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Carry-over of dioxins and PCBs from feed and soil to eggs at low contamination levels; Influence of mycotoxin binders on the carry-over from feed to eggs

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9 6 **Carry-over of dioxins and PCBs from feed and soil to eggs at low**
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12 7 **contamination levels - Influence of mycotoxin binders on the**
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15 8 **carry-over from feed to eggs**
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1
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3 **Abstract**
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5
6 Laying hens were fed with compound feed containing six different levels of dioxins, dioxin-
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8 like PCBs and indicator PCBs for a period of 56 days. This was followed by a period of 56
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10 days on clean feed. Dioxin levels in feed varied from background levels to three times the
11
12 current EU tolerance limit of 0.75 ng TEQ/kg. At all dose levels a rapid increase was
13
14 observed in the dioxin levels in eggs.. There was a clear linear dose-response relationship
15
16 between the dioxin levels in eggs and feed. The feed containing 0.4 ng TEQ dioxins per kg
17
18 resulted in egg levels just above the EU limit of 3 pg TEQ/g fat. Dioxin-like and indicator
19
20 PCB residues followed a pattern very similar to that of dioxins. Exposure to the highest
21
22 indicator PCB level of 32 µg/kg resulted in egg levels around 300 ng/g fat.
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27 Exposure to dioxins through contaminated soil, mixed at 10% into the feed, resulted in a
28
29 similar carry-over as from feed. Mycotoxin binders, mixed at 0.5% into the feed, had little
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31 effect on the carry-over of dioxins from the feed to the egg.
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34 It can be concluded that consumption of feed or soil with even moderate levels of dioxins
35
36 and dioxin-like PCBs rapidly results in increased levels in eggs. The current EU dioxin limit
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38 for feed cannot guarantee egg dioxin levels below the EU-limit.
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44 **Keywords:** dioxins, PCBs, carry-over, chickens, eggs, feed, soil
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45 Introduction

46 Incidents with residues of PCBs and dioxins have shown that these compounds pose a major
47 threat to the quality of edible products derived from food producing animals. As the exposure
48 of part of the populations in Western countries still exceeds the exposure limits set by e.g. the
49 Scientific Committee on Food (SCF 2001), the EU has developed a strategy for further
50 reducing the exposure to dioxins and dioxin-like PCBs. This includes the establishment of
51 residue limits for dioxins in food (EC 2001) and feed (EC 2002).

52 Since the dioxin crisis in 1999 in Belgium, the Dutch government established a number
53 of monitoring programmes for dioxins aiming at the early detection of contaminated feed and
54 food and subsequently the revelation and elimination of novel sources of dioxins. In 2001,
55 over 300 samples of animal origin were screened in the DR CALUX-assay and if suspected
56 positive for dioxins, analysed by HRGC/HRMS. In one case, eggs obtained from a small farm
57 with free-range hens were shown to contain dioxin levels above the then existing Dutch limit
58 of 5 pg TEQ/g fat (Traag *et al.* 2002). This initial observation was followed by a focussed
59 action on these types of eggs and showed a clear elevation in dioxin levels in eggs from free-
60 range hens, in particular those from farms producing eggs according to strict organic
61 standards. This was confirmed in a survey by The Dutch Food Inspection Service, showing
62 that eggs from six out of 68 farms would exceed the new EU dioxin limit for eggs of 3 pg
63 TEQ/g (as of July 2003), and three exceeded the then existing limit of 5 pg TEQ/g (De Vries,
64 2002). In addition some of the samples contained high levels of dioxin-like PCBs, with a
65 highest total TEQ level of 15 pg TEQ/g fat. Follow-up studies were performed during the
66 winter at the first farm, and the levels in eggs from hens that were kept inside, strongly
67 suggested that the source of the contamination was not the feed, but the outdoor environment
68 (unpublished). Similar has been observed in other studies, but in these cases the soil was
69 clearly contaminated with dioxins (Stephens *et al.* 1990, 1994, 1995, Schuler *et al.* 1997,

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3 70 Lovett *et al.* 1998, Harnly *et al.* 2000, Petreas *et al.* 2001, Air *et al.* 2002, Pussemier *et al.*
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5 71 2004). Another study was performed in the autumn of 2003, when a large number of farms
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8 72 producing organic eggs were visited and investigated for possible sources of dioxins and
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10 73 factors that may contribute to the exposure of hens to dioxins (Brandsma *et al.* 2004). This
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12 74 study again showed that most eggs would comply with the current dioxin limit of 3 pg TEQ/g
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14 75 fat, but that 13% of the eggs, produced on 26% of the farms would not be in compliance with
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16 76 this limit.

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19 77 In July 2003, new EU limits for dioxins in food and feed became official, including limits
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21 78 for eggs and feed, being respectively 3 pg TEQ/g fat and 0.75 ng TEQ/kg feed (EC 2001,
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23 79 2002). Eggs from free-ranging hens were excluded from these limits until 1-1-2004, later
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25 80 extended to 1-1-2005. At the same time, studies were started to investigate the carry-over of
26
27 81 dioxins and PCBs from feed and soil to eggs at relatively low levels, and to investigate
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29 82 possible ways for reducing the exposure and carry-over. The current paper describes a
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31 83 controlled study on the relationship between dioxin and PCB levels in eggs and feed (Study
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33 84 I), as well as soil (Study II). Contrary to previous studies 6 different levels in feed were used
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35 85 in a range around the current and proposed EU-limits for dioxins and dioxin-like PCBs. In
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37 86 addition we studied the depletion of these contaminants, which is relevant for risk
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39 87 management purposes in cases of crisis situations. As a potential tool to decrease the carry-
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41 88 over to eggs we also tested the effects of so-called mycotoxin binders on the carry-over of
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43 89 dioxins and PCBs (Study III). The results of the studies were used to develop a mathematical
44
45 90 model based on the physiology of the laying hen, that can be used for predicting the levels in
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47 91 eggs resulting from a certain feed contamination, and the effect of certain variables on this
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49 92 relationship (Van Eijkeren *et al.* 2005).
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3 93 **Materials and methods**
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8 95 *Preparation of feed*
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12 97 *Preparation of feed for Study I: relation between levels in feed and eggs*
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15 98 Feed was prepared with soy oil spiked with the different dioxin and PCB congeners. The
16
17 99 composition of the feed is given in Table 1. A blanc oil, obtained from Nutreco (Boxmeer,
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19 The Netherlands) was analysed by both CALUX and by GC/MS and shown to be low in
20 100
21 dioxin-like compounds. Subsequently, a stock solution of contaminated oil was prepared with
22 101
23 about 7500 pg TEQ/g of dioxins and dioxin-like PCBs. An aliquot of 200-gram oil was mixed
24 102
25 with the following PCB stock solutions: 7.5 ml PCB 105 at 100 ng/ml, 15 ml PCB 118 at 100
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27 ng/ml, 1.2 ml PCB 156 at 100 ng/ml, 7.5 ml PCB 28 at 100 ng/ml, 15 ml PCB 52 at 100
28 104
29 ng/ml, 30 ml PCB 101 at 100 ng/ml, 30 ml PCB 138 at 100 ng/ml, 30 ml PCB 153 at 100
30 105
31 ng/ml and 15 ml PCB 180 at 100 ng/ml. The organic solvent (nonane) was removed under
32 106
33 vacuum. Subsequently the following standard mixtures were added: 44 µl CIL EDF-4096
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35 dioxins (tetra's 2.5 ng/µl, penta's, hexa's and hepta's 6.25 ng/µl and octa's 12.5 ng/µl), 408 µl
36 108
37 CIL EC-4986 non-ortho PCBs (10 µg/ml) and 785 µl CIL EC-4987 mono-ortho PCBs (10
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39 µg/ml). The organic solvent again was removed under vacuum (1 hr, 63°C).
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45 111 The 200 gram oil was first diluted to 1500 gram, and subsequently diluted for
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47 incorporation into the different feeds, as follows: 30 gram + 1470 gram blank oil (b used for
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49 feed B), 60 gram + 1440 gram blank oil (c used for feed C), 265 gram + 3237 gram blank oil
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51 (d used for feed D), 685 gram + 3882 gram blank oil (e used for feed E), and 420 gram + 980
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53 gram blank oil (f used for feed F).
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57 116 Analysis of oil b by GC/MS showed a total TEQ content of 20 pg TEQ/g, with a
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59 contribution of 11 pg TEQ/g for dioxins, 5.3 pg TEQ/g for non-ortho PCBs and 3.7 pg TEQ/g
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3 118 for mono-ortho PCBs. The blank oil contained a total TEQ content of 0.4 pg TEQ/g fat
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5 119 (upperbound). Based on these results the Nutreco feed mill in Heyen, The Netherlands,
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7 120 prepared the feeds with 0.95% of oils (b) to (f), and the blank oil a in the following quantities:
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10 121 290 and 650 kg blank feed (prepared in 2 batches), 110 kg of feeds B, C, D and F, and 400 kg
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12 122 of feed E. Table 2 shows the analysed dioxin content of the different feeds
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17 124 *Preparation of feed for Study II: relation between levels in soil and eggs*

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19 125 Aim of this study was to assess the bioavailability of dioxins from contaminated soil.
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21 126 Therefore, feeds were prepared by mixing the blank feed with 10% of soil collected from two
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23 127 chicken farms with elevated dioxin and dioxin-like PCB levels in eggs. The soils had been
24
25 128 analysed previously showing dioxin levels of 5.88 ng TEQ/kg (soil A) and 1.94 ng TEQ/kg
26
27 129 (soil B). Pooled egg samples collected at the same time at these farms were shown to contain
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29 130 dioxin levels of 1.0 and 7.3 pg TEQ/g fat. An additional feed was prepared as a control, by
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31 131 mixing feed E (study I) with 10% of clean sand.
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37 133 *Preparation of feed for Study III: effect of binders on carry-over from feed to eggs*

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39 134 Aim of this study was to assess whether or not binders would influence the bioavailability of
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41 135 dioxins and PCBs in feed. A number of different binders were obtained for this study from the
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43 136 producers. These materials were claimed to prevent the absorption of mycotoxins and as such
44
45 137 might be potential candidates for reducing the absorption of dioxins as well. The materials
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47 138 were first screened by DR CALUX[®] and analysed by GC/MS, and shown not to contain
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49 139 elevated dioxins and PCBs. The following feeds were prepared: feed E mixed with 0.5% Exal
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51 140 H (Tessenderlo, Madrid, Spain), feed E mixed with 0.5% MycoAd A-Z (Jadis, Schiedam, The
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53 141 Netherlands), and feed E mixed with 0.5% Klinofeed (Unipoint, Truttikon, Switzerland). The
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55 142 level of the binders was based on the recommendations of the providers.
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3 143 *Animal studies*
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8 145 *Study I*
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10 146 Hens (Bovans Gold line, age about 45 weeks at the start) were housed in a three-tier battery
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12 147 with 3-4 hens per cage. The different groups contained 12, 12, 12, 13, 26 and 13 animals
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14
15 148 respectively. After an adaptation period on blank feed for 1 week, hens were fed with the
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17 149 different feeds A to F for 56 days, followed by another 56 days on blank feed A. During the
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20 150 whole 112-day period eggs were collected and pooled per day per feeding group. On days 56
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22 151 and 112, at least five hens from each group were slaughtered and abdominal fat, livers and the
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24 152 ovaries collected. The group fed with feed E was larger allowing the collection of tissues of
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27 153 another five animals on days 10 and 28.
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31 155 *Study II and III*
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34 156 Hens (ISA Brown Warren, age about 25 weeks at the start) were housed in a three-tier battery
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36 157 with 2-3 hens per cage. The seven groups of 5 animals followed an adaptation period of one
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38 158 week on clean feed, and then were fed with the different feeds for 32 days. During the whole
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40
41 159 32-day period eggs were collected and pooled per day per feeding group. On day 32 the hens
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43 160 from each group were slaughtered and abdominal fat, livers and the ovaries collected.
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48 162 *Analysis of dioxins and dioxin-like PCBs*
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50 163 Levels of dioxins, non-ortho and mono-ortho PCBs were determined by high resolution
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52 164 GC/MS, basically as described by Tuinstra *et al.* (1994). Eggs and ovaries were freeze-dried
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55 165 and subsequently fat was extracted using Accelerated Solvent Extraction (ASE). Abdominal fat
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57 166 was melted in an oven at 70°C. After extraction or sample pre-treatment ¹³C labeled dioxins and
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60 167 dioxin-like PCBs were added to the fat phase of the samples. Feed samples were extracted with

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3 168 ethyl acetate or, when minerals or soil was added to the feed (study II and III), with toluene using
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5 169 ASE. Prior to extraction ¹³C labeled dioxins and dioxin-like PCBs were added to the feed
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8 170 samples. Separation between dioxins and fat was carried out using gel permeation
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10 171 chromatography. The system consisted of an HPLC pump (Gilson, model 305, an autosampler
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12 172 (Gilson, model 231) equipped to inject 12,5 ml of sample solution, and a fraction collector
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14 173 (Gilson, model 202) adapted to collect 300 ml fractions using 500 ml glass collection flasks. The
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16 174 glass GPC column (Spectrum) (60 x 2.5 cm) was packed with Biobeads SX 3. After an
17
18 175 additional clean up with activated Al₂O₃, separation between planar compounds (dioxins and
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20 176 non-ortho PCBs) and non-planar compounds like other PCBs was carried out with porous
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22 177 graphitised carbon. The alumina (basic) clean-up was performed with an automatic sample
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24 178 preparation system using solid phase extraction columns (ASPEC, Gilson). The columns were
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26 179 packed with 1 gram deactivated alumina (7% water) shortly before use. Porous graphitised
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28 180 carbon clean up was performed using an HPLC system consisting of an HPLC pump (Gilson
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30 181 model 205), a column switching device (Gilson, valvemate), a solvent switching device (Gilson,
31
32 182 valvemate), an autosampler (Gilson model 231), equipped with a 5 ml loop and a fraction
33
34 183 collector (Gilson model 202) adapted to collect 100 ml fractions. The column used was
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36 184 Hypercarb (100 X 4.6 mm) (Shandon). The final extract with planar compounds was concen-
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38 185 trated to 10 µl and the extract containing the non-planar PCBs to 200 µl. Both extracts were
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40 186 analysed with gas chromatography-high-resolution-mass spectrometry (HRGC-HRMS)
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42 187 (Autospec, Micromass). The mass spectrometric method to determine the tetra through octa
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44 188 dioxins is based on United States Environmental Protection Agency protocols. Included in the
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46 189 analysis is a standard QA programme e.g. determination of recovery of internal standards,
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48 190 accuracy of spiked samples and blanks. Non-detectable congeners were quantified at the limit of
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50 191 detection (upperbound). Absolute levels were transferred to TEQ levels using the TEF values
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60 192 described by Van den Berg *et al.* (1998).

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3 193 *Analysis of indicator PCBs*
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5 194 The seven indicator PCBs (28, 52, 101, 118, 138, 153 and 180) were analysed by GC/MS
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7
8 195 following on-line clean-up over a silica HPLC column using a large volume injector (LVI). In
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10 196 short, 200 mg of the homogenised fat sample is mixed with 800 µl of the internal standard
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12 197 solution of ¹³C PCB 118 and PCB 198 (6.25 ng/ml). Subsequently, 50 µl is injected onto the
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14 198 silica column and after elution of the fat with hexane, the direction of the flow is switched to
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16 199 back-flush and the column is cleaned with 3 ml dichloromethane. The PCB containing
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18 200 fraction (approximately 1 ml) is directly transferred to the gas chromatograph, which is
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20 201 provided with an LVI. In the LVI the large amount of solvent was separated from the
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22 202 compounds of interest by an uncoated retention gap of 10 meters, which ends up in a retaining
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24 203 column of 1-meter coated with a non-polar phase. The temperature of the LVI, located in the
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26 204 oven of the GC, is slightly (80°C) above the boiling point of the mobile phase. Hexane is
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28 205 evaporated slowly and blown off via the Solvent Vapour Exit (SVE) while the PCB's are
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30 206 retained on the 1-meter retaining column. After a specific time the majority of the solvent was
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32 207 evaporated, the SVE closed and the temperature of the oven was raised in order to start
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34 208 chromatography. Detection was done by mass-spectrometry with a bench top system in
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36 209 Selected Ion Mode (SIM).
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45 211 *DR CALUX[®] analysis*
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47 212 The DR CALUX[®] test was carried out as described earlier by Bovee *et al.* 1998. An aliquot of
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49 213 0.5 g fat was mixed with hexane and extracted on acid silica as described previously. In each
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51 214 test series a blanc butter fat sample and butter fat samples containing 1, 2, 3 and 6 pg TEQ/g
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53 215 fat were included. The response obtained with the butter fat sample of 3 pg TEQ/g, containing
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55 216 1.5 pg TEQ/g dioxins and 1.5 pg TEQ/g non-ortho PCBs, was used as the reference signal.
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3 217 Samples showing a lower response were declared negative, samples showing a higher
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5 218 response suspected.

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8 219 Aliquots of 5 gram feed or binder were mixed with 15 ml methanol/water 85/15 (v/v) and
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10 220 extracted twice with 20 ml hexane/diethyl ether 97/3 (v/v). The extract was reduced to 5 ml
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12 221 and purified over acid silica columns as described above. Feed samples were included as
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15 222 control samples.

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1
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3 223 **Results and Discussion**
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8 225 *Carry-over from feed to eggs (study I)*
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12 227 *Dioxin and PCB levels in the feeds*
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15 228 Table 2 shows the total levels of dioxins, dioxin-like PCBs and indicator PCBs in the various
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17 229 feeds. Each feed was analysed 3 to 5 times, corresponding to the number of samples taken
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19 230 from the different bags. The variation coefficients were in general below 5%, demonstrating
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21 231 the good homogeneity of the feeds. The relative contribution of dioxins, non-ortho and mono-
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23 232 PCBs to the total TEQ level was as intended, being respectively 50, 30 and 20%. Table 3
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25 233 shows the levels of the various congeners in the two highest contaminated feeds. The overall
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27 234 levels were higher than intended, which cannot be explained by the background levels in the
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29 235 feed ingredients, as shown by the level in the blank feed (A). However, since a rather broad
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31 236 range of different concentrations in feed was used, this deviation had no consequences for the
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33 237 aim of the study.
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42 239 *Levels in eggs*
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44 240 Table 4 shows the average feed consumption and the productivity of the hens, both during and
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46 241 after the exposure period. There was no effect of the treatment on these parameters. The
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48 242 exposure did not affect the average egg weight, but in all groups a slight decrease was
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50 243 observed after 3.5 weeks on clean feed, resulting in weights around 48-51 grams per egg
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52 244 (without shell). There is no explanation for this decrease, or possible consequences for the
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54 245 residue levels in the eggs.
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57 246 Figure 1 presents the time-related levels of dioxins (A), non-ortho PCBs (B), mono-ortho
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59 247 PCBs (C) and total TEQ (D) in the eggs from the different exposure groups. These data show
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3 248 that even after 56 days on contaminated feed, levels in eggs continued to increase. In a similar
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5 249 study, Pirard and De Pauw (2005) exposed laying hens to a dioxin level ten-fold higher than
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8 250 our highest concentration. They observed a continuous increase in the levels for 4 weeks, at
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10 251 which time point the animals stopped laying eggs. As shown in Figure 1, after cessation of the
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12 252 exposure, there was initially a small increase in the residues, than a rapid decline, and
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15 253 subsequently a much slower decrease. A similar profile was observed previously in a study
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17 254 with much higher dioxin and PCB levels (Hoogenboom *et al.* 2002). The explanation lies in
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19 255 the fact that the production of a full-grown yolk requires about ten days. Thus yolks of eggs
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21 256 laid during the first days on clean feed were largely formed and contaminated during the
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23 257 exposure period. Redistribution of dioxins from abdominal fat to the general circulation and
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25 258 thus to eggs, may explain the slow decrease in residues during the latter part of the study.
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29 259 Figure 1A also shows that the current limit for eggs of 3 pg TEQ/g fat was rapidly exceeded,
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31 260 when exposed to feed containing dioxins just above the current limit for feed of 0.75 ng
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34 261 TEQ/kg.

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36 262 Figure 2 shows the levels of the 7 indicator PCBs in egg fat during the exposure and
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38 263 depletion period. In general the pattern was very similar to that of the dioxins and dioxin-like
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40 264 PCBs. Again, data indicate that egg levels continued to increase even after 56 days. At similar
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42 265 dose levels (1.8 or 6 $\mu\text{g}/\text{kg}$ feed), De Vos *et al.* (2005) observed steady-state levels after
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44 266 respectively 40 or 70 days. The highest level obtained at 56 days of exposure via the most
45
46 267 contaminated feed was around 325 ng/g fat. Based on 4% fat in the feed, the feeds E and F
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48 268 would contain respectively 350 and 800 ng/g fat, thereby exceeding e.g. the Belgium limit for
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50 269 feed of 200 ng/g. Only eggs from hens fed the highest contaminated feed F would actually
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52 270 exceed the Belgium limit for indicator PCBs of 200 ng/g fat, although it cannot be excluded
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54 271 that after longer exposure also eggs from hens fed with feed E might have approached the
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58 272 limit.
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3 273 The relationship between the dioxin levels in feed and eggs after different periods of
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5 274 exposure is graphically presented in Figure 3. There is a clear linear relationship between the
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8 275 feed levels and the levels in eggs at the different exposure times. This figure also clearly
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10 276 demonstrates that even after a short period on feed contaminated at the current EU-limit of
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12 277 0.75 ng TEQ/kg, the limit for eggs was exceeded.
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17 279 *Levels in abdominal and ovary fat*
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19 280 The time-related levels of the sum of dioxins and dioxin-like PCBs (A), as well as the 7
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21 281 indicator PCBs (B) in egg, abdominal and ovary fat sampled from the chickens fed with feed
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24 282 E are shown in Figure 4. During the 56-days exposure period, levels in eggs and ovary fat
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26
27 283 were comparable and generally higher than in the abdominal fat. This was particularly true for
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29 284 the indicator PCBs. This difference was also clear for either dioxins or dl-PCBs (data not
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31 285 shown). After another 56 days on clean feed, levels of dioxin-like compounds were very
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34 286 similar in ovary and egg fat and slightly lower than in the abdominal fat. However, levels of
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36 287 the indicator PCBs were clearly elevated in the abdominal fat. The combined results indicate
37
38 288 that the decrease of dioxins and PCBs is much slower in the body fat than in the eggs and
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40
41 289 related ovaries. From the model for carry-over (Van Eijkeren *et al.*) it appears that the levels
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43 290 in eggs possess a clearly bi-phasic nature, an initial phase with fast rising or, after cessation of
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45 291 contamination, fast declining levels, followed by a terminal phase with slowly increasing or
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48 292 decreasing levels. On the other hand, in abdominal fat, which acts as a great capacitor for
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51 293 these highly lipophilic compounds, the relative contribution of the fast rising phase is very
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53 294 low and the kinetics of contamination is almost mono-exponential with the rate of the slow
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55 295 terminal phase
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3 297 *Carry-over rates of individual congeners from feed to egg*
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5 298 To compare the carry-over of individual congeners from feed to eggs, it is possible to
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8 299 calculate the so-called Carry-Over Rate (COR), defined as the fraction of a certain congener
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10 300 excreted in the eggs, in relation to the amount ingested from the feed. Ideally, this should be
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12 301 done under steady-state conditions, but this was not achieved within the current study period.
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14 302 Therefore, the COR values should be regarded as minimum values. Based on the daily egg fat
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16 303 production of 5.1 g and daily feed intake of 116 g, CORs were calculated for feeds E and F in
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18 304 I (Table 3). These data suggest a COR for the more important lower chlorinated congeners
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20 305 around 40% and a lower COR for the higher chlorinated hepta and octa congeners. This is in
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22 306 line with the observation by Pirard and De Pauw (2005) who also showed a much higher
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24 307 excretion of the higher chlorinated PCDD/Fs into the feces. The non-ortho and mono-ortho
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26 308 PCBs showed similar CORs as the lower chlorinated dioxins although some mono-ortho PCB
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28 309 congeners appeared to have even higher CORs. These were present at relatively low
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30 310 concentrations and in practice do not contribute significantly to the TEQ levels. In the group
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32 311 of the indicator PCBs, the lower chlorinated PCBs 53 and 101 showed very low carry-over
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34 312 rates, contrary to PCB 28. Similar has been observed previously in laying hens (De Vos *et al.*
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36 313 2005) and broilers (Hoogenboom *et al.* 2004b, Maervoet *et al.* 2004). Based on excretion in
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38 314 the feces, De Vos *et al.* (2005) showed that active metabolism of these congeners must
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40 315 underly this observation.
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48 316 Before steady state conditions are reached, part of the absorbed compounds are still
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50 317 accumulated in the body fat. Based on the mathematical modelling it is expected that after
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52 318 reaching steady-state, COR values will be doubled, implying that the amounts excreted in the
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54 319 eggs will be close to the amounts ingested (Van Eijkeren *et al.* 2005). As for total TEQ levels,
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56 320 the absorbed fraction ranges between 90% and 100%, the COR for total TEQs at steady state
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58 321 is expected to range between the same limits.
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323 Carry-over from soil to eggs (study II)

324 Table 5 shows the levels of dioxins and PCBs in feed E, feed E mixed with 10% sand and the
325 two blank feeds mixed with 10% of the soils sampled at two chicken farms. The two soils
326 were reanalysed and shown to contain respectively 4.0 and 1.7 ng TEQ dioxins per kg and
327 only minor amounts of dioxin-like PCBs. The level in the feed containing soil A is in
328 agreement with the dilution, the level in the feed with soil B is relatively high which can best
329 be explained by the application of the upperbound principle. Lower bound levels (i.e. levels
330 of non-detects set at 0) in these two feeds were respectively 0.40 and 0.19 ng TEQ/kg,
331 reflecting the differences observed in the soil.

332 Figure 5 shows the dioxin and dioxin-like PCB levels in the eggs from the hens
333 obtaining these feeds. Time-related levels in the eggs from the hens fed with feed E, either
334 without or with 10% sand were very similar to the results obtained in study I. The dilution of
335 contaminated feed with 10% sand did not clearly influence the levels observed in the eggs and
336 abdominal fat. The results obtained with the blank feed mixed with 10% soil A or soil B
337 reflected the differences in the soil and feed levels. A plateau level appeared to be reached in
338 this study within a short period of exposure. Maximum dioxin and total TEQ levels measured
339 in eggs from hens exposed to soil A were 2.4 and 2.9 pg TEQ/g fat. In the case of soil B,
340 highest levels of 1.2 and 1.6 pg TEQ/g were observed. Again, levels in abdominal fat were
341 lower than those for egg fat (data not shown). For comparison, levels determined in pooled
342 egg samples from the farms where the soil was sampled were 7.3 and 1.0 pg TEQ/g fat.

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344 Effect of binders (study III)

345 Table 5 includes the levels of dioxins and PCBs in the four feeds supplemented with the
346 different binders. Levels in egg and abdominal fat of the hens fed with these feeds showed

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3 347 that the binders, at least at the levels used in this study, had little or no effect on the residue
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5 348 levels of dioxins and PCBs in the eggs (Table 6), although in the case of MycoAd AZ and
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7 349 especially Klinofeed, both aluminium silicates, there appeared to be a slight decrease in the
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10 350 levels of the different contaminants in both the eggs and the abdominal fat. It seems
11
12 351 worthwhile to investigate if higher levels may have a more profound effect, and whether this
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15 352 also works with the absorption of dioxins and PCBs from soil..
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22 355 **Conclusions**

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24 356 • Laying hens are sensitive indicators for dioxin exposure, meaning that relatively low
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26 357 intake levels result in high levels in the egg yolk. This is not only true for
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28 358 contaminated feed but also for contaminated soil.
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30 359 • The current EU limit for feed of 0.75 ng TEQ/kg, established in 2002, is insufficient to
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32 360 guarantee that levels in eggs will not exceed the limit of 3 ng TEQ/kg fat. A further
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34 361 decrease in the feed limit by a factor 4 is required to achieve this goal. The alternative
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36 362 is to increase the limit in eggs, but this is not in line with the further reduction in the
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38 363 exposure aimed at by the EU authorities.
- 39
40 364 • There are currently no Dutch or European limits for indicator PCBs in feed or eggs.
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42 365 However, the Belgium limit of 200 ng/g fat for feed does ensure that the limit for eggs
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44 366 of 200 ng/g fat is not exceeded.
- 45
46 367 • Addition of binders at the tested levels has minimal effects on the resulting residues of
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48 368 dioxins and PCBs in hens.
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51 369 **Acknowledgements**

52 370 The study was sponsored by the Dutch Ministry of Agriculture, Nature and Food Quality.
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3 472 **Legends**
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8 474 Figure 1. Levels of dioxins (A), non-ortho PCBs (B), mono-ortho PCBs (C) and total TEQ
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10 475 (D) in egg fat from hens fed with feeds containing 0.04 (A, +), 0.34 (B, Δ, open triangle), 0.58
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12 476 (C, ▲, closed triangle), 0.76 (D, ○, open circle), 1.85 (E, ◇, open diamond) and 3.95 (F, ▼,
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14 477 reversed triangles) ng TEQ/kg of dioxins and PCBs.
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19 479 Figure 2. Levels of indicator PCBs in egg fat from hens fed with feeds containing 0.2 (A, +),
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21 480 2.3 (B, Δ, open triangle), 4.3 (C, ▲, closed triangle), 6.0 (D, ○, open circle), 14.2 (E, ◇, open
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23 481 diamond) and 31.7 (F, ▼, reversed triangles) μg/kg feed of indicator PCBs.
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26 482
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28 483 Figure 3. Relation between dioxin levels in feed and egg fat after 10 (▼, reversed triangles),
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30 484 32 (▲, closed triangle) and 58 (●, closed circle) days of feeding contaminated feed. Solid
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32 485 lines represent the current limits in feed and eggs, dashed lines the action limits in the EU.
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37 487 Figure 4. Levels of dioxins and dioxin-like PCBs (A), and indicator PCBs in egg (▲, closed
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39 488 triangle), abdominal (▼, reversed triangles) and ovary fat (●, closed circle) from chickens fed
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41 489 with feed E.
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46 491 Figure 5. Levels of dioxins (A) and total TEQ (B) in egg fat from hens fed with feed 750 (●,
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48 492 closed circle), feed 750 mixed with 10% sand (▲, closed triangle), and blank feed mixed with
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50 493 10% soil A (◆, closed diamond) or soil B (■, closed square). For comparison the results
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52 494 obtained with feed 750 in the first study are included (○, open circle).
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495 Table 1 shows the ingredients of the feed, Topline extra.

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Ingredient	Amount (%)
Corn	32.53
Wheat	19.61
Corn-by-products	2.00
Maizeglutenfeed	3.52
Wheatglutenfeed	4.31
Soybeans, extracted	13.98
Sunflower seed, extracted	7.42
Rape seed extracted	2.33
Limestone fine <15 mm	2.00
Limestone 1-3 mm	7.55
Monocalcium phosphate	0.06
Sodium chloride	0.16
Sodium carbonate	0.12
Chicken fat	4.00
Methionin 98	0.09
Fytase 5000 L	0.01
Cholinchloride	0.02
Fish oil	0.10
Vitamin mix 44	0.30

497

498 Table 2. Levels of dioxins, non-ortho, mono-ortho and indicator PCBs in the different feeds used in
 499 study I, as determined by GC/MS. Results are expressed as upperbound* levels and are the mean of 3-
 500 5 analyses. The % CV of the TEQ levels is given in brackets.

501

Feed	Dioxins (ng TEQ/kg)	no-PCBs (ng TEQ/kg)	mo-PCBs (ng TEQ/kg)	total TEQ (ng TEQ/kg)	Indicator PCBs (µg/kg)
A	0.04 (10)	0.00 (16)	0.00 (3)	0.04 (10)	0.2 (14)
B	0.20 (18)	0.09 (4)	0.06 (4)	0.34 (12)	2.3 (1)
C	0.30 (2)	0.17 (3)	0.11 (1)	0.58 (2)	4.3 (7)
D	0.40 (2)	0.22 (2)	0.14 (1)	0.76 (1)	6.0 (4)
E	0.97 (1)	0.55 (2)	0.33 (1)	1.85 (1)	14.2 (2)
F	2.04 (2)	1.19 (1)	0.72 (2)	3.95 (1)	31.7 (3)

502
 503 * only in the case of feed A, lowerbound differed from upperbound levels, being respectively 0.01 and
 504 0.02 ng TEQ/kg for dioxins and total TEQ.

505 Table 3. Levels of the different dioxin and PCB congeners in feeds E and F and the eggs from
 506 chickens fed with these feeds and sampled at day 56. Feed was analyzed in five-fold, eggs once. Based
 507 on feed intake and egg production, carry-over rates for feed to eggs were determined for day 56.

508

	feed E	eggs E day 56	carry-over (%)	feed F	eggs F day 56	carry-over (%)
dioxins	ng/kg	pg/g fat		ng/kg	pg/g fat	
2,3,7,8-TCDF	0.12	1.10	40	0.25	2.33	40
1,2,3,7,8-PeCDF	0.33	2.86	39	0.66	7.15	48
2,3,4,7,8-PeCDF	0.33	2.83	38	0.67	6.76	44
1,2,3,4,7,8-HxCDF	0.34	3.33	43	0.70	7.00	44
1,2,3,6,7,8-HxCDF	0.33	2.98	40	0.68	6.44	42
2,3,4,6,7,8-HxCDF	0.43	3.03	31	0.84	6.80	35
1,2,3,7,8,9-HxCDF	0.32	2.85	39	0.67	6.28	41
1,2,3,4,6,7,8-HpCDF	0.40	1.60	18	0.82	3.26	18
1,2,3,4,7,8,9-HpCDF	0.32	1.44	20	0.66	3.06	21
OCDF	0.63	0.98	7	1.39	1.55	5
2,3,7,8-TCDD	0.14	1.31	41	0.30	3.26	47
1,2,3,7,8-PeCDD	0.37	3.39	41	0.79	8.04	45
1,2,3,4,7,8-HxCDD	0.40	3.80	42	0.84	8.38	44
1,2,3,6,7,8-HxCDD	0.35	3.45	44	0.74	7.49	44
1,2,3,7,8,9-HxCDD	0.40	2.87	32	0.84	6.49	34
1,2,3,4,6,7,8-HpCDD	0.42	2.15	22	0.84	3.97	21
OCDD	1.34	3.82	13	2.57	4.78	8
non-ortho-PCBs	ng/kg	pg/g fat		ng/kg	pg/g fat	
PCB 81	5.12	46.70	40	10.86	102.0	41
PCB 77	10.85	112.00	45	22.06	252.0	50
PCB 126	4.98	54.10	48	10.86	123.0	50
PCB 169	4.89	55.40	50	10.55	124.0	52
mono-ortho PCBs	ng/kg	ng/g fat		ng/kg	ng/g fat	
PCB 123	5	0.10	78	14	0.22	70
PCB 118	1576	17.10	48	3454	40.60	52
PCB 114	6	0.07	52	14	0.16	51
PCB 105	894	9.36	46	1940	23.00	52
PCB 167	10	0.20	84	24	0.41	76
PCB 156	139	1.75	55	307	4.03	58
PCB 157	13	0.15	51	26	0.39	65
PCB 189	10	0.14	61	21	0.29	61
indicator-PCBs	µg/kg	ng/g fat		µg/kg	ng/g fat	
PCB 28	0.98	8.4	38	2.13	20.7	43
PCB 52	1.30	1.6	5	2.91	2.9	4
PCB 101	2.88	3.5	5	6.33	7.5	5
PCB 138	2.99	34.6	51	6.72	83.1	54
PCB 153	2.79	36.1	57	6.27	86.2	61
PCB 180	1.89	25.6	59	4.27	58.5	41

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3 510 Table 4. Feed consumption and productivity of the hens during and after the exposure period. Data are
4 511 based on the overall feed consumption and egg production over the whole exposure or withdrawal
5 512 period.
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Feed	Exposure period			Withdrawal period		
	Feed consumption (g/day)	Egg production (%)	Egg weight* (g)	Feed consumption (g/day)	Egg production (%)	Egg weight* (g)
A	116	88	54	112	85	50
B	108	82	56	111	86	51
C	121	90	56	121	84	53
D	113	80	55	110	86	52
E	124	90	54	121	84	51
F	108	90	54	111	79	51

18 514 * average weight per egg without eggshell
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515
 516 Table 5. Levels of dioxins, non-ortho and mono-ortho PCBs in the different feeds used in studies II
 517 and III, as determined by GC/HRMS (mean of n=5, upperbound levels, lowerbound between brackets
 518 where different from upperbound).

519

Feed	Dioxins (ng TEQ/kg)	no-PCBs (ng TEQ/kg)	mo-PCBs (ng TEQ/kg)	total TEQ (ng TEQ/kg)
E	0.73	0.43	0.27	1.41
E + 10% sand	0.65	0.39	0.25	1.28
A + 10% soil A	0.44 (0.40)	0.10	0.05 (0.04)	0.64 (0.53)
A + 10% soil B	0.35 (0.19)	0.11	0.08 (0.07)	0.54 (0.37)
E + Exal H	0.87	0.53	0.33	1.73
E + MycoAd Z	0.93	0.55	0.34	1.81
E + Klinofeed	0.84	0.51	0.34	1.70

520

521 Table 6. Levels of dioxins, non-ortho, mono-ortho and indicator PCBs in the eggs and abdominal fat
 522 of chickens exposed in the absence or presence of mycotoxin binders. Samples collected at day 32
 523 were pooled from 5 hens or eggs and analyzed in single.

524

Feed	Dioxins (pg TEQ/g)	no-PCBs (pg TEQ/g)	mo-PCBs (pg TEQ/g)	total TEQ (pg TEQ/g)	Indicator PCBs ng/g fat
Eggs					
Feed E	7.4	5.3	3.1	15.8	117
E + Exal H	7.4	4.7	2.9	15.0	109
E + MycoAd Z	6.2	4.3	2.6	13.1	97
E + Klinofeed	6.8	4.3	2.6	13.7	100
Abdominal fat					
Feed E	6.2	4.5	3.0	13.7	84
E + Exal H	5.7	4.3	2.9	12.9	89
E + MycoAd Z	5.4	4.0	2.6	11.9	81
E + Klinofeed	4.8	3.4	2.3	10.4	71

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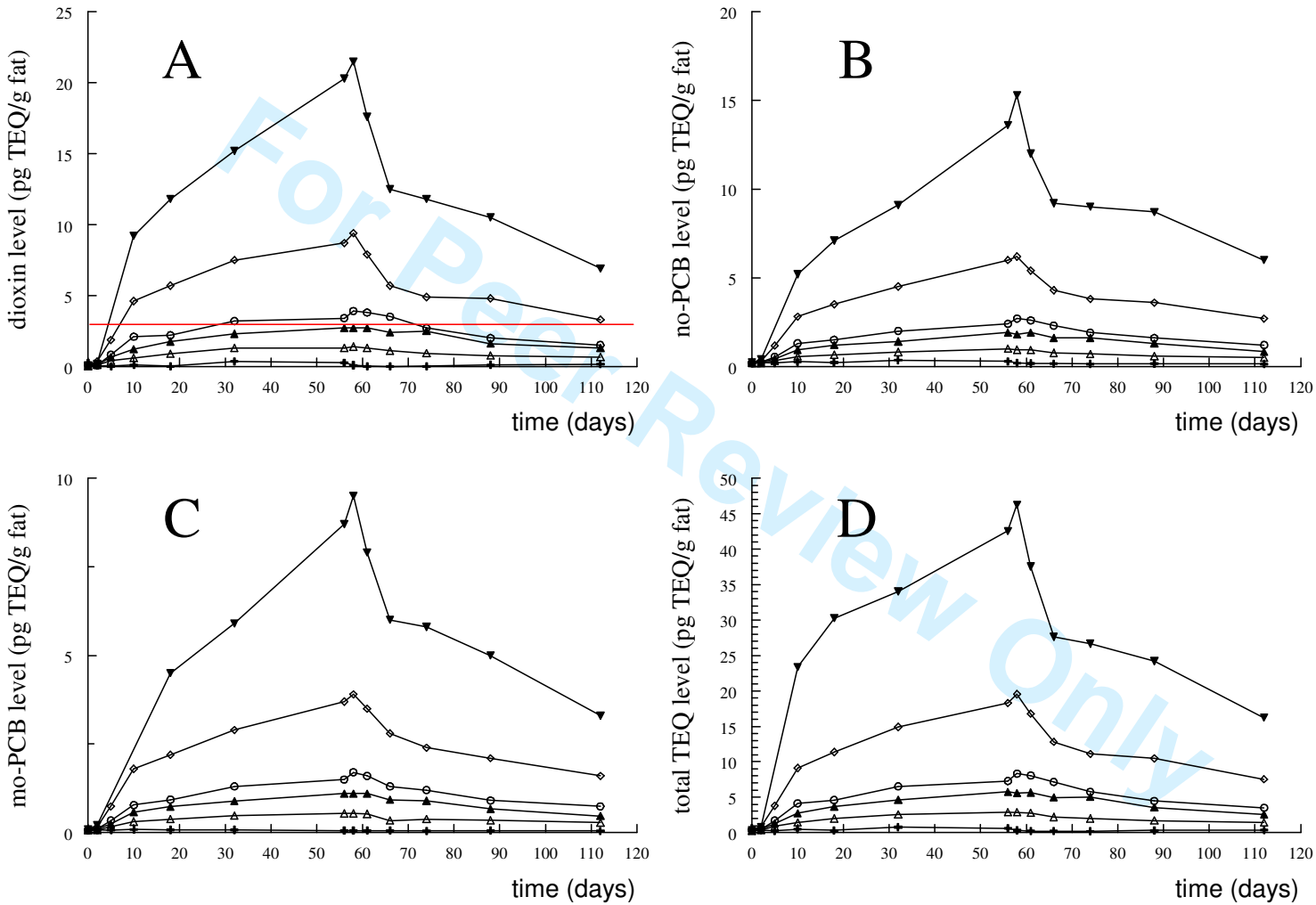


Figure 1. Levels of dioxins (A), non-ortho PCBs (B), mono-ortho PCBs (C) and total TEQ (D) in egg fat from hens fed with feeds containing 0.04 (A, +), 0.34 (B, Δ , open triangle), 0.58 (C, \blacktriangle , closed triangle), 0.76 (D, \circ , open circle), 1.85 (E, \diamond , open diamond) and 3.95 (F, \blacktriangledown , reversed triangles) ng TEQ/kg of dioxins and PCBs. Results from single analysis.

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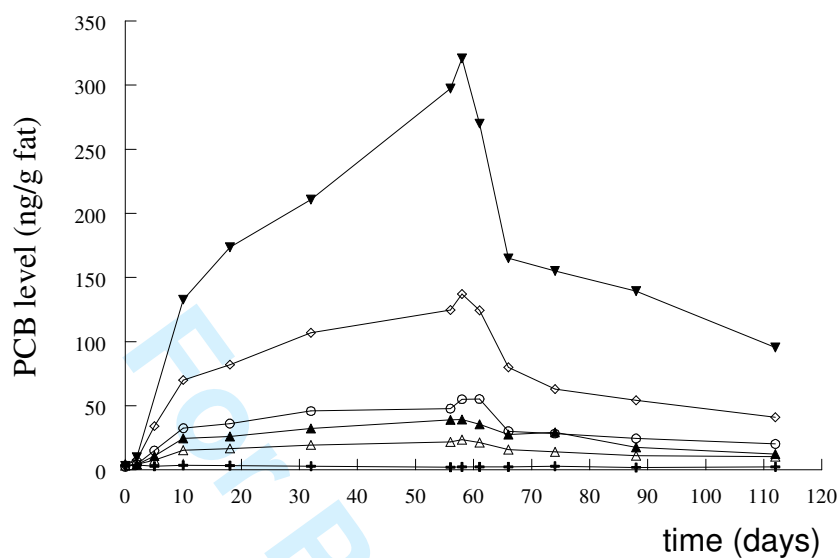


Figure 2. Levels of indicator PCBs in egg fat from hens fed with feeds containing 0.2 (A, +), 2.3 (B, Δ, open triangle), 4.3 (C, ▲, closed triangle), 6.0 (D, ○, open circle), 14.2 (E, ◇, open diamond) and 31.7 (F, ▼, reversed triangles) µg/kg feed of indicator PCBs. Results from single analysis.

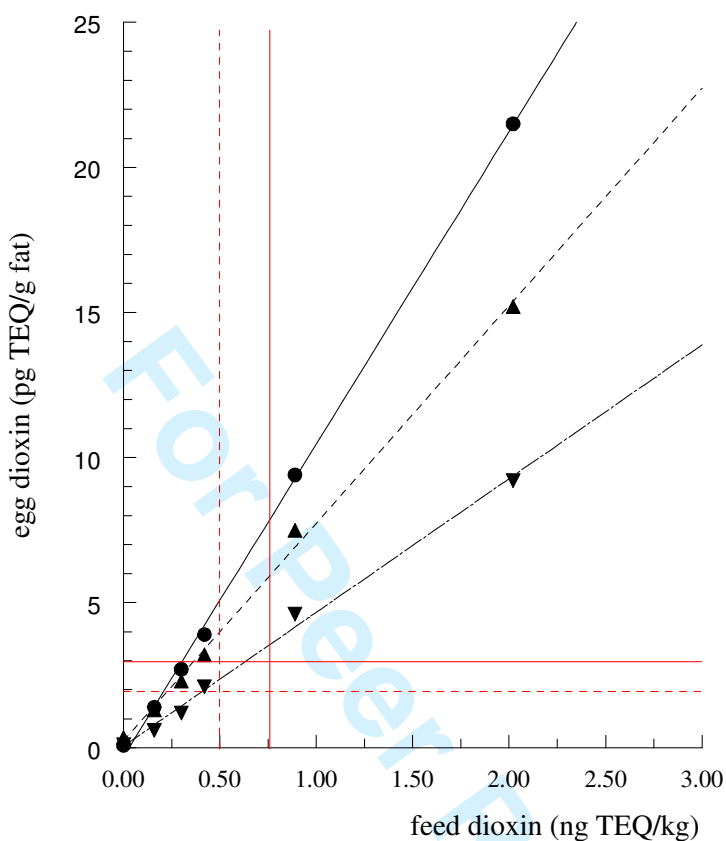


Figure 3. Relation between dioxin levels in feed and egg fat after 10 (▼, reversed triangles), 32 (▲, closed triangle) and 58 (●, closed circle) days of feeding contaminated feed. Solid lines represent the current limits in feed and eggs, dashed lines the action limits in the EU.

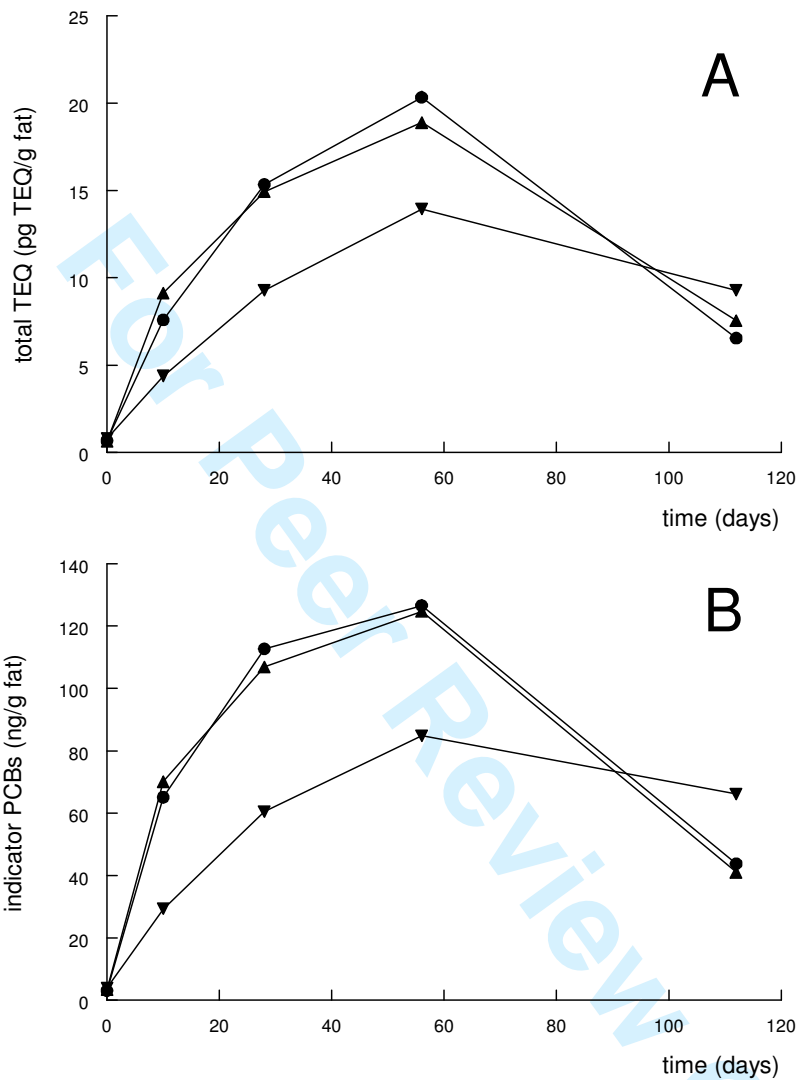


Figure 4. Levels of dioxins and dioxin-like PCBs (A), and indicator PCBs (B) in egg (▲, closed triangle), abdominal (▼, reversed triangles) and ovary fat (●, closed circle) from chickens fed with feed E. Results from single analysis.

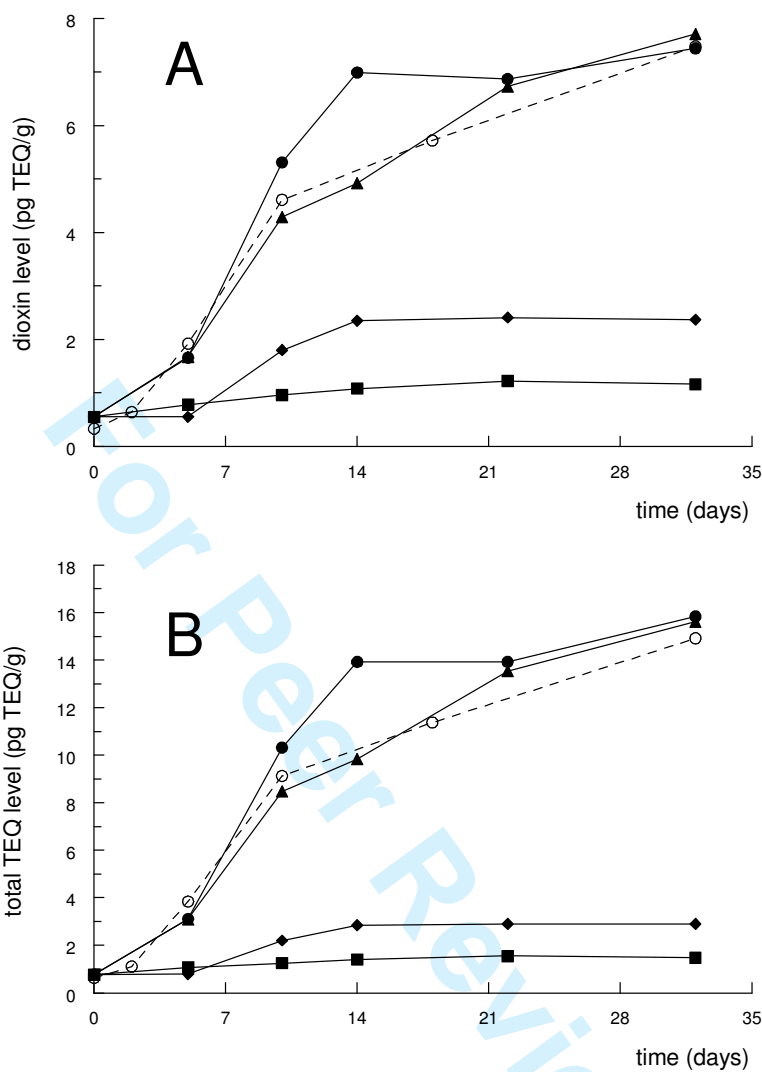


Figure 5. Levels of dioxins (A) and total TEQ (B) in egg fat from hens fed with feed E (●, closed circle), feed E mixed with 10% sand (▲, closed triangle), and blank feed A mixed with 10% soil A (◆, closed diamond) or soil B (■, closed square). For comparison the results obtained with feed E in the first study are included (○, open circle). Results from single analysis.