

httk : High-Throughput Toxicokinetics Virtual Training

Breakout Group Worksheet: Beginner **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

Goal

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: BeginnerFiles_toxsci.xlsx. (This file can be found in the Supplementary data provided in Wambaugh et al. (2018): <https://doi.org/10.1093/toxsci/kfy020>).

Hints

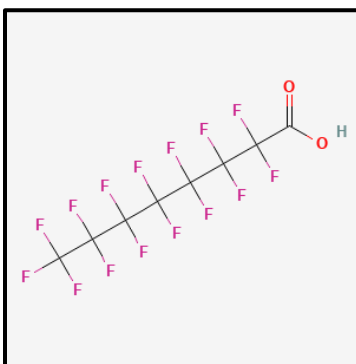
Navigate to the “help” tab in the pane in the bottom right corner of your Rstudio screen. Here, you can search for htk functions. You can also type “help([function])” into the consol. For example, type “help(solve_pbt)” into the console and you will see the help page for the pbt model.

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)
```

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, C₈-PFCA, FC-143, F-*n*-octanoic acid

Name in htk: Perfluorooctanoic acid

CASRN: 335-67-1

DSSTox substance identifier (DTXSID): DTXSID8031865

Molecular Formula: C₈HF₁₅O₂

SMILES: OC(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F

Directions

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

```
chem.cas="335-67-1"
get_chem_id(chem.cas=chem.cas)

## $chem.cas
## [1] "335-67-1"
## $chem.name
## [1] "Perfluorooctanoic acid"
## $dtxsid
## [1] "DTXSID8031865"

dtxsid=get_chem_id(chem.cas=chem.cas)$dtxsid
chem.cas=get_chem_id(chem.cas=chem.cas)$chem.cas
chem.name=get_chem_id(chem.cas=chem.cas)$chem.name
```

2. htkk provides a variety of models. Depending on your goal, some models might be more appropriate to use than others. The following questions will investigate some of the model differences. [Hint: search solve_ 'modelname' in the help file to see a diagram of the compartment models for each model.]

- a. Say we wish to simulate the concentration of Perfluorooctanoic acid in the liver.

Which of the following models would be appropriate for this: 1comp, 3comp, pbtk?

(3comp and pbtk)

- b. Which of the aforementioned models could we use if we needed to investigate chemical kidney concentrations?

(Pbtk)

- c. What compartments does the pbtk model have? What compartments does 3comp have?

(pbtk: kidney, liver, gut lumen, rest, lung, blood (plasma));

3comp: gut, liver, rest, blood (plasma))

3. Find chemical and physical parameters within htkk.

- a. Find logHenry and logP for this chemical [Hint: Search chem.physical_and_invitro.data or get_physchem_param]

(logP = 3.11, logHenry = -9.72)

```
# Multiple ways to achieve this. Examples are:  
# i.  
our.subset <- subset(chem.physical_and_invitro.data,  
Compound == "Perfluorooctanoic acid")  
our.subset[,c("logP", "logHenry")]  
# ii.  
get_physchem_param(param=c('logP', 'logHenry'),  
chem.cas = "335-67-1")
```

- b. Find the ratio of blood to plasma ("Rblood2plasma") for humans. [Hint: Use chem.physical_and_invitro.data.]

(0.6436)

```
our.subset <- subset(chem.physical_and_invitro.data,  
Compound == "Perfluorooctanoic acid")  
our.subset[, "Human.Rblood2plasma"]]
```

- 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?

Error: missing metabolic clearance data for species

```
parameterize_pbtk(chem.cas= "335-67-1", species="Rabbit",  
suppress.messages = TRUE)
```

```
Error in get_clint(dt xsid = dt xsid, chem.name = chem.name,  
chem.cas = chem.cas, : Missing metabolic clearance data for given  
species.
```

set default.to.human to true to substitute human value.

- 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]

(0.004779)

```
parameterize_pbtck(chem.cas="335-67-1", species="Rabbit",
  default.to.human = TRUE, suppress.messages = TRUE)$Funbound.plasma
```

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)`]

```
tox.data <- data.frame(read_excel("BeginnerFiles_toxsci.xlsx"))
```

```
PFOA.subset <- subset(tox.data, CAS == "335-67-1")
```

First 10 columns and 7 rows of the subset

	Compound	DSSTox_Substance_Id	CAS	Reference	Species	Species.Weight	Species.Weight.Units	Dose	Dose.Units.and.Type	Time
1619	Pentadecafluorooctanoic acid	DTXSID8031865	335-67-1	NHEERL 2015	rat	NA	kg	1.0	mg/kg po	0.25000000
1620	Pentadecafluorooctanoic acid	DTXSID8031865	335-67-1	NHEERL 2015	rat	NA	kg	1.0	mg/kg po	0.50000000
1621	Pentadecafluorooctanoic acid	DTXSID8031865	335-67-1	NHEERL 2015	rat	NA	kg	1.0	mg/kg po	1.00000000
1622	Pentadecafluorooctanoic acid	DTXSID8031865	335-67-1	NHEERL 2015	rat	NA	kg	1.0	mg/kg po	2.00000000
1623	Pentadecafluorooctanoic acid	DTXSID8031865	335-67-1	NHEERL 2015	rat	NA	kg	1.0	mg/kg po	4.00000000
1624	Pentadecafluorooctanoic acid	DTXSID8031865	335-67-1	NHEERL 2015	rat	NA	kg	1.0	mg/kg po	8.00000000
1625	Pentadecafluorooctanoic acid	DTXSID8031865	335-67-1	NHEERL 2015	rat	NA	kg	1.0	mg/kg po	12.00000000

- 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.]

(1 mg/kg)

```
PFOA.oral <- subset(PFOA.subset, Route == "po" &
                    Subject == 81)
unique(PFOA.oral$Units)
```

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

(Plasma concentration, ug/mL, rat)

```
unique(PFOA.oral[,c("Media", "Media.Units")])
unique(PFOA.oral$Species)
```

- iii. How long did the experiment last?

(72 hours)

```
max(PFOA.oral$Time)
unique(PFOA.oral$Time.Units)
```

- 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: htkk has units in days. use `days = number-of-hours-in-experiment/24`. When plotting, make sure to convert htkk output units into hours.
- Note that `ug/mL = mg/L`
- Make sure to use `iv.dose=FALSE`
- When plotting overlaid items, you can use the following commands (and make sure units of time are the same):

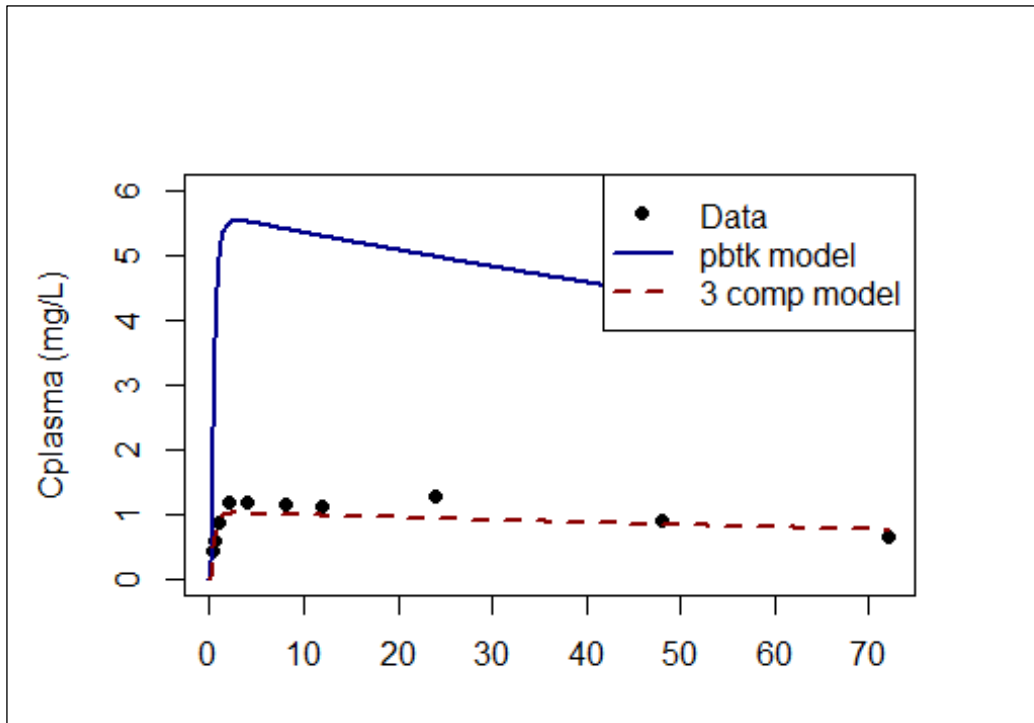
```
plot(time.experimental, conc.experimental) # will plot
points for experimental data
lines(time.simulation1, conc.simulation1) # will plot a
line for simulation 1
lines(time.simulation2, conc.simulation2) # will plot a
line for simulation 2
```

```
pbtok.soln_oral <- solve_pbtok(chem.cas = "335-67-1", species=
"Rat",
                                dose = 1, input.units = "mg/kg",
                                output.units = "mg/L",
                                days = 72/24, iv.dose = FALSE,
                                suppress.messages=TRUE)

comp3.soln_oral <- solve_3comp(chem.cas="335-67-1", species=
"Rat",
                                dose = 1, input.units =
"mg/kg", output.units =
"mg/L",
                                days = 72/24, iv.dose =
FALSE, suppress.messages =
TRUE)

# Plotting

plot(PFOA.oral[, "Time"], PFOA.oral[, "Value"], ylim = c(0,6),
      xlab = "Time (hr)", ylab = "Cplasma (mg/L)", pch =
19)
lines(pbtok.soln_oral[, "time"]*24,
      pbtok.soln_oral[, "Cplasma"],
      col = "darkblue", lwd = 2, lty = 1)
lines(comp3.soln_oral[, "time"]*24,
      comp3.soln_oral[, "Cplasma"],
      col = "darkred", lwd = 2, lty = 2)
legend("topright", legend=c("Data", "pbtok model", "3 comp
model"),
      col = c("black", "darkblue", "darkred"), pch =
c(19, NA, NA),
      lwd = c(NA, 2, 2), lty = c(NA, 1, 2))
```



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

Answer: The 3comp model fits the data better than the pbtk model. The oral absorption is described by sending the stomach contents to the liver first, accounting for a first pass effect. Remaining chemical after clearance is sent to the rest of the body, which represents a large volume. Since concentration is amount/volume, the venous concentration will be smaller when compared to solve_pbtk. The solve_pbtk also accounts for first pass effect, but doesn't lump the same compartments into the rest of the body. There is also an additional kidney and lung compartments, where chemical will be partitioned differently from the rest of the body. The more complex pbtk model contributes to venous amount divided by a much smaller volume than that of the 3comp rest of the body. The result will be a higher concentration simulation when using solve_pbtk.

- c. Our second goal is to compare the IV dose experimental data pertaining to subject 85 with IV dose htk simulations. Extract the experiment specifications for IV .
 - i. Find the IV dose and IV dose units for subject 85.
(0.2 mg/kg)

```
PFOA.iv <- subset(PFOA.subset, Route == "iv" & Subject == 85)
unique(PFOA.iv$Dose)
unique(PFOA.iv$Units)
```



```
unique(PFOA.iv[,c("Media","Media.Units")])
unique(PFOA.iv$Species)
```

iii. How long did the experiment last?

(72 hours)

```
max(PFOA.iv$Time)
unique(PFOA.iv$Time.Units)
```

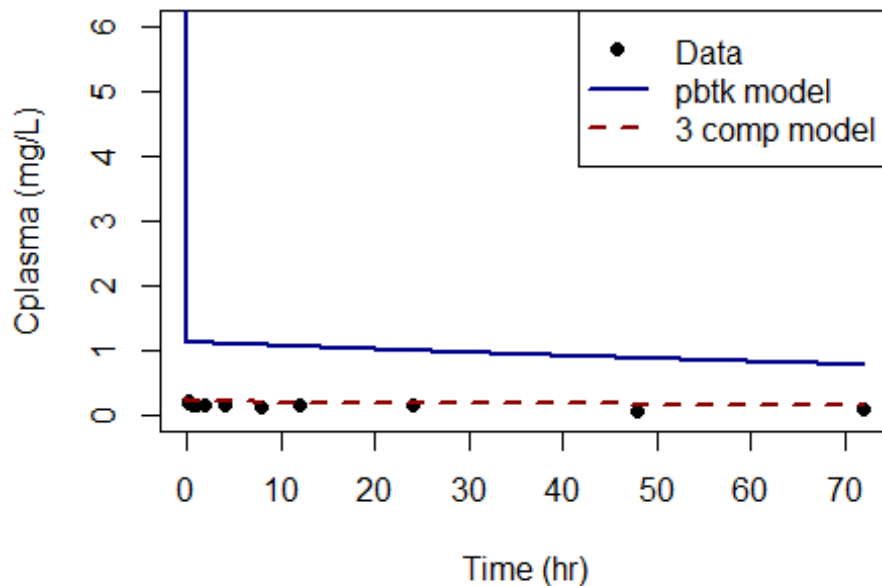
d. Using these specs, generate iv dose results using both the 3comp and pbtk models and plot them against the experimental data. [Hint: Make sure to use `iv.dose=TRUE`]

```
pbtk.soln_iv <- solve_pbtk(chem.cas = "335-67-1", species= "Rat",
  dose = 0.2, input.units = "mg/kg",
  output.units = "mg/L",
  days = 72/24,
  iv.dose = TRUE, suppress.messages=TRUE)

comp3.soln_iv <- solve_3comp(chem.cas = "335-67-1", species= "Rat",
  dose = 0.2, input.units = "mg/kg",
  output.units = "mg/L",
  days = 72/24,
  iv.dose = TRUE, suppress.messages=TRUE)

# Plotting

plot(PFOA.iv[, "Time"], PFOA.iv[, "Value"], ylim=c(0,6), xlab = "Time (hr)",
  ylab = "Cplasma (mg/L)", pch = 19)
lines(pbtk.soln_iv[, "time"]*24, pbtk.soln_iv[, "Cplasma"],
  col = "darkblue", lwd = 2, lty = 1)
lines(comp3.soln_iv[, "time"]*24, comp3.soln_iv[, "Cplasma"],
  col = "darkred", lwd = 2, lty = 2)
legend("topright", legend = c("Data", "pbtk model", "3 comp model"),
  col=c("black", "darkblue", "darkred"), lty = c(NA, 1, 2),
  pch = c(19, NA, NA), lwd=c(NA, 2, 2))
```



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

Answer: The 3comp model fits the data better than the pbtk model. The difference between 3comp and pbtk consists on where the iv injection is made. For the 3comp model, blood is part of the rest of the body compartment, which is the largest compartment in the model. The pbtk model separates blood from the rest of the body into smaller venous and arterial blood separated by a lung between them. Since concentration is amount/volume, the venous pbtk concentration will be larger at the initial injection time, due to smaller venous volume used by pbtk when compared to 3comp. The result will be a higher concentration simulation when using solve_pbtk. Compare the oral dose and IV dose time curves. Do they make sense?

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

(3 comp oral = 2.649 mg/L/d,

Pbtk oral = 14 mg/L/d,

3 comp iv = 0.5329 mg/L/d

Pbtk iv = 2.815 mg/L/d)

```
tail(comp3.soln_oral, n = 1)[, "AUC"]
tail(pbtk.soln_oral, n = 1)[, "AUC"]
tail(comp3.soln_iv, n = 1)[, "AUC"]
tail(pbtk.soln_iv, n = 1)[, "AUC"]
```

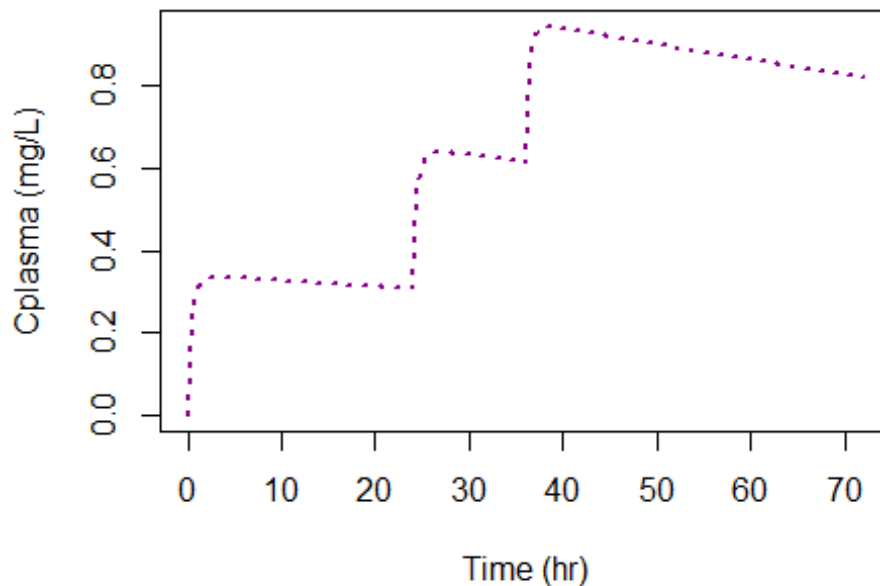
5. Some chemical exposure occurs frequently. In the case of PFOS, a subject might be exposed to multiple doses over a period of time rather than just one single initial exposure. Say you wish to investigate how multiple exposures affect the concentration of the substance in the plasma by splitting the 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) and calculate solutions. Plot the concentration in plasma over time. How does the solution differ from that in 4b?

```
#Create dosing matrix
dm <- matrix(c(0,24/24,36/24,0.33,0.33,0.33), nrow = 3)
colnames(dm) <- c("time","dose")

comp3.soln_oralbolus <- solve_3comp(chem.cas="335-67-1",
                                   species= "Rat",
                                   dose = NULL,
                                   dosing.matrix = dm,
                                   input.units = "mg/kg",
                                   output.units = "mg/L",
                                   days = 72/24, iv.dose=FALSE,
                                   suppress.messages = TRUE)

plot(comp3.soln_oralbolus[, "time"]*24,
      comp3.soln_oralbolus[, "Cplasma"], col="darkmagenta", lwd = 2, xlab =
      "time (hr)", ylab = "Cplasma (mg/L)", type="l", lty=3)
```



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

(0.0409)

```
convert_units(input.units = "ppmv", output.units = "uM", chem.cas =
"335-67-1")
```

7. htk 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?

(Human and rat)

```
subset(chem.physical_and_invitro.data, CAS == 335-67-1)$All.Species
```

8. Find the following parameters using the pbt model [Hint: Use `parameterize_pbt()`]:
- Liver to plasma partition coefficient for PFOA. [Hint: partition coefficients have the suffix "2pu".]

(154)

```
parameterize_pbt(chem.name = "Perfluorooctanoic acid",
suppress.messages = TRUE)$kliver2pu
```

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

(Vkidneyc = fractional kidney volume = 0.00419 L/kg
Qkidneyf = fractional flow rate to kidney = 0.2214 L/h)

```
parameterize_pbt(chem.cas = chem.cas, species="Human",  
suppress.messages = TRUE)$vkidneyc  
  
parameterize_pbt(chem.cas = chem.cas, species="Human",  
suppress.messages = TRUE)$Qkidneyf
```