

# ICE IVIVE workflow

## Description

The workflow allows the flexibility to select from five different rat and human PK models: a one-compartment model that incorporates Monte Carlo simulation to simulate the population variance (1c), and four models leveraging the EPA's httk package which include a 3 compartment model (solve\_3comp), a multi-compartment PBPK model for modeling oral and i.v. exposure (solve\_pbtk), a multi-compartment PBPK model for modeling inhalation (gas) route of exposure (solve\_gas\_pbtk), and a multi-compartment human PBPK model that includes both maternal and fetal compartments, and a placenta modeled as a joint organ shared by mother and fetus (solve\_fetal\_pbtk). The workflow is to predict the equivalent administered dose (EAD) that would lead to steady state plasma concentration or maximum plasma concentration (Cmax) equal to the bioactive concentration from in vitro assays. For inhalation exposures using the (solve\_gas\_pbtk) EAD is generated in uM or ppmv if using "concentration" exposure vs a dose.

Two example files are included: \* ChemicalData\_Rnotebook.txt \* InvitroData\_Rnotebookv.txt

## Load libraries

```
#load libraries
library(tidyverse)    #needed for read-delim()
library(deSolve)
library(doParallel)
library(httk)         #this is needed for models: solve_3comp, solve_pbtk, solve_gas_pbtk, solve_fetal_p
                     #The code is compatible with httk_2.2.2
library(readxl)       #for read_excel() #2025
#library(xlsx)        #for writing the excel file that is user output
date <- format(Sys.Date(), format="%y%m%d") #added to add information on date
```

## Input data and output file path

There are several input variables needed to run the code. Some variables are model specific, as detailed. Adjust the file paths to point to the file in your directory.

chemFile: the file containing chemical data from ICE. this has the CASRN as the first field assayFile: the file with the in vitro bioactivity data. The first column is the CASRN and the subsequent columns are the bioactivity values output\_file: this is the file for the outputs

```
chemFile <- "ChemicalData_Rnotebook.txt" #chemicals data from ICE, include CASRN field as identifier
assayFile <- "InvitroData_Rnotebook.txt" #invitro data from ICE, includes CASRN field as identifier the
#chemFile <- "Template_ICEData.txt"
#assayFile <- "InvitroData_Rnotebook_10chem.txt"
Userout <- "User_Results.xlsx"           #file name (and path) for the output file for the tissue; conc
```

```
EADplot_file <- "User_plot.pdf"           #the file for the plot of the EAD plot
invivo <- NULL                             #invivo data provided by user
#invivoFile <- "InvivoData_Rnotebook.txt"
#invivo <- as.data.frame(read_delim(invivoFile, delim = "\t"))
```

## Model variables

Details about what model, route, and dose need to be specified. There are differences in what is needed if a 1 compartment model is used vs the PBPK models

## What model?

Models type is limited to 5 different models. Currently, species in ICE is limited to human or rat. Using this notebook one can expand the species with minor editing of the code provided the parameters are available.

```
targetTissue <- 'Cplasma'
#1c: 'Cplasma'
#solve_3comp: 'Cplasma', 'Cliver'
#solve_pbt: 'Cplasma', 'Cliver', 'Cgut', 'Clung', 'Ckidney', 'Crest'
#solve_gas_pbt: 'Cplasma', 'Cliver', 'Cgut', 'Clung', 'Ckidney', 'Cmuc', 'Crest'
#solve_fetal_pbt: 'Cplasma', 'Cliver', 'Cgut', 'Clung', 'Ckidney', 'Cadipose', 'Crest', 'Cplacenta',
targetTissue_fetal <- 'Cfplasma'
#solve_fetal_pbt: 'Cfplasma', 'Cfliver', 'Cfgut', 'Cflung', 'Cfkidney', 'Cfbrain', 'Cfthyroid', 'Cfr
species <- "human"           #human or rat
bWeight <- 70                #the body weight of simulated subject
modelType <- "1c"            #"1c", "solve_3comp", "solve_pbt", "solve_gas_pbt", "solve_fetal_pbt"; only
```

For the 1 compartment model, values are needed to parameterize the Monte Carlo simulation.

```
nsamples <- 300              #user-provided value for the mc simulations, any number between
```

For the PBPK models some additional parameters can be modified. An inhalation exposure has additional parameters The route determines where the chemical will enter the system and route, interval, and days are needed for all PBPK models.

```
route <- "oral"              #oral, iv, or inhalation needed for PBPK models; "solve_gas_pbt
interv <- 24                 #dosing interval, hours
ndays <- 3                   #number of days dosing is done
expDose <- 1                 #current calculations assume 1mg/kg/dose for "1c", "solve_3comp
expConc <- 1                 #Dosing for "solve_gas_pbt" model
ConcentrationUnit <- "uM"    #Renamed as Output Conc. Units in PBPK tool since ICE4.0. Opti
#the unit for in vitro activity concentration is consistent wi
gestationDays <- 91          #gestational days when dose starts, range is 91 days (14 weeks
```

## Inhalation specific parameters

For an inhalation route of exposure, there are 2 different ways that the exposure can be modeled. “expDose” models a set bolus dose like the exposure for an oral or IV exposure. The “expConc” is more typical of a gas

exposure. This models an inhaled concentration over a duration of time, set by “expLength”. The “expDose” option is currently unavailable.

```
gasDosing <- "expConc"                                #two dosing option methods for solve_gas_pbt model, "expConc"  
                                                    #but for ICE3.3 release, "expConc" is the only option due to a  
                                                    #This is only needed if gasDosing == "expConc" because otherwise  
if(gasDosing == "expConc" & (!exists("expConc") || is.null(expConc))){  
  expConc <- 1                                         #current calculations assume 1uM of air concentration  
}  
gasInputUnit <- "ppmv"                                #Input concentration unit for gas model (httkv2.1.0 and later)  
expLength <- 0.25                                     #length of the gas exposure, hours
```

## Gestation model specific parameters

```
gestationDays <- 91                                  #gestation model specific parameter; gestational days when dose starts
```

## Argument for EADboxplot.R

```
chemDisplay <- "ChemicalName"                        #choice is "CASRN" or "ChemicalName"  
if(modelType != "solve_gas_pbt"){                    #"mg/kg/dose" for "1c", "solve_3comp", "solve_pbt"; uM or ppm  
  EADUnit <- "mg/kg/dose"  
}else{EADUnit <- gasInputUnit}
```

## Load functions

```
source("steadyState.R")                              #All required R scripts and input files should be in the working directory  
source("CalcEAD.R")                                  #required for "1c", "solve_3comp", "solve_pbt", "solve_gas_pbt"  
source("EADboxplot.R")
```

## Load data

Notice that the chem input has the source of the FU and Clint data. ICE now has 2 different sources of these values so the source is important for tracking this information.

```
#there are single quotes in chemical names that need to be addressed, the tidyR handles well  
#chemical <- as.data.frame(read_delim(chemFile, delim = "\t"))  
chemical <- as.data.frame(read_delim(chemFile, trim_ws = TRUE, na = c("", "NA"), delim = "\t", quote = "\""))  
#chemical[1:2,]
```

```
#invitro <- as.data.frame(read_delim(assayFile, delim = "\t"))  
invitro <- as.data.frame(read_delim(assayFile, trim_ws = TRUE, na = c("", "NA"), delim = "\t", quote = "\""))  
invitro <- semi_join(invitro, chemical, by = "CASRN")                                     #2025  
#invitro[1:2,]
```

## Preparing data

Minor prep work is done on the data that comes from ICE Partitioning coefficients are obtained by internal function from the htk package using the provided phys chem parameters. Compare with the example input file for naming Note that a few checks are done. These will generate flags that can be used to follow up as needed.

```
##load data
chemical <- as.data.frame(read_delim(chemFile, trim_ws = TRUE, na = c("", "NA"), delim = "\t", quote = "\"")
invitro <- as.data.frame(read_delim(assayFile, trim_ws = TRUE, na = c("", "NA"), delim = "\t", quote = "\"")
invitro <- semi_join(invitro, chemical, by = "CASRN") #2025

##column names should be labeled correctly, see the example file
##cleaning the names for taking from an ICE Query-stricter matching is used to handel including the ADM
colnames(chemical)<-gsub(".*Name.*", "ChemicalName", colnames(chemical))
colnames(chemical)<-gsub(".*Parameters fu.*", "fu", colnames(chemical), ignore.case =TRUE)
colnames(chemical)<-gsub(".*funbound.*", "fu", colnames(chemical), ignore.case =TRUE)
colnames(chemical)<-gsub(".*fu Source.*", "fu Source", colnames(chemical), ignore.case =TRUE)
colnames(chemical)<-gsub(".*MW.*", "MW", colnames(chemical))
colnames(chemical)<-gsub(".*HL.*", "HL", colnames(chemical))
colnames(chemical)<-gsub(".*Parameters Clint.*", "Clint", colnames(chemical), ignore.case = TRUE)
colnames(chemical)<-gsub(".*Clint Source.*", "Clint Source", colnames(chemical), ignore.case = TRUE)
colnames(chemical)<-gsub(".*LogP.*", "LogP", colnames(chemical))
colnames(chemical)<-gsub(".*pKa, Basic.*", "pka_Accept", colnames(chemical))
colnames(chemical)<-gsub(".*pKa, Acidic.*", "pka_Donor", colnames(chemical))

#Converting units for internsic clearance values
#this moves from log10 ul/ml/10^6 cells to just ul/ml/10^6 cells
#chemical$Clint<-10^chemical$Clint
chemical$logPwa<--1*(chemical$HL+1.61) #this gets the water-air partition coefficient, as the unit for
chemical$Flag <-"
#catch for low Fu
chemical$Flag[chemical$fu<=1e-10]<-"fu is zero,likely due to rounding. Setting to 1e-5"
chemical$fu[chemical$fu<=1e-10]<-1e-5
```

## 1c modeling

If modelType given by the user is “1c” This will run a simple, 1 compartment model. In this model the C steady state is determined vs the Cmax from the other models.

## Calculating the EADs

The 1c model has a monte carlo simulation that calculates the C<sub>ss</sub> at the 50th and the 95th percentile The first step is generating the CSS object with the call to “steadState”.

```
### starting to model
p.out_all <- NULL

### model "1c"
if(modelType == "1c"){
```

```

chemInput <- chemical
#chemInput <- chemical[,c("CASRN", "ChemicalName", "Clint", "fu", "MW", "Flag")]
set.seed(1234)
CSS<- steadyState(inputData = chemInput, nsamples = nsamples, species = species, ConcentrationUnit = "mg/kg")
head(CSS)

#There are 2 different steady state predictions, 50 and 95 percentile.
#The EAD is calculated for each using the "CalcEAD" function. The data is then formatted into a single data frame.

EAD.out50 <- CalcEAD(Css = CSS[,c("CASRN", "50%", "fu")], inVitro = invitro, adj.fu = "fu")
colnames(EAD.out50)<-gsub("EAD","EAD.50", colnames(EAD.out50))
#add in the flag column
EAD.out50<-left_join(chemInput[,c("CASRN","Flag")],EAD.out50)
EAD.out95 <- CalcEAD(Css = CSS[,c("CASRN","95%", "fu")], inVitro = invitro, adj.fu = "fu")
colnames(EAD.out95)<-gsub("EAD","EAD.95", colnames(EAD.out95))
EAD.out<-left_join(EAD.out50,EAD.out95)
EAD.out<-EAD.out[,setdiff(colnames(EAD.out), c("adj.fu", "fu", "adj.arm","arm"))] # remove the columns
#creating output file for user, should have EAD values and the parameters for calculating the circular dichroism
CSS2<-as.data.frame(CSS, stringsAsFactors=FALSE); colnames(CSS2)<-gsub("50%", "Css, 50%ile", colnames(CSS2))
CSS2$Species<-species; CSS2$model<-modelType; CSS2$Dose, mg/kg<-expDose; CSS2$Route<-route
CSS2$nsimulations <- nsamples
colnames(CSS2)<-gsub("Css_Unit","Css, Units", colnames(CSS2))

CSS2<-left_join(CSS2, chemical %>% select(any_of( c("CASRN","Clint Source", "fu Source"))))

#reformatting columns to match the other models:
CSS2<-CSS2 %>% select(any_of(c("CASRN", "ChemicalName", "Css, 50%ile", "Css, 95%ile", "Css, Units", "EAD.50", "EAD.95")))
ssEAD.out<-EAD.out[,c("CASRN", setdiff(colnames(EAD.out), c(colnames(invivo), "50%", "95%")))]
outputData<-full_join(CSS2,ssEAD.out)
colnames(outputData)<-gsub("Clint$", "Clint, ul/ml/10^6 cells", colnames(outputData))#add units
p.out_all <- CSS2 %>% select(any_of(c("CASRN", "ChemicalName", "LogP", "LogP_InputSource", "Clint", "fu Source")))
p.out_all<- p.out_all %>% rename("Log of Henry's Law constant" = "HL")

#The output file is an excel workbook. This has 2 tabs, one for the EAD results and the other for the PK parameters.
xlsx::write.xlsx(file=Userout, x=outputData, sheetName ="EADResults", append=FALSE, row.names = FALSE)
xlsx::write.xlsx(file=Userout, x=invivo, sheetName ="inVitroData", append=TRUE, row.names = FALSE, sheetIndex = 2)
xlsx::write.xlsx(file=Userout, x=p.out_all, sheetName ="PKparameters", append=TRUE, row.names = FALSE, sheetIndex = 3)

#To view the results, the "EADboxplot" function plots the values.
EADplot <- EADboxplot(EAD.out = outputData, invivo = invivo, label="EAD", EADUnit = EADUnit, species = species)
#Arguments for EADplot functions include "EAD.out, invivo, label, chemDisplay, EADUnit, modelType, species, units"
EADplot
}

```



```

chemical$DTXSID2<-paste0(chemical$DTXSID, "_n")
if(species_1=="Human"){
  chem.physical_and_invitro.data <- add_chemtable(chemical, current.table = chem.physical_and_invitro
}else{
  chem.physical_and_invitro.data <- add_chemtable(chemical, current.table = chem.physical_and_invitro
  chem.physical_and_invitro.data <- add_chemtable(chemical, current.table = chem.physical_and_invitro
}

# to allow the user to change the body weight for rat and human
if(species_1=="Human"){
  physiology.data[4,6] <- bWeight
}else{
  physiology.data[4,4] <- bWeight #Average rat body weight,changed from [2,6] to [4,4]
}

if(route != "iv"){
  iv.dose = FALSE
}else{iv.dose =TRUE}

dpd <- 24/interv #calculating the doses per day
cmaxall <- NULL
}

```

## solve\_3comp model

The solve\_3comp model is a 3 compartment model that has the gut,gut lumen, liver, and rest of the body compartments with the plasma equivalent to the liver plasma concentrations.

```

# note that dose=0 stops the initial dosing of the compartments and is needed to accurately model the s
if(modelType == "solve_3comp"){
  for(this.cas in chemical[, "CASRN"]) {
    set.seed(1234)
    Kliver2pu <- chemical$Kliver2pu[chemical$CASRN == this.cas]
    Krest2pu <- chemical$Krest2pu[chemical$CASRN == this.cas]
    Kgut2pu <- chemical$Kgut2pu[chemical$CASRN == this.cas]
    param <- parameterize_3comp(chem.cas = this.cas, species = species_1, default.to.human = TRUE)
    print(param$BW) # to make sure that the body weight had been changed.
    param$Kliver2pu <- ifelse(!is.na(Kliver2pu), Kliver2pu , param$Kliver2pu) #2025June
    param$Kliver2pu_Source <- ifelse(!is.na(Kliver2pu), "CUSTOM", "httkCalculated") #2025June
    param$Krest2pu <- ifelse(!is.na(Krest2pu), Krest2pu, param$Krest2pu) #2025June
    param$Krest2pu_Source <- ifelse(!is.na(Krest2pu), "CUSTOM", "httkCalculated") #2025June
    param$Kgut2pu <- ifelse(!is.na(Kgut2pu), Kgut2pu, param$Kgut2pu) #2025June
    param$Kgut2pu_Source <- ifelse(!is.na(Kgut2pu), "CUSTOM", "httkCalculated") #2025June
    p.out0 <- as.data.frame(param) #automatically resets row names #for output #2025
    colnames(p.out0) <- paste0("ModelParam_", colnames(p.out0)) #for output #2025
    rownames(p.out0)<- this.cas #for output #2025
    p.out <- rownames_to_column(p.out0, var = "CASRN") #for output #2025
    concMax <- max(solve_3comp(chem.cas = this.cas, parameters = param, doses.per.day = dpd, days = nday
    cmax_temp <- as.data.frame(cbind(this.cas, concMax, ConcentrationUnit))
    cmaxall <- rbind(cmaxall, cmax_temp)
    p.out_all <- rbind(p.out_all, p.out) #2025
  }
}

```



```
}
}
```

## solve\_pbtck model

the solve\_pbtck model is a PBPK model that has gutlumen, gut, liver, kidneys, veins, arteries, lungs, and the rest of the body compartments.

```
if(modelType == "solve_pbtck"){
  for(this.cas in chemical[, "CASRN"]) {
    set.seed(1234)
    Kkidney2pu <- chemical$Kkidney2pu[chemical$CASRN == this.cas]
    Kliver2pu <- chemical$Kliver2pu[chemical$CASRN == this.cas]
    Krest2pu <- chemical$Krest2pu[chemical$CASRN == this.cas]
    Kgut2pu <- chemical$Kgut2pu[chemical$CASRN == this.cas]
    param <- parameterize_pbtck(chem.cas = this.cas, species = species_1, default.to.human = TRUE)
    print(param$BW) # to make sure that the body weight had been changed.
    param$Kkidney2pu <- ifelse(!is.na(Kkidney2pu), Kkidney2pu, param$Kkidney2pu) #2025June
    param$Kkidney2pu_Source <- ifelse(!is.na(Kkidney2pu), "CUSTOM", "httkCalculated") #2025June
    param$Kliver2pu <- ifelse(!is.na(Kliver2pu), Kliver2pu, param$Kliver2pu) #2025June
    param$Kliver2pu_Source <- ifelse(!is.na(Kliver2pu), "CUSTOM", "httkCalculated") #2025June
    param$Krest2pu <- ifelse(!is.na(Krest2pu), Krest2pu, param$Krest2pu) #2025June
    param$Krest2pu_Source <- ifelse(!is.na(Krest2pu), "CUSTOM", "httkCalculated") #2025June
    param$Kgut2pu <- ifelse(!is.na(Kgut2pu), Kgut2pu, param$Kgut2pu) #2025June
    param$Kgut2pu_Source <- ifelse(!is.na(Kgut2pu), "CUSTOM", "httkCalculated") #2025June
    p.out0 <- as.data.frame(param) #automatically resets row names #for output #2025
    colnames(p.out0) <- paste0("ModelParam_", colnames(p.out0)) #for output #2025
    rownames(p.out0) <- this.cas #for output #2025
    p.out <- rownames_to_column(p.out0, var = "CASRN") #for output #2025
    output0 <- solve_pbtck(chem.cas = this.cas, parameters = param, doses.per.day = dpd, days = ndays,
    trim_output <- as.data.frame(output0) #store output

    if(route == "iv"){
      #ignore possible computational errors
      to_ignore <- 2 #<- time point 2 will always be anomalous
      n_to_ignore <- max(1, floor((24*ndays)/interv))
      if((24/interv) == 1){
        steps_between_doses <- 97
      }else if(24/interv > 1){
        steps_between_doses <- floor(97 / (24/interv))+1
      }else if(24/interv < 1){
        steps_between_doses <- floor(97 / (24/interv))
      }
      for(i in 1:n_to_ignore){
        to_ignore[i+1] = to_ignore[i] + steps_between_doses
      }
      trim_output <- trim_output[-to_ignore,]
    }

    concMax <- max(trim_output[, targetTissue])
    cmax_temp <- as.data.frame(cbind(this.cas, concMax, ConcentrationUnit))
    cmaxall <- rbind(cmaxall, cmax_temp)
  }
}
```



```

    p.out_all <- rbind(p.out_all, p.out)    #2025
  }
}

```

## solve\_gas\_pbt model

The solve\_gas\_pbt is a PBPK model similar to the “solve\_pbt” model but it uses an inhalation route of exposure assuming the chemical is volatile (gas). As a result, it has some additional checks to see if the assumption of gas exposure is reasonable but will still proceed with a flag warning. It also includes 2 different dosing approaches, a concentration over time (expConc) or a single dose (expDose)

```

if(modelType == "solve_gas_pbt"){
  #check the assumption of volatility
  chemical$Flag[chemical$HL <= -7.80388 & chemical$Flag!=""] <-"fu is zero,likely due to rounding. Set to 1"
  chemical$Flag[chemical$HL <= -7.80388 & chemical$Flag==""] <-"Chemical likely nonvolatile, consider a gas"

  for(this.cas in chemical[, "CASRN"]) {
    set.seed(1234)
    Kkidney2pu <- chemical$Kkidney2pu[chemical$CASRN == this.cas]
    Kliver2pu <- chemical$Kliver2pu[chemical$CASRN == this.cas]
    Krest2pu <- chemical$Krest2pu[chemical$CASRN == this.cas]
    Kgut2pu <- chemical$Kgut2pu[chemical$CASRN == this.cas]
    # --- expDose option has currently been disabled due to a bug in the httk package ---
    # if(modelType=="solve_gas_pbt" & gasDosing == "expDose"){
    #   ConcentrationUnit <- "uM" # output unit only has one option of "uM" for solve_gas_pbt model
    #   concMax <- max(solve_gas_pbt(chem.cas = this.cas, parameters = NULL, doses.per.day = dpd, days
    # }

    if(gasDosing == "expConc"){
      param <- parameterize_gas_pbt(chem.cas = this.cas, species = species_1, default.to.human = TRUE)
      print(param$BW) # to make sure that the body weight had been changed.
      param$Kkidney2pu <- ifelse(!is.na(Kkidney2pu), Kkidney2pu, param$Kkidney2pu) #2025June
      param$Kkidney2pu_Source <- ifelse(!is.na(Kkidney2pu), "CUSTOM", "httkCalculated") #2025June
      param$Kliver2pu <- ifelse(!is.na(Kliver2pu), Kliver2pu, param$Kliver2pu) #2025June
      param$Kliver2pu_Source <- ifelse(!is.na(Kliver2pu), "CUSTOM", "httkCalculated") #2025June
      param$Krest2pu <- ifelse(!is.na(Krest2pu), Krest2pu, param$Krest2pu) #2025June
      param$Krest2pu_Source <- ifelse(!is.na(Krest2pu), "CUSTOM", "httkCalculated") #2025June
      param$Kgut2pu <- ifelse(!is.na(Kgut2pu), Kgut2pu, param$Kgut2pu) #2025June
      param$Kgut2pu_Source <- ifelse(!is.na(Kgut2pu), "CUSTOM", "httkCalculated") #2025June
      p.out0 <- as.data.frame(param) #automatically resets row names #for output #2025
      colnames(p.out0) <- paste0("ModelParam_", colnames(p.out0)) #for output #2025
      rownames(p.out0) <- this.cas #for output #2025
      p.out <- rownames_to_column(p.out0, var = "CASRN") #for output #2025
      concMax <- max(solve_gas_pbt(chem.cas = this.cas, parameters = param, doses.per.day = NULL, days
      #note for v2.2.2, must specify total "daily.dose" when "doses.per.day" is not set to NULL.
    }
    cmax_temp <- as.data.frame(cbind(this.cas, concMax, ConcentrationUnit))
    cmaxall <- rbind(cmaxall, cmax_temp)
    p.out_all <- rbind(p.out_all, p.out)    #2025
  }
}

```

## solve\_fetal\_pbtok model

The solve\_fetal\_pbtok is a human PBPK model that includes both maternal and fetal compartments and a placenta modeled as a joint organ shared by mother and fetus. It simulated gestation day of 91-280 days. A flag warning will show if the gestation day is out of this range. The exposure routes for this model are oral and IV.

```
if(modelType == "solve_fetal_pbtok"){
  if(species_1 != "Human"){
    stop("Gestation model is for human only")
  }
  if(gestationDays < 91 || gestationDays > 280) {
    print("Warning: the gestation day is the day when dosing starts, which shall be within the range of
  }

  for(this.cas in chemical[, "CASRN"]) {
    set.seed(1234)
    Kkidney2pu <- chemical$Kkidney2pu[chemical$CASRN == this.cas]
    Kliver2pu <- chemical$Kliver2pu[chemical$CASRN == this.cas]
    Krest2pu <- chemical$Krest2pu[chemical$CASRN == this.cas]
    Kgut2pu <- chemical$Kgut2pu[chemical$CASRN == this.cas]
    param <- parameterize_fetal_pbtok(chem.cas = this.cas, species = "human", default.to.human = TRUE)
    print(param$BW) # to make sure that the body weight had been changed
    param$Kkidney2pu <- ifelse(!is.na(Kkidney2pu), Kkidney2pu, param$Kkidney2pu) #2025June
    param$Kkidney2pu_Source <- ifelse(!is.na(Kkidney2pu), "CUSTOM", "httkCalculated") #2025June
    param$Kliver2pu <- ifelse(!is.na(Kliver2pu), Kliver2pu, param$Kliver2pu) #2025June
    param$Kliver2pu_Source <- ifelse(!is.na(Kliver2pu), "CUSTOM", "httkCalculated") #2025June
    param$Krest2pu <- ifelse(!is.na(Krest2pu), Krest2pu, param$Krest2pu) #2025June
    param$Krest2pu_Source <- ifelse(!is.na(Krest2pu), "CUSTOM", "httkCalculated") #2025June
    param$Kgut2pu <- ifelse(!is.na(Kgut2pu), Kgut2pu, param$Kgut2pu) #2025June
    param$Kgut2pu_Source <- ifelse(!is.na(Kgut2pu), "CUSTOM", "httkCalculated") #2025June
    p.out0 <- as.data.frame(param) #automatically resets row names #for output #2025
    colnames(p.out0) <- paste0("ModelParam_", colnames(p.out0)) #for output #2025
    rownames(p.out0) <- this.cas #for output #2025
    p.out <- rownames_to_column(p.out0, var = "CASRN") #for output #2025

    output0 <- solve_fetal_pbtok(chem.cas = this.cas, parameters = param, times=seq(gestationDays, ge
    #initial.values = NULL; #times: time sequence in days. Default is from 13th week of pregnancy to
    #store output
    trim_output <- as.data.frame(output0)

    if(route == "iv"){
      #ignore computational errors
      to_ignore <- 2 #<- time point 2 will always be anomalous
      n_to_ignore <- max(1, floor((24*ndays)/interv))
      if((24/interv) == 1){
        steps_between_doses <- 41
      }else if(24/interv > 1){
        steps_between_doses <- floor(41 / (24/interv))+1
      }else if(24/interv < 1){
        steps_between_doses <- floor(41 / (24/interv))
      }
      for(i in 1:n_to_ignore){
```

```

    to_ignore[i+1] = to_ignore[i] + steps_between_doses
  }
  trim_output <- trim_output[-to_ignore,]
}

concMax <- max(trim_output[,targetTissue])
fconcMax <- max(trim_output[,targetTissue_fetal])
#initial.values = NULL; #times: time sequence in days. Default is from 13th week of pregnancy to .
cmax_temp <- as.data.frame(cbind(this.cas, concMax, fconcMax, ConcentrationUnit))
cmaxall <- rbind(cmaxall, cmax_temp)
p.out_all <- rbind(p.out_all, p.out) #2025
}
}

```

## Calc EAD from PBPK models except for “solve\_fetal\_pbtk”

The PBPK models currently give estimates based on the median (50%ile) of the population. As such there is minor formatting that needs to be done to generate the EAD predictions and make the output file

```

if(modelType %in% c("solve_3comp", "solve_pbtk", "solve_gas_pbtk", "solve_fetal_pbtk")){
  cmaxall$concMax <- as.numeric(cmaxall$concMax) #they are bound as characters so converting to numeric
  Cmax <- merge(chemical, cmaxall, by.x="CASRN", by.y="this.cas")
  names(Cmax) <- gsub("concMax", "Cmax", names(Cmax) )
}

```

*#Calculating the EADs from non- solve\_fetal\_pbtk*

```

if(modelType == "solve_3comp" | modelType == "solve_pbtk" | modelType == "solve_gas_pbtk"){
  EAD.out_max <- CalcEAD(Css = Cmax[,c("CASRN", "Cmax")], inVitro = invitro)

  #creating output file for user, should have EAD values and the parameters for calculating the circula
  Cmax2<-as.data.frame(Cmax, stringsAsFactors=FALSE)
  #adding in the source of the parameters
  Cmax2<-left_join(Cmax2, chemical %>% select(any_of(c("CASRN","Clint Source", "fu Source"))))
  Cmax2$Species<-species; Cmax2$Model<-modelType; Cmax2$Dose, mg/kg<-expDose;
  ssEAD.out<-EAD.out_max[,c("CASRN", setdiff(colnames(EAD.out_max), c(colnames(invintro), "Cmax")))]
  colnames(Cmax2) <- gsub("ConcentrationUnit", "Cmax, Units", names(Cmax2) )
  Cmax2$Route <- route
  Cmax2$Dose Interval, hrs" <- interv
  Cmax2$Length of Dosing, Days" <- ndays
  Cmax2$Target Tissue" <- targetTissue
  Cmax2$Target Fetal Tissue" <- targetTissue_fetal
  if(modelType == "solve_gas_pbtk" && gasDosing == "expConc"){ #for if inhalation/gas
    Cmax2$Exposure Concentration<-expConc
    Cmax2$Exposure Concentration Unit<-gasInputUnit
    Cmax2$Exposure Length, hr"<-expLength
    Cmax2$Exposure Period, hr"<-interv
    Cmax2<- Cmax2[, setdiff(colnames(Cmax2), c("Dose, mg/kg"))]
  }

  #reformatting columns to match the other models:

```

```

Cmax2<-Cmax2 %>% select(any_of(c("CASRN", "ChemicalName", "Target Tissue", "Cmax", "Cmax, Units", "FL

outputData<-full_join(Cmax2,ssEAD.out)
colnames(outputData)<-gsub("Clint$", "Clint, ul/ml/106 cells", colnames(outputData)) #add units

#The output file is an excel workbook. This has 2 tabs, one for the EAD results and the other for the
xlsx::write.xlsx(file=Userout,x=outputData, sheetName = "EADResults", append=FALSE, row.names = FALSE,
xlsx::write.xlsx(file=Userout,x=invitro, sheetName = "inVitroData", append=TRUE, row.names = FALSE, sh
xlsx::write.xlsx(file=Userout,x=p.out_all, sheetName = "PKparameters", append=TRUE, row.names = FALSE,

#to view the results, the "EADboxplot" function plots the values.
#Note that if the gas model is used EADUnit needs to be changed to "uM"
#plotting
EADplot <- EADboxplot(EAD.out = outputData, invivo = invivo, label="EAD", EADUnit = EADUnit, species =
#Arguments for EADplot functions include "EAD.out, invivo, label, chemDisplay, EADUnit, modelType, sp
EADplot
}

```

## Calc EAD from solve\_\_fetal\_\_pbtk

The PBPK models currently give estimates based on the median (50%ile) of the population. As such there is minor formatting that needs to be done to generate the EAD predictions and make the output file

```

#Calculating the EADs corresponding to maternal and fetal plasma Cmax
if(modelType == "solve_fetal_pbtk"){
  EAD.out_max <- CalcEAD(Css = Cmax[,c("CASRN", "Cmax")], inVitro = invitro)
  EAD.out_fmax <- CalcEAD(Css = Cmax[,c("CASRN", "fCmax")], inVitro = invitro)

  #creating output file for UI. Only Sciome need to use this file
  EAD.out50<-EAD.out_max
  colnames(EAD.out50)<-gsub("EAD", "EAD.50_Cmax", colnames(EAD.out50))
  EAD.out95<-cbind(EAD.out50[1], matrix(NA, nrow=nrow(EAD.out50), ncol = ncol(EAD.out50)-1))
  colnames(EAD.out95)<-gsub("EAD.50", "EAD.95_Cmax", colnames(EAD.out50)) #creating a blank data object
  #add in the flag after creating the "95" object
  EAD.out50<-left_join(chemical[,c("CASRN", "Flag")], EAD.out50)
  EAD.out<-left_join(EAD.out50, EAD.out95)
  EAD.out<-EAD.out[,setdiff(colnames(EAD.out), c("adj.fu", "fu", "arm", "adj.arm"))]

  EAD.out50_fmax<-EAD.out_fmax
  colnames(EAD.out50_fmax)<-gsub("EAD", "EAD.50_fCmax", colnames(EAD.out50_fmax)) #rename EAD to fetal E
  EAD.out95_fmax<-cbind(EAD.out50_fmax[1], matrix(NA, nrow=nrow(EAD.out50_fmax), ncol = ncol(EAD.out50_
  colnames(EAD.out95_fmax)<-gsub("EAD.50", "EAD.95", colnames(EAD.out50_fmax)) #creating a blank data ob
  #add in the flag after creating the "95" object
  EAD.out50_fmax<-left_join(chemical[,c("CASRN", "Flag")], EAD.out50_fmax)
  EAD.out_fmax<-left_join(EAD.out50_fmax, EAD.out95_fmax)
  EAD.out_fmax<-EAD.out_fmax[,setdiff(colnames(EAD.out_fmax), c("adj.fu", "fu", "arm", "adj.arm"))]

  EAD.out_both<-left_join(EAD.out, EAD.out_fmax)

  #creating output file for user, should have EAD values and the parameters for calculating the circula
  Cmax2<-as.data.frame(Cmax, stringsAsFactors=FALSE);
  #adding in the source of the parameters

```

```

Cmax2<-left_join(Cmax2, chemical %>% select(any_of( c("CASRN","Clint Source", "fu Source"))))
Cmax2$Species<-species; Cmax2$Model<-modelType; Cmax2$Dose, mg/kg<-expDose;
ssEAD.out<-EAD.out_both[,c("CASRN", setdiff(colnames(EAD.out_both), c(colnames(invivo), "Cmax")))]
colnames(Cmax2) <- gsub("ConcentrationUnit", "Cmax, Units", names(Cmax2) )
Cmax2$Route <- route
Cmax2$Dose Interval, hrs" <- interv
Cmax2$Length of Dosing, Days" <- ndays
Cmax2$Target Tissue" <- targetTissue
Cmax2$Target Fetal Tissue" <- targetTissue_fetal
#reformatting columns to match the other models:
Cmax2<-Cmax2 %>% select(any_of(c("CASRN", "ChemicalName", "Target Tissue", "Cmax", "Target Fetal Tissue")))
outputData<-full_join(Cmax2,ssEAD.out)
colnames(outputData)<-gsub("Clint$", "Clint, ul/ml/10^6 cells", colnames(outputData))#add units

#The output file is an excel workbook. This has 2 tabs, one for the EAD results and the other for the
xlsx::write.xlsx(file=Userout,x=outputData, sheetName ="EADResults", append=FALSE, row.names = FALSE,
xlsx::write.xlsx(file=Userout,x=invivo, sheetName ="inVitroData", append=TRUE, row.names = FALSE, sh
xlsx::write.xlsx(file=Userout,x=p.out_all, sheetName ="PKparameters", append=TRUE, row.names = FALSE,

#to view the results, the "EADboxplot" function plots the values.
#Note that if the gas model is used EADUnit needs to be changed to "uM" or "ppmv"
#plotting
EADplot <- EADboxplot(EAD.out = outputData, invivo = invivo, label="EAD.50_Cmax", EADUnit = EADUnit, s
fEADplot <- EADboxplot(EAD.out = outputData, invivo = invivo, label="EAD.50_fCmax", EADUnit = EADUnit
#Arguments for EADplot functions include "EAD.out, invivo, label, chemDisplay, EADUnit, modelType, sp
print(EADplot)
print(fEADplot)
}

```

## Information on the session

```
sessionInfo()
```

```

## R version 4.4.3 (2025-02-28 ucrt)
## Platform: x86_64-w64-mingw32/x64
## Running under: Windows 11 x64 (build 26100)
##
## Matrix products: default
##
## locale:
##  [1] LC_COLLATE=English_United States.utf8
##  [2] LC_CTYPE=English_United States.utf8
##  [3] LC_MONETARY=English_United States.utf8
##  [4] LC_NUMERIC=C
##  [5] LC_TIME=English_United States.utf8
##
## time zone: America/New_York
## tzcode source: internal
##

```

```

## attached base packages:
## [1] parallel stats      graphics grDevices utils      datasets  methods
## [8] base
##
## other attached packages:
## [1] scales_1.3.0      readxl_1.4.5      http_2.2.2        doParallel_1.0.17
## [5] iterators_1.0.14  foreach_1.5.2     deSolve_1.40      lubridate_1.9.4
## [9] forcats_1.0.0     stringr_1.5.1     dplyr_1.1.4       purrr_1.0.4
## [13] readr_2.1.5       tidyr_1.3.1       tibble_3.2.1      ggplot2_3.5.2
## [17] tidyverse_2.0.0   knitr_1.50
##
## loaded via a namespace (and not attached):
## [1] gtable_0.3.6      xfun_0.52         rJava_1.0-11      lattice_0.22-6
## [5] tzdb_0.5.0        vctrs_0.6.5       tools_4.4.3       Rdpack_2.6.3
## [9] generics_0.1.3    pkgconfig_2.0.3   Matrix_1.7-2      data.table_1.17.0
## [13] lifecycle_1.0.4   farver_2.1.2      compiler_4.4.3    textshaping_1.0.0
## [17] munsell_0.5.1     mitools_2.4       codetools_0.2-20  survey_4.4-2
## [21] htmltools_0.5.8.1 yaml_2.3.10       pillar_1.10.1     crayon_1.5.3
## [25] tidyselect_1.2.1  digest_0.6.37     mvtnorm_1.3-3     stringi_1.8.7
## [29] splines_4.4.3     fastmap_1.2.0     grid_4.4.3        colorspace_2.1-1
## [33] expm_1.0-0        cli_3.6.4         magrittr_2.0.3    xlsxjars_0.6.1
## [37] survival_3.8-3    withr_3.0.2       bit64_4.6.0-1     timechange_0.3.0
## [41] rmarkdown_2.29    bit_4.6.0         cellranger_1.1.0  ragg_1.3.3
## [45] hms_1.1.3         msm_1.8.2          evaluate_1.0.3    rbibutils_2.3
## [49] rlang_1.1.5       Rcpp_1.0.14       glue_1.8.0        DBI_1.2.3
## [53] xlsx_0.6.5        rstudioapi_0.17.1 vroom_1.6.5       R6_2.6.1
## [57] systemfonts_1.2.1

```