearce, 2017), see
## calc_fup_correction.
```

```
## Warning in available_rblood2plasma(chem.cas = chem.cas, species = species, :
## Human in vivo measured Rblood2plasma substituted.

#To read the BW directly from the excel sheet, we can use unique to select on e value, since it lists the same BW multiple times. If you prefer, you can define the BW as we have done for previous examples.
BW<-unique(alprazolam_oral12mgperkg$Species.Weight)
param$BW<-BW
param$Fgutabs<-0.15
out.alp.12<-solve_3comp(chem.name="alprazolam",
                        parameters=param,
                        species="Rat",
                        iv.dose= FALSE,
                        dose = 12.5,
                        input.units = "mg/kg",
                        output.units = "mg/L", days=6/24)

## None of the monitored components undergo unit conversions (i.e. conversion factor of 1).
##
## AUC is area under the plasma concentration curve in uM*days units with Rblood2plasma = 0.78.
## The model outputs are provided in the following units:
## mg: Aintestine, Atubules, Ametabolized
## mg/l: Cliver, Csyscomp
## mg/l*days: AUC

param$Fgutabs<-0.2
out.alp14<-solve_3comp(chem.name="alprazolam",
                       parameters=param,
                       species="Rat",
                       iv.dose= FALSE,
                       dose = 12.5,
```

```

        input.units = "mg/kg",
        output.units = "mg/L", days=6/24)

## None of the monitored components undergo unit conversions (i.e. conversion factor of 1).
##
## AUC is area under the plasma concentration curve in uM*days units with Rblood2plasma = 0.78.
## The model outputs are provided in the following units:
## mg: Aintestine, Atubules, Ametabolized
## mg/l: Cliver, Csyscomp
## mg/l*days: AUC

plot(alprazolam_oral12mgperkg$Time/24,alprazolam_oral12mgperkg$Value,xlab = "Time, h",ylim=c(0,.7),ylab="Plasma concentration, mg/L",pch=19)

lines(out.alp.12[,"time"], out.alp.12[,"Cplasma"], col="darkcyan",lwd=2)

title(main="Alprazolam oral dosing in Rats, 12.5 mg/kg vs 7 mg/kg")

lines(out.alp14[,"time"],out.alp14[,"Cplasma"],col="orange",lwd=2)

# overlay 7 mg points and solution

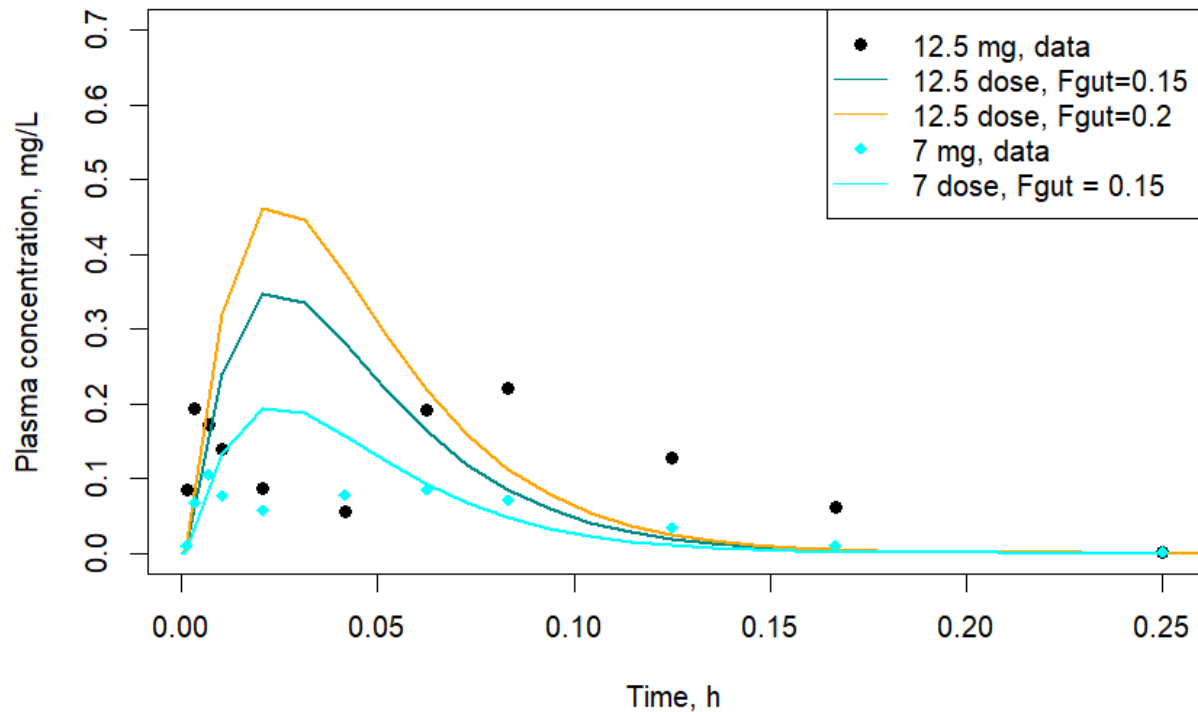
points(alprazolam_oral7mgperkg$Time/24,alprazolam_oral7mgperkg$Value, pch =18, col = "cyan")

lines(out.alp5[,"time"],out.alp5[,"Cplasma"],col="cyan",lwd=2)

legend(x="topright",legend=c("12.5 mg, data" ,"12.5 dose, Fgut=0.15","12.5 dose, Fgut=0.2", "7 mg, data", "7 dose, Fgut = 0.15"),col=c("black","darkcyan","orange", "cyan","cyan"), pch=c(19, NA, NA, 18, NA), lty=c(NA,1,1,NA,1))

```

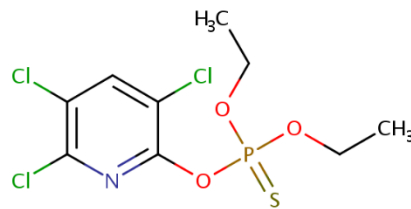

Alprazolam oral dosing in Rats, 12.5 mg/kg vs 7 mg/kg



Result: Certainly, we expect that the concentration of the drug in the plasma increases if dose increases, which both experimental data and the solution reflect. From problem 2, we found that using $F_{gutabs} = 0.15$ for the 7 mg/kg dose gave a much better fit to the data than a higher value. Indeed, when using the larger dose of 12.5 mg/kg, $F_{gutabs} > 0.15$ (i.e. $F_{gutabs} = 0.2$) still overpredicts the solution. Thus, the ideal F_{gutabs} will not change even if the dose changes. That is, a larger dose does not stimulate the body to absorb more of the chemical.

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_pbtck`.
 - c) Optional: consider why we might initially use the `pbtck` model (over the `3comp` model) for this chemical. Plot the experimental data vs the `htkk` solution for plasma concentration. Does the solution fit the data well? Why or why not do you think this may be the case?

```
#Let's first determine some physicochemical properties for Chlorpyrifos
get_physchem_param(param = c('logP', 'MW', 'logHenry'), chem.cas = "2921-88-2")

## $logP
## [1] 4.96
##
## $MW
## [1] 350.6
##
## $logHenry
## [1] -5.53
```

#If the logHenry is larger than glycerol (-7.8), then the compound is volatile. The logHenry is -5.53, probably a semivolatile. Also, chlorpyrifos is very lipophilic.

```
chlorpyrophos_po_50mgperkh <- read_excel("chlorpyrophos_po_50mgperkh.xlsx")
```

```
View(chlorpyrophos_po_50mgperkh)
```

```
time.Chlorpyrophos<-chlorpyrophos_po_50mgperkh$Time/24
```

```
conc.Chlorpyrophos<-chlorpyrophos_po_50mgperkh$Value
```

```
p<-parameterize_pbtk(chem.cas = "2921-88-2",species="Rat" )
```

```
## Warning in apply_clint_adjustment(Clint.point, Fu_hep = Fu_hep,  
## suppress.messages = suppress.messages): Clint adjusted for in vitro partitioning
```

```
## (Kilford, 2008), see calc_hep_fu.
```

```
## Warning in calc_ma(chem.cas = chem.cas, chem.name = chem.name, dtxsid =  
## dtxsid, : Membrane affinity (MA) predicted with method of Yun and Edginton
```

```
## (2013), see calc_ma.
```

```
## Warning in apply_fup_adjustment(fup.point, fup.correction = fup.adjustment,  
: :
```

```
## Fup adjusted for in vivo lipid partitioning (Pearce, 2017), see
```

```
## calc_fup_correction.
```

```
## Warning in available_rblood2plasma(chem.cas = chem.cas, species = species,  
: :
```

```
## Human in vivo measured Rblood2plasma substituted.
```

```
p$Fgutabs<-0.15
```

```
out<-solve_pbtk(chem.cas = "2921-88-2",  
                parameters=p,  
                species="Rat",  
                iv.dose= FALSE,  
                dose = 50,  
                input.units = "mg/kg",  
                output.units = "mg/L", days= 12/24)
```

```
## None of the monitored components undergo unit conversions (i.e. conversion factor of 1).
```

```
##
```

```
## AUC is area under the plasma concentration curve in uM*days units with Rblood2plasma = 0.733.
```

```
## The model outputs are provided in the following units:
```

```
## mg: Agutlumen, Atubules, Ametabolized
```

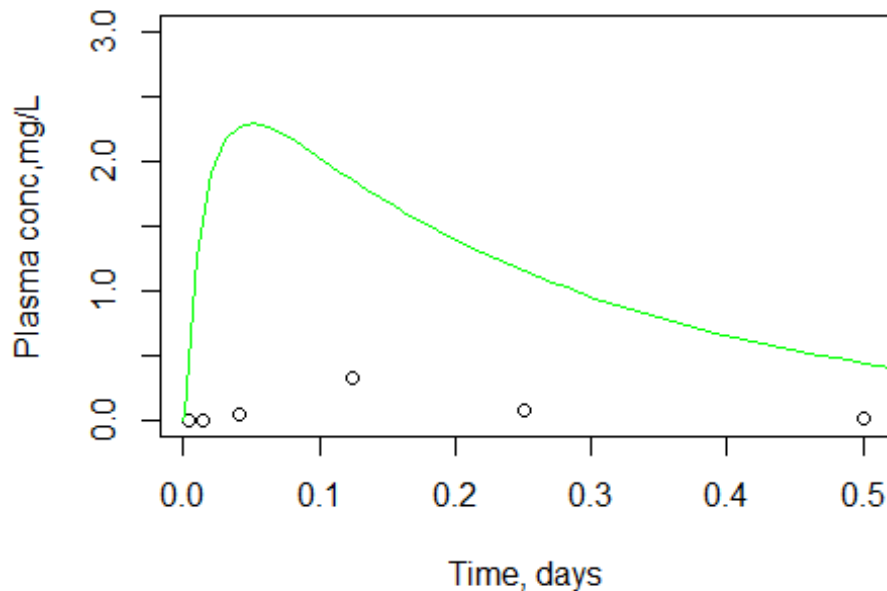
```
## mg/l: Cgut, Cliver, Cven, Clung, Cart, Crest, Ckidney, Cplasma
```

```
## mg/l*days: AUC
```

```
plot(time.Chlorpyrophos,conc.Chlorpyrophos,ylim=c(0,3),main="Oral Chlorpyrophos dosing in Rat,50 mg/kg", xlab="Time, days", ylab="Plasma conc,mg/L")
```

```
lines(out[, "time"],out[, "Cplasma"],col="green")
```

Oral Chlorpyrophos dosing in Rat, 50 mg/kg



Result:

Chlorpyrophos' $\log Henry = -5.53 > -7.8$, indicating it is probably a semi-volatile. Also, chlorpyrophos is very lipophilic.

Optional: The pbtk model includes a lung compartment without exhalation. A semi-volatile chemical can be inhaled (and exhaled even if taken orally). The pbtk model does not include exhalation, but we still wish to see how the fit compares to the data.

Even with reduced oral absorption, the results overpredict the data! Perhaps this is due to the need for a lung compartment that has exhalation.

- 2) htk also include an inhalation model (`solve_gas_pbtk`). Due to the modular approach designed in htk, 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 chlorpyrophos (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?

#We already have the data. We can parameterize the gas inhalation model for an oral dose.

```
outinh<-solve_gas_pbtk(chem.cas = "2921-88-2",  
  exp.conc = 0,  
  # doses.per.day = 1,  
  # daily.dose=1,  
  species="Rat",  
  input.units="mg/kg",  
  output.units="mg/L",  
  days=12/24,dose = 50,  
  iv.dose=FALSE,  
  plots=FALSE)
```

```
## Warning in apply_clint_adjustment(Clint.point, Fu_hep = Fu_hep,  
## suppress.messages = suppress.messages): Clint adjusted for in vitro partitioning
```

```
## (Kilford, 2008), see calc_hep_fu.
```

```
## Warning in parameterize_gas_pbtk(chem.cas = "2921-88-2", chem.name =  
## "Chlorpyrifos", : Funbound.plasma adjusted for in vitro partitioning (Pearce,  
## 2017). Set adjusted.Funbound.plasma to FALSE to use original value.
```

```
## Warning in parameterize_gas_pbtk(chem.cas = "2921-88-2", chem.name =  
## "Chlorpyrifos", : Cannot calculate saturable metabolism with Vmax = 0.  
## Defaulting to first-order metabolic clearance.
```

```
## None of the monitored components undergo unit conversions (i.e. conversion factor of 1).
```

```
##
```

```
## AUC is area under the plasma concentration curve in uM*days units with Rblood2plasma = 0.733.
```

```
## The model outputs are provided in the following units:
```

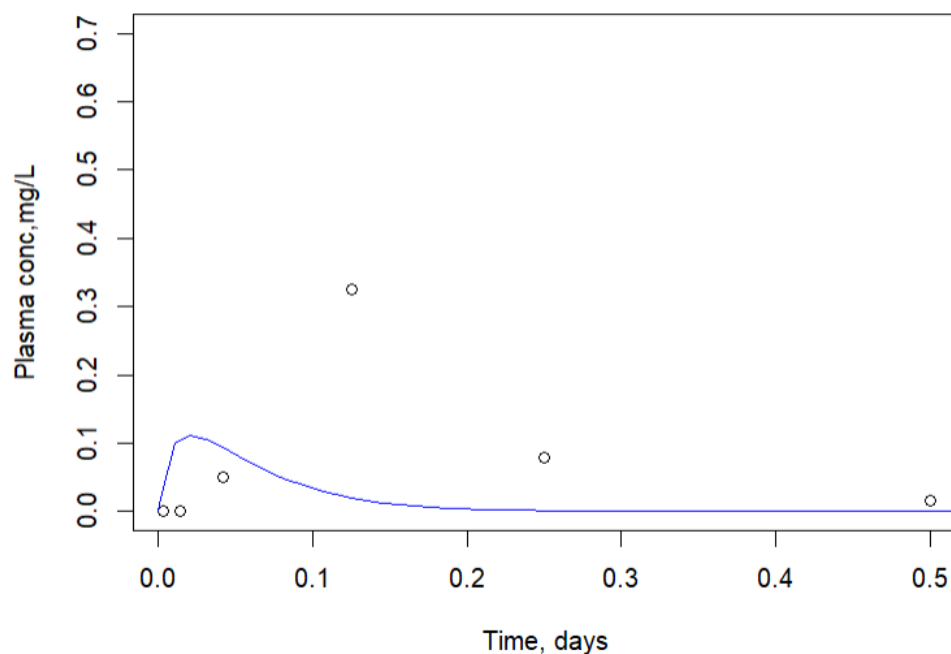
```
## mg: Agutlumen, Atubules, Ametabolized
```

```
## mg/l: Cgut, Cliver, Cven, Clung, Cart, Crest, Ckidney, Cplasma, Calvppmv, Cendexhppmv, Cmixexhppmv, Cmuc
```

```
## mg/l*days: AUC
```

```
plot(time.Chlorpyrophos,conc.Chlorpyrophos,ylim=c(0,0.7),main="Oral Chlorpyrophos dosing in Rat,50 mg/kg",  
xlab="Time, days", ylab="Plasma conc,mg/L")  
lines(outinh[, "time"],outinh[, "Cplasma"],col="blue")
```

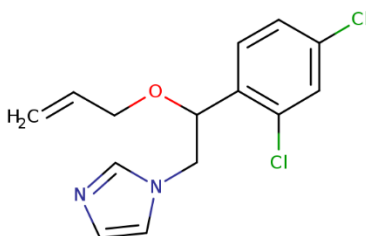
Oral Chlorpyrophos dosing in Rat, 50 mg/kg



Result: Using the gas_pbt model with oral exposure (i.e. incorporating exhalation in the lung) seems to underpredict solutions while using the pbt model overpredicts solutions.

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 httk: 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_pbtck()`. Hint: use `iv.dose = TRUE`.
- c) run the gas pbtck model?
- d) Plot the plasma concentration /time for rat at 1 mg/kg IV

```
imazalil_iv_1mgperkg <- read_excel("imazalil_iv_1mgperkg.xlsx")
time.imazalil<-imazalil_iv_1mgperkg$Time/24
conc.imazalil<-imazalil_iv_1mgperkg$Value
get_physchem_param(param = c('logP', 'MW', 'logHenry', 'pKa_Accept'), chem.cas =
"35554-44-0")

## $logP
## [1] 3.82
##
## $MW
## [1] 297.2
##
## $logHenry
## [1] -4.88
##
## $pKa_Accept
## [1] "8.12"

par<-parameterize_gas_pbtck(chem.cas = "35554-44-0", species="Rat" )
```

```

## Warning in apply_clint_adjustment(Clint.point, Fu_hep = Fu_hep,
## suppress.messages = suppress.messages): Clint adjusted for in vitro partitioning
## (Kilford, 2008), see calc_hep_fu.

## Warning in parameterize_gas_pbtk(chem.cas = "35554-44-0", species = "Rat")
:
## Funbound.plasma adjusted for in vitro partitioning (Pearce, 2017). Set
## adjusted.Funbound.plasma to FALSE to use original value.

## Warning in parameterize_gas_pbtk(chem.cas = "35554-44-0", species = "Rat")
:
## Cannot calculate saturable metabolism with Vmax = 0. Defaulting to first-order
## metabolic clearance.

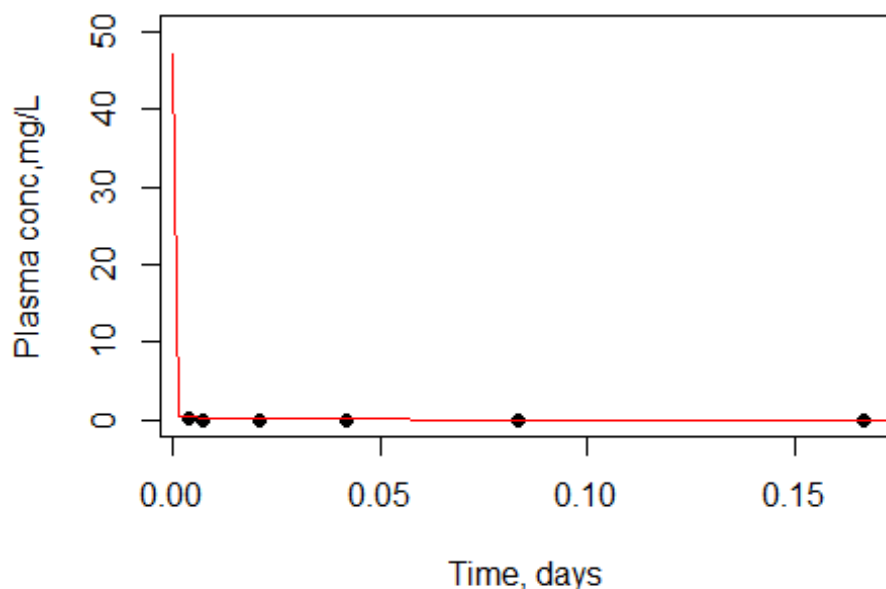
par$Fgutabs<-0.15
outinh2<-solve_gas_pbtk(chem.cas = "Imazalil",
                        exp.conc = 0,
                        parameters = par,
                        # doses.per.day = 1,
                        # daily.dose=1,
                        species="Rat",
                        input.units="mg/kg",
                        output.units="mg/L",
                        days=4/24,dose = 1,
                        iv.dose=TRUE,
                        plots=FALSE)

## None of the monitored components undergo unit conversions (i.e. conversion factor of 1).
##
## AUC is area under the plasma concentration curve in uM*days units with Rblood2plasma = 0.733.
## The model outputs are provided in the following units:
## mg: Aven, Atubules, Ametabolized
## mg/l: Cgut, Cliver, Cven, Clung, Cart, Crest, Ckidney, Cplasma, Calvppmv, Cendexhppmv, Cmixexhppmv, Cmuc
## mg/l*days: AUC

plot(time.imazalil,conc.imazalil,ylim=c(0,50),main="Imazalil iv dosing in Rat",1mg/kg", xlab="Time, days", ylab="Plasma conc,mg/L",pch=19)
lines(outinh2[, "time"],outinh2[, "Cplasma"],col="red")

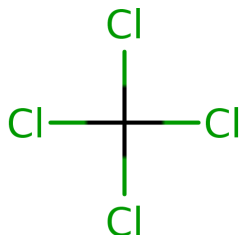
```


Imazalil iv dosing in Rat, 1mg/kg



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 htk: 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_pbtok()` to find the solution.
 - i. Hints: 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"`.
 - ii. `solve_gas_pbtok` defaults to linear clearance unless "vmax" and "km" are input. Use `vmax = 0.25` and `km = 0.25`.
 - b. Plot the experimental data and `httk` solutions.

```
ccl4_Rat_1000ppm <- read_excel("IntermediateFiles_toxsci.xlsx", "ccl4_Rat_1000ppm")  
  
## New names:  
## * `` -> `...3`  
  
time.ccl4<-ccl4_Rat_1000ppm$time_hr_corr/24  
conc.ccl4<-ccl4_Rat_1000ppm$conc  
dm<-matrix(c(0.0,2/24,1000,0),nrow=2)  
colnames(dm)<-c("time","forcing_values")  
#ALL parameter values are default  
out1<-solve_gas_pbtok(chem.cas="56-23-5",  
                      species="Rat",  
                      forcings=dm,  
                      vmax=0.25,  
                      km=0.25,  
                      input.units="ppmv",  
                      output.units="mg/L",  
                      time=seq(0,12/24,length=100),  
  
                      )  
  
## Warning in apply_clint_adjustment(Clint.point, Fu_hep = Fu_hep,  
## suppress.messages = suppress.messages): Clint adjusted for in vitro partit
```

```

ioning
## (Kilford, 2008), see calc_hep_fu.

## Warning in parameterize_gas_pbtck(chem.cas = "56-23-5", chem.name = "Carbon
## tetrachloride", : Funbound.plasma adjusted for in vitro partitioning (Pear
ce,
## 2017). Set adjusted.Funbound.plasma to FALSE to use original value.

## None of the monitored components undergo unit conversions (i.e. conversio
n factor of 1).
##
## AUC is area under the plasma concentration curve in uM*days units with Rbl
ood2plasma = 0.695.
## The model outputs are provided in the following units:
## mg/l: Cgut, Cliver, Cven, Clung, Cart, Crest, Ckidney, Cplasma, Calvppmv,
Cendexhppmv, Cmixexhppmv, Cmuc
## mg: Atubules, Ametabolized
## mg/l*days: AUC

plot(time.ccl4,conc.ccl4,ylim=c(0,25),main="CCl4 inhalation 1000ppm",xlab="Ti
me,days",ylab = "Arterial conc mg/L",pch=19)
lines(out1[, "time"],out1[, "Cart"])

```

CCl4 inhalation 1000ppm

