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Ministry of Health, Welfare and Sport

**Transfer model for dioxins (total TEQ)
in laying hens, version 1.1 - model
documentation.**

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Colophon

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Contents

Summary—4

| | |
|----------------------------|-------------------------------|
| 1 | Introduction—5 |
| 2 | Model description—6 |
| 2.1 | General overview—6 |
| 2.2 | Model equations—6 |
| 2.3 | Model assumptions—8 |
| 2.4 | Generic parameters—8 |
| 2.5 | System-dependent parameters—8 |
| 3 | Software details—10 |
| 4 | Model applicability—11 |
| 5 | References—12 |
| Annex I: R-Code —13 | |

Summary

The fundamentals of a feed-food transfer model for dioxin (PCDD/F and dl-PCB) concentrations (total TEQ) in body fat and egg yolk fat in laying hens are presented. The model, which is available as a webtool application (<https://feedfoodtransfer.nl/>), can be used to compare simulated concentrations with regulatory limits for these food matrices.

1 Introduction

Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (dl-PCBs) (dioxins) are persistent environmental pollutants (POPs) that may accumulate in fatty tissues and the liver (“hepatic sequestration”) of animals and humans. One of the main sources of human exposure to PCDD/Fs and dl-PCBs is the consumption of animal products, such as meat, milk and eggs. In turn, animal feed is an important contributor to the accumulation of PCDD/Fs and dl-PCBs in animal products used for human consumption.

The concentration of the sum of PCDD/Fs and dl-PCBs is expressed as total toxic equivalents of 2,3,7,8-TCDD (total TEQ) using Toxic Equivalence Factors (TEFs) for the different PCDD/F and dl-PCB congeners (van den Berg et al. 2006).

This report presents the transfer model that estimates the total TEQ concentration in body fat and egg yolk fat of laying hens after exposure to PCDD/Fs and/or dl-PCBs (expressed as TEQ) via feed. The estimated concentrations can be compared to the regulatory limits that have been defined by regulatory agencies in various countries. The model is available as a webtool application (<https://feedfoodtransfer.nl/>).

For simulations based concentrations of individual PCDD/F and dl-PCB congeners, a congener specific model is also available as a webtool application. The calibration of this latter model is based on individual congener concentrations.

2 Model description

2.1 General overview

The transfer model used to estimate TEQ concentrations in egg yolk comprises two compartments representing a central compartment and a fat compartment (Fig 1). Intake of PCDD/Fs and dl-PCBs, expressed as TEQ, is modelled as a constant intake amount, A_i , of which a fraction (F_{abs}) is absorbed over the gut wall into the central compartment on a daily basis. PCDD/Fs and dl-PCBs that have been absorbed by the central compartment can migrate to the fat compartment (q_c), and vice versa (q_f). PCDD/Fs and dl-PCBs can leave the laying hen's body through two processes. The first is excretion to egg yolk fat (εy), with ε being the laying efficiency, and y being the excretion rate constant. The second is hepatic clearance (k).

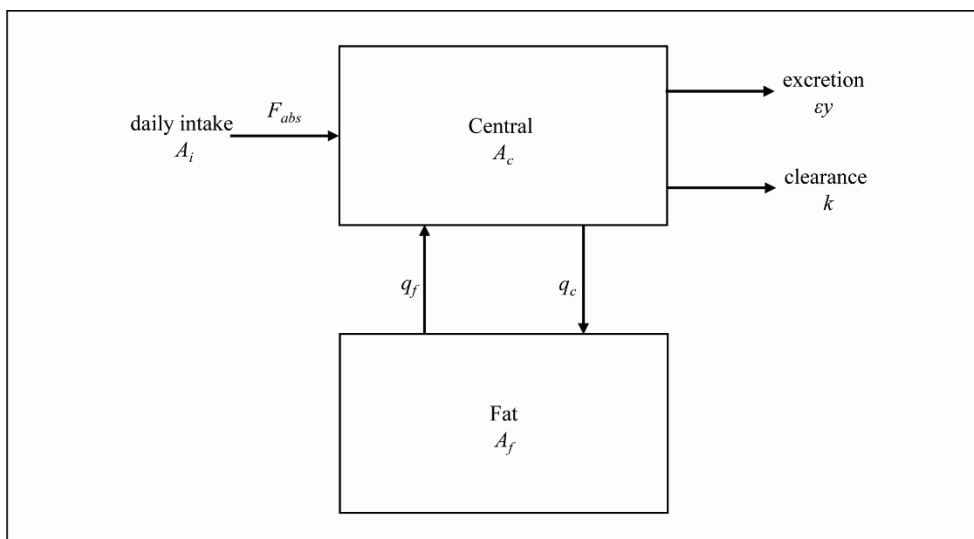


Figure 1. Two compartments comprised kinetic model for the PCDD/F and dl-PCB toxic equivalent (TEQ), in the laying hen (Figure taken from Notenboom et al. 2023).

2.2 Model equations

The transfer model described in this report consists of a set of coupled ordinary differential equations (ODEs). These ODEs describe the distribution of total TEQ between the central compartment and the fat compartment over time. For both compartments of the models, an ODE was formulated. Specifically, equations 1 and 2 describe the change in the amount of total TEQ in the central compartment and the fat compartment, respectively.

$$\frac{dA_c(t)}{dt} = F_{abs}A_i - (q_c + \varepsilon y + k)A_c(t) + q_f A_f(t) \quad (1)$$

$$\frac{dA_f(t)}{dt} = q_c A_c(t) - q_f A_f(t) \quad (2)$$

$A_c(t)$ [pg]: Amount of substance in the central compartment at time t

$A_f(t)$ [pg]: Amount of substance in the fat compartment at time t

F_{abs} [-]: Absorption fraction

A_i [pg day⁻¹]: Daily intake amount of substance

q_c [day⁻¹]: Transfer rate constant from central compartment to fat compartment

q_f [day⁻¹]: Transfer rate constant from fat compartment to central compartment

ε [-]: Laying efficiency

y [day⁻¹]: Excretion rate constant from central compartment to egg yolk fat

k [day⁻¹]: Hepatic clearance rate constant

The transfer rate parameters used in equations 1 and 2 can be expressed as unknown model parameters (van Eijkeren et al., 2006):

$$q_c = \frac{Q}{P_c V_c} \quad (3)$$

$$q_f = \frac{Q}{P_f V_f} \quad (4)$$

$$y = \frac{P_{y,f} W_{y,f}}{P_c V_c} \quad (5)$$

$$k = \frac{CL}{P_c V_c} \quad (6)$$

Here, Q represents the compartment blood flow. V_c and V_f represent the volumes of the central and fat compartment, respectively. P_c and P_f represent the blood-body partition coefficient and the blood-fat partition coefficients, respectively. CL represents the hepatic clearance rate. $W_{y,f}$ is the amount of fat in the egg yolk, $P_{y,f}$ is the partition coefficient between the blood and the egg yolk fat.

The amount of total TEQ in the body and fat compartment can be converted into concentrations by dividing the amount by the volume of the respective compartments, V_c and V_f .

Finally, the change in the amount of total TEQ in eggs at a given time, $A_{yf}(t)$, can be calculated as follows:

$$\frac{dA_{yf}(t)}{dt} = \varepsilon y A_c(t) - A_{yf}(t) \quad (7)$$

2.3 Model assumptions

The following assumptions were made in the presented model:

- The kinetic behavior of all PCDD/Fs and dl-PCBs in laying hens is equal.
- PCDD/Fs and dl-PCBs homogeneously distribute over the fat compartment.
- PCDD/F and dl-PCB concentrations measured in abdominal fat are representative of the PCDD/F and dl-PCB concentrations in the fat compartment.
- Hepatic sequestration was neglected
- The amount of fat in the egg yolk, $W_{y,f}$ is constant over time and can be measured.
- The laying hens are assumed to weigh 1840 grams (V_{total}). The sizes of the two compartments (central and fat) are calibrated.
- It is assumed that the hens get fed once a day with a constant amount of feed per day.
- The volume of the central compartment can be derived by taking the difference between the total volume and the volume of the fat compartment (which was calibrated (see section 2.5)).
- It is assumed that the density of the compartments is 1 g/cm³. The volume of the model compartments, V_c and V_f , are thus equal to the weights of the compartments (W_c and W_f).

2.4 Generic parameters

The remaining parameters (*Table 1*) are not specific to PCDD/Fs or dl-PCBs and can all be obtained from literature.

Table 1. Generic parameter values

| Parameter | Value | Unit | Source |
|-------------|-------|-------------------|-------------------------------------------------------------|
| ϵ | 0.9 | day ⁻¹ | Mean of exposure groups in Kan and Jonker-Den Rooyen (1978) |
| V_{total} | 1840 | cm ³ | expert opinion |
| W_{egg} | 60 | g | Kan and Jonker-Den Rooyen (1978) |
| W_y | 19.2 | g | Gilbert (1971) |
| $W_{y,f}$ | 5.76 | g | Gilbert (1971) |

2.5 System-dependent parameters

As explained by van Eijkeren et al., (2006), the presented transfer model contains two parameters, q_c and q_f , that can be identified unconditionally from the available experimental data, whereas it contains four parameters, y , k , F_{abs} , and W_f , that are conditionally identifiable. The four conditionally identifiable parameters cannot be directly fitted to the data. However, they can be determined in relation with each other (van Eijkeren et al., 2006):

$$c_1 = r = \epsilon y + k \quad (8)$$

$$c_2 = y F_{abs} \quad (9)$$

$$c_3 = \frac{F_{abs}}{W_f} \quad (10)$$

Here, parameters c_1 , c_2 , and c_3 can be fitted to the available experimental data. Although the four conditionally identifiable parameters cannot be directly fitted to the data and it is thus impossible to find a single value they should take on, it is possible to find the possible range of values they might take on. This can be done by defining boundary conditions on the parameter values. More specifically, the hepatic clearance, k , cannot be lower than 0 day^{-1} , whereas the absorption fraction, F_{abs} cannot exceed 1. If the model is fitted with the first boundary condition, i.e., $k \geq 0$, then it follows that $y \leq \frac{c_1}{\varepsilon}$, $F_{abs} \geq \frac{c_2}{y}$, and $W_f \geq \frac{F_{abs}}{c_3}$. Similarly, from the second boundary condition, i.e., $F_{abs} \leq 1$, it follows that $y \Rightarrow c_2$, $k \leq c_1 - \varepsilon c_2$ and $W_f \leq \frac{1}{c_3}$.

The true parameter values are thus mathematically bound to be within the boundaries described above. However, in order to find definitive parameter values for the transfer model, assumptions were necessary. Therefore, as a conservative estimate for the substance concentrations in egg yolk fat, an hepatic clearance of 0 was assumed, i.e., $k = 0$. From this assumption, it follows that $y = \frac{c_1}{\varepsilon}$, $F_{abs} = \frac{\varepsilon c_2}{c_1}$ and $W_f = \frac{\varepsilon c_2}{c_1 c_3}$. These assumptions were first described by van Eijkeren et al. (2006). Notenboom et al. (2023) calibrated these model parameters using the experimental data reported by Hoogenboom et al. (2006) and Traag et al. (2006) for the PCDD/F and dl-PCB toxic equivalent (TEQ) using the TEFs from van den Berg et al. (2006). The calibrated parameters used in the transfer model are shown in Table 2.

Table 2. Calibrated parameter values used in the transfer model for PCDD/F and dl-PCB (total TEQ)

| Parameter | Description | Value | Unit |
|-----------|-----------------------------------------------------------------------------------------|-------|-----------------------|
| q_c | Mass transfer rate from central compartment to fat compartment (Notenboom et al., 2023) | 0.14 | [day^{-1}] |
| q_f | Mass transfer rate from fat compartment to central compartment (Notenboom et al., 2023) | 0.06 | [day^{-1}] |
| F_{abs} | Absorption fraction. Calibrated (Notenboom et al., 2023) | 0.78 | [-] |
| y | Excretion rate constant to egg yolk fat | 0.057 | [day^{-1}] |
| k | Hepatic clearance rate | 0 | [day^{-1}] |
| V_f | | 230 | [g] |

3 Software details

The transfer model simulations were developed and run using the R modelling language and using the deSolve package. Specifications on the programming packages are listed below:

Name software: R (tested with version 4.0.5)
Manufacturer: The R Foundation for Statistical Computing
Place of manufacture: online
Year of manufacture: 2021
Description: A programming language for statistical computing

Name software: DeSolve (tested with version 1.30)
Manufacturer: Karline Soetaert, Thomas Petzoldt and R. Woodrow Setzer
Place of manufacture: online
Year of manufacture: 2023
Description: Package to solve systems of differential equations
url: <https://cran.r-project.org/web/packages/deSolve/index.html>

Name software: dplyr (tested with version 1.1.4)
Manufacturer: Hadley Wickham, Romain François, Lionel Henry, Kirill Müller, Davis Vaughan, Ryan Dickerson, Posit Software, PBC
Place of manufacture: online
Year of manufacture: 2023
Description: A fast, consistent tool for working with data frame like objects, both in memory and out of memory.
url: <https://cran.r-project.org/web/packages/dplyr/index.html>

4 Model applicability

The transfer model presented in this report can be used to simulate the carry-over of PCDD/Fs and dl-PCBs (expressed as total TEQ) from feed to body fat and egg yolk fat of laying hens. As such, the model enables comparison of the estimated concentration to regulatory limits of these food matrices. Similarly the model can be used to estimate the wash-out period needed to comply with regulatory limits in case the concentrations exceeded such regulatory limits.

An example of a model application is given in Figure 2. Exposure to 1.9 ng TEQ/ kg feed was simulated for an exposure duration of 56 days. Feed intake was 0.113 kg per day. After the 56 days of exposure to contaminated feed, an additional 200 days were simulated in which only clean feed was provided (i.e. feed not containing any PCDD/F and dl-PCB congeners).

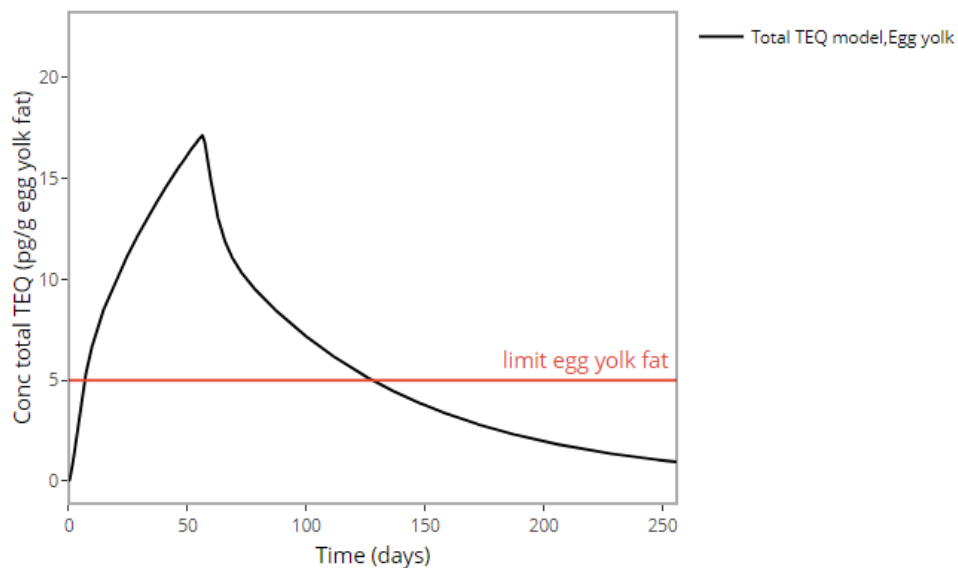


Figure 2 Simulated total TEQ concentration in egg yolk of laying hens fed contaminated feed (1,9 ng TEQ/kg feed) during 56 days followed by clean feed for 200 days. Laying hens were fed 0.113 kg dry feed per day.

5 References

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Traag, WA, Kan, CA, van der Weg, G, Onstenk, C, Hoogenboom, LAP. (2006). Residues of dioxins (PCDD/Fs) and PCBs in eggs, fat and livers of laying hens following consumption of contaminated feed. *Chemosphere* 65:9, 1518-1525. <https://doi.org/10.1016/j.chemosphere.2006.04.001>

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van Eijkeren JCH, Zeilmaker MJ, Kan CA, Traag WA, Hoogenboom LAP. (2006) A toxicokinetic model for the carry-over of dioxins and PCBs from feed and soil to eggs, *Food Additives & Contaminants*, 23:5, 509-517. <https://doi.org/10.1080/02652030500512045>.

Annex I: R-Code

The code below illustrates the basic implementation of the transfer model for PCDD/Fs and dl-PCBs (dioxins)(total TEQ) in laying hens, v1.1. This code can be used freely and is provided "as is" without warranty of any kind. The National Institute for Public Health and the Environment (RIVM) expressly rejects all liability for the accuracy, completeness, or suitability of the information provided. Use of the information is entirely at your own risk.

Model code (dioxinModel.R):

```
# INITIAL

assign_parameters = function()
{
  # model parameters
  tef = 1
  Fabs = 0.78
  qcentral = 0.14
  qfat = 0.06
  e = 0.9
  yy = 0.057
  k = 0
  Vf = 230
  Vc = 1840 - Vf
  Vtotal = 1840

  # dosing parameters
  contaminationLevel = input$Cfeed #ng total teq per kg feed
  feedIntake = input$feedIntake #kg feed per day
  TDOSEOFF <- input$tdoseoff

  # timing commands
  TSTOP <- input$tstop # length of experiment (days)
  POINTS <- 1000 # number of points in plot
  CINT <- 0.1 # communication interval

  # return all variables in this function's environment
  as.list(sys.frame(sys.nframe()))
}

calculate_variables = function(parameters)
{
  with(parameters,
  {
    dose = Fabs*contaminationLevel*1000*feedIntake*tef
    # exposure definitions
```

```

CINT <- TSTOP / POINTS # Communication interval

# return all variables in this function's environment
as.list(sys.frame(sys.nframe()))

}) # end with
}

# END!INITIAL

# DYNAMIC

# DERIVATIVE

derivative = function(t, y, parameters, ...)
{
  with(parameters,
  {
    D<- y[1]
    DoseMassBal<-y[2]
    Ac <- y[3]
    Af <- y[4]
    AEgg <- y[5]
    AEggClearance<- y[6]
    AMetabolized <- y[7]

    Cf = Af/Vf
    Cc = Ac/Vc
    Cegg = AEgg/5.76

    dD<- -D
    dDoseMassBal<- 0 #recording of the dose for the mass balance
    calculation
    dAc <- D -(qcentral+k+e*yy)*Ac + qfat*Af
    dAf <- qcentral*Ac-qfat*Af
    dAEgg <- (e*yy)*Ac-AEgg
    dAEggClearance = AEgg
    dAMetabolized <-k*Ac

    #{ Mass Balance}
    Total = DoseMassBal
    Calculated = D+Ac+Af+AEgg+AEggClearance+AMetabolized
    ERROR=((Total-Calculated)/Total+1E-30)*100
    MASSBBAL=Total-Calculated + 1

    list(c(dD, dDoseMassBal, dAc, dAf,
           dAEgg, dAEggClearance,
           dAMetabolized),
         c(ERROR = ERROR,
           MASSBBAL = MASSBBAL,
           Cf = Cf,

```

```

        Cc = Cc,
        Cegg = Cegg)
    )# end list
  }) # end with
}

run_model <- function(parameters)
{
  parameters <- calculate_variables(parameters)

  with(parameters, {

    dosing <- data.frame(var = c(rep("D", TDOSEOFF),
rep("DoseMassBal", TDOSEOFF)),
                        time = c(seq(0,TDOSEOFF-1, by =
1),seq(0,TDOSEOFF-1, by = 1)),
                        value = rep(dose, TDOSEOFF*2),
                        method = rep("add", TDOSEOFF*2))
    TSTART <- 0.0
    times <- seq.int(TSTART, TSTOP+TDOSEOFF, CINT)

    y <- c(
      D = 0,
      DoseMassBal = 0,
      Ac = 0,
      Af = 0,
      AEgg = 0,
      AEggClearance = 0,
      AMetabolized = 0
    )

    solution <- deSolve::ode(
      y,
      times,
      derivative,
      parameters,
      events = list(data = dosing),
      method = "Isodes"
    )

    return(as.matrix(unclass(solution)))
  })
}

```

Running the model (example)

```
library(dplyr)
source("dioxinModel.R")

### User input ###
input <- c(feedIntake = list(0.113),
           tstop = list(200),
           tdoseoff = list(56),
           Cfeed = list(1.9))

### Run model ###
p <- assign_parameters()
p <- calculate_variables(p)
solution <- as.data.frame(run_model(p))
```
