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Wim Traag

► **To cite this version:**

Ron Laurentius Hoogenboom, Jan van Eijkeren, Marco Zeilmaker, Kees Kan, Wim Traag. A toxicokinetic model for the carry-over of dioxins and PCBs from feed and soil to eggs. *Food Additives and Contaminants*, 2006, 23 (05), pp.509-517. 10.1080/02652030500512045 . hal-00577578

HAL Id: hal-00577578

<https://hal.science/hal-00577578>

Submitted on 17 Mar 2011

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A toxicokinetic model for the carry-over of dioxins and PCBs from feed and soil to eggs

Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2005-177.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	02-Dec-2005
Complete List of Authors:	Hoogenboom, Ron; RIKILT-Institute of Food Safety, Safety and Health van Eijkeren, Jan; RIVM Zeilmaker, Marco; RIVM Kan, Kees; Animal Science Group of Wageningen UR, Animal Husbandry Traag, Wim; RIKILT-Institute of Food Safety
Methods/Techniques:	Regulations, Toxicology - pharmacokinetics
Additives/Contaminants:	Dioxins, PCBs
Food Types:	Animal feed, Eggs

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12 6 **A toxicokinetic model for the carry-over of dioxins and PCBs**
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15 7 **from feed and soil to eggs**
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1
2
3 **Abstract**
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6 28 A mathematical model for the kinetics of carry-over of dioxins and dioxin-like PCBs from
7
8 29 feed mixed with contaminated oil to eggs has been developed. This model incorporates
9
10 30 uptake of the compounds over the gut wall and their subsequent transport by blood,
11
12 31 distribution over the body, hepatic metabolism and excretion through egg yolk fat. The
13
14 32 model is analysed with respect to the possibility of identifying yet unknown model
15
16 33 parameters by fitting these to the experimental data. The model was fitted to the
17
18 34 experimental data on the carry-over from feed to eggs. The calibrated model was applied to
19
20 35 calculate the steady-state concentrations in eggs which were compared to European
21
22 36 Maximum Residue Levels for dioxins in feed and eggs, showing that these limits do not
23
24 37 match. The feed limit of 0.75 ng TEQ/kg should be reduced to about 0.17 ng TEQ/kg in
25
26 38 order to guarantee egg levels below the residue limit of 3 pg TEQ/g fat. Experimental
27
28 39 results of carry-over from contaminated soil were used to estimate the absorption of
29
30 40 dioxin-like compounds from soils as compared to the absorption from feed, resulting in a
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32 41 value around 40 to 60% absorption from soil as compared to around 90% absorption from
33
34 42 feed.
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45 **Key words:-** Dioxins, PCBs, chicken, feed, soil, PBPK-model
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46 Introduction

47 Controlled exposure studies under laboratory conditions have been performed to examine
48 carry-over of dioxins and dioxin-like PCBs to eggs from feed mixed with contaminated oil
49 and from clean feed mixed with contaminated soil (Hoogenboom *et al.*, 2005). The
50 experiments with contaminated feed not only served the determination of steady state
51 carry-over, but the study of the kinetics of carry-over as well. The work also aimed to
52 develop a computer model to describe carry-over. Only a few authors have until now
53 described a model to relate contamination levels of eggs to the contamination of ingested
54 soil or feed. Schuler *et al.* (1997) presented the calculation of total TEQ contamination of
55 eggs from ingestion of soil, based on soil contamination levels, congener specific transfer
56 efficiencies and background feed contamination levels. Their model did not contain time as
57 a variable and implicitly assumed steady state contamination of the eggs. However, egg
58 contamination caused by uptake from contaminated soil or feed is a kinetic process and the
59 apparent contamination levels may not represent steady state. Huygebaert *et al.* (2002)
60 developed a kinetic model for the excretion of PCBs in egg yolks from contaminated feed.
61 Their model assumption was that excretion is governed by the hydraulics of, and
62 absorption in the animal intestinal tract. However, not only absorption of contaminants
63 over the intestinal wall is an important aspect for the kinetics of egg contamination, but
64 also the distribution of the contaminant over body tissues, its metabolism and the
65 characteristics of excretion, i.e. laying efficiency and, for highly lipophilic contaminants
66 like dioxins, egg lipid content.

67 This study concerned the modelling of transfer of dioxins and dioxin-like PCBs from
68 contaminated feed to eggs and their accumulation in fat tissue. A kinetic model was
69 developed that considers the uptake of the contaminant, i.e. its absorption over the
70 intestinal wall after oral intake, the distribution of the contaminant over the body and its

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3 71 elimination by hepatic metabolism and excretion by egg yolk fat. Experimental data on
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5 72 carry-over from feed to eggs provided by Hoogenboom *et al.* (2005) were used to calibrate
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8 73 the model, relating residues in eggs to exposure through feed at different contamination
9
10 74 levels. Data obtained from Hoogenboom *et al.* (2002), obtained from a study with much
11
12 75 higher dioxin and PCB levels, served model verification. The calibrated and validated
13
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15 76 model has been applied to estimate uptake from another matrix, *i.e.*, from contaminated
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17 77 soil added to clean feed. Moreover, the model was applied for comparing the European
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20 78 Maximum Residue Limits for dioxin residues (expressed as TEQ values) in feed and eggs.
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79 **Materials and method**

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81 Experimental

82 All data for calibrating the model were obtained from the experiment described in the
83 accompanying paper by Hoogenboom *et al.* (2005). Verification data were obtained from
84 the study by Hoogenboom *et al.* (2002).

85

86 Modelling approach

87 The underlying physiologically based pharmacokinetic (PBPK-)model describing the
88 kinetics of carry-over of contaminants from feed to eggs is depicted in figure 1 (upper
89 panel). The corresponding compartment model in figure 1 (lower panel) can be formulated
90 mathematically as a set of two mass balances, representing the changes in absolute
91 amounts of contaminants in respectively the central and the fat compartment.

$$\begin{aligned} \frac{dA_c}{dt} &= F_{abs}D - (q_c + \varepsilon y + k)A_c + q_f A_f \\ \frac{dA_f}{dt} &= q_c A_c - q_f A_f \end{aligned} \quad (1)$$

93 Here, the reduced parameters expressed in terms of the unknown PBPK-model parameters

$$q_c = \frac{Q}{P_c V_c}, \quad q_f = \frac{Q}{P_f V_f}, \quad y = \frac{P_{y,f} W_{y,f}}{P_c V_c} \quad \text{and} \quad k = \frac{CL}{P_c V_c} \quad (2)$$

95 are the rate constants of mass transfer from the central to the fat compartment and *vice*
96 *versa*, excretion with egg yolk fat and hepatic clearance, respectively.

97 The time course of the concentration in egg yolk, expressed as pg TEQ/g yolk fat
98 and in abdominal fat (pg TEQ / g fat) is observed. If the latter is representative of the
99 residue levels in the fat compartment, than the models for these observations, can be
100 derived from equation (1) to be

$$C_{y,f}(t) = \frac{y}{W_{y,f}} \cdot \frac{F_{abs} D}{r} \left(1 - \left(\frac{(\lambda_2 + r)e^{\lambda_1 t} - (\lambda_1 + r)e^{\lambda_2 t}}{\lambda_2 - \lambda_1} \right) \right) \quad (3)$$

for the concentration in egg yolk fat, and

$$C_f(t) = \frac{1}{W_f} \cdot \frac{q_c}{q_f} \frac{F_{abs} D}{r} \left(1 - \left(\frac{\lambda_2 e^{\lambda_1 t} - \lambda_1 e^{\lambda_2 t}}{\lambda_2 - \lambda_1} \right) \right) \quad (4)$$

for the concentration in abdominal fat. Here, $r = \varepsilon y + k$ represents the total elimination of the compounds and

$$\lambda_1 = -\frac{1}{2} \left(q_c + r + q_f - \sqrt{(q_c + r + q_f)^2 - 4q_f r} \right)$$

$$\lambda_2 = -\frac{1}{2} \left(q_c + r + q_f + \sqrt{(q_c + r + q_f)^2 - 4q_f r} \right) \quad (5)$$

are the exponential rates of the slow, long lasting, terminal phase ($\lambda_1 \approx q_f r / (q_c + q_r + r)$) and the fast, short lasting, initial phase $\lambda_2 \approx q_c + q_r + r$.

Note, that the experimental data necessitate the introduction of a few additional parameters with respect to the model in equation (1), such as the amount of fat in egg yolk, which is known, and the weight of the fat compartment, which is unknown. As a result, the model contains the four unknown kinetic parameters for transport and elimination, q_c , q_f , k and y , the unknown absorbed fraction F_{abs} and the unknown fat compartment weight, W_f .

Straightforward analysis shows that the inter compartment transfer rate parameters q_c and q_f can be identified unconditionally from the data. Therefore, these parameters can be freely varied to fit the data. However, the elimination rate parameters y and k , the fraction absorbed and the fat compartment weight W_f can only be determined in relation to each other, i.e., the parameters

$$c_1 = r = \varepsilon y + k, \quad c_2 = y \cdot F_{abs} \quad \text{and} \quad c_3 = F_{abs} / W_f \quad (6)$$

could be varied freely to fit the data, but not the parameters y , k , F_{abs} , and W_y separately.

Note that c_1 denotes the total elimination from the system of the amount that is absorbed

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3 122 over the gut wall, c_2 denotes the elimination through eggs of the amount that is ingested by
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6 123 the hens and c_3 relates the experimentally determined abdominal fat concentrations to the
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9 124 unknown fraction of the contaminant that absorbed.

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11 125 Without introducing these new parameters of equation (6) into the computer model, this
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13 126 problem can be resolved as follows. The fraction absorbed is 1 at maximum, while the
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16 127 metabolism rate is 0 at minimum. If the model is fit to the data while the metabolism rate is
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18 128 kept constant, $k = 0$, then the maximum value for the excretion rate $y_{max} = c_1$ can be
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20 129 determined from the data, as well as the minimum fraction absorbed, $F_{abs, min}$, and the
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22 130 minimum weight of the fat compartment $W_{y, min}$. This would represent the case that
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25 131 elimination by metabolism is negligible compared to elimination through eggs. Once these
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27 132 parameters are identified under this restrictive condition, then for $F_{abs, max} = 1$, *i.e.*,
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29 133 representing the case that the amount ingested is totally absorbed over the gut wall, the
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31 134 minimum value for the excretion rate is found to be the product $y_{min} = y_{max} \cdot F_{abs, min}$ of the
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33 135 values found for the case $k = 0$. From this minimum, the maximum value for the
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35 136 metabolism rate $k_{max} = y_{max} - y_{min}$ can be found. The maximum value of the fat compartment
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37 137 weight follows from $W_{f, max} = W_{f, min} / F_{abs, min}$.

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41 138 It is assumed that after ovulation, the yolk is not any more contaminated during egg
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43 139 white formation and deposition. Therefore, one should provide for a one-day delay
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45 140 between ovulation of the yolk and laying of the corresponding egg. Besides, while in the
46
47 141 long range, the daily loss by excretion is the product of laying efficiency and excretion per
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49 142 day, *i.e.* ϵy , the amount to be found in an excreted egg is proportional to y only. Moreover,
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51 143 one should provide for the time, τ , between the start of feeding contaminated feed and the
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53 144 first yolk ovulation thereafter, which is apparent as an off-set in the time course of egg
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55 145 contamination. This latter parameter is estimated from the data too.
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146 **Results and Discussion**

147 The underlying physiologically based pharmacokinetic (PBPK-)model describing the
148 kinetics of carry-over of contaminants from feed to eggs is depicted in figure 1. The fat
149 compartment comprises abdominal fat, subcutaneous fat, fat fraction of the skin, bone
150 marrow and intermuscular fat. This compartment is characterized by its storage capacity,
151 because of the extreme lipophilicity of the compounds, and by its relatively poor blood
152 flow that may even be further limited by intra-tissue diffusion. Therefore, equilibrium
153 between the concentration in this compartment and the concentration in blood is likely to
154 be reached much slower, typically in the order of a few days, than equilibrium between the
155 concentration in blood and other tissues.

156 The central compartment comprises all the other tissues, which will reach
157 equilibrium with blood in the order of hours rather than in the order of days. After
158 absorption, the contaminants enter the systemic blood circulation via the *vena porta*, i.e.,
159 into the central compartment ($F_{abs}D$). Biotransformation of the contaminants is assumed to
160 take place in the liver, i.e. the central compartment, which is characterized by the
161 parameter CL (for clearance). Excretion by egg yolk is from the blood, i.e., from the
162 central compartment into yolk. The concentration in the growing yolks is assumed to be
163 proportional to the concentration in blood (partition coefficient $P_{y,f}$). A model, based on
164 the work of Donoghue and co-workers (Donoghue *et al.*, 1996, 1997a, 1997b; Donoghue
165 and Meyers, 2000; Donoghue, 2001) that incorporated also yolk growth as determinant of
166 the concentration had to be abandoned, because it systematically under-estimated yolk
167 contamination levels during the initial phase. After ovulation, the yolk is assumed to be
168 excreted from the central system, because the lipophilic contaminants are not likely to
169 interact between system and yolk during the phase of white formation. This is in contrast

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3 170 with non-lipophilic drugs. Yolk fat of weight $W_{y,f}$ is produced with an efficiency of ε per
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6 171 day. Laying efficiency is determined experimentally and thus is a known model parameter.
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8 172 Eggs in the trial weighed about 60 grams in the mean, of which according to Gilbert (1971)
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10 173 32% was assumed to be yolk weight) and 30% of yolk weight to be egg yolk fat weight,
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12 174 amounting to 5.8 g of yolk fat per egg. Laying performance during the experiment was
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14 175 about 90%. Data on total TEQs, i.e., the sum TEQs of dioxins, furans, mono- and non-
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16 176 ortho PCBs, as presented in the accompanying article (Hoogenboom *et al.* 2005), were
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18 177 used for further calibrating the model. This consisted of fitting the two transfer rate
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20 178 constants, q_c and q_f , the egg yolk excretion rate y , the fraction absorbed F_{abs} and the fat
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22 179 compartment weight W_f , while keeping the elimination constant $k = 0$. The model was
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24 180 fitted to both the data on egg yolk and abdominal fat levels of all the different feed
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26 181 contamination levels simultaneously. Therefore, all the available data served model fitting
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28 182 in only one optimization run, optimizing the log likelihood of the fitting parameter set. As
29
30 183 yolks ovulate the day before the eggs are sampled, eggs yolk levels analyzed from time t
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32 184 (day), were compared to computed levels at time $t - 1$ (day). Moreover, as ovulation after
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34 185 the start of applying contaminated feed, considered to be $t = 0$, takes place at an unknown
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36 186 time, another parameter, $\tau < 1$ (day), was fitted to the data. Data fitting was performed with
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38 187 ACSL Optimize, optimizing the log likelihood of the parameter values. Fitted values were
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40 188 automatically converted to values for the other extreme case when $F_{abs} = 1$, meaning
41
42 189 complete absorption of the contaminants. Parameter values and ranges of values found by
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44 190 fitting are:

$$\begin{aligned} q_c &= 0.17 \text{ [-/day]}, & q_f &= 0.078 \text{ [-/day]}, & \tau &= 0.19 \text{ [day]} \\ 0.043 &\leq y \leq 0.055 \text{ [-/day]}, & 0 &\leq k \leq 0.012 \text{ [-/day]} \\ 0.78 &\leq F_{abs} \leq 1 \text{ [-]} & \text{and} & & 230 &\leq W_f \leq 290 \text{ [g]} \end{aligned}$$

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3 192 of which the first line shows unconditional results, while the resulting interval estimates in
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5 193 the second and third line are mutually dependent. Taking e.g. the minimum possible value
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8 194 for metabolism, $k = 0$, the corresponding value for excretion is maximal and corresponding
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10 195 values for absorption and fat compartment weight are minimal. The value for the mean
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12 196 ovulation time τ for the population was 0.19 day after the start of feeding contaminated
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15 197 feed, being about four and a half hours. Clearly, considering the values for y and k , the
16
17 198 main route of elimination of dioxins from the body is by egg yolk fat and not metabolism.
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19 199 The fact that the transfer parameter from the central to the fat compartment q_c is higher
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21 200 than q_f implies that the distribution volume ($P_f V_f$) of the fat compartment, i.e., its
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23 201 physiological volume corrected for its storage capacity as compared to the same volume of
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25 202 blood, is greater than the distribution volume ($P_c V_c$) of the central compartment.
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29 203 Figure 2 shows the residue data and the computed concentration-time curves for the
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31 204 total TEQ levels in both egg yolk and abdominal fat based on the fitted parameter values.
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33 205 Figure 3 shows similar curve fits for the total TEQ, but also the TEQ levels derived from
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35 206 the dioxins and furans, the non-ortho and the mono-ortho PCBs. Notice the clear bi-phasic
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37 207 time course of egg residue levels, while abdominal fat levels seem to be mono-exponential.
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39 208 The (short-lasting) fast first phase has a half-life of about 2.5 days. The (long-lasting) slow
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41 209 terminal phase appeared to have a terminal half-life of about 50 days. Fat storage behaves
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43 210 like a large capacitor, with slowly reacting kinetics, while egg levels, that are proportional
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45 211 to blood levels, reflect the small capacitance, fast reaction kinetics of blood levels. This
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47 212 phenomenon appears not only during the period of feeding contaminated feed, but also
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49 213 during the stage thereafter. The apparent difference in the kinetics of residues in eggs and
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51 214 abdominal fat is also depicted in Figure 4 for the 1.85 ng TEQ/kg feed contamination
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53 215 group. Note that during the contamination period, abdominal fat residue levels are lower
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55 216 then egg residue levels, and *vice versa* during the depletion period on clean feed. After
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3 217 prolonged feeding with contaminated feed, the steady state concentrations would reach
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5 218 about the same level (lower panel).
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8 219 Based on the model parameters found by fitting the model to the data, the
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10 220 concentration-time courses for total TEQ, total dioxin TEQ, non-ortho PCB TEQ and
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12 221 mono-ortho PCB TEQ were calculated for the experiment with highly contaminated feed
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14 222 from Hoogenboom *et al.* (2002). Note that in this study the relative contribution of
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16 223 dioxins, non-ortho PCBs, and mono-ortho-PCBs to the total TEQ level was quite different
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18 224 from the gross composition of the contamination in the current experiment, being
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20 225 respectively 31, 12 and 58%, as compared to 53, 29 and 18%. In addition, the total TEQ
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22 226 level of about 200 ng TEQ/kg feed was 50 times higher than the maximum level in this
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24 227 experiment: 3.95 ng TEQ/kg feed. The result of the verification is depicted in figure 5,
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26 228 showing an quite satisfactory fit of the data.
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31 229 The model was subsequently used to compare EC Maximum Residue Limits for
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33 230 dioxin TEQ residue levels in feed (0.75 ng TEQ/kg feed) and in eggs (3 pg TEQ/g fat).
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35 231 From the former, the corresponding steady-state egg contamination level was calculated to
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37 232 be 13 pg TEQ/g fat, to be compared with the MRL value of 3 pg TEQ/g fat. On the other
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39 233 hand, maintaining the MRL value of 3 pg TEQ/g fat in eggs, feed levels should not exceed
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41 234 0.17 ng TEQ/kg feed, being about 4 times lower than the MRL value of 0.75 ng TEQ/kg
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43 235 feed. Based on current levels in battery eggs, this seems to be quite achievable.
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48 236 Furthermore, the model was employed to estimate the fraction of dioxins absorbed
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50 237 from two contaminated soils incorporated in the feed as described by Hoogenboom *et al.*
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52 238 (2005). Keeping all other parameters at the value of the calibrated model, the fraction
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54 239 absorbed was derived from the data. Absorption from the two soils appeared to be less
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56 240 efficient than absorption from contaminated oil mixed into the feed being respectively 40%
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3 241 for one of the soils and 60% for the other, as compared to about 90% (estimated range: 80
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5 242 to 100%) for the oil contaminated feed.

6
7 243 In the current study, indicator PCBs (PCBs 28, 53, 101, 118, 138, 153 and 180)
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9 244 were also spiked to the oil used for preparing the feed. Figure 6 shows the data for total
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11 245 indicator PCB levels in eggs and abdominal fat together with a curve fit. Parameters had to
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13 246 be slightly modified, since without modification there was a clear overestimation of the
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15 247 egg levels (Table 1). The reduced value of notably q_f shows that the distribution volume
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17 248 of the fat compartment for these indicator PCBs is greater than for dioxins and dioxin-like
18
19 249 PCBs, resulting in a greater storage capacity and reduction of residue levels.
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21 250 Corresponding half-lives of the initial and terminal phase are 2.8 days and 55 days,
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23 251 respectively, ten percent increased as compared to the values of 2.5 and 50 days for dioxins
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25 252 and dioxin-like PCBs.
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32 253

33 34 254 **Conclusions**

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37 255 • A model was developed for the kinetics of total sum TEQ carry-over of dioxins and
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39 256 dioxin-like PCBs from feed to eggs. The model could not only predict total sum
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41 257 TEQ, but also the results for sum TEQs of separate groups (dioxins and furans,
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43 258 mono-ortho PCBs and non-ortho PCBs). The model was not tested for individual
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45 259 congeners. The model could successfully be applied for data on the kinetics of total
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47 260 and group sum TEQs of contaminated feed with a much higher level of
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49 261 contamination and a quite different contaminant composition
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53 262 • Absorption of the sum TEQ of the dioxin-like compounds from feed prepared with
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55 263 contaminated oil is quite efficient, being around 90%; the main elimination route is
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57 264 by yolk fat excretion. Residues in eggs show clearly bi-phasic kinetics, indicating a
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3 265 fast response after contamination in the order of days followed by a much slower
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6 266 response in the order of months
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8 267 • In contrast, residues in abdominal fat did not show bi-phasic kinetics: the short-
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10 268 lasting fast response phase lacks almost completely. Differences in residue levels in
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12 269 eggs and abdominal fat, expressed as pg TEQ/ g fat can be attributed to differences
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14
15 270 in the kinetics of the corresponding compartments. At steady state, after prolonged
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17 271 exposure to contaminated feed, residue levels are expected to be about the same.
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20 272 • The model was applied to carry-over of dioxins from two soils incorporated in feed
21
22 273 to eggs. The range of absorbed fractions from the two soils was still high but lower
23
24 274 than the absorption of dioxins from oil incorporated into feed.
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26
27 275 • From model calculations, it is evident that EC MRL values for dioxin-derived TEQ
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29 276 levels in laying hens feed and in eggs are not in accordance/compliance. At least a
30
31 277 fourfold reduction is required to guarantee egg levels below the MRL. This should
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33 278 be verified experimentally.
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38 280 **Acknowledgements**

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41 281 This study was sponsored by the Dutch Ministry of Agriculture, Nature Management and
42
43 282 Food Quality.
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310 dioxins and dibenzofurans from soil into eggs of foraging chicken. *Chemosphere*, 34;
311 711-718.

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2
3 312 Table 1. Optimized parameters for the model for dioxin-like compounds and indicator
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5 313 PCBs. The parameters q_c and q_f represent the transfer from the central compartment to the
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7 314 fat compartment and vice versa, y and k the elimination via yolk fat and clearance, F_{abs} the
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9 315 absorbed fraction, and W_f the weight of the fat compartment.
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	q_c	q_f	y	k	F_{abs}	W_f
Dioxin and PCB TEQ	0.17	0.078	$0.043 < y < 0.055$	$0 < k < 0.011$	$0.78 < F_{abs} < 1$	$230 < W_f < 290$
Indicator- PCBs	0.14	0.046	$0.051 < y < 0.075$	$0 < k < 0.022$	$0.68 < F_{abs} < 1$	$220 < W_f < 320$

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8 321 Figure 1. Two compartment PBPK model (A) for the disposition of lipophilic compounds
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10 322 in egg yolk fat of laying hens. A denotes the amounts of contaminant, V the compartments
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12 323 volumes, P partition with respect to blood. The fat compartment serves as a storage for the
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14 324 highly lipophilic contaminants. Fraction F of the dose is absorbed over the gut wall into
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16 325 the central compartment. Elimination is through liver clearance and through excretion with
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18 326 egg yolk fat that is produced with weight $W_{y,f}$ per day with a laying efficiency of ε . These
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20 327 processes can also be described by the set of reduced parameters (B) derived from the PBPK
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22 328 model parameters (see Materials and Methods), with k for hepatic clearance, y for
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24 329 excretion through the yolk and q_c and q_f being the compartment transfer parameters from
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26 330 central to fat compartment and *vice versa*.
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34 332 Figure 2. Measured levels (symbols) and computed concentration-time curves based on
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36 333 fitted parameter values for egg yolk (A), and abdominal fat (B). From lower to upper, data
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38 334 and computations correspond to increasing feed contamination levels of 0.34, 0.58, 0.76,
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40 335 1.85 and 3.95 ng of total TEQ (dioxins, furans, non- and mono-ortho PCBs) / kg feed.
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42 336 Hens were fed contaminated feed during the first 56 days of the experiment.
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48 338 Figure 3. Levels and calculations for the different groups of contaminants in the yolk fat,
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50 339 based on the data obtained with feed contamination 1.85 ng TEQ/kg feed. Upper line (*):
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52 340 total sum TEQ model fit. Next lower line (+): sum TEQ of the group of dioxins and furans;
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54 341 next lower line (x): sum TEQ of the group of non-ortho PCBs; next lower line (o): sum
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56 342 TEQ of the group of mono-ortho PCBs. The last three model calculations were based on
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58 343 the same parameter values that fitted the total TEQ data. At day 55, levels of ten individual
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3 344 eggs instead of a pooled sample are shown. Hens were fed contaminated feed during the
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5 345 first 56 days of the experiment.
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12 348 Figure 4. Kinetics of TEQ contamination in eggs (*, upper line) and abdominal fat (+,
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15 349 lower line) during the experimental period of the group fed the diet containing 1.85 ng
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17 350 TEQ/kg feed (A) and during prolonged feeding with contaminated feed (B). Hens were fed
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19 351 contaminated feed during the first 56 days of the experiment.
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26 354 Figure 5. Contamination levels in egg yolk after administration of ten-fold diluted feed
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29 355 from the Belgian dioxin crisis (Hoogenboom *et al.* 2002). Hens were fed contaminated
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31 356 feed for 7 days followed by 27 days on clean feed. Model calculations were based on the
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33 357 same parameter values that fitted the total TEQ data. Verification shows the total sum TEQ
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35 358 level of all congener groups (upper line, *), mono-ortho-PCB sum TEQs (next upper line,
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37 359 x), dioxin sum TEQs (next lower line, o) and non-ortho-PCB sum TEQs (lower line, +).
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39 360 The steps of the staircase show daily contamination level of eggs.
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45 362 Figure 6. Levels of indicator PCBs in egg fat (A) and body fat (B) for the 5 different feed
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47 363 levels, and the curve-fit based on the model. Corresponding feed levels from bottom to top
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49 364 were respectively 0.2, 2.3, 4.3, 6.0, 14.2 and 31.7 $\mu\text{g}/\text{kg}$ feed. Hens were fed contaminated
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51 365 feed during the first 56 days of the experiment.
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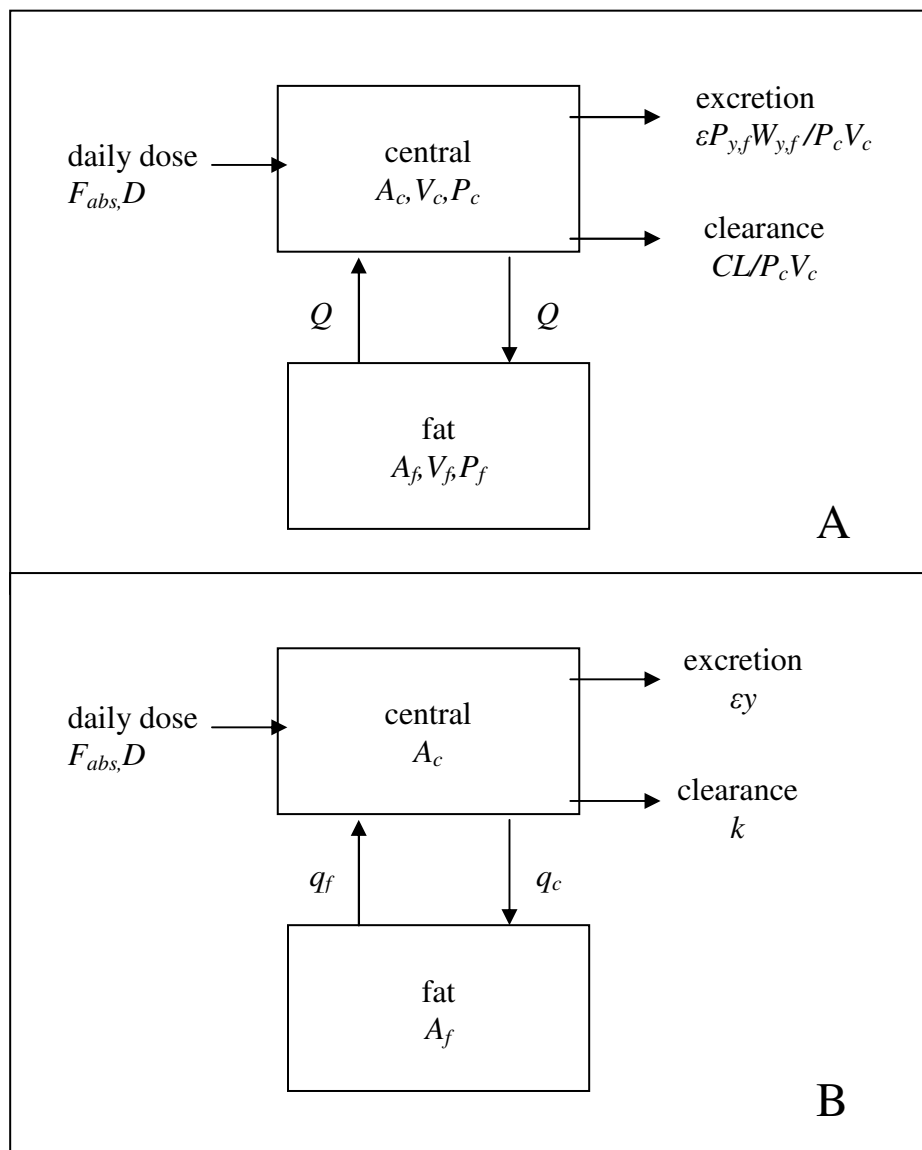
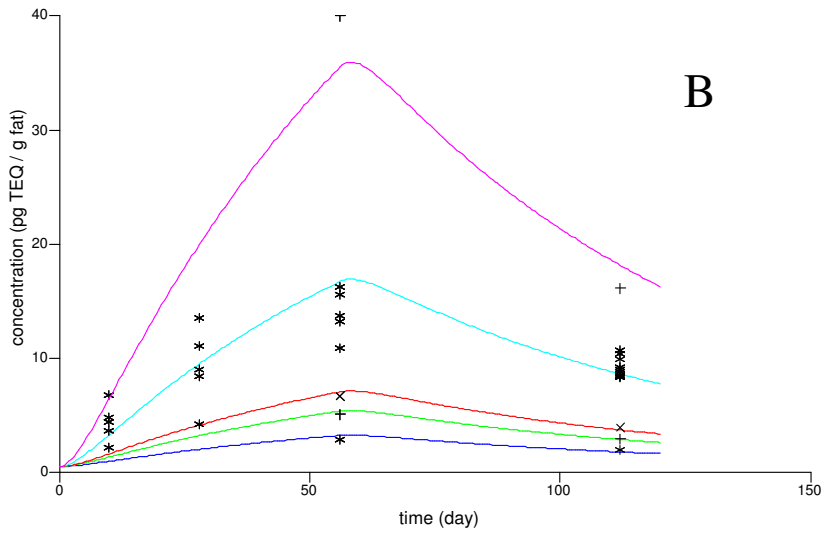
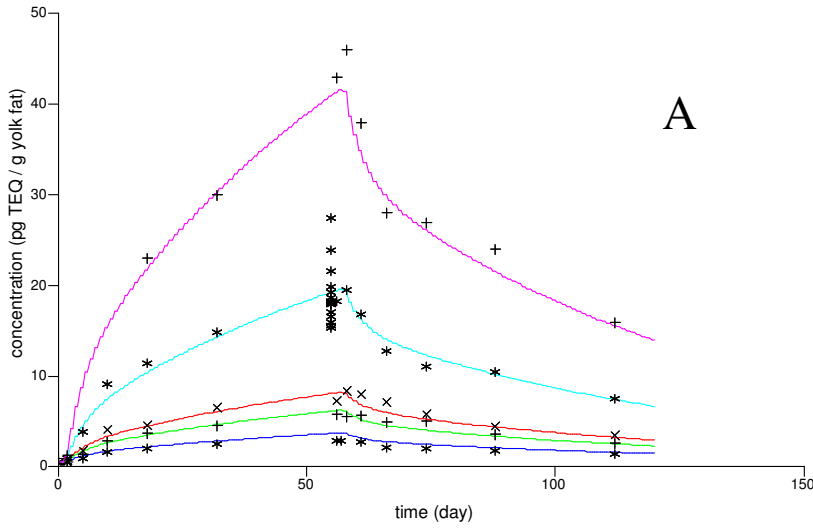
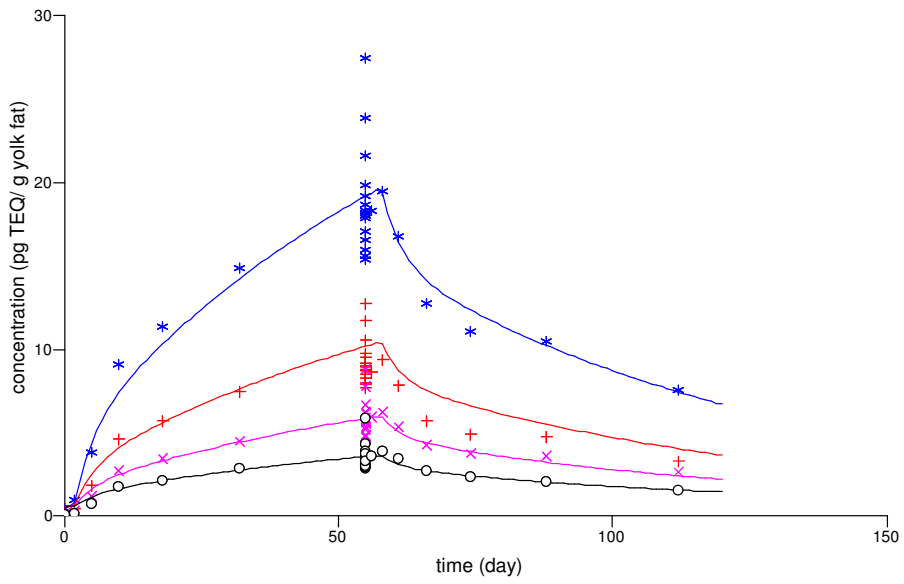


Figure 1



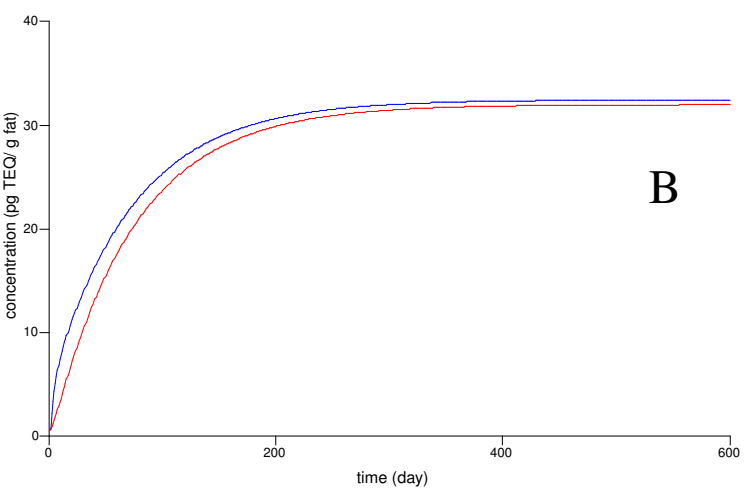
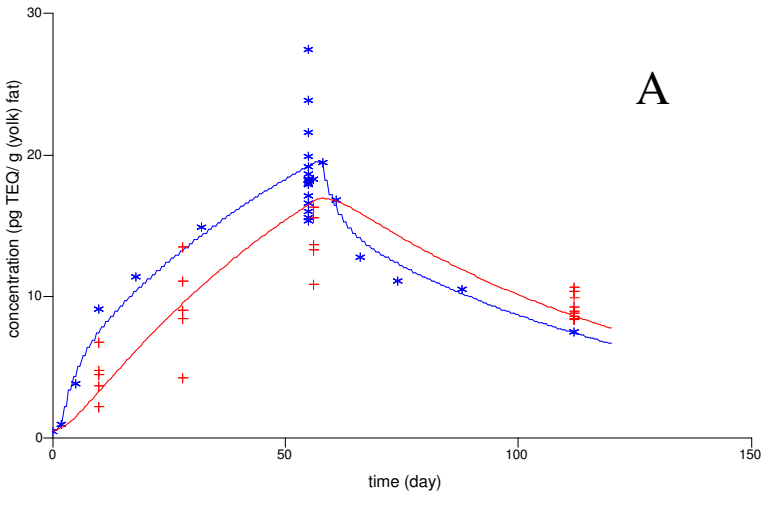
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395 Figure 2.



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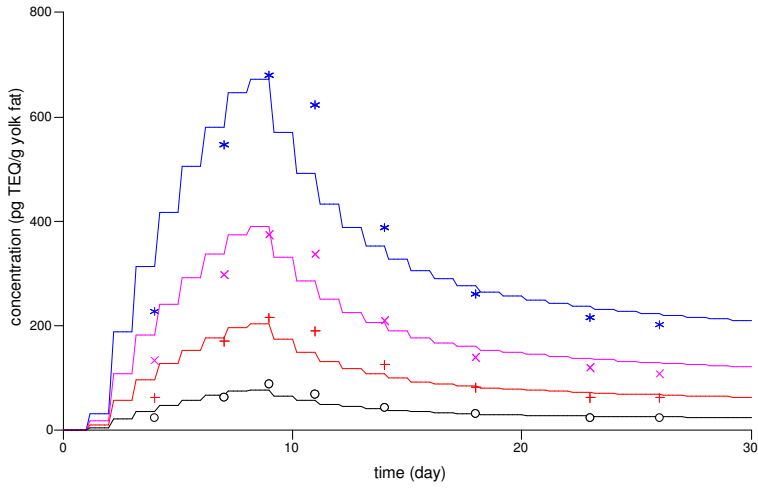
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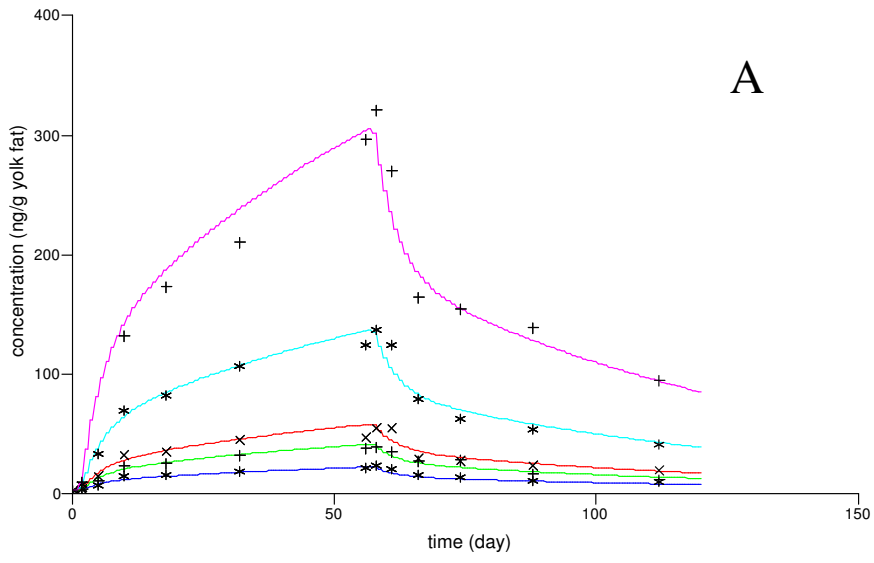
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Figure 5.

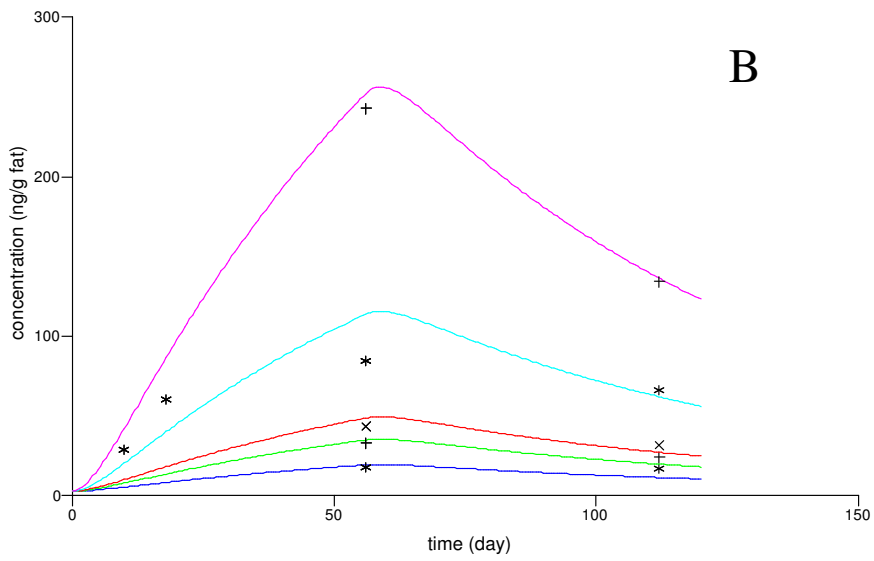
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409 Figure 6.

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