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

Tissue compartment	Equation
Gut lumen	$\frac{dM_{lumen}}{dt} = Q_{food} * \left(C_{food} - C_{faeces}\right) + f_1\left(\frac{V_{max} * C_{liver}}{K_m + C_{liver}} * V_{liver}\right)$
Gut tissue	$\frac{dM_{gut}}{dt} = Q_{gut} * \left(C_{art} - \frac{C_{gut}}{P_{gut}}\right) + k_a * M_{lumen}$
Liver Metabolism Clearance	$\frac{dM_{liver}}{dt} = Q_{liver} * \left(C_{art} - \frac{C_{liver}}{P_{liver}}\right) - \frac{V_{max} * C_{liver}}{K_m + C_{liver}} * V_{liver} + Q_{gut} * \left(\frac{C_{gut}}{P_{gut}}\right) - Q_{gut} * \left(\frac{C_{liver}}{P_{liver}}\right)$ $\frac{dM_{liver}}{dt} = Q_{liver} * \left(C_{art} - \frac{C_{liver}}{P_{liver}}\right) - \left(C_{liver} * Cl_{hepatic}\right) + Q_{gut} * \left(\frac{C_{gut}}{P_{gut}}\right) - Q_{gut} * \left(\frac{C_{liver}}{P_{liver}}\right)$
Heart, Brain, Bone, Adipose tissue, Muscle, Lung	$\frac{dM_{tissue}}{dt} = Q_t * \left(C_{art} - \frac{C_t}{P_t}\right)$
Kidney	$\frac{dM_{kidney}}{dt} = Q_{kidney} * \left(C_{art} - \frac{C_{kidney}}{P_{kidney}}\right) - \left(C_{kidney} * Cl_{renal}\right)$
Venous blood	$\frac{dM_{ven}}{dt} = \sum_{T}^{not\ liver+gut} Q_t * \left(\frac{C_t}{P_t}\right) + \left(Q_{gut} + Q_{liver}\right) * \left(\frac{C_{liver}}{P_{liver}}\right) - Q_{tot} * C_{ven}$

Arterial blood	$\frac{dM_{art}}{dt} = Q_{tot} * C_{ven} * \left(\frac{Q_{tot}}{Q_{tot} + Q_{exhale} * P_{air}}\right) - Q_{tot} * C_{art}$
Milk	$\frac{dM_{mgland}}{dt} = Q_{mgland} * \left(C_{art} - \frac{C_{mgalnd}}{P_{milk}}\right) - Q_{milk} * C_{mgland}$
Egg	$\frac{dM_{reprod}}{dt} = Q_{reprod} * \left(C_{art} - \frac{C_{reprod}}{P_{egg}}\right) - Q_{egg} * C_{reprod}$

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).

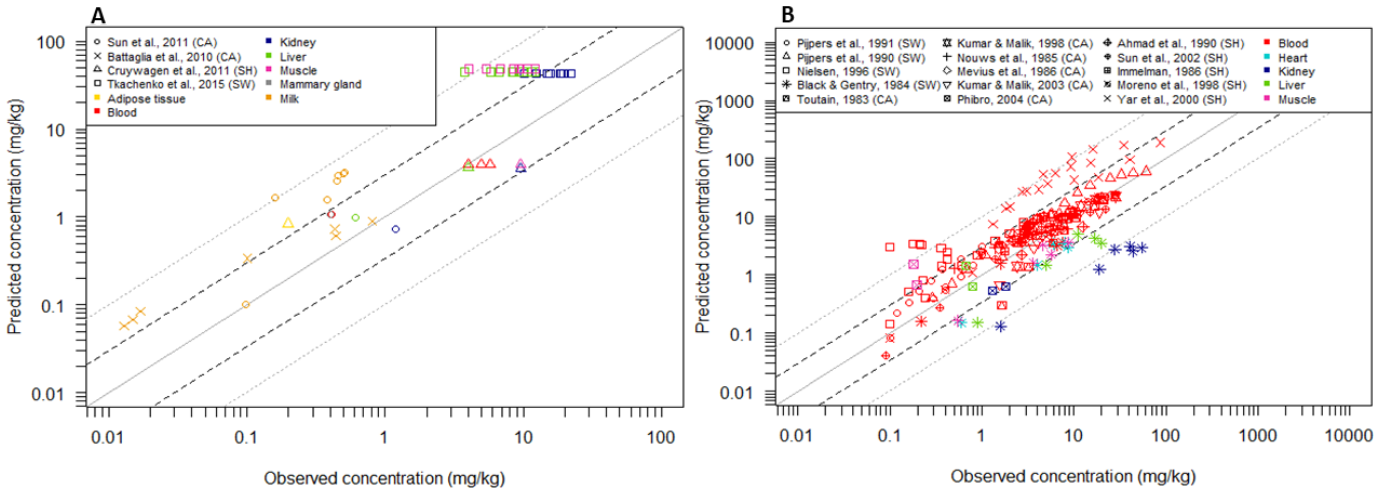

Figure 1. Comparison between concentrations measured in various organs of cattle (CA), sheep (SH), and swine (SW) and PBK model predictions for A) melamine and B) oxytetracycline. Dotted lines represent the 3-fold and 10-fold changes. Organs, species, and references of experimental datasets are indicated in legend: colours and shapes represent organs and studies, respectively.

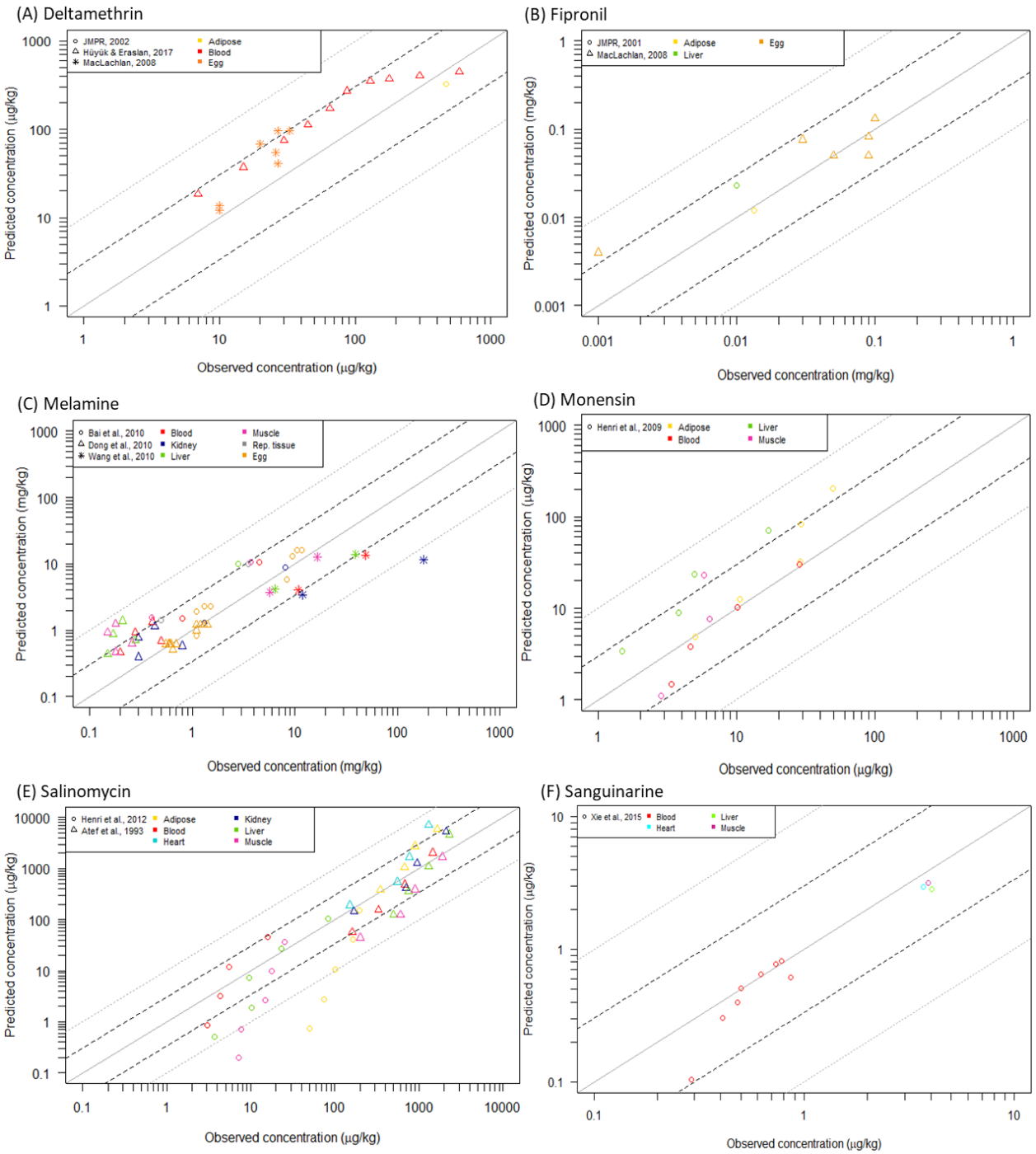

Figure 2. Comparison between quantities measured in chicken and PBK model predictions for six chemicals in various organs. Dotted lines represent the 3-fold and 10-fold changes. Organs, species, and references of experimental datasets are indicated in legend: colours and shapes represent organs and studies, respectively (Lautz et al., 2020c).

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' 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.

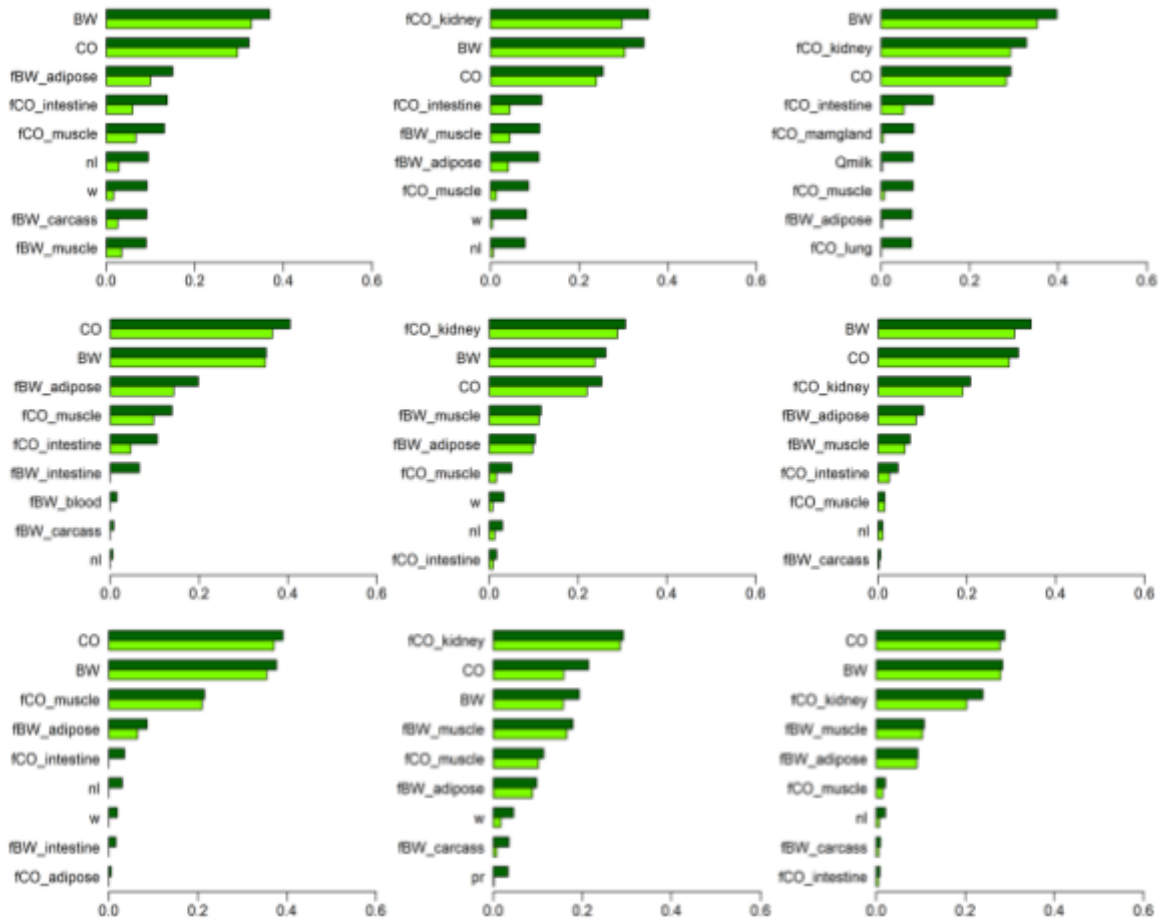

Figure 3. Sensitivity analysis of the cattle (upper panels), sheep (middle panels) and swine (lower panels) PBK model applied to oxytetracycline. Sobol's sensitivity indices were estimated for the blood concentration in the three species at three time points (from left to right): 1.25, 9.5, and 24 hours 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.

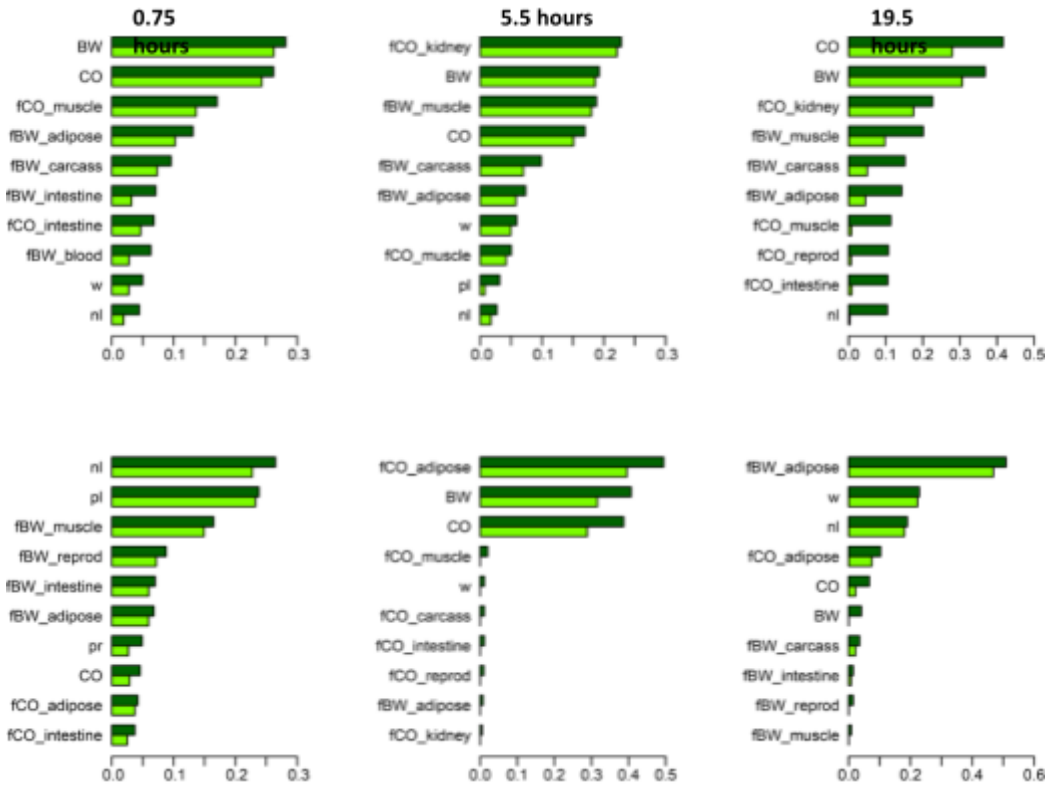

Figure 4. Sensitivity analysis of the chicken PBK model applied to melamine (upper panel) and deltamethrin (lower panel). Sobol' sensitivity analysis indices were estimated for the blood concentrations at three time points: 0.75, 5.5, and 19.5 hours. Sobol's total indices (TI) are presented in dark green and Sobol's first-order indices (FOI) in light green. Parameters were ordered according to the TI. The eleven most influencing parameters according to the total sensitivity indices are shown. BW: body weight, CO: cardiac output, fCO_tissue: fraction cardiac output of a specific tissue; fBW_tissue: fraction body weight of a specific tissue; tissue constituents neutral lipids (nl), polar lipds (pl), proteins (pr) and water (w) (Lautz et al., 2020c).

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

```
    ### 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
    rates <- read.csv("data_rates_animals.csv",stringsAsFactors = FALSE)  #physiological rates
    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,"BW"],

fBW[fBW$species==species,colnames(fBW)%in%substr(Names_fix[grep('fBW',Names_fix)],start=5,stop=nchar(Names_fix[grep('fBW',Nam
es_fix)]))],
                        fCO[fCO$species==species,"CO"],

fCO[fCO$species==species,colnames(fCO)%in%substr(Names_fix[grep('fCO',Names_fix)],start=5,stop=nchar(Names_fix[grep('fCO',Nam
es_fix)]))],
                        rates[rates$species==species,colnames(rates)%in%Names_fix],
                        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])
        colnames(fix_in) <- Names_fix

        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="_"),
```

```
                      "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',Nam
es_fix)]))],

fCO[fCO$species==species,colnames(fCO)%in%substr(Names_fix[grep('fCO',Names_fix)],start=5,stop=nchar(Names_fix[grep('fCO',Nam
es_fix)]))],
                        rates[rates$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
        X1 <- as.data.frame(X1)
        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=""))

    #Input model function
      for (j in 1:nrow(par_in)) {
        print(paste0("Running loop: ", j))
        print(paste("Current time: ", Sys.time()))

        SimRes[j,] <- multi_tool(
          par_in = par_in[j,], #input data frame
            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

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

```
    #==================================#
    # probabilistic                    #
    # multi-compartment model function  #
    # for farm animal species          #
    #==================================#
    dMdt <- function(i, M, C, h, physnames,
                     Qin, Qout, CO, PC, OW,
                     E_bolus, E_cont, E_iv, kgastric,
                     Qbolus, Qingest, Qiv, Qexhale,
                     kabs, Vmax, Km, Cl, fbile, fbact, Qmilk) {
      #input via food
      dMfood <- E_bolus[i] * Qbolus + E_cont[i] * Qingest
      #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"]
      dMart[physnames=="art"] <- -sum(dMart)
      #delivery to venous blood and passage through portal vein
      dMven <- Qout * (C / PC)
      dMart[physnames=="liver"] <- dMart[physnames=="liver"] - dMven[physnames=="intestine"]
        dMven[physnames=="liver"]  <-  dMven[physnames=="liver"]  +  Qout[physnames=="intestine"]  *  (C[physnames=="liver"]  /
PC[physnames=="liver"])
      dMven[physnames=="ven"] <- -sum(dMven[physnames!="intestine"])
      #metabolism an enterohepatic circulation and retransformation
      dMmet <- ifelse(!is.na(Vmax)&!is.na(Km),(Vmax*(C/PC))/(Km+(C/PC))*OW,0)
      dMmet[physnames=="liver"] <- ifelse(!is.na(Cl[physnames=="liver"]),Cl[physnames=="liver"]*C[physnames=="liver"])
      dMmet[physnames=="lumen"] <- -dMmet[physnames=="liver"] * fbile * fbact
      dMmet[physnames=="metab"] <- -dMmet[physnames=="liver"] - dMmet[physnames=="lumen"]
      #transport over lung and exhalation
      dMexh <- -CO * C
      dMexh[physnames!="ven"] <- 0
      dMexh[physnames=="art"] <- -dMexh[physnames=="ven"] * (CO / (CO + Qexhale * PC[physnames=="air"]))
         dMexh[physnames=="air"]   <-   -dMexh[physnames=="ven"]   *   ((Qexhale   *   PC[physnames=="air"])/(CO   +   Qexhale   *
PC[physnames=="air"]))
      #excretion to urine, milk, feces
      dMexc <- M[physnames=="lumen"] * kgastric
      dMexc[physnames=="kidney"] <- C[physnames=="kidney"] * Cl[physnames=="kidney"]
      dMexc[physnames=="mamgland"] <- C[physnames=="mamgland"] * Qmilk[physnames=="mamgland"]
      dMexc[physnames=="urine"] <- -dMexc[physnames=="kidney"]
      dMexc[physnames=="milk"] <- -dMexc[physnames=="mamgland"]

      dMdt <- dMfood + dMiv + (dMabs + dMart + dMven + dMmet + dMexh + dMexc) * (h / 60)

      M <- M + dMdt
      C <- ifelse(OW==0,0,M/OW)

      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) {

      #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","feces","metab","feed","iv")

      #Organ weights
      fBW <- c(t(par_in[comp]),rep(0,11))
      fBW[physnames=="ven"] <-  2/3 * fBW[physnames=="blood"]
      fBW[physnames=="art"] <-  1/3 * fBW[physnames=="blood"]
      fBW <- fBW[physnames!="blood"]
      OW <- BW*fBW/sum(fBW) #organ volums (L), based on normalized weight fractions and overall assumed density of 1 L/kg

      #Blood flows Q
      comp <- gsub('fBW','fCO',comp)
      fCO <- c(t(par_in[comp]),rep(0,11))
      fCO <- (fCO[physnames!="blood"])
      Qin <- fCO*CO/sum(fCO) #blood flows (L/min), normalized to CO
      Qout <- -Qin

      #Tissue-blood partitioning
                                                                                        comp                                    <-
c(colnames(par_in[grep('_nl',colnames(par_in))]),colnames(par_in[grep('_pl',colnames(par_in))]),colnames(par_in[grep('_pr',co
lnames(par_in))]),colnames(par_in[grep('_w',colnames(par_in))]))
      comp <- comp[order(comp)]

      PCcomp <- comp[c(-grep('exp_',comp),-grep('int_',comp))] #all tissue composition -names
      fPC_old <- c(t(par_in[PCcomp])) #'old fractions'
      fPC_new <- fPC_old*c(par_in$nl,par_in$pl,par_in$pr,par_in$w) #new fractions

        #normalisation to mean sum
        sumold <- rep(tapply(fPC_old,rep(seq(length(fPC_old)/4),each=4),sum),each=4)
        sumnew <- rep(tapply(fPC_new,rep(seq(length(fPC_old)/4),each=4),sum),each=4)
```

```
    fPC_new <- fPC_new*(sumold/sumnew)

  PCexps <- comp[grep('exp_',comp)]
  fPC_exp <- c(t(par_in[PCexps]))   #all QSAR exponents
  PCints <- comp[grep('int_',comp)]
  fPC_int <- c(t(par_in[PCints]))   #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[match(tissnames,physnames)] <- PC1
  PC[physnames=="air"] <- (Pv/S)/(R*Temp)
  PC[is.na(PC)] <- PC[physnames=="blood"]
  PC <- PC/PC[physnames=="blood"]
  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))
  kgastric <- rep(0,length(physnames))
  Qmilk <- rep(0,length(physnames))
  fbile <- par_in$fbile #fraction of metabolites reentering lumen with bile (-)

  Qingest[physnames=="lumen"] <- par_in$Qingest / (24*60) #ingestion rate kgfeed/min
  Qingest[physnames=="feed"] <- -par_in$Qingest / (24*60)
  Qiv[physnames=="ven"] <- 1
  Qiv[physnames=="iv"] <- -1
  Qbolus[physnames=="lumen"] <- 1
  Qbolus[physnames=="feed"] <- -1
  kgastric[physnames=="feces"] <- par_in$kgastric
  kgastric[physnames=="lumen"] <- -par_in$kgastric           #gastric emptying rate constant (1/min)
  Qmilk[physnames=="mamgland"&Qin!=0] <- -par_in$Qmilk       #milk production rate (L/min)

  #TK parameters
  kabs <- rep(0,length(physnames))
  Vmax <- rep(NA,length(physnames))
  Km <- rep(NA,length(physnames))
  Cl <- rep(0,length(physnames))

  fbact <- par_in$fbact
  kabs[physnames=="intestine"] <- par_in$kabs
  kabs[physnames=="lumen"] <- -par_in$kabs
  Vmax[physnames=="liver"] <- -par_in$Vmax_tot
  Km[physnames=="liver"] <- par_in$Km_tot
  Cl[physnames=="liver"] <- -par_in$Cl_hepatic * BW
  Cl[physnames=="kidney"] <- -par_in$Cl_renal * BW


  #Time vector for exposure ----
  h <- 3                                      #stepsize in seconds
  t <- seq(3600*t_start,3600*t_end,by=h)      #vector with timepoints (seconds)
  E_iv <- rep(0,times=length(t))
  E_bolus <- rep(0,times=length(t))
  E_cont <- rep(0,times=length(t))

  if (route=="oral" & regime == "bolus") {
    E_bolus[t>=E_start*3600 & t<3600*E_end & t%%(3600*E_int)==0] <- (E_dose/MW)*BW
  } 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
  }

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

#Create output sheet ----
  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("heart_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")
  }

#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, Cl=Cl, fbile=fbile, fbact=fbact, Qmilk=Qmilk)

    M <- output$M
    C <- output$C

#Write to results for t_A
if (chem_fix & any(t[i]==round(t_A*3600))) {
    results[,paste("blood_t",t[i]/3600,sep="")] <- C[physnames=="ven"] * MW
    results[,paste("fat_t",t[i]/3600,sep="")] <- C[physnames=="adipose"]* MW
    results[,paste("liver_t",t[i]/3600,sep="")] <- C[physnames=="liver"] * MW
    results[,paste("kidney_t",t[i]/3600,sep="")] <- C[physnames=="kidney"] * MW
    results[,paste("muscle_t",t[i]/3600,sep="")] <- C[physnames=="muscle"] * MW
    results[,paste("heart_t",t[i]/3600,sep="")] <- C[physnames=="heart"] * MW
    results[,paste("brain_t",t[i]/3600,sep="")] <- C[physnames=="brain"] * MW
    results[,paste("carcass_t",t[i]/3600,sep="")] <- C[physnames=="carcass"] * MW
    results[,paste("lung_t",t[i]/3600,sep="")] <- C[physnames=="lung"] * MW
```

```
      } 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)
}
```

Part II Checklist for model evaluation

PBK Model Evaluation Checklist	Checklist assessment	Comments
Name of the PBK model (as in the reporting template)		
Model developer and contact details		
Name of person reviewing and contact details		
Date of checklist assessment		
A. Context/Implementation		
A.1. Regulatory Purpose		
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?)		
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 *in vitro* data vs. no experimental data)?		
A.2. Documentation		
3. Is the model documentation adequate, i.e. does it address the essential content of model reporting template, including the following:		
● Clear indication of the chemical, or chemicals, to which the model is applicable?		
● Is the model being applied for the same scientific purpose as it was developed, or has it been repurposed somehow?		
● Model assumptions?		
● Graphical representation of the proposed mode of action, if known?		
● Graphical representation of the conceptual model?		
● Supporting tabulation for parameters (names, meanings, values, mean and standard deviations, units and sources)?		
● Relevance and reliability of model parameters?		
● Uncertainty and sensitivity analysis?		
● Mathematical equations?		
● PBK model code?		
● Software algorithm to run the PBK model code?		
● Qualification of PBK software platform?		
A.3 Software Implementation and Verification		
4. Does the model code express the mathematical model?		
5. Is the model code devoid of syntactic and mathematical errors?		
6. Are the units of input and output parameters correct?		
7. Is the chemical mass balance respected at all times?		
8. Is the cardiac output equal to the sum of blood flow rates to the tissue compartments?		

9. Is the sum total of tissue volumes equal to total body volume?		
10. Is the mathematical solver a well-established algorithm?		
11. Does the mathematical solver converge on a solution without numerical error?		
12. Has the PBK modelling platform been subjected to a verification process (for a different use, for instance, in the pharmaceutical domain)?		
A.4 Peer engagement (input/review)		
13.a Has the model been used previously for a regulatory purpose?		
● Is prior peer engagement in the development and review of the model sufficient to support the envisaged application?		
● 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.		
B. Assessment of Model Validity		
B.1 Biological Basis (Model Structure and Parameters)		
14. Is the model consistent with known biology?		
● Is the biological basis for the model structure provided?		
● Is the complexity of the model structure appropriate to address the regulatory application?		
● Are assumptions concerning the model structure and parameters clearly stated and justified?		
● Is the choice of values for physiological parameters justified?		
● Is the choice of methods used to estimate chemical-specific ADME parameters justified?		
● Saturable kinetics		
B.2 Theoretical Basis of Model Equations		
15. Are the underlying equations based on established theories, .e.g. Michaelis-Menten kinetics, Fick's laws of diffusion?		
● 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?		
B.3. Reliability of input parameters		
16. Has the uncertainty (individual variability, experimental reproducibility and reliability) in the input parameters been characterised?		
B.4. Uncertainty and Sensitivity Analysis		

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