Generic PBK model for farm animal species: Cattle (*Bos taurus*), Swine (*Sus scrofa*), Sheep (*Ovis aries*) and Chicken (*Gallus gallus domesticus*)

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Part I. PBK model reporting template

A. Name of model

Generic PBK model for farm animal species: cattle (*Bos taurus*), swine (*Sus scrofa*), sheep (*Ovis aries*) and chicken (*Gallus gallus domesticus*)

B. Contact details

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C. Summary of model characterisation, development, validation, and potential applicability

Animal risk assessment of chemicals is important for the protection of various animal species, as well as food safety. This package highlighted available PBK models for various farm animal species and identified the need to develop and/or refine generic models for these species (Lautz et al., 2019a). Generic PBK models for cattle (*Bos taurus*), swine (*Sus scrofa domesticus*), sheep (*Ovis aries*) and chicken (*Gallus gallus domesticus*) have been developed in the open source freeware R. For each species, the PBK model has been implemented for regulated compounds and environmental contaminants to predict the concentration in a range of body compartments and organs (blood, liver, kidney etc), milk (sheep and cattle) and eggs (chicken). Global sensitivity analyses have been performed using the function “soboljansen” in the “sensitivity” package to identify parameters which have the most impact on the model’s output. Predictions of concentrations in tissues and milk for two chemicals in cattle, swine and sheep; and for 6 chemicals in chicken were compared to the available experimental data. The differences between model predictions and data were within a 3-fold factor for 71% of the predictions. It is acknowledged that the performance of these generic PBK models in farm animals with regards to the accuracy of tissue and milk concentration predictions for different chemicals still needs improvement before full confidence can be built towards their use in routine regulatory risk assessment. The collection of species-specific and chemical-specific kinetic data is needed to reduce uncertainties and to fill in data gaps in the regulatory framework of pesticides and other substances, such as feed additives and contaminants.

D. Model characterisation (modelling workflow)

**Step 1 – Scope and purpose of the model (problem formulation)**

Animal risk assessment of chemicals for the protection of various animal species, as well as food safety, can benefit from using PBK modelling approaches. PBK modelling approaches can also be used to harmonise human and environmental risk assessment.

**Step 2 – Model conceptualisation (model structure, mathematical representation)**
The generic models, physiological data, R codes and case studies are available in references (Lautz et al., 2019b,c; 2020a,b,c,d). The model includes eleven compartments (arterial and venous blood, gastrointestinal tract (gut lumen, gut tissue), liver, heart, brain, adipose tissue, kidney, muscle, lung, bone). Milk is included as the twelfth compartment for cattle and sheep, while this compartment is replaced by eggs in chicken. All the organs/tissues and mixed compartments are modelled a blood flow-limited distribution. The venous blood that flows out of the gastrointestinal tract in the portal vein and enters the liver. The model equations and parameter abbreviations are provided in the supporting information of the manuscripts (Lautz et al., 2020b, c).

**Step 3 – Model parameterisation (parameter estimation and analysis)**

An extensive literature search was performed to collect physiological parameters and their inter-individual variability (mean, coefficient of variation, sample size) for four farm animal species: cattle (*Bos taurus*), swine (*Sus scrofa*), and sheep (*Ovis aries*) and chicken (*Gallus gallus domesticus*). These physiological parameters were estimated based on the results of extensive literature searches described in Lautz et al., (2019b; 2020a,c,d). Relative tissue volumes and blood flows were mostly assessed using data on mature animals.

**Step 4 – Computer implementation (solving the equations)**

Model was developed using R software (version 3.3.3). The physiological input parameters and R codes for the PBK models and its application to the four farm animal species are also available on the EFSA knowledge junction (see Lautz et al., 2019 b,c, Lautz et al., 2020d in references) with a Creative Common Attribution 4.0 license. Physiological input parameters are available on the EFSA knowledge junction under the DOI 10.5281/zenodo.3433224 with a Creative Common Attribute 4.0 license and published elsewhere (Lautz et al., 2019).

**Differential equations describing the rate of change of chemical mass in each compartment**

<table>
<thead>
<tr>
<th>Tissue compartment</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gut lumen</td>
<td>$\frac{dM_{\text{lumen}}}{dt} = Q_{\text{food}} \times (C_{\text{art}} - C_{\text{faccess}}) + f_1 \left( \frac{V_{\text{max}} \times C_{\text{liver}}}{K_m \times C_{\text{liver}} + V_{\text{liver}}} \right)$</td>
</tr>
<tr>
<td>Gut tissue</td>
<td>$\frac{dM_{\text{gut}}}{dt} = Q_{\text{gut}} \times (C_{\text{art}} - C_{\text{gut}}) + k_a \times M_{\text{lumen}}$</td>
</tr>
<tr>
<td>Liver Metabolism</td>
<td>$\frac{dM_{\text{liver}}}{dt} = Q_{\text{liver}} \times (C_{\text{art}} - C_{\text{liver}}) - \frac{V_{\text{max}} \times C_{\text{liver}}}{K_m \times C_{\text{liver}} + V_{\text{liver}}} \times Q_{\text{gut}} + Q_{\text{gut}} \times (C_{\text{gut}}) - Q_{\text{gut}} \times (C_{\text{liver}})$</td>
</tr>
<tr>
<td>Clearance</td>
<td>$\frac{dM_{\text{liver}}}{dt} = Q_{\text{liver}} \times (C_{\text{art}} - C_{\text{liver}}) - (C_{\text{liver}} \times C_{\text{hepatic}}) + Q_{\text{gut}} \times (C_{\text{gut}}) - Q_{\text{gut}} \times (C_{\text{liver}})$</td>
</tr>
<tr>
<td>Heart, Brain, Bone, Adipose tissue, Muscle, Lung</td>
<td>$\frac{dM_{\text{tissue}}}{dt} = Q_t \times \left( C_{\text{art}} - \frac{C_t}{P_t} \right)$</td>
</tr>
<tr>
<td>Kidney</td>
<td>$\frac{dM_{\text{kidney}}}{dt} = Q_{\text{kidney}} \times \left( C_{\text{art}} - \frac{C_{\text{kidney}}}{P_{\text{kidney}}} \right) - (C_{\text{kidney}} \times C_{\text{renal}})$</td>
</tr>
<tr>
<td>Venous blood</td>
<td>$\frac{dM_{\text{ven}}}{dt} = \sum_t \left( Q_t \times \left( \frac{C_t}{P_t} \right) + (Q_{\text{gut}} + Q_{\text{liver}}) \times \left( \frac{C_{\text{liver}}}{P_{\text{liver}}} \right) - Q_{\text{tot}} \times C_{\text{ven}} \right)$</td>
</tr>
</tbody>
</table>
Arterial blood
\[
\frac{dM_{\text{art}}}{dt} = Q_{\text{tot}} \times C_{\text{ven}} \times \left( \frac{Q_{\text{tot}}}{Q_{\text{art}} + Q_{\text{exhale}} \times P_{\text{art}}} \right) - Q_{\text{tot}} \times C_{\text{art}}
\]

Milk
\[
\frac{dM_{\text{mglnd}}}{dt} = Q_{\text{mglnd}} \times \left( C_{\text{art}} - \frac{C_{\text{mglnd}}}{P_{\text{milk}}} \right) - Q_{\text{milk}} \times C_{\text{mglnd}}
\]

Egg
\[
\frac{dM_{\text{reprod}}}{dt} = Q_{\text{reprod}} \times \left( C_{\text{art}} - \frac{C_{\text{reprod}}}{P_{\text{egg}}} \right) - Q_{\text{egg}} \times C_{\text{reprod}}
\]

**Step 5 – Model Performance**

Validation of the model: comparison of predicted blood and tissues concentrations with measured data. Due to the extremely limited availability of experimental data for partition coefficients, the partitioning behaviour of a chemical in the included species was estimated using a QSAR model based on several tissue constituents (e.g. polar and non-polar lipids, water, and proteins) and the octanol/water partition coefficient. The PBK model was evaluated through comparing model predictions of tissue concentrations with experimental data measured in farm animal species and available from the literature for melamine, oxytetracycline, deltamethrin, fipronil, monensin, salinomycin, and sanguinarine (Figure 1 & 2).

![Figure 1. Comparison between concentrations measured in various organs of cattle (CA), sheep (SH), and swine (SW) and PBK model predictions for A) melamine and B) oxytetracycline. Dotted lines represent the 3-fold and 10-fold changes. Organs, species, and references of experimental datasets are indicated in legend: colours and shapes represent organs and studies, respectively.](image-url)
For melamine, a data gap for melamine was identified with regards to absorption rate in the digestive tract of ruminants. However, differences between exposure and excreted concentrations in ruminants suggest that the absorption of melamine is higher than 75%. In monogastric animals, such as swine, the absorption of melamine is nearly 100% and unchanged melamine is detected in urine only. Since absorption rates for melamine in the included species were not available in literature, melamine absorption rates were extrapolated from chicken, leading to uncertainty in the PBK models for cattle and sheep.
Overall, literature data on melamine in various tissues of the included species were very limited, so the quality of the included papers is of high relevance for the reliability of the model performance. Milk concentrations were overpredicted by the model in most cases when only about 2% of ingested melamine has been reported to be excreted in milk. For oxytetracycline, a veterinary antibiotic administered orally and intravenously, absorption is only partial in the swine’s intestine and was not characterised in adult ruminants, i.e. cattle and sheep, and may vary compared to monogastric species. For other substances, such differences in absorption between monogastric and ruminants have been observed. Oxytetracycline undergoes no metabolism and is excreted in urine unchanged. Overall, measured blood concentrations were often available in literature, whereas tissue concentrations were scarce. For oxytetracycline, model predictions were reliable compared to observed data.

Global Sensitivity analyses were performed using the variance-based Sobol method (Saltelli et al., 2008; Sobol et al., 2007). First order and total Sobol sensitivity indices were estimated for oxytetracycline in cattle, swine, and sheep; and melamine and deltamethrin in chicken. Sensitivity was assessed at three time points for the concentration in blood and kidneys. Parameter values and exposure scenario characteristics are described in detail in the supplementary material of Lautz et al. (2019d,e, 2020c). Global sensitivity analysis of the PBK models for cattle, sheep, and swine with oxytetracycline shows that body weight (BW), cardiac output (CO) and renal blood flow (fCO_kidney) were the main contributors to the overall variance of the model output (Figure 3). However, the relative contribution of each of those varied among cattle, sheep and swine. During the absorption phase, the model output for the blood concentration was impacted by the intestinal blood flow (fCO_intestine) as well as distribution of the chemical towards other organs such as muscle and adipose tissue (fBW_adipose, fCO_muscle). In the elimination phase, renal blood flow was the most sensitive parameter and had a strong influence on model outputs. The results of the global sensitivity analysis for model outputs with regards to kidney concentrations were similar to that for blood concentrations. In chicken, BW and CO were also parameters that contributed most to the variation of the model outcome, as well as intestinal blood flow (fCO_intestine) during the absorption phase (Figure 4). In the elimination phase renal blood flow (fCO_kidney) was the most sensitive parameter for melamine. For the lipophilic compound deltamethrin the neutral fraction of the tissue (nl), the adipose tissues relative volume (fBW_adipose), blood flow to the adipose tissue (fCO_adipose) and the lipid content of the tissues contributed the most to the overall variance and predictions of internal concentrations.
Figure 3. Sensitivity analysis of the cattle (upper panels), sheep (middle panels) and swine (lower panels) PBK model applied to oxytetracycline. Sobol’s sensitivity indices were estimated for the blood concentration in the three species at three time points (from left to right): 1.25, 9.5, and 24 hours for cattle; 0.5, 6.5, and 21 hours for sheep; 0.5, 5, and 21 hours for swine. Estimation of the Sobol’ total sensitivity indices (TI) are presented in dark green and estimation of the Sobol’ first-order indices (FOI) in light green. The nine most influencing parameters according to the total sensitivity indices are shown.
Step 6 – Model Documentation

The current generic PBK models have been published in the peer reviewed literature (Lautz et al., 2020a,b,c) (see reference below).

E. Identification of uncertainties

Model structure

Metabolism, excretion and ingestion routes, which are chemical-specific, were the main sources of uncertainty in model structure.

Input parameters

Farm animal physiology (including blood flow, chemical uptake) were not fully available for the included species and should be measured to improve the mechanistic aspects of the PBK models. Values for all the sheep physiological parameters were available, in contrast to cattle and swine for which interspecies extrapolations were performed. The uncertainty due to species extrapolation has not been quantified, but the global sensitivity analysis highlighted the parameters for which extrapolation may critically affect model outputs. However, the lack of data relative to the tissue constituents in farm animal species limits the level of complexity of the QSAR models that can be used to predict partition coefficients.

Model output
Predictions of tissue concentrations for two chemicals were compared to experimental data and were accurate with 71% of predictions within a 3-fold factor. At the tissue or organ level, 58-84% of the predictions in blood, kidney, and muscle were within 3-FC, whereas 67% of the predicted concentrations in liver were between 3-FC to 10-FC. From an inter-species perspective, the model predictions showed relatively low variability with 82%, 76%, and 63% of predictions within a 3-FC for cattle, sheep, and swine respectively. Model predictions exceeded 10-FC in only 3% of cases in sheep and cattle and 9% in swine. In chicken, predictions of tissue concentrations for six chemicals were compared to experimental data and were accurate with 71% of predictions within a 3-fold factor. At the tissue or organ level, 57-97% of the predictions were within 3-FC, whereas 33-35% of the predicted concentrations in adipose tissue, liver and muscle were between 3-FC and 10-FC.

Other uncertainties (e.g. model developed for different substance and/or purpose)
None to report

Overall evaluation of uncertainties: Overall, the model accuracy did not depend on the species to a large extent with PBK model predictions from an inter-species perspective; the PBK model prediction was within a 3-fold change of 82%, 76%, 71%, and 63% for cattle, sheep, chicken, and swine, respectively. Model predictions exceeded 10-FC in only 3% of cases in sheep and cattle and 9% in swine. However, literature data on chemical data in various tissues of the included species were very limited, so the quality of the included papers is of high relevance for the reliability of the model performance (Lautz et al., 2019d, 2020c).

F. Model implementation details

- software (version no): The code was written and executed in in R. 3.3.3
- availability of code: Yes, code is accessible via the peer reviewed publication.
- software verification / qualification: was done by peer reviewed publication.

G. Peer engagement (input/review)
Done by publishing the model in peer reviewed journals, see references

Strategy for reducing overall uncertainty
1. Peer-reviewed literature providing tissue residues, milk residues, and egg residues for a range of regulated compounds (pesticides, feed additives) and anthropogenic or naturally-occurring contaminants (e.g. persistent organic pollutants, mycotoxins) are still relatively scarce. Extensive literature searches and data collection from pre-market dossiers should be performed to develop kinetic databases to further explore the predictability of the models for a larger group of compounds.
2. The PBK models for cattle, sheep, and swine are validated for chemicals which are excreted only via the kidney, while the chicken model includes metabolism. Chemicals which may undergo transport and phase I and/or Phase II metabolism are not included in the model case studies. Integration of the in vitro metabolism, kinetic data and generic enzyme activities can provide the basis to further develop quantitative in vitro to in vivo extrapolation models, which can be implemented in PBK models for animal risk assessment and ultimately limit in vivo testing in farm animal species. More case studies would need to be conducted on chemicals of relevance to animal risk assessment in the three animal species.

PBK model code
Model was developed using R software (version 3.3.3). The full data collection and R codes of the PBK models for cattle, swine, sheep and chicken including case studies are described in Lautz et al. and are available for download (Lautz et al, 2019a,b,c; Lautz et al, 2020d) on EFSA knowledge junction under the Creative Commons attribution 4.0 international.

### PBK model input for cattle, sheep and swine

```r
# Model PBK (cattle, sheep, swine) generic
# 01/2019
# L.S. Lautz, S. Hoeks, R. Oldenkamp

# probabilistic multi-compartment model for farm animal species

# source functions
source("singlechemical_dmdt.R")

# Loading physiology data
fBW <- read.csv("data_fBW_animals.csv", stringsAsFactors = FALSE) # organ fractions, incl distribution parameters
fCO <- read.csv("data_fCO_animals.csv", stringsAsFactors = FALSE) # blood flow fractions, incl distribution parameters
fPC <- read.csv("data_pc_tissue.csv", stringsAsFactors = FALSE) # tissue composition

# Loading TK data
TK <- read.csv("data_TK.csv", stringsAsFactors=FALSE) # toxicokinetic parameters

# Loading physicochemical data
chem <- read.csv("data_chem.csv", stringsAsFactors = FALSE) # physicochemical parameters

# Inputs ----
# Defining the exposure scenario
species <- "species" # cattle/d/cattle_b/sheep/swine
chemical <- "chemical" # add chemical name
regime <- "bolus" # exposure regime (bolus)
route <- "route" # exposure route (oral/iv)
E_dose <- 0 # exposure dose (mg/kgbw)
E_start <- 0 # start of exposure phase (h)
E_end <- 24 # end of exposure phase (h)
E_int <- 24 # interval between doses (h)

# Simulation parameters
A_type <- "single" # type of probabilistic analysis ("single" or "VA")
chem_fix <- TRUE # fixing the chemical and TK parameters or not (TRUE/FALSE)
n_sim  <- 1000 # number of iterations
n_out  <- 9 # number of compartments to output (blood, total body)
t_start <- 0 # start of simulation (h)
t_end <- 24 # end of simulation (h)
t_A <- c(seq(0.025,0.225,by=0.025),seq(0.25,24,by=0.25)) # time points (h), only relevant when chem_fix=TRUE

# Setup single animal simulation-----
if(A_type == "single") {
  Names_fix <- c("BW", paste("fBW",colnames(fBW[2+3*c(1:19)]),sep=" "), 
                 "CO", paste("fCO",colnames(fCO[2+3*c(1:19)]),sep=" "), 
                 colnames(rates[2+3*c(0:4)]), 
                 colnames(fPC[2:57]), 
                 "nl","pl","pr","w", 
                 colnames(TK[3:8]), 
                 colnames(chem[2:5]))
  NP_fix <- length(Names_fix) # number of fixed parameters
  fix_in <- cbind(fBW[fBW$species==species,],fCO[fCO$species==species,],
                  rates[rates$species==species,],
                  fPC[fPC$species==species,],
                  TK[TK$species==species&TK$chemical==chemical,],
                  chem[chem$chemical==chemical,])
  par_in <- fix_in
}

# Setup probabilistic simulations-----
if(A_type == "VA") {
  # Variability analysis with fixed chemical and toxicokinetic parameters
  Names_var <- c("BW", 
                 paste("fBW",colnames(fBW[2+3*c(1:19)]),sep=" "),
                 paste("fCO",colnames(fCO[2+3*c(1:19)]),sep=" "),
                 .....
                 paste("rates",colnames(rates[2+3*c(0:4)]),sep=" "),
                 .....
                 paste("TK",colnames(TK[3:8]),sep=" "),
                 .....
                 paste("chem",colnames(chem[2:5]),sep=" ")).
  # Fixing physiological and physicochemical parameters
  chem_fix <- TRUE
  n_sim  <- 1000
  n_out  <- 9
  t_start <- 0
  t_end <- 24
  t_A <- c(seq(0.025,0.225,by=0.025),seq(0.25,24,by=0.25))
  # Loading physiology data
  fBW <- read.csv("data_fBW_animals.csv", stringsAsFactors = FALSE)
  fCO <- read.csv("data_fCO_animals.csv", stringsAsFactors = FALSE)
  fPC <- read.csv("data_pc_tissue.csv", stringsAsFactors = FALSE)
  TK <- read.csv("data_TK.csv", stringsAsFactors=FALSE)
  chem <- read.csv("data_chem.csv", stringsAsFactors = FALSE)
  # Inputs ----
  # Defining the exposure scenario
  species <- "species" # cattle/d/cattle_b/sheep/swine
  chemical <- "chemical" # add chemical name
  regime <- "bolus" # exposure regime (bolus)
  route <- "route" # exposure route (oral/iv)
  E_dose <- 0 # exposure dose (mg/kgbw)
  E_start <- 0 # start of exposure phase (h)
  E_end <- 24 # end of exposure phase (h)
  E_int <- 24 # interval between doses (h)
  # Simulation parameters
  A_type <- "single" # type of probabilistic analysis ("single" or "VA")
  chem_fix <- TRUE # fixing the chemical and TK parameters or not (TRUE/FALSE)
  n_sim  <- 1000 # number of iterations
  n_out  <- 9 # number of compartments to output (blood, total body)
  t_start <- 0 # start of simulation (h)
  t_end <- 24 # end of simulation (h)
  t_A <- c(seq(0.025,0.225,by=0.025),seq(0.25,24,by=0.25)) # time points (h), only relevant when chem_fix=TRUE
  # Setup single animal simulation-----
  if(A_type == "single") {
    Names_fix <- c("BW", 
                   paste("fBW",colnames(fBW[2+3*c(1:19)]),sep=" "),
                   "CO", paste("fCO",colnames(fCO[2+3*c(1:19)]),sep=" "),
                   .....
                   colnames(rates[2+3*c(0:4)]),
                   .....
                   colnames(TK[3:8]),
                   .....
                   colnames(chem[2:5]))
    NP_fix <- length(Names_fix) # number of fixed parameters
    fix_in <- cbind(fBW,fCO,rates,fPC,TK,chem)
    par_in <- fix_in
  }
  # Setup probabilistic simulations-----
  if(A_type == "VA") {
    # Variability analysis with fixed chemical and toxicokinetic parameters
    Names_var <- c("BW", 
                   paste("fBW",colnames(fBW[2+3*c(1:19)]),sep=" "),
                   paste("fCO",colnames(fCO[2+3*c(1:19)]),sep=" "),
                   .....
                   paste("rates",colnames(rates[2+3*c(0:4)]),sep=" "),
                   .....
                   paste("TK",colnames(TK[3:8]),sep=" "),
                   .....
                   paste("chem",colnames(chem[2:5]),sep=" ")).
    # Fixing physiological and physicochemical parameters
    chem_fix <- TRUE
    n_sim  <- 1000
    n_out  <- 9
    t_start <- 0
    t_end <- 24
    t_A <- c(seq(0.025,0.225,by=0.025),seq(0.25,24,by=0.25))
  }
```
"CO",
paste("fCO",colnames(fCO[2+3*c(1:19)]),sep="_"),
colnames(rates[2+3*c(0:4)])
#initial list with mean + sd values of all varying parameters
Means <- cbind(fBW[fBW$species==species,2+3*c(0:19)],
fCO[fCO$species==species,2+3*c(0:19)],
rates[rates$species==species,2+3*c(0:4)])
SDs <- cbind(fBW[fBW$species==species,3+3*c(0:19)],
fCO[fCO$species==species,3+3*c(0:19)],
rates[rates$species==species,3+3*c(0:4)])
Distributions <- cbind(fBW[fBW$species==species,4+3*c(0:19)],
fCO[fCO$species==species,4+3*c(0:19)],
rates[rates$species==species,4+3*c(0:4)])
colnames(Means) = colnames(SDs) = colnames(Distributions) = Names_var
Names_var_not <- colnames(SDs[which(is.na(SDs))])
Means <- Means[!(colnames(Means)%in%Names_var_not)] #exclude all parameters without SD
SDs <- SDs[!(colnames(SDs)%in%Names_var_not)] #exclude all parameters without SD
Distributions <- Distributions[!(colnames(Distributions)%in%Names_var_not)] #exclude all parameters without SD
Names_var <- colnames(SDs)
NP_var <- length(Names_var) #number of varying parameters
#list with names of all fixed parameters (including 'varying parameters' that are 0 or NA)
Names_fix <- c(colnames(fPC[2:57]),
"nl","pl","pr","w",
colnames(TK[3:8]),
colnames(chem[2:5]),
Names_var_not)
NP_fix <- length(Names_fix) #number of fixed parameters
fix_in <- cbind(fPC[fPC$species==species,colnames(fPC)%in%Names_fix],
data.frame(nl=1,pl=1,pr=1,w=1),
TK[TK$species==species&TK$chemical==chemical,colnames(TK)%in%Names_fix],
chem[chem$chemical==chemical,colnames(chem)%in%Names_fix],
fBW[fBW$species==species,colnames(fBW)%in%substr(Names_fix[grep('fBW',Names_fix)],start=5,stop=nchar(Names_fix[grep('fBW',Names_fix)]))],
fCO[fCO$species==species,colnames(fCO)%in%substr(Names_fix[grep('fCO',Names_fix)],start=5,stop=nchar(Names_fix[grep('fCO',Names_fix)]))],
rates[rates$species==species,colnames(rates)%in%Names_fix])
colnames(fix_in) <- Names_fix
#create data frames with random samples
X1 <- matrix(NA, nrow = n_sim, ncol = NP_var)
colnames(X1) <- Names_var
SARes <- X1
for(i in 1:NP_var){
if (Distributions[i] == "N") {
SARes[,i] <- rnorm(n_sim, mean = Means[,i], sd = SDs[,i])
} else if (Distributions[i] == "B") {
alpha <- ((1 - Means[,i]) / SDs[,i]^2 - 1 / Means[,i]) * Means[,i] ^ 2
beta <- alpha * (1 / Means[,i] - 1)
SARes[,i] <- rbeta(n_sim, shape1 = alpha, shape2 = beta)
} else if (Distributions[i] == "LN") {
CV <- SDs[,i]/Means[,i]
mlog <- log(Means[,i]/sqrt(1+CV^2)) #mean of log values
slog <- sqrt(log(1+CV^2)) #sd of log values
SARes[,i] <- rlnorm(n_sim, meanlog = mlog, sdlog = slog)
}
}
var_in <- SARes
par_in <- cbind(var_in,fix_in)
write.csv(par_in,"par_in.csv",row.names = FALSE)

#===========================================================================
#Model application-----
#setup table model results
SimRes <- matrix(NA, nrow = nrow(par_in), ncol = n_out*length(t_A))
colnames(SimRes) <- c(paste("blood_t",t_A,sep=""),
paste("fat_t",t_A,sep=""),
paste("liver_t", t_A, sep=""),
paste("kidney_t",t_A,sep=""),
paste("muscle_t",t_A,sep=""),
paste("heart_t", t_A, sep=""),
paste("brain_t",t_A,sep=""),
paste("carcass_t",t_A,sep=""),
paste("lung_t", t_A, sep=""))
for(j in 1:nrow(par_in)){
print(paste("Running loop: ", j))
print(paste("Current time: ", Sys.time()))
SimRes[j,] <- multi_tool(par_in = par_in[j],
 species = species, regime = regime, route = route, E_dose = E_dose, E_start = E_start, E_end = E_end, E_int = E_int,
n_out = n_out, t_start = t_start, t_end = t_end, t_A = t_A, chem_fix = chem_fix)
}
write.csv(SimRes,"results.csv",row.names = FALSE)

PBK model function with integrated QSAR for tissue:blood partition coefficients
dMdt <- function(i, M, C, h, physnames, Qin, Qout, CO, PW, Kgastric, Qbolus, Qinest, Qiv, Qexhale, kabs, Vmax, Km, Cl, fbile, fbact, Qmilk)
{
# input via food
dMfood <- E_bolus[i] * Qbolus + E_cont[i] * Qinest
# input via iv
dMiv <- E_iv[i] * Qiv
# absorption over intestinal wall
dMabs <- M[physnames == "lumen"] * kabs
# delivery via arterial blood
dMart <- Qin * C[physnames == "art"]
# delivery to venous blood and passage through portal vein
dMven <- Qout * (C / PC)
# metabolism and enterohepatic circulation and retransformation
dMmet <- ifelse(!is.na(Vmax) & !is.na(Km), (Vmax * C / PC) / (Km + C / PC) * OW, 0)
# transport over lung and exhalation
dMexh <- -CO * C
# excretion to urine, milk, feces
dMexh <- -dMexh[physnames != "ven"]
dMexc <- M[physnames == "lumen"] * kgastric
# Function to run model
multi_tool <- function(par_in, species, regime, route, E_dose, E_start, E_end, E_int, n_out, t_start, t_end, t_A, chem_fix) {
# Physicochemical properties ----
MW <- par_in$MW
Kow <- par_in$Kow # Kow
S <- (0.001 * par_in$S) / MW  # solubility
Temp <- 298  # Temperature 298 K = 25 degC
R <- 8.314   # Gas constant (J/mol/K)
Pv <- par_in$Pv # vapor pressure
# General physiology ----
BW <- par_in$BW            # bodyweight (kg)
CO <- par_in$CO         # cardiac output (L/min)
Qexhale <- (0.499 * (BW^0.81))      # ventilation mammalian (L/min)
# one matrix per relevant phys parameter [in right order]
comp <- colnames(par_in[grep('fBW', colnames(par_in))])
comp <- comp[order(comp)]
physnames <- c(substr(comp, start=5, stop=nchar(comp)), "ven", "art", "lumen", "milk", "urine", "air", "feeces", "metab", "feed", "iv")
# Organ weights
EBW <- c(par_in[comp], rep(0, 11))
EBW[physnames == "ven"] <- 2/3 * EBW[physnames == "blood"]
EBW[physnames == "art"] <- 1/3 * EBW[physnames == "blood"]
EBW[physnames == "lumen"] <- EBW[physnames == "blood"]
CM <- EBW * sum(c(EBW[physnames == "ven"], EBW[physnames == "art"], EBW[physnames == "lumen"]))
# Body flows Q
Qin <- Qin * (Qout / (Qout + Qexhale * PC[physnames == "air"]))
Qout <- Qin + Qin * PC[physnames == "air"]
# Body flows (L/min), normalized to CO
Qin <- Qin + PC[physnames == "air"]
Qout <- Qin + PC[physnames == "air"]
return(list(M=M, C=C))
}
# Function to run model
multi_tool <- function(par_in, species, regime, route, E_dose, E_start, E_end, E_int, n_out, t_start, t_end, t_A, chem_fix) {
fPC_new <- fPC_new*(sumold/sumnew)
PCexps <- comp[grep('exp_',comp)]  #all QSAR exponents
PCints <- comp[grep('int_',comp)]  #all QSAR intercepts
tissnames <- c(substr(PCcomp[grep('_nl',PCcomp)],start=1,stop=nchar(PCcomp[grep('_nl',PCcomp)])-3))
PC1 <- fPC_int*fPC_new*Kow^fPC_exp
PC1 <- tapply(fPC_int*fPC_new*Kow^fPC_exp,rep(seq(length(PC1)/4),each=4),sum)  #partitioning coefficients tissue-water
PC <- rep(PC1[tissnames=='blood'],length(physnames))
PC[physnames=='air'] <- (Pv/S)/(R*Temp)
PC[is.na(PC)] <- PC[physnames=='blood']
PC <- PC/PC[physnames=='blood']

#Rates and flows (not chemical-dependent)
physnames <- physnames[physnames=='blood']
Qingest <- rep(0,length(physnames))
Qiv <- rep(0,length(physnames))
Qbolus <- rep(0,length(physnames))
Qmilk <- rep(0,length(physnames))
fiile <- rep(0,length(physnames))

if (route=='oral' & regime == "bolus") {
  E_bolus[(t>=E_start*3600 & t<3600*E_end & t%%(3600*E_int)==0)] <- (E_dose/MW)*BW  #bolus
} else if (route=='oral' & regime == "continuous") {
  E_cont[(t>=E_start*3600 & t<3600*E_end)] <- ((E_dose/MW)/par_in$Qingest)*BW  #mmol/kgfeed
} else {
  E_iv[t==E_start*3600] <- (E_dose/MW)*BW  #intravenous injection
}

M <- rep(0,length(physnames))  #mass per compartment (mmol)
C <- rep(0,length(physnames))  #concentration per compartment (mmol/L)

#Model simulation ----
for (i in 1:length(t)) {
  output <- dMdt(i=i, M=M, C=C, h=h, physnames=physnames, Qin=Qin, Qout=Qout, CO=CO, PC=PC, OW=OW, E_bolus=E_bolus, E_cont=E_cont, E_iv=E_iv, kgastric=kgastric, Qbolus=Qbolus, Qingest=Qingest, Qiv=Qiv, Qexhale=Qexhale, kabs=kabs, Vmax=Vmax, KM=KM, CI=CI, fiile=fiile, Qmilk=Qmilk)
  M <- output$M
  C <- output$C

  if (chem_fix) {
    results <- matrix(NA,nrow=1,ncol=n_out*length(t_A))
    colnames(results) <- c(paste("blood_t",t_A,sep=""), paste("fat_t",t_A,sep=""), paste("liver_t",t_A,sep=""), paste("kidney_t",t_A,sep=""), paste("muscle_t",t_A,sep=""), paste("brain_t",t_A,sep=""), paste("carcass_t",t_A,sep=""), paste("lung_t",t_A,sep=""))
  } else {
    results <- matrix(0,nrow=1,ncol=3)
    colnames(results) <- c("Cmax","tmax","AUC_24h")
  }

#Write to results for t_A
if (chem_fix & any(t[i]==round(t_A*3600))) {
  results[,paste("blood_t",t_A[is.na(t_A)])] <- C[physnames=='blood']  #blood
  results[,paste("fat_t",t_A[is.na(t_A)])] <- C[physnames=='fat']  #fat
  results[,paste("liver_t",t_A[is.na(t_A)])] <- C[physnames=='liver']  #liver
  results[,paste("kidney_t",t_A[is.na(t_A)])] <- C[physnames=='kidney']  #kidney
  results[,paste("muscle_t",t_A[is.na(t_A)])] <- C[physnames=='muscle']  #muscle
  results[,paste("brain_t",t_A[is.na(t_A)])] <- C[physnames=='brain']  #brain
  results[,paste("carcass_t",t_A[is.na(t_A)])] <- C[physnames=='carcass']  #carcass
  results[,paste("lung_t",t_A[is.na(t_A)])] <- C[physnames=='lung']  #lung
}
} else if (!chem_fix) {
    results[,"tmax"] <- ifelse(C[physnames=="ven"]>results[,"Cmax"],t[i]/3600,results[,"tmax"])
    results[,"Cmax"] <- ifelse(C[physnames=="ven"]>results[,"Cmax"],C[physnames=="ven"],results[,"Cmax"])
    results[,"AUC_24h"] <- results[,"AUC_24h"] + C[physnames=="ven"]
}
return(results)
### PBK Model Evaluation Checklist

<table>
<thead>
<tr>
<th>PBK Model Evaluation Checklist</th>
<th>Checklist assessment</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name of the PBK model (as in the reporting template)</td>
<td></td>
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<tr>
<td>Model developer and contact details</td>
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<td></td>
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<tr>
<td>Name of person reviewing and contact details</td>
<td></td>
<td></td>
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<tr>
<td>Date of checklist assessment</td>
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</tr>
<tr>
<td><strong>A. Context/Implementation</strong></td>
<td></td>
<td></td>
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<tr>
<td><strong>A.1. Regulatory Purpose</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. What is the acceptable degree of confidence/uncertainty (e.g. high, medium or low) for the envisaged application (e.g. priority setting, screening, full assessment?)</td>
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<tr>
<td>2. Is the degree of confidence/uncertainty in application of the PBK model for the envisaged purpose greater or less than that for other assessment options (e.g. reliance on PBK model and \textit{in vitro} data vs. no experimental data)?</td>
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<tr>
<td><strong>A.2. Documentation</strong></td>
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<tr>
<td>3. Is the model documentation adequate, i.e. does it address the essential content of model reporting template, including the following:</td>
<td></td>
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<tr>
<td>- Clear indication of the chemical, or chemicals, to which the model is applicable?</td>
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<tr>
<td>- Is the model being applied for the same scientific purpose as it was developed, or has it been repurposed somehow?</td>
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<tr>
<td>- Model assumptions?</td>
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<tr>
<td>- Graphical representation of the proposed mode of action, if known?</td>
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<tr>
<td>- Graphical representation of the conceptual model?</td>
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<tr>
<td>- Supporting tabulation for parameters (names, meanings, values, mean and standard deviations, units and sources)?</td>
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<td></td>
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<tr>
<td>- Relevance and reliability of model parameters?</td>
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<tr>
<td>- Uncertainty and sensitivity analysis?</td>
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<td></td>
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<tr>
<td>- Mathematical equations?</td>
<td></td>
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<tr>
<td>- PBK model code?</td>
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<tr>
<td>- Software algorithm to run the PBK model code?</td>
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<tr>
<td>- Qualification of PBK software platform?</td>
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<tr>
<td><strong>A.3 Software Implementation and Verification</strong></td>
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<tr>
<td>4. Does the model code express the mathematical model?</td>
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<tr>
<td>5. Is the model code devoid of syntactic and mathematical errors?</td>
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<tr>
<td>6. Are the units of input and output parameters correct?</td>
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<tr>
<td>7. Is the chemical mass balance respected at all times?</td>
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<tr>
<td>8. Is the cardiac output equal to the sum of blood flow rates to the tissue compartments?</td>
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<tr>
<td>9.</td>
<td>Is the sum total of tissue volumes equal to total body volume?</td>
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<tr>
<td>10.</td>
<td>Is the mathematical solver a well-established algorithm?</td>
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<tr>
<td>11.</td>
<td>Does the mathematical solver converge on a solution without numerical error?</td>
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<tr>
<td>12.</td>
<td>Has the PBK modelling platform been subjected to a verification process (for a different use, for instance, in the pharmaceutical domain)?</td>
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<tr>
<td>A.4 Peer engagement (input/review)</td>
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<tr>
<td>13.a</td>
<td>Has the model been used previously for a regulatory purpose?</td>
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<td></td>
<td>• Is prior peer engagement in the development and review of the model sufficient to support the envisaged application?</td>
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<td></td>
<td>• Is additional review required? Peer engagement includes input/review by experts on specific aspects of model development, individual reviews of the model by experts, or collective reviews by peer review panels. Availability of the comments and tracking of revisions to the model in response to peer input contributes to increased confidence in the model for potential application.</td>
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<tr>
<td>B. Assessment of Model Validity</td>
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<tr>
<td>B.1 Biological Basis (Model Structure and Parameters)</td>
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<tr>
<td>14.</td>
<td>Is the model consistent with known biology?</td>
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<td></td>
<td>• Is the biological basis for the model structure provided?</td>
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<td></td>
<td>• Is the complexity of the model structure appropriate to address the regulatory application?</td>
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<tr>
<td></td>
<td>• Are assumptions concerning the model structure and parameters clearly stated and justified?</td>
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<td></td>
<td>• Is the choice of values for physiological parameters justified?</td>
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<tr>
<td></td>
<td>• Is the choice of methods used to estimate chemical-specific ADME parameters justified?</td>
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<tr>
<td></td>
<td>• Saturable kinetics</td>
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<tr>
<td>B.2 Theoretical Basis of Model Equations</td>
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<tr>
<td>15.</td>
<td>Are the underlying equations based on established theories, e.g. Michaelis-Menten kinetics, Fick's laws of diffusion?</td>
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<tr>
<td></td>
<td>• In the case of PBK models for particles, does the model take into consideration the properties of particles, e.g. particle size ranges, (poor) solubility, aggregation, partitioning and diffusion/sedimentation behaviour?</td>
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<tr>
<td>B.3. Reliability of input parameters</td>
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<tr>
<td>16.</td>
<td>Has the uncertainty (individual variability, experimental reproducibility and reliability) in the input parameters been characterised?</td>
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<tr>
<td>B.4. Uncertainty and Sensitivity Analysis</td>
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</tr>
</tbody>
</table>
17. Has the impact of uncertainty (individual variability, experimental reproducibility and reliability) in the parameters on the chosen dose metric been estimated?

- Local sensitivity analysis?
- Global sensitivity analysis?

18. Is confidence in influential input parameter estimates (i.e., based on comparison of uncertainty and sensitivity) reasonable (within expected values; similar to those of analogues) in view of the intended application?

B.5. Goodness-of-Fit and Predictivity

19. For PBK models for which there are sufficient in vivo data for the chemical of interest:

- Suitability as analogue (chemical and biological similarity)?
- Reliable estimation of chosen dose metric for analogue?
- In general is the biological Variability of in vivo reference data (from analogue) established?

NA = not applicable, NR = not reported