National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Transfer model for organochlorine pesticides in laying hens, version 1.1 - model documentation

2024

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Summary

The fundamentals of a feed-food transfer model to simulate organochlorine pesticide concentrations in egg yolk fat of laying hens are presented. The model, which is available as a webtool application (https://feedfoodtransfer.nl/), can be used to compare simulated concentrations in egg yolk fat with regulatory limits.

1 Introduction

The presence of pesticide residues in the feed of laying hens can lead to the accumulation of these pesticides in the egg yolk fat. Consumption of these contaminated eggs will result in exposure of consumers to these pesticide residues.

This report presents a transfer model that estimates the concentrations of several (banned) organochlorine pesticides in laying hens' egg yolk fat. The model is specifically developed for the pesticides dieldrin, hexachlorobenzene (HCB), α-hexachlorocyclohexane (α-HCH), β-hexachlorocyclohexane (β-HCH), γ-hexachloro-cyclohexane (γ-HCH, lindane), heptachlor (β-HCE) and total dichlorodiphenyltrichloroethane (total DDT, the sum of p,p'-DDT and its metabolite dichlorodiphenyldichloroethylene, p,p'-DDE). A single model topology is presented which is calibrated separately for each pesticide by fitting the model to experimental data. 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/).

2 Model description

2.1 General overview

The transfer of pesticides from feed to eggs of laying hens is described using a transfer model with a single compartment – the total body compartment (Figure 1). The pesticide is absorbed in the body of the laying hen and leaves the body either via liver clearance, or via excretion into the egg yolk fat.

A simple model was defined due to the primary interest in the long-term trends of the pesticide levels in eggs. This model is a single compartment version of the model structure presented by (Van Eijkeren et al., 2006) for the carry-over of PCDD/F's and dioxin-like PCBs from feed to eggs. That model consists of two compartments and allows for a fast elimination phase with a half-life time of about 2.5 days and a slow elimination phase with a half-life time of about 50 days. Here, the fast phase is omitted resulting in a model suitable for long term kinetics as measured by (Kan & Jonker-Den Rooyen, 1978).

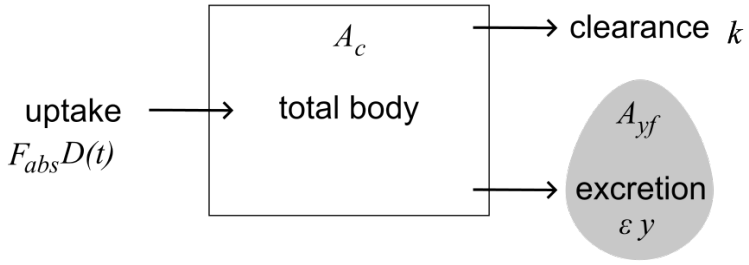

Figure 1. One compartment model describing a pesticide level (A_c) in the laying hen. A fraction (F_{abs}) of the given dose $D(t)$ is absorbed via the intestines (left) and either cleared via the liver with rate k (top right) or excreted into the egg yolk fat. The amount of pesticides in the egg yolk fat (A_{yf}) is the product of the pesticide level (A_c), laying efficiency ε and the excretion rate y.

2.2 Model equations

The transfer model described in this report essentially consists of an ordinary differential equation (ODE) that describes the absorption and clearance of pesticides from the total body compartment. The change in the pesticide amount in the body compartment, A_c, over time is described by

$$\frac{dA_c}{dt} = F_{abs}D(t) - (k + \epsilon y)A_c; \qquad (1)$$

with F_{abs} the absorption fraction, $D(t)$ the amount of pesticide intake per day, k the hepatic rate constant (per day), ε the laying efficiency (eggs per day), and y the excretion rate to the yolk fat (per day).

The pesticide level in the yolk fat, A_{yf}, is described by:

$$\frac{dA_{yf}}{dt} = \epsilon y A_c - A_{yf}. \quad (2)$$

The concentrations of pesticide in the body and yolk fat are calculated as: $C_c = \frac{A_c}{V_c}\frac{P_{yf}}{P_c}$ and $C_{yf} = \frac{A_{yf}}{W_{egg}F_yF_{yf}}$ with W_{egg} the egg weight, F_y the yolk fraction of an egg and F_{yf} the fraction of fat in the yolk.

The pesticide dose is described by:
$D_{in}(t) = \begin{cases} I \cdot L_{pest}, \ t \in \{0, 1, \dots, t_{off}\} \\ I \cdot D_{bg}, \ t \in \{t_{off}+1, \dots, t_{end}\} \end{cases}$.
Where I denotes the amount of feed intake per day, L_{pest} the total fraction of pesticide in the feed, t_{off} the last day of intake of contaminated feed and D_{bg} the background fraction of the pesticide in the feed or environment. The daily dose is fully passed in one day, so the pesticide intake can be described as: $\frac{dD}{dt} = -D(t) + D_{in}(t)$.

2.3 Model assumptions

The following assumptions were made in the presented model:

- The pesticide is either cleared via the liver or excreted via egg yolk.
- Any background levels of pesticides are negligible if the feed is contaminated.
- For dieldrin, α-hexachlorocyclohexane (α-HCH), β-hexachlorocyclohexane (β-HCH), γ-hexachloro-cyclohexane (γ-HCH, lindane) and heptachlor (β-HCE) the initial pesticide level is considered to be 0 and the background level in the feed and environment (D_{bg}) is considered to be 0 as well. For HCB, the background concentration in egg yolk fat is equal to 0.19 µg/g fat.

2.4 Generic parameters

The remaining parameters (Table 1*)* are not specific to the organochlorine pesticides 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 & Jonker-Den Rooyen, 1978)
Vc	1840	cm^3	expert opinion
W_{egg}	60	g	(Kan & Jonker-Den Rooyen, 1978)
F_y	0.32	-	(Gilbert, 1971)
F_{yf}	0.30	-	(Gilbert, 1971)

2.5 System-dependent parameters

All system-dependent parameters, except the absorption fraction F_{abs}, are obtained by fitting the model to the data reported by Kan & Jonker-Den Rooyen (1978). The experiment consisted of two distinct intervals. First, the laying hens were fed one of four – control, low, medium or high - contamination level diets for 16 weeks, followed by a 12-week period on clean feed. During the experiment pooled eggs obtained at the time points 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 17, 18, 19, 20, 21, 22, 24, 26 and 28 weeks were analyzed. Hens were slaughtered and abdominal fat was sampled and analyzed at the time points 0, 4, 6, 8, 10, 12, 14, 20, 24 and 28 weeks.

The model was fitted to both the data on egg yolk and abdominal fat levels of all three feed contamination levels simultaneously. As hens ovulate the day before the eggs are sampled, egg yolk levels analyzed at time t were compared to computed levels at day $t-1$ during the fitting of the model. All available data were used in a single optimization run, maximizing the log-likelihood of the parameter values. Data fitting was performed with ACSL Optimize (Johnson, 2003). All parameters were set to be positive, with no upper boundaries. The resulting parameters are listed in Table 2 and the simulation results obtained with these parameter values are shown in Figure 2 and Figure 3.

It must be noted that all pesticides concentrations in the clean feed were below the detection limits. Nevertheless, since HCB was detected in eggs of laying hens in the control group (Kan & Jonker-Den Rooyen, 1978), it was deduced that a background level of HCB had to be present in the clean feed. Therefore, it was assumed that the background level of HCB was slightly below the detection limit (see Table 2).

Table 2. Values for the rate of excretion through egg yolk fat (y), hepatic elimination (k), ratio of partition coefficients P_{yf}/P_c, initial egg residue level (C_0), feed background level (D_{bg}) and fraction absorbed F_{abs}.

	y (day^{-1})	k (day^{-1})	P_{yf}/P_c (-)	C_0 (mg/g yolk fat)	D_{bg} (mg/kg feed)	F_{abs}[a] (-)
Dieldrin	0.0176	0.0043	5.8	0	0	0.94
HCB	0.021	0[b]	6.2	0.00019	0.0049[c]	0.95
α-HCH	0.010	0.101	3.3	0	0	1.00
β-HCH	0.019	0[b]	6.8	0	0	0.91
γ-HCH	0.010	0.089	3.0	0	0	1.00
β-HCE	0.008	0.012	2.7	0	0	0.98
Total DDT	0.015	0.006	4.7	0.00042	0	0.965

[a] from (Kan & Jonker-Den Rooyen, 1978); [b] the fitted value was 3 (HCB) and 5 (β-HCH) orders of magnitude smaller than for excretion through egg yolk fat; [c] detection limit 0.005 mg/kg feed.

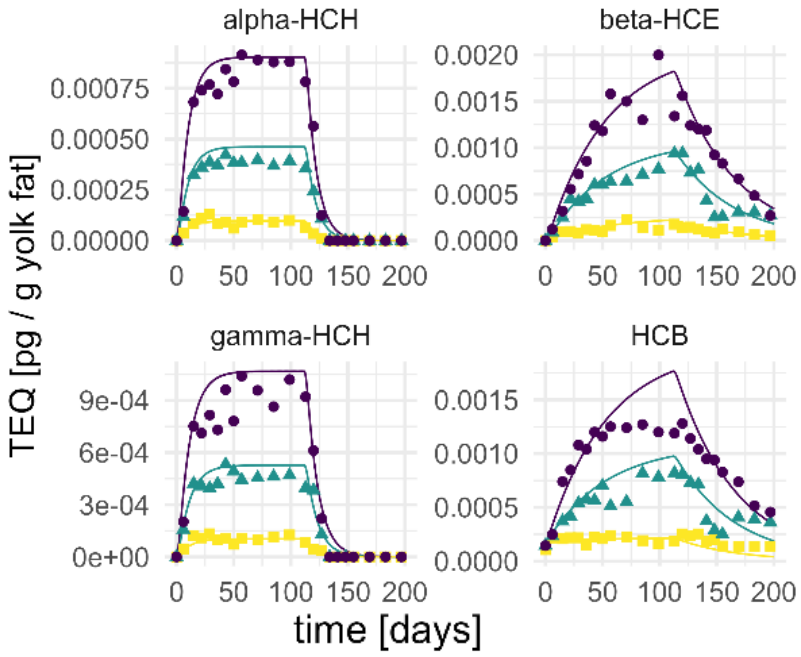

Figure 2. Model simulations with parameters fitted for alpha-HCH, beta-HCE, gamma-HCH and HCB to the corresponding pesticide data (Kan & Jonker-Den Rooyen, 1978).

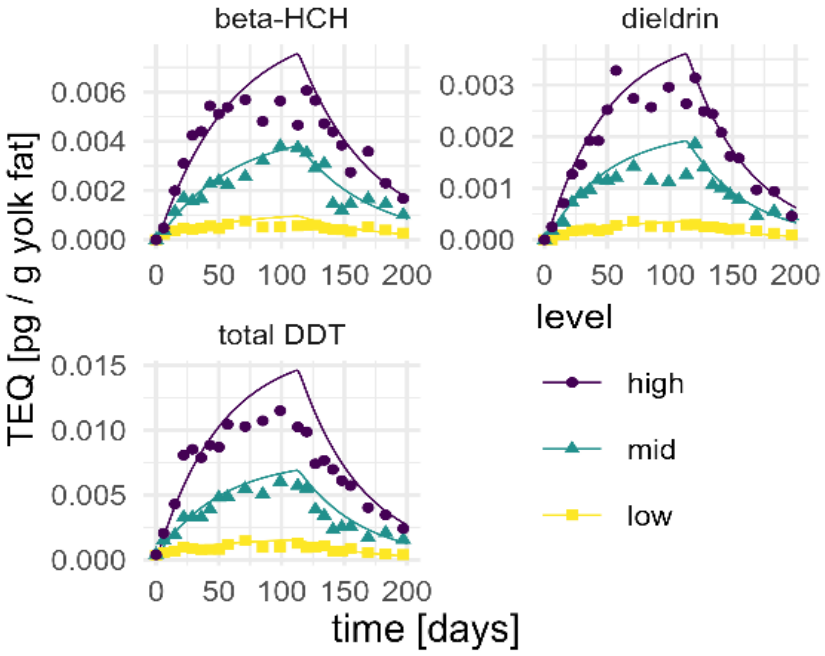

Figure 3. Model simulations with parameters fitted for beta-HCH, dieldrin and total DDT pesticide to the corresponding data (Kan & Jonker-Den Rooyen, 1978).

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 v. 4.2.2)
Manufacturer: The R Foundation for Statistical Computing
Place of manufacture: online
Year of manufacture: 2022
Description: A programming language for statistical computing

Name software: DeSolve (tested with v. 1.35)
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 transfer of organochlorine pesticides 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 determined by regulatory agencies in various countries. 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 3. The model was used to simulate dieldrin levels in eggs over time after a 20-day period of intake of contaminated feed (Figure).

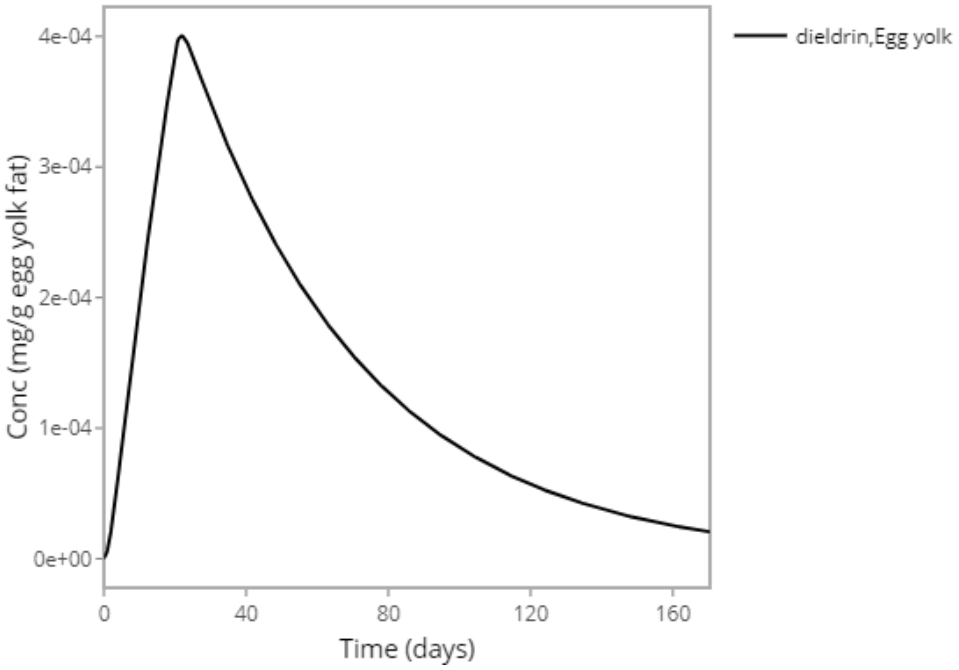

Figure 4. Simulated dieldrin level in egg yolk fat of laying hens fed contaminated feed (0.086 mg dieldrin per kg feed) during 20 days followed by clean feed for 150 days. The total feed intake was 0.113 kg per day.

5 References

Gilbert, A. (1971). The egg: its physical and chemical aspects. In “Physiology and Biochemistry of the Domestic Fowl” Vol. 3, eds. Bell, DJ, and Freeman, BM. In: Academic, New York.

Johnson, H. A. (2003). Using Advanced Continuous Simulation Language (ACSL) to simulate, solve, and fit mathematical models in nutrition. *Adv Exp Med Biol*, *537*, 371-388. https://doi.org/10.1007/978-1-4419-9019-8_24

Kan, C. A., & Jonker-Den Rooyen, J. C. (1978). Accumulation and depletion of some organochlorine pesticides in high-producing laying hens. *Journal of Agricultural and Food Chemistry*, *26*(4), 935-940.

Van Eijkeren, J. C., Zeilmaker, M. J., Kan, C. A., Traag, W. A., & Hoogenboom, L. A. (2006). A toxicokinetic model for the carry-over of dioxins and PCBs from feed and soil to eggs. *Food Addit Contam*, *23*(5), 509-517. https://doi.org/10.1080/02652030500512045

6 Annex I: R-Code

The code below illustrates the basic implementation of the transfer model for organochlorine pesticides in laying hens, version 1.1. 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 (dieldrinHCBlindaneModel.R):

```
# INITIAL

modelParameters<- read.csv("pesticide_param.csv", header =
TRUE)

assign_parameters = function()
  {

  pesticide = input$compound
  param = modelParameters[modelParameters[,
'name']==pesticide,]

  # Pesticide specific parameters
  yy = param$yy # Excretion rate to egg yolk fat [day-1]
  k = param$k # Hepatic clearance rate [day-1]
  ratP = param$ratP # Ratio of partition coefficient Pf/P [-]
  C0 = param$C0 # initial egg residue level [mg/g yolk fat]
  Dbg = param$Dbg # background dose level [mg per kg feed]
  Fabs = param$Fabs # Absorption rate [-]
  L0 = 0

  # Generic parameters
  e = 0.91 # laying efficiency [-]
  rhofat = 0.9 # Density of fat
  V = 1850 # Volume of the hen (unit?)
  Wegg = 57.5 # Average weight of an egg [g]
  Fyolk = 0.32 # fraction of yolk in an egg [-]
  Fyolkfat = 0.3 # fraction of fat in the yolk of an egg [-]

  # Dosing parameters
  contaminationLevel = input$Cfeed #mg per kg feed
  feedIntake = input$feedIntake #kg feed per day

  # Dosing timing
  TDOSEOFF <- input$tdoseoff

  # timing commands
  TSTOP <- 0.5+input$tstop # length of experiment after
stopping the dosing (days)
  POINTS <- 400 # number of points in plot
  CINT <- 0.5 # communication interval

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

calculate_variables = function(parameters)
```

```
{
  with(parameters,
  {
    Wyolkfat = Wegg * Fyolk * Fyolkfat # Weight of the yolk
fat in the egg
    Yeff = e*yy # excretion through yolk
    dose = Fabs*feedIntake*((1-L0)*contaminationLevel +
L0*Dbg) # Absorbed dose

    Ayel0 = Wyolkfat*C0 # Initial amount of contaminant
excreted to yolk fat
    A0 = Ayel0/yy # Initial amount of contaminant excreted
to?
    Ay = Ayel0
    A = A0

    # 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]
    AEgg <- y[4]
    AEggClearance<- y[5]
    AMetabolized <- y[6]

    Cc = Ac/(V/ratP)
    Cegg = AEgg/Wyolkfat

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

    #{Mass Balance}
    Total = DoseMassBal
    Calculated = D+Ac+AEgg+AEggClearance+AMetabolized
    ERROR=((Total-Calculated)/Total+1E-30)*100
    MASSBBAL=Total-Calculated + 1
    list(c(dD, dDoseMassBal, dAc,
            dAEgg, dAEggClearance,
            dAMetabolized),
          c(ERROR = ERROR,
            MASSBBAL = MASSBBAL,
            Cc = Cc,
```

```
            Cegg = Cegg,
            Cf.Af = 0)
    )# 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 = A0,
      AEgg = Ayel0,
      AEggClearance = 0,
      AMetabolized = 0
      )

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

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

Model parameters (pesticide_param.csv):

```
Compound,name,yy,k,ratP,C0,Dbg,Fabs
C1,dieldrin,0.0176,0.0043,5.8,0,0,0.94
C2,HCB,0.021,0,6.2,0.00019,0.0049,0.95
C3,alpha-HCH,0.01,0.101,3.3,0,0,1
C4,beta-HCH,0.019,0,6.8,0,0,0.91
C5,gamma-HCH,0.01,0.089,3,0,0,1
C6,beta-HCE,0.008,0.012,2.7,0,0,0.98
C7,total DDT,0.015,0.006,4.7,0.00042,0,0.965
```

Run model (example.R)

```
source("dieldrinHCBlindaneModel.R")

library(dplyr)

### User input ###

compound <- c("dieldrin")
feedIntake <- 0.113 # kg
Cfeed <- c("C1"=0.086) # mg/kg
tstop <- 150 # number of simulation days after stopping the
exposure
tdoseoff <- 20 # number of days until exposure stops

### Run model ###

input <- c(compound = compound, feedIntake = feedIntake,
tstop = tstop, tdoseoff = tdoseoff, Cfeed = list(Cfeed))
p <- assign_parameters()
p <- calculate_variables(p)
solution <- as.data.frame(run_model(p))
```