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Carry-over of dioxins and PCBs from feed and soil to eggs at low contamination levels

Influence of binders on the carry-over from feed to eggs

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Summary

In order to study the relationship between dioxin and PCB levels in feed and eggs, laying hens were fed with compound feed containing six different levels of dioxins, dioxin-like PCBs and indicator PCBs for a period of 56 days. This was followed by a period of another 56 days on clean feed. Dioxin levels varied from background to about three times the current tolerance limit of 0.75 ng TEQ/kg. At the higher dose levels a rapid increase was observed in the egg levels, followed by a more gradual increase. Steady state was not yet obtained after 56 days of exposure. Exposure to a feed containing 2.0 ng TEQ/kg resulted in a rapid increase in the levels in eggs with a maximum of 20 pg TEQ/g fat, which decreased to levels around 7 pg TEQ/g after 56 days on clean feed. Exposure to feed at 1.0 ng TEQ/kg resulted in egg levels above the EU-limit of 3 pg TEQ/g fat within 10 days, with a maximum level around 9 pg TEQ/g after 56 days. The feed containing 0.4 ng TEQ/kg resulted in egg levels just above the limit. There was a clear linear relationship between the dioxin levels in eggs and feed.

Dioxin-like and indicator PCBs followed a very similar pattern as dioxins. Exposure to the highest indicator PCB level of 32 µg/kg feed resulted in egg levels of 300 ng/g fat.

Most dioxin congeners showed a very similar carry-over rate with the exception of the hepta and especially the octa congeners, where the carry-over was low. Dioxin-like PCBs showed a similar or even better carry-over as the lower chlorinated dioxins. Most of the indicator PCBs showed a similar good carry-over with the exception of PCBs 52 and 101.

Exposure through contaminated soil, mixed at 10% into feed, resulted in similar carry-over as feed. A number of different so-called binders, mixed at 0.5% into the feed, had no effect on the carry-over of dioxins and PCBs from the feed to the egg.

Data were successfully modelled in order to predict the levels after different exposure scenario's. Overall, these data show that exposure to dioxins and dioxin-like PCBs rapidly results in increased levels in eggs and that the current EU limit for feed cannot guarantee egg levels below the EU-limit. Therefore, dioxin levels in chicken feed should be as low as possible and the current limit for dioxins in feed should be further reduced, at least in the case of chicken feeds. More in general, it is important to further harmonize the tolerance limits for feed and edible products like meat, eggs and milk. Information about the carry-over of contaminants in farm animals is essential for this purpose.

Samenvatting

Om het verband te bestuderen tussen dioxine- en PCB-gehaltes in voer van kippen en hun eieren, werden 6 groepen legkippen gedurende 56 dagen gevoerd met voeders met verschillende gehaltes aan dioxines, dioxine-achtige PCBs en indicator PCBs. Vervolgens kregen de kippen gedurende 56 dagen schoon voer. Dioxinegehaltes in het voer varieerden tussen een laag achtergrondniveau en bijna drie keer de huidige dioxinenorm van 0,75 ng TEQ/kg. Bij de hogere doseringen werd een snelle toename in de dioxinegehaltes in eieren waargenomen, gevolgd door een periode met een meer geleidelijke toename. Steady-state condities werden echter niet bereikt binnen de proefperiode van 56 dagen. Blootstelling aan een voer met 2 ng TEQ/kg resulteerde in eieren met een maximum gehalte van 20 pg TEQ/g vet, hetgeen daarna weer afnam in de periode op schoon voer naar gehaltes rond 7 pg TEQ/g vet. Zo resulteerde blootstelling aan voer van 1 ng TEQ/kg, dus iets boven de norm, binnen 10 dagen in gehaltes boven de dioxine-norm voor eieren van 3 pg TEQ/g vet. Het hoogste gehalte in deze groep was 9 pg TEQ/g vet. Zelfs het voer van 0,4 ng TEQ/kg resulteerde uiteindelijk in eieren met dioxine-gehaltes boven de norm. Er was een lineair verband tussen de gehaltes in voer en eieren.

Het verloop in de gehaltes van dioxine-achtige en indicator-PCBs in de eieren was vergelijkbaar met dat van de dioxines. Het hoogste indicator-PCB gehalte van 32 µg/kg voer resulteerde in een gehalte in eieren van 300 ng/g vet.

De meeste dioxine-congeneren vertoonden een vergelijkbare overdracht van voer naar ei, met uitzondering van de hepta en vooral de octa-congeneren, die een lage carry-over lieten zien. De dioxine-achtige PCBs toonden een vergelijkbare hoge overdracht als dioxines, evenals de meeste indicator-PCBs, met uitzondering van PCBs 52 en 101.

Blootstelling aan verontreinigde grond, gemengd door het voer (10%), leidde tot een vergelijkbare goede overdracht als bij de voeders. Toevoeging van een aantal zogenaamde binders aan verontreinigd voer leidde niet tot een lagere overdracht van dioxines en PCBs.

Op basis van de data en fysiologische gegevens over legkippen, werd een PB-PK model gemaakt, waarmee de gevolgen van een dioxinebesmetting via voer of grond voor de gehaltes in eieren op basis van blootstellingniveau en tijdsduur kunnen worden voorspeld.

De huidige studie laat zien dat verhoogde blootstelling van legkippen aan dioxines en PCBs leidt tot een snelle toename van de gehaltes in eieren. De huidige EU-normen voor dioxines in voer kunnen niet garanderen dat de gehaltes in eieren beneden de norm blijven. Derhalve moet er gestreefd worden naar zo laag mogelijke gehaltes van dioxines in voeders en zouden de huidige limieten voor dioxines in voeders, in elk geval voor kippen, verlaagd moeten worden. Het is daarnaast van groot belang om te streven naar een betere afstemming van dergelijke normen in voer en eindproduct, waarbij informatie over overdracht van contaminanten bij landbouwhuisdieren van essentieel belang is.

1 INTRODUCTION

1.1 Properties of dioxins and PCBs

Incidents with PCBs and dioxins (Figure 1) have shown that these compounds pose a major threat to edible products derived from food producing animals. Dioxins are produced as by-products in the synthesis of certain chemicals, like pentachlorophenol and 2,4,5-trichlorophenoxyacetic acid, and during incineration of waste. These compounds bind to the so-called Ah-receptor present in mammalian cells, thus resulting in the transcription of a large number of genes. In laboratory animals exposure results in tumours in the liver, and at even lower levels in effects on the immune and reproductive systems, as well as impaired learning. Based on these studies the World Health Organisation (WHO) and Scientific Committee on Food (SCF) have set very low exposure limits of respectively 1-4 pg TEQ/kg bw/day (WHO 2000) and 14 pg TEQ/kg bw/week (SCF 2001). These limits include the 17 toxic dioxins as well as 12 so-called planar PCBs which have similar properties to dioxins. Several studies have subsequently shown that at present, the exposure of part of the populations in Western countries exceeds these limits. Therefore, the EU has developed a strategy for further reducing the exposure. This includes the establishment of residue limits for food products, being 1 pg TEQ/g fat for pork, 2 pg TEQ/g fat for poultry, and 3 pg TEQ/g fat for beef, milk and eggs (EC 2001). In addition limits have been set for feed (0.75 ng TEQ/kg) and feed ingredients (0.75-6 ng/kg TEQ) (EC 2002). TEQ (Toxic Equivalents) refers to the use of the so-called TEQ principle based on the fact that the concentrations of the different congeners are multiplied by a Toxic Equivalency Factor (TEF), expressing their relative toxicity to the most toxic congener 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Berg *et al* 1998).

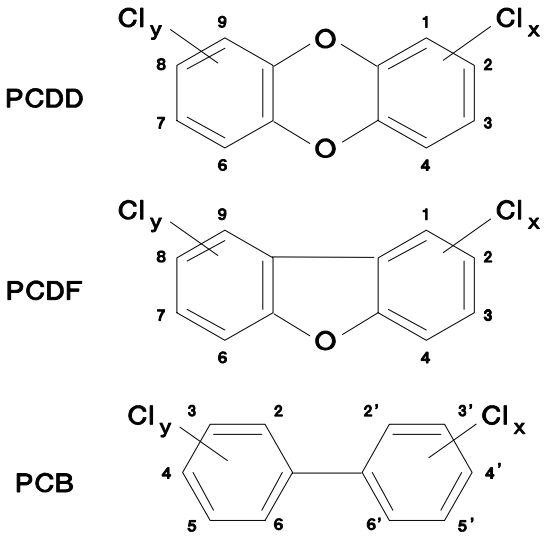

Figure 1. Structures of dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and polychlorobifenyls (PCBs).

PCBs are a group of 209 different congeners, which have been produced as technical mixtures (like Arochlor 1254) and have been used in large amounts as e.g. heat transfer fluids, hydraulic lubricants and dielectric fluids for capacitors and transformers. Twelve of these congeners have a planar structure and have similar properties to dioxins. At this stage planar dioxin-like PCBs are included in the

exposure limits of WHO and SCF but not yet in the EU food and feed limits. Based on more detailed information on current levels of these PCBs, the EU limits will be adapted in the near future. Other non-dioxin-like PCBs have been shown to affect brain development (Schmidt 1999) and appear to be responsible for the tumour promotion effects of these mixtures (Plas *et al* 2001). In addition metabolites of non-dioxin-like PCBs interfere with the homeostasis of vitamin A and thyroid hormones (Safe 1994). In practice, the consumer will be exposed to a mixture of both dioxins, dioxin-like PCBs and other PCBs and the toxicology of these mixtures is even more complex. As a result, no exposure limits for non-dioxin-like PCBs have been established thus far, contrary to limits for PCBs in food. In order to avoid difficulties in analysing all 209 congeners, it is customary to analyse only seven so-called indicator PCBs that represent the different technical mixtures. These include PCBs 28, 53, 101, 138, 153 and 180 but also PCB 118, which is a dioxin-like mono-ortho PCB.

1.2 Dioxins in eggs from organic farms in the Netherlands

Since the dioxin crisis in 1999 in feed and food in Belgium, RIKILT has been involved in a number of monitoring programmes aiming at the early detection of contaminated feed and food ingredients and the discovery of possible new sources of dioxins. In one of these programmes, at least 400 animal feeds and feed ingredients are screened annually with the CALUX-bioassay to select possibly positive samples. Suspected samples are further investigated by GC/MS, as well as 5-10% of the negative samples. In general samples showing a positive response are fish oil and fish meal, and clay minerals. In 2001 a new programme was started, aiming at investigating products of animal origin, like meat and eggs. Over 300 samples, primarily originating from the National Plan for controlling products of animal origin, were screened in the CALUX-assay and suspected samples were further investigated by GC/MS. This part of the programme resulted in an estimation of the fraction of samples non-compliant with the legal limits. In addition samples collected in each quarter of the year were pooled per type of sample and analysed by GC/MS, in order to assess the average background level of dioxins and co-planar PCBs in these products. Results are presented on the Internet site: www.rikilt.wur.nl/dioxinen. The approach was continued in 2002.

In 2001 this strategy resulted in the discovery that an egg obtained from a farm with free-range hens, contained dioxin levels above the then existing Dutch limit of 5 pg TEQ/g fat (Traag et al. 2002). The initial observation was followed by a focussed action on these types of eggs and showed a clear elevation in dioxin levels from free-range hens, in particular in eggs from farms producing eggs according to organic standards. The Dutch Food Inspection Service (KvW) pursued on these observations by sampling eggs from most of the organic farms in the Netherlands during the fall of 2001. A total number of 68 egg samples were screened with the CALUX bioassay and 9 suspected samples were found. Further investigation by GC/MS resulted in six samples that exceeded the current EU limit for eggs of 3 pg TEQ/g (since July 2003), and three that exceeded the existing limit at that time of 5 pg TEQ/g (De Vries, 2002). In addition some of the samples contained high levels of dioxin-like non-ortho PCBs, with a highest total TEQ level of 15 pg TEQ/g fat.

Follow-up studies were performed during the wintertime at the first farm, and these strongly suggested that the source of the contamination was not the feed, but the outdoor environment. Dioxin levels decreased when hens were kept inside, although not to the levels normally observed in eggs from hens kept in batteries.

In July 2003, new EU limits for dioxins in food and feed became official, including limits for eggs and feed, being respectively 3 pg TEQ/g fat and 0.75 ng TEQ/kg feed. However, eggs from free-ranging hens were excluded from these limits until 1-1-2004. At the same time, studies were started to investigate the carry-over rates of dioxins and PCBs from feed and soil to eggs at relatively low levels,

and to investigate possible ways for lowering the exposure and carry-over rates. In the autumn of 2003 ASG, in cooperation with RIKILT, performed a study (Brandsma *et al.* 2004). A large number of farms with organic eggs were visited and investigated for possible sources of dioxins and factors that may contribute to the exposure of chickens to dioxins. This study shows that most eggs would comply with the current limit of 3 pg TEQ/g fat, but that 13% of the eggs, coming from 26% of the farms would not be able to meet this limit.

This report describes the results, being the relationship between dioxin and PCB levels in feed and eggs, and the effects of contaminated soil and of binders on the carry-over. A possible problem is that the experimentally determined carry-over as such cannot be extrapolated beyond the applied experimental protocol. This feature hampers the application of the experimental findings to all kinds of situations, which may be encountered in practical situations such as an accidental high-peak exposure. This limitation may be overcome by combining an experimental approach with mechanistically based mathematical modelling. In this case the kinetics, i.e. the combined process of uptake, distribution and elimination resulting in the accumulation of dioxins in eggs of the laying hen, are described in terms of a mathematical model, with model parameters estimated on the basis of the aforementioned experimental results. The results of the studies were used to develop such a mathematical model based on the physiology of the laying hen, that can now be used for predicting the levels in eggs resulting from a certain feed contamination, and the effect of certain variables on this relationship.

1.3 Data from other studies

Chickens have been involved in some major feed incidents with dioxins and PCBs, leading to clear effects like decreased hatching of eggs and symptoms called chicken edema disease (reviewed by Hoogenboom, 2004a). With respect to the relatively low exposure and contamination levels, which are observed in the eggs of free-range chickens, a limited amount of information is available. In the early nineties German investigators observed elevated levels of dioxins in eggs from free-range chickens (EAE Technology, 1999). Two different studies showed ranges of 0.4-11.4 and 0.5-22.8 pg I-TEQ/g as compared to 0.6-2.3 and 0.2-6.0 for eggs from battery-housed chickens. Shuler *et al.* (1997) performed a field survey on chickens from five farms (A-E) in Switzerland. Soil samples, taken from a depth of 0-10 cm were analysed and shown to contain 11 (A), 13 (B), 1.8 (C), 1.3 (D) and 1.4 (E) ng I-TEQ/kg dry weight. Corresponding levels in single eggs were respectively 3.1 and 6.1 (A), 19 and 12 (B), 4.6 and 2.3 (C), 6.1 (D) and 3.5 (E) ng I-TEQ/kg fat. The relatively low levels in eggs from farm A were hypothesised to be due to the high density of chickens on this particular farm as compared to the other farms, resulting in the disappearance of soil organisms. More recent results from a Swiss monitoring study on samples collected in 1999 and 2000, confirm that eggs often contain elevated dioxin levels (average 2.9, maximum 13 pg TEQ/g fat), with 6 out of 18 eggs with levels higher than 3 pg TEQ/g fat (Schmid et al. 2002). According to the authors, part of the elevated levels might be explained by the use of contaminated kaolinic clay in feed. Harnly *et al.* (2000) described a more controlled study in a contaminated area close to a pentachlorophenol wood treatment facility in Oroville, California, USA. Soil concentrations of around 6 ng I-TEQ/kg (range 1.5-46) resulted in egg levels of 20-50 ng I-TEQ/kg fat (range 0.8-140). The fraction of eggs exceeding 10 ng I-TEQ/kg was around 70-90%. Modelling of the data indicated that for confined hens, a soil level of 2.7 ng TEQ/kg was required to result in an egg level of 10 ng I-TEQ/kg fat. However, a soil level of only 0.4 ng TEQ/kg was required for hens that had a larger area to forage. Again, this suggests a role for worms and insects, since their presence is more likely at lower densities. Air et al. (2002) reported dioxin levels around 15 pg TEQ/g fat (range 1-56) in eggs from hens reared on allotments in Newcastle upon Tyne. The contamination resulted from the use of incinerator ash for footpaths. Twenty months after removal of the ash, levels

decreased to an average of 19 pg TEQ/g (range 2-26) in eggs from chickens that were already contaminated. Levels in eggs from chickens newly introduced to the allotments were on average 8 pg TEQ/g (range 0-31). The pattern in the contaminated eggs indicated that the complete removal of the ash was not achieved. Rose et al. (2003), in studying the possible effects of the FMD animal pyres, observed in a small number of farms dioxin levels in eggs up to 7 pg TEQ/g (range 1-7) with total TEQ levels up to 34 pg TEQ/g (range 1-34). The eggs were not produced for commercial activities. The authors concluded that the pyres did not contribute to the contamination, also based on comparison with previous data showing similar levels in eggs sampled between 1994 and 1996 (FSA 2000). Average levels in hen eggs were 6.3 pg TEQ/g fat (range 1-22). Recently the Food Standards Agency of Ireland (FSAI, 2004) investigated dioxins and PCBs in battery, free-range, barn and organic eggs. Average dioxin levels were respectively 0.29, 0.32, 0.27 and 1.43 pg TEQ/g fat, average total TEQ levels respectively 0.65, 0.79, 0.57 and 2.73 pg TEQ/g fat. Only four samples were analysed for the organic eggs, but one showed a dioxin level above the EU-limit of 3, with a total TEQ level of 6.6 pg TEQ/g. These data clearly demonstrate that the problem is more widespread and that even higher levels might be expected at contaminated sites.

Only few studies are available on the possible role of worms in the carry-over of dioxins and PCBs. Wågman *et al* (2001) showed that worms fed contaminated food are able to accumulate in particular the more chlorinated PCBs, mainly due to a slower elimination of these congeners. Matscheko *et al* (2002) studies levels in soil and worms and observed that the accumulation of certain PCBs from soil to worms is higher than that of 2,3,7,8-substituted dioxins. In general the ratio's between worm lipids and soil organic matter were in the order of 1 to 20 for PCBs and 0.1 to 1 for dioxins, with higher accumulation of the tetra and penta chlorinated PCDD/Fs. Regarding the lipid content of worms (1-2%) and percentage of organic matter in soil (2-6%), levels of PCBs in worms on a wet weight base in general exceeded those in soil. For dioxins this seems to be less the case.

In general these studies confirm that very low soil levels already may result in high levels in eggs, which appear to be a very sensitive product.

2 MATERIALS AND METHODS

2.1 Preparation of feed

2.1.1 Preparation of feed for Study A

Feed was prepared with soy oil spiked with the different dioxin and PCB congeners. A blanc oil, obtained from Nutreco (Boxmeer, The Netherlands) was analysed by both CALUX and by GC/MS and shown to be low in dioxin-like compounds. Subsequently, a stock oil was prepared with about 7500 pg TEQ/g of dioxins and dioxin-like PCBs. An aliquot of 200-gram oil was mixed with the following PCB stock solutions:

- 7.5 ml PCB 105 100 ng/ml
- 15 ml PCB 118 100 ng/ml
- 1.2 ml PCB 156 100 ng/ml
- 7.5 ml PCB 28 100 ng/ml
- 15 ml PCB 52 100 ng/ml
- 30 ml PCB 101 100 ng/ml
- 30 ml PCB 138 100 ng/ml
- 30 ml PCB 153 100 ng/ml
- 15 ml PCB 180 100 ng/ml

The organic solvent (nonane) was removed under vacuum. Subsequently the following standard mixtures were added:

- 44 µl CIL EDF-4096 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 CIL EC-4986 non-ortho PCBs (10 µg/ml)
- 785 µl CIL EC-4987 mono-ortho PCBs (10 µg/ml)

The organic solvent was again removed under vacuum (1 hr, 63ºC). The 200 ml oil was first diluted to 1500 ml, and subsequently diluted for incorporation into the different feeds, as follows:

- 30 gram + 1470 gram blank oil (oil b used for feed B)
- 60 gram + 1440 gram blank oil (oil c used for feed C)
- 265 gram + 3237 gram blank oil (oil d used for feed D)
- 685 gram + 3882 gram blank oil (oil e used for feed E)
- 420 gram + 980 gram blank oil (oil f used for feed F)

Analysis of oil (b) by GC/MS showed a total TEQ content of 20 pg TEQ/g, with a contribution of 11 pg TEQ/g for dioxins, 5.3 pg TEQ/g for non-ortho PCBs and 3.7 pg TEQ/g for mono-ortho PCBs. The blank oil contained a total TEQ content of 0.4 pg TEQ/g fat (upperbound). Based on these results the Nutreco feed mill in Heyen, The Netherlands, prepared the feeds with 0.95% of oils (b) to (f), and the blank oil a in the following quantities: 290 and 650 kg blank feed (prepared in 2 batches), 110 kg of feeds B, C, D and F, and 400 kg of feed E.

Table 1 shows the ingredients of the feed, Topline extra.

Ingredient	Amount (%)
Corn	32.53
Wheat	19.61
Corn-byproducts	2.00
Maizeglutenfeed	3.52
Wheatglutenfeed	4.31
Soyabeans, 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
Sodiumchloride	0.16
Sodiumcarbonate	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
Total	100.00

2.1.2 Preparation of feed for Study B

Aim of this study was to assess the bioavailability of dioxins from contaminated soil. Therefore, feeds were prepared by mixing the blank feed with 10% of soil collected from two chicken farms with elevated dioxin and PCB levels in eggs. The soils were analysed and shown to contain dioxin levels of 1.94 ng TEQ/kg (soil A) and 5.88 ng TEQ/kg (soil B). Pooled egg samples collected at the same time at these farms were shown to contain dioxin levels of 1.0 and 7.3 pg TEQ/g fat. As a control an additional feed was prepared by mixing feed E with 10% of clean sand.

2.1.3 Preparation of feed for Study C

Aim of this study was to assess whether or not binders would influence the bioavailability of dioxins and PCBs in feed. For this study a number of different binders were obtained from the producers. These materials were claimed to prevent the absorption of mycotoxins and as such might be potential candidates for reducing the absorption of dioxins as well. The materials were first screened by CALUX and analysed by GC/MS, and shown not to contain dioxins and PCBs above the limits. The following feeds were prepared:

- feed E mixed with 0.5% Exal H
- feed E mixed with 0.5% MycoAd A-Z
- feed E mixed with 0.5% Klinofeed
- feed E mixed with 0.5% Humac

2.2 Animal studies

2.2.1 Study A

Hens (Bovans Gold line, age about 45 weeks at the start) were housed in a three-tier battery with 3-4 hens per cage. The different groups contained 12, 12, 12, 13, 26 and 13 animals respectively. After an adaptation period on blank feed for 1 week, hens were fed with the different feeds A to F for 56 days, followed by another 56 days on blank feed A. During the whole 112-day period eggs were collected and pooled per day per feeding group. On days 56 and 112, at least five hens from each group were slaughtered and abdominal fat, livers and the ovaries collected. The group fed with feed E was larger allowing the collection of tissues of another five animals on days 10 and 28.

2.2.2 Study B and C

Hens (ISA Brown Warren, age about 25 weeks at the start) were housed in a three-tier battery with 2-3 hens per cage. The seven groups of 5 animals followed an adaptation period of one week on clean feed, and then were fed with the different feeds for 32 days. During the whole 32-day period eggs were collected and pooled per day per feeding group. On day 32 the hens from each group were slaughtered and abdominal fat, livers and the ovaries collected.

2.3 Analysis of dioxins and dioxin-like PCBs

Levels of dioxins, non-ortho and mono-ortho PCBs were determined by high resolution GC/MS, basically as described by Tuinstra et al. (1994). Eggs and ovaries were freeze dried and subsequently fat was extracted using Accelerated Solvent Extraction (ASE). Abdominal fat was melted in an oven at 70 °C After extraction or sample pretreatment ^{13}C labelled dioxins and dioxin-like PCBs were added to the fat phase of the samples. Feed samples were extracted with ethylacetate or, when minerals or soil was added to the feed (study B and C), with toluene using ASE. Prior to extraction ^{13}C labeled dioxins and dioxin-like PCBs were added to the feed samples

Separation between dioxins and fat was carried out using gel permeation chromatography. The system consisted of an HPLC pump (Gilson, model 305, an autosampler (Gilson, model 231) equipped to inject 12,5 ml of sample solution, and a fraction collector (Gilson, model 202) adapted to collect 300 ml fractions using 500 ml glass collection flasks. The glass GPC column (Spectrum) (60 x 2.5 cm) was packed with Biobeads SX 3. After an additional clean up with activated Al_2O_3, separation between planar compounds (dioxins and non-ortho PCBs) and non-planar compounds like other PCBs was carried out with porous graphitised carbon. The alumina (basic) clean-up was performed with an automatic sample preparation system using solid phase extraction columns (ASPEC, Gilson). The columns were packed with 1 gram deactivated alumina (7% water) shortly before use. Porous graphitised carbon clean up was performed using a HPLC system consisting of an HPLC pump (Gilson model 205), a column switching device (Gilson, valvemate), a solvent switching device (Gilson, valvemate), an autosampler (Gilson model 231), equipped with a 5 ml loop and a fraction collector (Gilson model 202) adapted to collect 100 ml fractions. The column used was Hypercarb (100 X 4.6 mm) (Shandon). The final extract with planar compounds was concentrated to 10 µl and the extract containing the non-planar PCBs to 200 µl. Both extracts were analysed with gas chromato-graphy-high-resolution-mass spectrometry (HRGC-MS) (Autospec, Micromass). The mass spectrometric method to determine the tetra through octa dioxins is based on United States Environmental Protection Agency protocols. Included in the analysis is a standard QA programme e.g. determination of recovery of internal standards, accuracy of spiked samples and

blanks. Absolute levels were transfered to TEQ levels using the TEF values described by van den Berg *et al.* (1998).

2.4 Analysis of indicator PCBs

The seven indicator PCBs (28, 52, 101, 118, 138, 153 and 180) were analysed by GC/MS following on-line clean-up over a silica HPLC column using a large volume injector (LVI). In short, 200 mg of the homogenised fat sample is mixed with 800 µl of the internal standard solution of ^{13}C PCB 118 and PCB 198 (6.25 ng/ml). Subsequently, 50 µl is injected onto the silica column and after elution of the fat with hexane, the direction of the flow is switched to back-flush and the column is cleaned with 3 ml dichloromethane. The PCB containing fraction (approximately 1 ml) is directly transferred to the gas chromatograph, which is provided with an LVI. In the LVI the large amount of solvent was separated from the compounds of interest by an uncoated retention gap of 10 meters, which ends up in a retaining column of 1-meter coated with a non-polar phase. The temperature of the LVI, located in the oven of the GC, is slightly (80°C) above the boiling point of the mobile phase. Hexane is evaporated slowly and blown off via the Solvent Vapour Exit (SVE) while the PCB's are retained on the 1-meter retaining column. After a specific time the majority of the solvent was evaporated, the SVE closed and the temperature of the oven was raised in order to start chromatography. Detection was done by mass-spectrometry with a bench top system in Selected Ion Mode (SIM).

2.5 CALUX analysis

An aliquot of 0.5 g fat was mixed with hexane and extracted on acid silica as described previously. In each test series a blanc butter fat sample and butter fat samples containing 1, 2, 3 and 6 pg TEQ/g fat were included. The response obtained with the butter fat sample of 3 pg TEQ/g, containing 1.5 pg TEQ/g dioxins and 1.5 pg TEQ/g non-ortho PCBs, was used as the reference signal. Samples showing a lower response were declared negative, samples showing a higher response suspected.

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

2.6 Mathematic modelling

The transfer of total dioxin Toxic EQuivalents (TEQs) in laying hens, based on the physiology of laying hens and the physicochemical properties of dioxin-like substances was quantified by means of mathematical modelling. The mathematical model herein, which is described in detail in the accompanying Technical Information, was calibrated on the basis of the experimental results (time-course of the TEQ in eggs and abdominal fat at 6 different contamination levels) mentioned above.

3 RESULTS

3.1 Experimental

3.1.1 Study A

3.1.1.1. Dioxin and PCBs in feeds

Appendix 1 shows the levels of the individual dioxins and dioxin-like PCBs in the various feeds, appendix 2 the levels of indicator PCBs. Each feed was analysed 3 to 5 times, corresponding to the number of samples taken from the different bags. The low standard deviation and variation coefficient demonstrates the good homogeneity of the feeds. Table 2 shows the intended and analysed dioxin, PCB and total TEQ levels. The relative contribution of dioxins, non-ortho and mono-PCBs was as intended, being respectively 50, 30 en 20%. However, the overall levels were in general higher than intended, which cannot be explained by the background levels in the feed ingredients. Since a rather broad range of different concentrations in feed was used, this deviation had no consequences for the aim of the study. Indicator PCB levels were respectively 0.2, 2.3, 4.3, 6.0, 14.2 and 31.7 µg/kg feed.

Table 2. Intended and analysed levels of dioxins, non-ortho and mono-ortho PCB levels in the different feeds, expressed in ng TEQ/kg.

Feed	Dioxins		no-PCBs		mo-PCBs		Total	
	Intended	Analysed	Intended	Analysed	Intended	Analysed	Intended	Analysed
A	0	0.04	0	0.00	0	0.00	0	0.04
B	0.10	0.20	0.06	0.09	0.04	0.06	0.20	0.34
C	0.20	0.30	0.12	0.17	0.08	0.11	0.40	0.58
D	0.38	0.40	0.23	0.22	0.15	0.14	0.75	0.76
E	0.75	0.97	0.45	0.55	0.30	0.33	1.50	1.85
F	1.50	2.04	0.90	1.19	0.60	0.72	3.00	3.95

3.1.1.2. Levels in eggs

Table 3 shows the average feed consumption and the productivity of the hens, both during and after the exposure period. There was no effect of the exposure on these parameters. The exposure did not affect the average egg weight, but in all groups a slight decrease was observed after 3.5 weeks on clean feed, resulting in weights around 48-51 grams per egg. At present there is no explanation for this decrease, or possible consequences for the residue levels in the eggs.

Appendix 3 (A-G) presents the levels of dioxins and PCBs in eggs and tissues, Figure 2 the levels of dioxins (A), non-ortho PCBs (B), mono-ortho PCBs (C) and total TEQ (D) in eggs. These data show that even after 8 weeks on contaminated feed, levels in eggs continued to increase. After cessation of the exposure, there was initially a small increase in the residues, and than a rapid decline, which was followed by a slower decrease. A similar profile was observed previously in a study with much higher dioxin and PCB levels (Hoogenboom *et al.* 2002). The formation of a full-grown yolk requires about ten days, meaning that eggs laid during the first days on clean feed were formed and contaminated still during the exposure period. Redistribution of dioxins from abdominal fat to the general circulation and thus to eggs, may explain the slow decrease in residues during the latter part of

the study. Figure 2A also shows that the current limit for eggs of 3 pg TEQ/g fat is rapidly exceeded when exposed to feed containing dioxins at the current feed limit of 0.75 ng TEQ/kg.

Table 3. Feed consumption and productivity of the hens during and after the exposure period

Feed	Exposure			Clean feed		
	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

* average weight per egg without eggshell

Appendix 4 (A-G) and Figure 3 show the levels of indicator PCBs in egg fat during the exposure and depletion period. In general the pattern was very similar to that of the dioxins and dioxin-like PCBs. The maximum level obtained at 56 days of exposure via the highest contaminated feed was around 325 ng/g fat. Based on 4% fat in the feed, the feeds E and F would contain respectively 350 and 800 ng/g fat, thereby exceeding the Belgium limit for feed of 200 ng/g. Only eggs from hens fed feed F actually exceeded the Belgium limit for indicator PCBs of 200 ng/g fat, although it cannot be excluded that after longer exposure also eggs from hens fed with feed E might approach the limit.

Figure 4 shows the relationship between the dioxin levels in feed and eggs after different periods of exposure. There is a clear linear relationship between the feed levels and the levels in eggs at the different exposure times. This Figure also clearly demonstrates that even after a short period on feed contaminated at the current EU-limit of 0.75 ng TEQ/kg, the limit for eggs is exceeded.

3.1.2 Study B

Appendix 5 shows the levels of dioxins and PCBs in feed E, feed E mixed with 10% sand and the two blank feeds mixed with 10% of the soils sampled at two chicken farms. Appendix 6 and 7 show the dioxin and PCB levels in the eggs from the hens fed with these feeds, appendix 8 the dioxin and dioxin-like PCB levels in the abdominal fat. As shown in Figure 5, time-related levels in the eggs from the hens fed with feed E and E mixed with 10% sand were very similar to the results obtained in study A (Figure 5).

The dilution of contaminated feed with sand did not clearly influence the levels observed in the eggs and abdominal fat. The results obtained with the blank feed mixed with 10% soil A or soil B reflected the differences in the soil and feed levels. In this case, a plateau level appeared to be reached within a short period of exposure. Maximum dioxin and total TEQ levels measured in eggs from hens exposed to soil A were 2.4 and 2.9 pg TEQ/g fat. In the case of soil B, highest levels of 1.2 and 1.6 pg TEQ/g were observed. Again, levels in abdominal fat were lower than those for egg fat. For comparison, levels determined in pooled egg samples from the farms where the soil was sampled were 7.3 and 1.0 pg TEQ/g fat (Appendix 9).

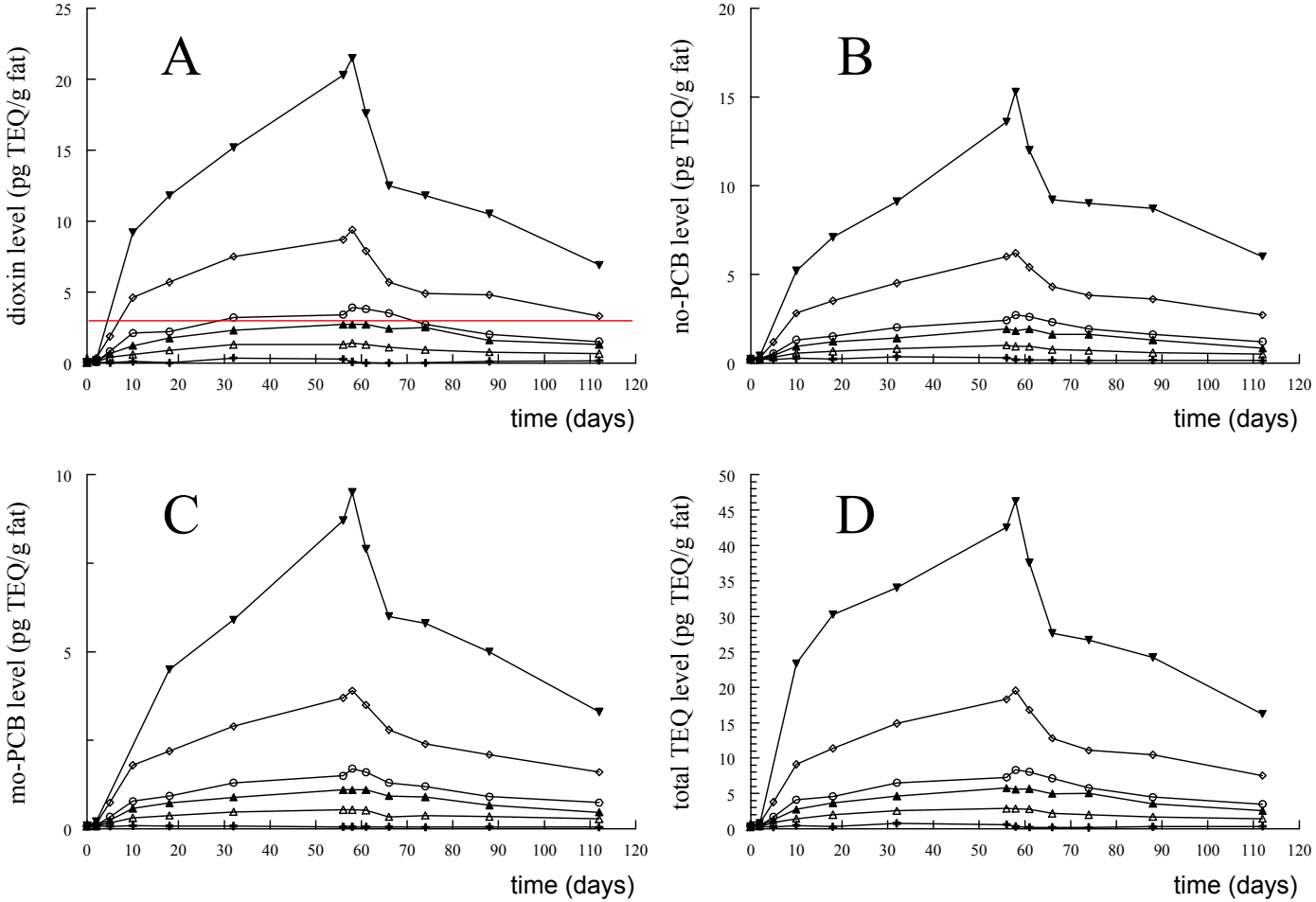

Figure 2. Levels of dioxins (A), non-ortho PCBs (B), mono-ortho PCBs (C) and total TEQ (D) in egg fat from hens fed with feeds containing 0.04 (A, +), 0.34 (B, Δ), 0.58 (C, ▲), 0.76 (D, ○), 1.85 (E, ◊) and 3.95 (F, ▼) ng TEQ/kg of dioxins and PCBs.

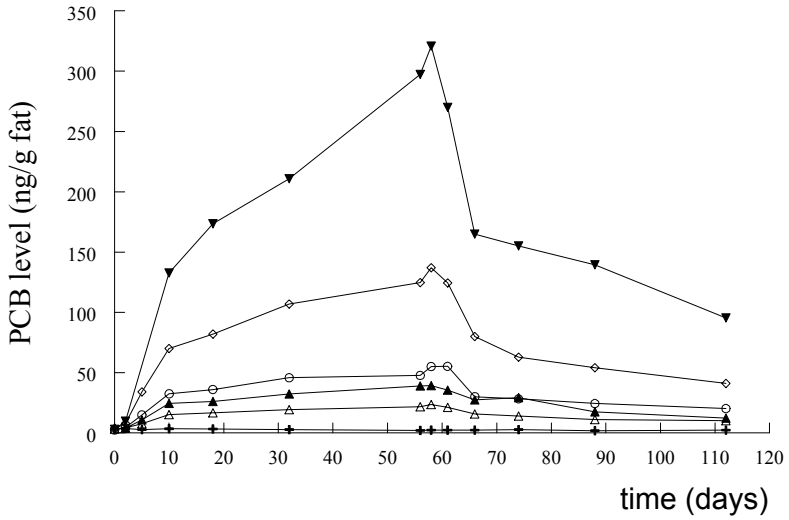

Figure 3. Levels of indicator PCBs in egg fat from hens fed with feeds containing 0.2 (A, +), 2.3 (B, Δ), 4.3 (C, ▲), 6.0 (D, ○), 14.2 (E, ◊) and 31.7 (F, ▼) µg/kg feed of indicator PCBs.

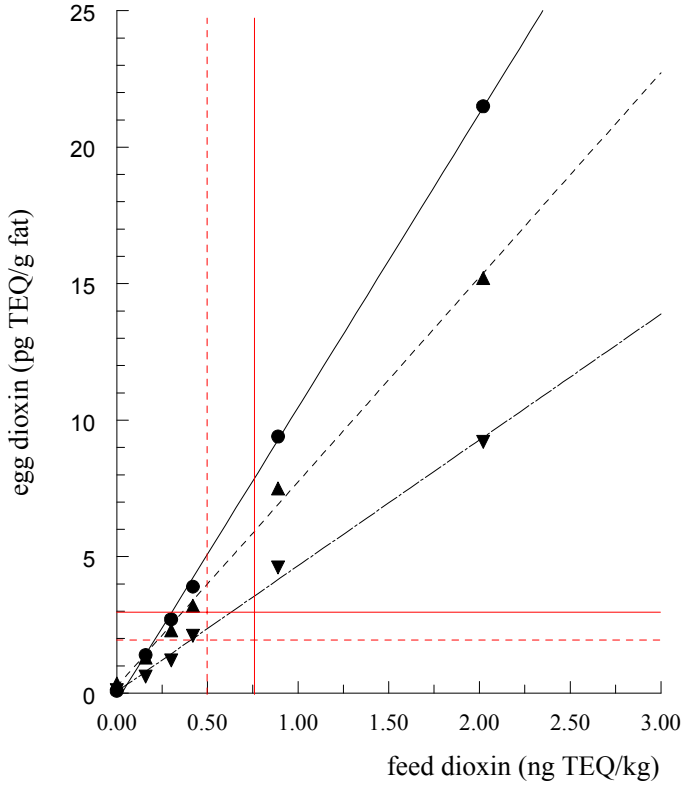

Figure 4. Relation between dioxin levels in feed and egg fat after 10 (▼), 32 (▲) and 58 (●) days of feeding contaminated feed. Solid lines represent the current limits in feed and eggs, dashed lines the action limits in the EU.

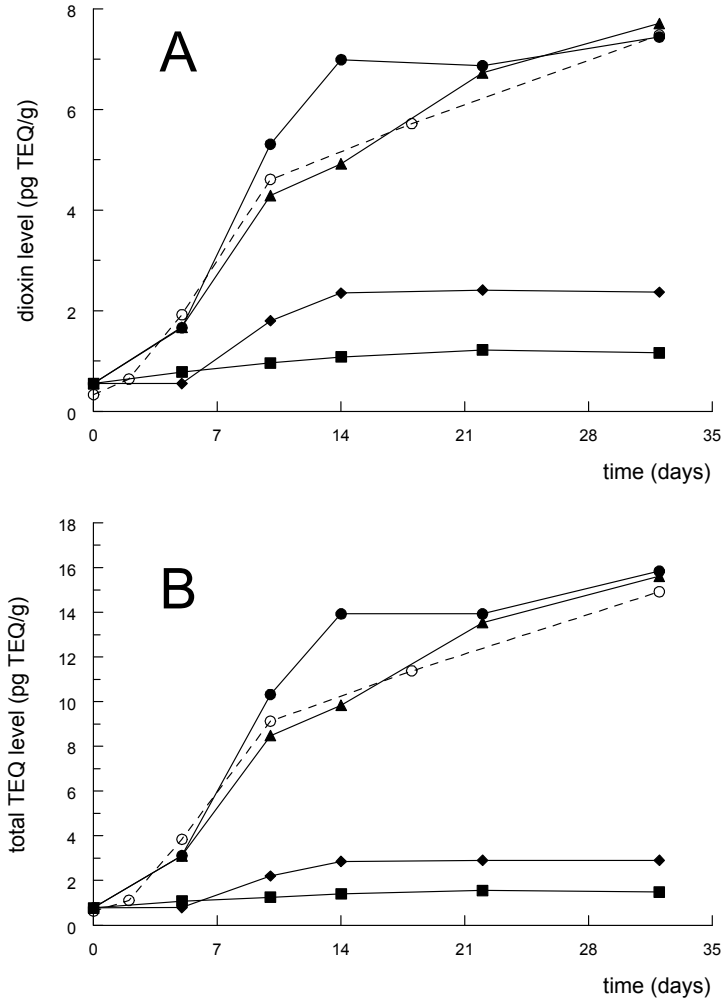

Figure 5. Levels of dioxins (A) and total TEQ (B) in egg fat from hens fed with feed 750 (●), feed 750 mixed with 10% sand (▲), and blank feed mixed with 10% soil A (♦) or soil B (■). For comparison the results obtained with feed 750 in the first study are included (○, open circle).

3.1.3 Study C

Appendix 10 shows the levels of dioxins and PCBs in the four feeds supplemented with the different binders. Appendix 11 and 12 show the dioxin and PCB levels in egg and abdominal fat of the hens fed with these feeds as compared to the feed E. These data show that the binder had little or no effect on the residue levels of dioxins and PCBs in the eggs

3.2 Mathematical modelling

3.2.1 Study A

The outcome of the calibration of the mathematical model is shown in Figure 6. As can be seen, the model could describe the accumulation of dioxins in hens and hen's eggs quite well. The model indicates that the half-life time of dioxins in these laying hens is about 1.7 months or 7 weeks. This means that continuous administration of a certain dose level will result in steady state, i.e. constant concentration levels, after about 8 months. In non-laying hens the half-life was calculated to be 14 months, indicating once again that excretion by egg yolk fat is the main route of elimination. In the range of the administered dose levels the model predicts a linear dose-response relation (dose levels in feed-concentration levels in eggs, see also Figure 3), i.e. the model predicts a constant transfer factor between the dose levels in feed and the resulting steady state levels in egg yolk fat, or:

"steady state concentration" in egg yolk fat = 17.6 x concentration in feed

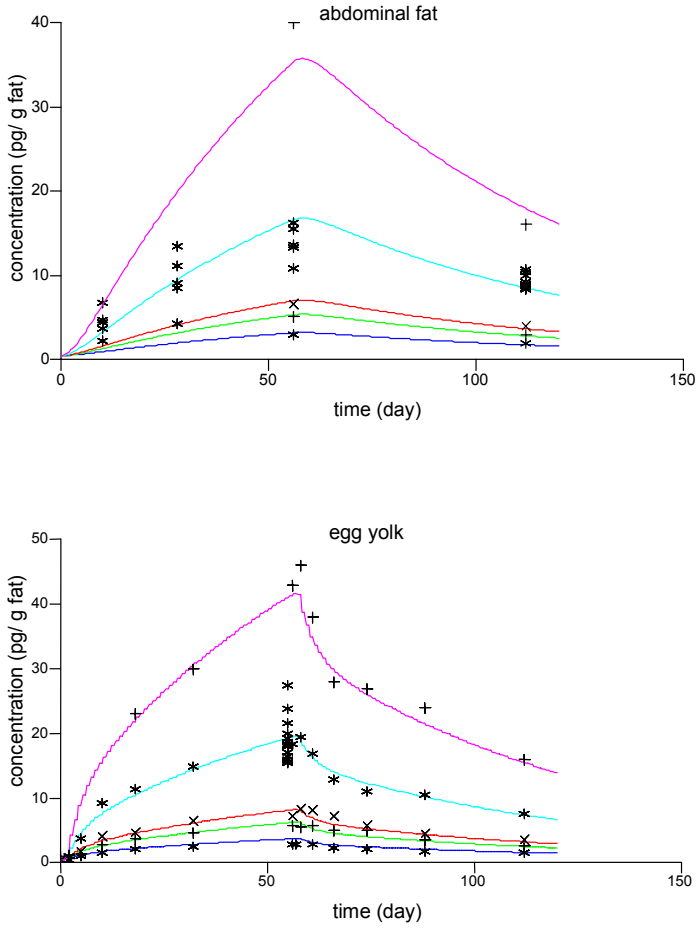

Figure 6. Concentration in abdominal fat (upper panel) and egg yolk (lower panel). The lines show the calculated concentration-time curves, the symbols denote experimental data. Five contamination levels: 0.34 ng/kg feed (B, lower line, *), 0.58 ng/kg feed (C, next lower line, +), 0.76 ng/kg feed (D, middle line, x), 1.85 ng/kg feed (E, next upper line, *) and 3.95 ng/kg feed (F, upper line, +).

with the steady state concentrations in egg yolk fat and feed in respectively pg TEQ/g fat and ng TEQ/kg feed. Applying this equation to the experimentally used feed levels of 0.34, 0.58, 0.76, 1.85 and 3.95 ng TEQ/kg feed leads to calculated steady state levels in eggs of 6, 10, 13, 33 and 70 pg TEQ/g fat. On the average these steady state levels are about two times higher than the levels observed after 56 days of exposure (3, 6, 7, 19 and 43 pg TEQ/g egg yolk fat). Similarly, a feed level of 0.76 ng TEQ/kg leads to a calculated steady state level of 13 pg TEQ/g egg yolk fat. This level clearly exceeds the current EU limit of 3 pg TEQ/g egg yolk fat. Further model calculation showed that, if steady state levels in egg yolk fat not exceeding 10, 5 or 3 pg TEQ/g fat are required, the corresponding contamination levels of feed should not exceed 0.57, 0.29 or 0.17 ng TEQ/kg feed. So, according to the model, when steady state levels of dioxins in egg yolk fat are not to exceed 3 pg TEQ/g fat the concentration should not exceed 0.17 ng TEQ/kg feed, being about 4 times lower than the current EU-limit.

The model also indicates that, contrary to current beliefs, abdominal fat and egg yolk fat display different dioxin kinetics during the period of contaminated feed administration. During the period of contaminated feed administration the concentration in egg yolk fat exceeds the levels in abdominal fat. However, soon after administration of clean feed, concentration levels in abdominal fat exceed those in egg yolk fat (see figure 7).

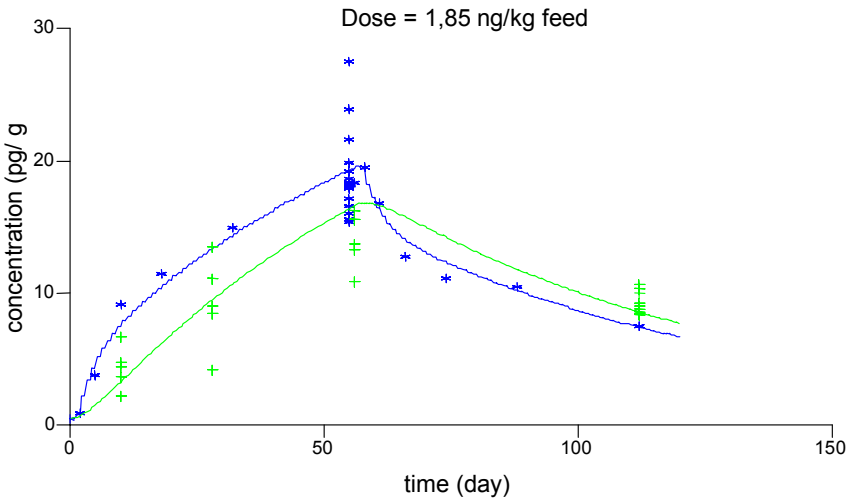

Figure 7. Concentration in egg yolk fat and abdominal fat. The lines show the calculated concentration-time curves, the symbols denote experimental data. Data: egg yolk fat (*) and abdominal fat (+).

3.2.2 Study B

The model, as calibrated under part A, readily predicted the accumulation of dioxins, furans and PCB's from feed to which 10% contaminated soil had been added, assuming the absorption fraction to be 60% (in the case of soil from farm A) and 40% (in the case of soil from farm B; to be compared with an absorption fraction of near 100 % used in part A). The modelling also suggests that the apparent plateau levels observed for soil, are merely due to the short exposure period, and that maximum levels reached after several months of exposure might actually be two to three-fold higher.

Together with the experimental observations the mathematical analysis confirms observations published elsewhere (Stephens *et al.*, 1990, 1994, 1995; Petreas *et al.*, 1991; Schuler *et al.*, 1997; Byrne and Ferrario, 2003) that the absorption of dioxins, furans and PCB's from soil, and subsequent carry-over to the egg, by laying hens might be substantial.

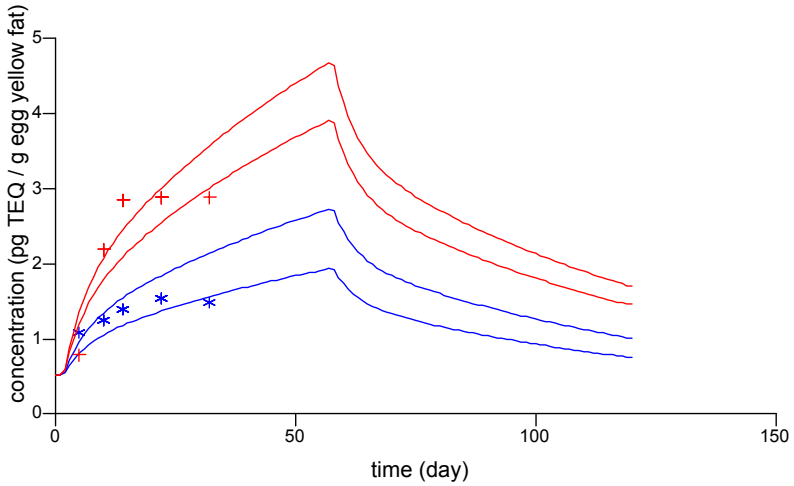

Figure 8. Total TEQ levels in eggs from hens fed with clean feed mixed with 10% of contaminated soil. Two upper lines: contamination levels representing range from 0.53 to 0.64 ng TEQ/ kg feed (upper and lowerbound levels), absorption fraction 60%, data + (soil A). Two lower lines: contamination levels representing range from 0.37 to 0.54 ng TEQ/ kg feed, absorption fraction 40%, data * (soil B).

3.2.3 Study C

As already shown feed containing a nominal concentration of 1.85 ng TEQ/kg feed led to a rapid accumulation of dioxins, furans and PCBs in egg yolk fat. When binders were added to the feed in a concentration of 0.1-1%, the experimentally observed accumulation fell within the accumulation as expected from feed containing a concentration $\pm$ 10% of the nominal value (see Figure 9; ± 10% was taken to represent the uncertainty in the nominal concentration). This result confirms that in these experiments none of the added binders showed any effectiveness in reducing the absorption of dioxins, furans or PCB's from the hen's gastro-intestinal tract.

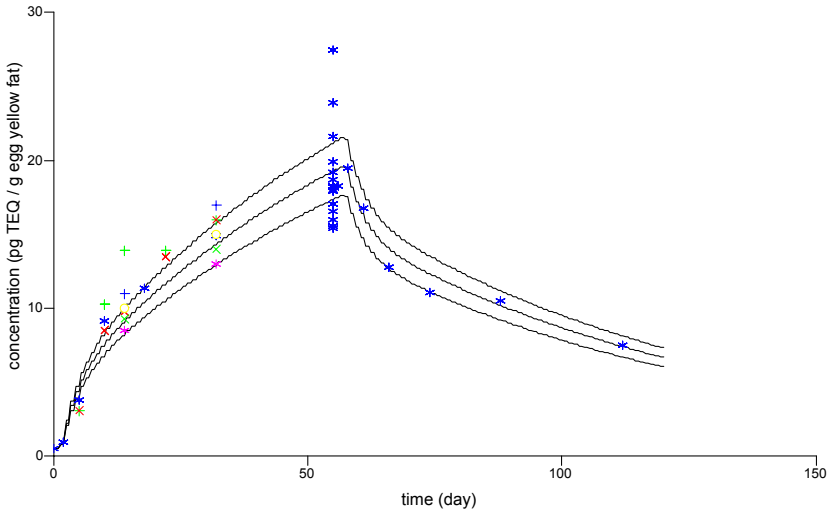

Figure 9. Predicted total TEQ levels in eggs from hens fed with feed containing 1.85 ng TEQ/kg feed (calculated, line in middle, data *), plus 10% (calculated, upper line), minus 10% (calculated, lower line). Other data: feed + clean sand, feed + different "binders" (Exal H, MycoAd A-Z, Humac, Klinofeed).

3.3 Carry-over rates of individual congeners

In order to compare the relative carry-over of individual congeners from feed to eggs, it is best to calculate the so-called Carry-over rate (COR), defined as the percentage of the amount of a certain congener excreted in the eggs, as compared to the amount ingested from the feed. Ideally, this should be done under steady-state conditions. Assuming a daily fat excretion of 5.1 g and daily feed intake of 116 g, CORs were calculated for feeds E and F in study A (Appendix 13). These data suggest a COR for the more important lower chlorinated congeners around 40% and a lower COR for the higher chlorinated hepta and octa congeners. The non-ortho and mono-ortho PCBs showed similar CORs as the lower chlorinated dioxins although some mono-ortho PCB congeners appeared to have even higher CORs. These were present at relatively low concentrations and in practice do not contribute significantly to the TEQ levels. In the case of the indicator PCBs, the lower chlorinated PCBs 53 and 101 showed very low carry-over rates, contrary to PCB 28. Similar has been observed previously in broilers (Hoogenboom *et al.* 2004).

It is important to realize that after reaching steady-state, CORs are expected to be doubled, implying that the amounts excreted in the eggs will be close to the amounts ingested. Before steady state conditions are reached, part of the absorbed compounds are still accumulated in the body fat. As for total TEQ levels, the absorbed fraction ranges between about 90% and 100%, the COR for total TEQs at steady state would range between the same limits.

4 CONCLUSIONS

- Laying hens are sensitive indicators for dioxin contamination of feed and environment, meaning that relatively low exposure levels may result in high levels in the egg yolk. This is not only true for contaminated feed but also (to a lesser extent) for contaminated soil.

- The new EU limit for feed of 0.75 ng TEQ/kg, established in 2002, is insufficient to guarantee that levels in eggs will not exceed the limit of 3 ng TEQ/kg fat. A further decrease in the feed limit by a factor 4 is required to achieve this goal.

- There are currently no Dutch or European limits for indicator PCBs in feed or eggs. The Belgium limit of 200 ng/g fat for feed does ensure that the limit for eggs of 200 ng/g fat is not exceeded.

- Addition of binders at the tested levels has no observable effect on the resulting residues of dioxins and PCBs in hens.

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Appendix 1. Levels of dioxins, non-ortho and mono-ortho PCBs in the different feeds. Results are expressed as the mean of 3 to 5 analyses, with the standard deviation and the variation coefficient. Individual levels in ng/kg. Total levels expressed in ng TEQ/kg.

	Feed A			**Feed B**			**Feed C**			**Feed D**			**Feed E**			**Feed F**		
	mean	**SD**	**%CV**	**mean**	**SD**	**%CV**	**mean**	**SD**	**%CV**	**mean**	**SD**	**%CV**	**mean**	**SD**	**%CV**	**mean**	**SD**	**%CV**
Dioxins																		
2,3,7,8-TCDF	0.01	0.00	24	0.04	0.01	35	0.04	0.00	3	0.06	0.00	2	0.12	0.01	4	0.25	0.01	2
1,2,3,7,8-PeCDF	0.02	0.01	41	0.06	0.01	11	0.10	0.00	4	0.14	0.01	4	0.33	0.00	1	0.66	0.02	2
2,3,4,7,8-PeCDF	0.01	0.00	23	0.06	0.01	11	0.10	0.00	3	0.13	0.01	5	0.33	0.01	3	0.67	0.02	3
1,2,3,4,7,8-HxCDF	0.01	0.00	24	0.06	0.00	7	0.11	0.00	1	0.15	0.00	2	0.34	0.01	3	0.70	0.01	1
1,2,3,6,7,8-HxCDF	0.01	0.00	10	0.05	0.01	9	0.11	0.00	3	0.14	0.00	2	0.33	0.01	3	0.68	0.04	5
2,3,4,6,7,8-HxCDF	0.01	0.00	12	0.06	0.01	11	0.14	0.02	16	0.15	0.03	17	0.43	0.02	5	0.84	0.14	16
1,2,3,7,8,9-HxCDF	<0.01			0.06	0.01	12	0.10	0.00	3	0.14	0.00	3	0.32	0.01	3	0.67	0.01	1
1,2,3,4,6,7,8-HpCDF	0.02	0.01	22	0.08	0.01	6	0.14	0.01	4	0.18	0.01	3	0.40	0.01	2	0.82	0.02	2
1,2,3,4,7,8,9-HpCDF	0.01	0.01	10	0.05	0.00	6	0.10	0.01	5	0.14	0.00	3	0.32	0.01	3	0.66	0.02	3
OCDF	0.05	0.02	47	0.12	0.01	8	0.23	0.01	5	0.29	0.01	4	0.63	0.01	2	1.39	0.14	10
2,3,7,8-TCDD	<0.01			0.05	0.02	46	0.05	0.00	6	0.06	0.00	3	0.14	0.00	2	0.30	0.01	2
1,2,3,7,8-PeCDD	0.01	0.00	13	0.07	0.01	9	0.11	0.00	4	0.16	0.00	2	0.37	0.00	1	0.79	0.01	1
1,2,3,4,7,8-HxCDD	0.01	0.00	3	0.07	0.01	6	0.13	0.01	5	0.17	0.00	1	0.40	0.01	2	0.84	0.01	1
1,2,3,6,7,8-HxCDD	0.01	0.00	9	0.06	0.00	7	0.12	0.00	3	0.15	0.00	3	0.35	0.01	4	0.74	0.01	1
1,2,3,7,8,9-HxCDD	0.01	0.00	12	0.07	0.01	9	0.13	0.00	2	0.17	0.01	3	0.40	0.01	3	0.84	0.01	1
1,2,3,4,6,7,8-HpCDD	0.06	0.02	28	0.11	0.01	5	0.18	0.01	3	0.22	0.01	4	0.42	0.01	3	0.84	0.02	2
OCDD	0.58	0.16	28	0.74	0.22	30	0.84	0.04	5	0.92	0.07	7	1.34	0.05	4	2.57	0.63	24
Total level [ub]	**0.04**	**0.00**	**10**	**0.20**	**0.04**	**18**	**0.30**	**0.01**	**2**	**0.40**	**0.01**	**2**	**0.97**	**0.01**	**1**	**2.04**	**0.03**	**2**
non-ortho-PCBs																		
PCB 81	0.04	10	22	0.84	0.04	5	1.61	0.03	2	2.17	0.03	1	5.12	0.14	3	10.86	0.17	2
PCB 77	0.56	131	24	1.99	0.04	2	3.82	0.21	6	4.75	0.07	2	10.85	0.16	1	22.06	0.63	3
PCB 126	0.03	5	17	0.77	0.03	4	1.50	0.04	3	2.01	0.04	2	4.98	0.10	2	10.86	0.13	1
PCB 169	0.02	9	52	0.75	0.01	2	1.51	0.02	1	2.04	0.01	0	4.89	0.07	1	10.55	0.12	1
Total level [ub]	**0.00**	**0.00**	**16**	**0.09**	**0.00**	**4**	**0.17**	**0.00**	**3**	**0.22**	**0.00**	**2**	**0.55**	**10**	**2**	**1.19**	**0.01**	**1**

Appendix 1 continued

	Feed A			Feed B			Feed C			Feed D			Feed E			Feed F		
	mean	**SD**	**%CV**	**mean**	**SD**	**%CV**	**mean**	**SD**	**%CV**	**mean**	**SD**	**%CV**	**mean**	**SD**	**%CV**	**mean**	**SD**	**%CV**
mono-ortho PCBs																		
PCB 123	<1			2	1	54	2	0	4	2	0	2	5			14	1	10
PCB 118	6	0	5	265	4	1	509	3	0	657	4	1	1576	15	1	3454	66	2
PCB 114	<1			2	1	50	2	0	6	3	0	4	6			14	1	8
PCB 105	2	0	5	147	3	2	287	3	1	372	2	1	894	14	2	1940	63	3
PCB 167	<1			2	1	50	2	0	2	3	0	8	10	2	20	24	1	6
PCB 156	1	0	11	24	1	4	45	1	1	59	1	1	139	2	1	307	10	3
PCB 157	<1			3	1	36	4	0	2	5	0	6	13	1	8	26	2	9
PCB 189	<1			2	1	37	3	0	2	4	0	2	10	0	3	21	1	4
Total level [ub]	**0.00**	**0.00**	**3**	**0.06**	**0.00**	**4**	**0.11**	**0.00**	**1**	**0.14**	**0.00**	**1**	**0.33**	**0.00**	**1**	**0.72**	**0.02**	**2**
Sum [ub]	**0.04**	**0.00**	**10**	**0.34**	**0.04**	**12**	**0.58**	**0.01**	**2**	**0.76**	**0.01**	**1**	**1.85**	**0.01**	**1**	**3.95**	**0.06**	**1**

Appendix 2. Levels of indicator PCBs in the various feeds. Results are expressed as the mean of 3 to 5 analyses, with the standard deviation and the variation coefficient. Levels expressed in µg/kg.

	Feed A			Feed B			Feed C			Feed D			Feed E			Feed F		
	mean	**SD**	**%CV**	**mean**	**SD**	**%CV**	**mean**	**SD**	**%CV**	**mean**	**SD**	**%CV**	**mean**	**SD**	**%CV**	**mean**	**SD**	**%CV**
PCB 28	0.02	0.02	80	0.20	0.03	13	0.33	0.02	7	0.44	0.03	7	0.98	0.01	1	2.13	0.02	1
PCB 52	0.02	0.01	51	0.21	0.01	3	0.40	0.03	7	0.55	0.03	5	1.30	0.02	2	2.91	0.05	2
PCB 101	0.03	0.00	11	0.45	0.02	4	0.84	0.07	8	1.20	0.04	3	2.88	0.09	3	6.33	0.18	3
PCB 118	0.01	0.00	8	0.21	0.01	4	0.41	0.03	6	0.58	0.03	6	1.37	0.03	2	3.07	0.10	3
PCB 138	0.04	0.01	11	0.48	0.02	3	0.91	0.07	7	1.28	0.05	4	2.99	0.07	2	6.72	0.29	4
PCB 153	0.03	0.00	9	0.44	0.01	2	0.83	0.06	7	1.17	0.04	3	2.79	0.06	2	6.27	0.21	3
PCB 180	<0.01			0.28	0.01	3	0.54	0.04	8	0.77	0.04	5	1.89	0.05	3	4.27	0.16	4
Sum	**0.16**	**0.02**	**14**	**2.27**	**0.03**	**1**	**4.25**	**0.30**	**7**	**5.98**	**0.24**	**4**	**14.20**	**0.30**	**2**	**31.70**	**0.93**	**3**

Appendix 3A.. Levels of dioxins. non-ortho and mono-ortho PCBs in eggs, abdominal fat and ovaries from hens fed with blank feed A. Individual levels expressed in pg/g fat (dioxins, non-ortho PCBs) and ng/g fat (mono-ortho PCBs). Total levels expressed as pg TEQ/g fat.

							Eggs							abdominal fat		ovaries	
Day	**0**	**2**	**5**	**10**	**18**	**32**	**56**	**58**	**61**	**66**	**74**	**88**	**112**	**56**	**112**	**56**	**112**
Dioxins																	
2,3,7,8-TCDF	< 0.50	< 0.50	< 0.50	*	<0.25	*	0,20	0.14	*	*	<0.05	0.12	0.15	0.16	0.12	0.21	0.09
1,2,3,7,8-PeCDF	0.13	< 0.10	< 0.10	0,11	<0.10	*	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	0,13	<0.10	<0.10	<0.10	<0.10
2,3,4,7,8-PeCDF	0.20	0.11	< 0.10	<0.10	<0.10	*	0.14	0.11	<0.10	<0.10	<0.10	0,10	0,12	<0.10	0,10	0,10	<0.10
1,2,3,4,7,8-HxCDF	< 0.10	< 0.10	< 0.10	<0.10	<0.10	0.16	*	<0.10	<0.10	<0.10	<0.10	0.25	0,14	<0.10	0,11	<0.10	<0.10
1,2,3,6,7,8-HxCDF	< 0.10	< 0.10	< 0.10	<0.10	<0.10	0.13	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
2,3,4,6,7,8-HxCDF	< 0.10	< 0.10	< 0.10	<0.10	<0.10	*	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
1,2,3,7,8,9-HxCDF	< 0.10	< 0.10	< 0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
1,2,3,4,6,7,8-HpCDF	0.38	< 0.25	< 0.25	*	*	0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	0,37	<0.25	0,27	<0.25	<0.25
1,2,3,4,7,8,9-HpCDF	< 0.25	< 0.25	< 0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25
OCDF	1.99	0.58	< 0.50	<0.50	<0.50	1.49	<0.50	0.52	<0.50	<0.50	<0.50	1.84	0.53	<0.50	<0.50	<0.50	<0.50
2.3.7.8-TCDD	0.11	< 0.05	< 0.05	<0.05	<0.05	0.13	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	0.08	<0.05	0.08	<0.05
1.2.3.7.8-PeCDD	< 0.10	< 0.10	< 0.10	0.126	<0.10	0.15	0.15	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
1.2.3.4.7.8-HxCDD	< 0.10	< 0.10	< 0.10	<0.10	<0.10	*	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
1.2.3.6.7.8-HxCDD	0.10	< 0.10	< 0.10	*	<0.10	0.19	0.14	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
1.2.3.7.8.9-HxCDD	< 0.10	< 0.10	< 0.10	<0.10	<0.10	0.12	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
1.2.3.4.6.7.8-HpCDD	1.80	0.66	0.60	0.72	0.54	0.70	1.07	0.95	0.71	*	0.47	1.55	0.69	0.46	0.54	0.71	0.98
OCDD	71.1	12.5	4.87	5.67	4.21	7.76	12.2	12.4	7.02	5.08	4.00	18.0	8.77	3.77	4.71	5.86	20.01
Total level [ub]	**0.46**	**0.34**	**0.34**	**0.32**	**0.36**	**0.44**	**0.38**	**0.31**	**0.29**	**0.29**	**0.29**	**0.33**	**0.32**	**0.33**	**0.30**	**0.40**	**0.30**
non-ortho PCBs																	
PCB 81	1.24	0.97	1.17	1.8	1.44	3.38	2.93	1.57	1.55	0.90	0.67	1.03	1.29	1.79	1.13	1.43	0.83
PCB 77	13.4	15.6	17.1	18.3	29.2	24.9	25.3	22.4	20.1	11.5	15.3	13.2	16.4	24.36	15.53	18.68	11.81
PCB 126	2.36	2.05	1.85	2.55	2.17	3.48	2.75	1.85	1.63	1.59	1.35	1.5	1.34	2.35	1.39	1.91	1.10
PCB 169	0.42	0.28	0.36	*	0.36	*	0.78	0.35	*	*	0.50	0.30	*	0.48	0.29	0.32	0.22
Total level [ub]	**0.24**	**0.21**	**0.19**	**0.26**	**0.22**	**0.35**	**0.29**	**0.19**	**0.17**	**0.16**	**0.14**	**0.15**	**0.14**	**0.24**	**0.14**	**0.20**	**0.10**

							Eggs							abdominal fat		ovaries	
Day	**0**	**2**	**5**	**10**	**18**	**32**	**56**	**58**	**61**	**66**	**74**	**88**	**112**	**56**	**112**	**56**	**112**
mono-ortho PCBs																	
PCB 123	< 0.01	< 0.01	< 0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PCB 118	0.38	0.36	0.35	0.43	0.41	0.42	0.28	0.30	0.27	0.26	0.25	0.28	0.25	0.39	0.28	0.35	0.47
PCB 114	< 0.01	< 0.01	< 0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PCB 105	0.10	0.10	0.09	0.11	0.11	0.12	0.08	0.09	0.07	0.06	0.06	0.08	0.07	0.10	0.07	0.09	0.17
PCB 167	0.02	0.02	0.02	*	*	*	*	*	*	*	*	*	*	*	*	*	0.02
PCB 156	0.06	0.06	0.06	0.07	0.06	0.06	0.05	0.05	0.05	0.05	0.04	0.04	0.04	0.07	0.05	0.05	0.04
PCB 157	0.01	< 0.01	< 0.01	0.01	*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01
PCB 189	< 0.01	< 0.01	< 0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	*	<0.01	<0.01
Total level [ub]	**0.09**	**0.09**	**0.08**	**0.10**	**0.10**	**0.10**	**0.07**	**0.07**	**0.07**	**0.07**	**0.06**	**0.07**	**0.06**	**0.09**	**0.07**	**0.08**	**0.10**
Sum [ub]	**0.80**	**0.64**	**0.61**	**0.68**	**068**	**0.88**	**0.74**	**0.57**	**0.53**	**0.52**	**0.50**	**0.55**	**0.52**	**0.66**	**0.51**	**0.68**	**0.50**

Appendix 3B. Levels of dioxins. non-ortho and mono-ortho PCBs in eggs, abdominal fat and ovaries from hens fed with feed B. Individual levels expressed in pg/g fat (dioxins, non-ortho PCBs) and ng/g fat (mono-ortho PCBs). Total levels expressed as pg TEQ/g fat.

							eggs							abdominal fat		ovaries	
Day	**0**	**2**	**5**	**10**	**18**	**32**	**56**	**58**	**61**	**66**	**74**	**88**	**112**	**56**	**112**	**56**	**112**
Dioxins																	
2,3,7,8-TCDF	< 0.5	< 0.5	< 0.5	<0.25	<0.25	0.27	0.26	0.26	0.25	0.25	0.29	0.19	0.16	0.37	0.19	0.32	0.18
1,2,3,7,8-PeCDF	< 0.10	< 0.10	*	<0.10	0.32	0.37	0.53	*	0.38	0.39	*	0.33	0.29	0.50	0.28	*	0.21
2,3,4,7,8-PeCDF	< 0.10	0.14	0.183	0.28	0.48	0.53	0.47	0.47	0.42	0.39	0.32	0.30	0.29	0.51	0.32	0.48	0.26
1,2,3,4,7,8-HxCDF	< 0.10	< 0.10	0.183	0.49	0.42	0.55	0.59	0.47	0.47	0.24	0.21	0.28	0.22	0.34	0.24	0.50	0.13
1,2,3,6,7,8-HxCDF	< 0.10	< 0.10	0.161	*	0.37	0.43	0.47	0.43	*	0.21	*	*	0.13	0.26	0.18	0.46	0.11
2,3,4,6,7,8-HxCDF	< 0.10	< 0.10	0.249	*	0.38	0.45	0.47	0.45	0.41	*	0.16	*	*	0.23	0.15	0.47	0.12
1,2,3,7,8,9-HxCDF	< 0.10	< 0.10	*	0.29	*	0.40	0.38	0.42	*	*	0.18	0.17	0.13	0.28	0.16	0.37	<0.10
1,2,3,4,6,7,8-HpCDF	0.32	< 0.25	0.29	0.53	*	0.79	0.37	0.39	*	<0.25	<0.25	<0.25	0.26	<0.25	<0.25	0.35	<0.25
1,2,3,4,7,8,9-HpCDF	< 0.25	< 0.25	< 0.25	*	*	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25
OCDF	< 0.50	< 0.50	< 0.50	0.60	<0.50	<0.50	<0.50	0.71	0.56	<0.50	<0.50	1.12	*	<0.50	<0.50	<0.50	<0.50
2.3.7.8-TCDD	< 0.05	< 0.05	0.05	<0.05	0.15	0.13	0.19	0.23	0.15	0.24	0.22	0.16	0.13	0.27	0.13	0.19	0.11
1.2.3.7.8-PeCDD	< 0.10	< 0.10	0.13	0.23	0.34	0.48	0.51	0.53	0.69	0.47	0.39	0.32	0.26	0.55	0.33	0.55	0.26
1.2.3.4.7.8-HxCDD	< 0.10	< 0.10	0.19	0.42	*	0.57	0.60	0.50	0.47	0.30	0.28	*	0.17	0.35	0.27	0.53	0.17
1.2.3.6.7.8-HxCDD	< 0.10	0.116	0.25	0.42	0.47	0.48	0.58	0.50	0.45	0.33	0.26	0.19	0.14	0.35	0.26	0.58	0.18
1.2.3.7.8.9-HxCDD	< 0.10	< 0.10	0.17	0.36	0.39	0.42	0.44	0.49	0.38	0.21	0.17	0.11	<0.10	0.22	0.14	0.48	0.11
1.2.3.4.6.7.8-HpCDD	0.69	0.60	0.62	1.02	0.86	0.84	0.71	1.99	1.84	0.65	0.81	1.99	0.82	0.47	0.33	0.68	0.38
OCDD	4.48	3.67	3.17	5.56	3.91	4.09	4.03	21.90	18.50	4.72	7.58	40.80	11.90	1.66	1.29	3.54	3.59
Total level [ub]	**0.34**	**0.36**	**0.46**	**0.68**	**1.00**	**1.27**	**1.35**	**1.38**	**1.36**	**1.10**	**0.95**	**0.80**	**0.69**	**1.35**	**0.80**	**1.37**	**0.63**
non-ortho PCBs																	
PCB 81	0.98	1.14	2.08	3.41	4.19	5.41	7.96	7.95	8.18	7.27	6.64	5.66	4.72	9.78	5.78	7.10	4.41
PCB 77	14.80	17.90	26.30	30.20	27.40	29.60	33.90	37.10	35.10	28.70	27.20	25.10	19.20	39.50	19.70	27.30	17.20
PCB 126	1.92	1.82	2.96	5.08	5.87	7.35	9.17	8.53	8.56	7.17	6.57	5.53	4.67	9.44	6.84	8.57	4.56
PCB 169	0.33	0.45	2.32	4.87	6.82	7.16	7.89	7.97	6.90	4.15	3.50	2.86	2.25	6.15	5.41	7.75	2.39
Total level [ub]	**0.20**	**0.19**	**0.32**	**0.56**	**0.66**	**0.81**	**1.00**	**0.94**	**0.93**	**0.76**	**0.70**	**0.58**	**0.49**	**1.01**	**0.74**	**0.94**	**0.48**

							eggs							abdominal fat		ovaries	
Day	**0**	**2**	**5**	**10**	**18**	**32**	**56**	**58**	**61**	**66**	**74**	**88**	**112**	**56**	**112**	**56**	**112**
mono-ortho PCBs																	
PCB 123	< 0.01	< 0.01	< 0.01	<0.01	0.01	0.01	0.02	0.02	0.02	*	0.01	0.01	0.01	0.02	0.01	0.02	<0.01
PCB 118	0.34	0.38	0.78	1.40	1.79	2.22	2.54	2.57	2.51	1.68	1.87	1.77	1.44	2.76	2.23	2.45	1.37
PCB 114	< 0.01	< 0.01	< 0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	<0.01	0.02	0.01	0.01	<0.01
PCB 105	0.09	0.11	0.30	0.61	0.82	1.09	1.32	1.34	1.33	0.90	1.04	0.94	0.75	1.38	1.08	1.23	0.70
PCB 167	0.02	0.02	0.03	*	*	*	*	*	*	*	0.03	*	*	*	0.04	0.04	0.02
PCB 156	0.05	0.06	0.11	0.17	0.20	0.24	0.25	0.25	0.24	0.13	0.16	0.14	0.11	0.25	0.23	0.26	0.12
PCB 157	< 0.01	< 0.01	0.02	0.02	0.02	0.03	0.03	0.02	0.03	0.01	0.02	0.02	0.01	0.03	0.02	0.03	0.01
PCB 189	< 0.01	< 0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	<0.01	0.01	<0.01	<0.01	0.02	0.02	0.02	<0.01
Total level [ub]	**0.08**	**0.09**	**0.18**	**0.31**	**0.38**	**0.48**	**0.54**	**0.54**	**0.53**	**0.34**	**0.38**	**0.35**	**0.29**	**0.56**	**0.47**	**0.52**	**0.28**
Sum [ub]	**0.62**	**0.64**	**0.96**	**1.55**	**2.04**	**2.55**	**2.89**	**2.86**	**2.82**	**2.20**	**2.03**	**1.74**	**1.47**	**2.93**	**2.01**	**2.83**	**1.39**

Appendix 3C. Levels of dioxins. non-ortho and mono-ortho PCBs in eggs, abdominal fat and ovaries from hens fed with feed C. Individual levels expressed in pg/g fat (dioxins, non-ortho PCBs) and ng/g fat (mono-ortho PCBs). Total levels expressed as pg TEQ/g fat.

						Eggs							abdominal fat		ovaries	
Day	**0**	**2**	**5**	**10**	**18**	**32**	**56**	**58**	**61**	**66**	**74**	**88**	**112**	**56**	**112**	**56**	**112**
Dioxins																	
2,3,7,8-TCDF	< 0.50	< 0.50	< 1.0	<0.50	0.35	0.33	0.46	0.42	0.41	0.47	0.50	0.28	*	0.50	0.26	0.41	0.23
1,2,3,7,8-PeCDF	< 0.10	< 0.10	0.24	*	0.62	0.82	*	0.94	*	0.75	0.86	0.74	0.53	0.85	0.44	*	0.34
2,3,4,7,8-PeCDF	0.11	0.16	0.28	0.52	0.76	0.82	0.94	0.95	1.00	0.74	0.90	0.63	0.46	0.83	0.44	0.98	0.40
1,2,3,4,7,8-HxCDF	< 0.10	< 0.10	0.25	0.76	0.85	0.98	0.99	1.06	0.90	0.58	0.57	0.48	0.31	0.58	0.36	1.06	0.22
1,2,3,6,7,8-HxCDF	< 0.10	< 0.10	0.28	0.62	0.82	0.81	0.95	0.93	0.78	0.50	0.49	0.34	0.24	0.47	0.29	0.94	0.24
2,3,4,6,7,8-HxCDF	< 0.10	0.14	0.31	0.67	0.77	0.89	0.92	0.90	0.75	0.49	0.46	0.31	*	0.43	0.24	0.99	0.29
1,2,3,7,8,9-HxCDF	< 0.10	< 0.10	0.20	*	*	0.75	0.87	0.85	0.81	0.49	0.49	0.34	0.25	0.49	0.24	0.90	0.16
1,2,3,4,6,7,8-HpCDF	<0.25	< 0.25	0.40	0.61	*	0.73	0.61	0.62	0.62	<0.25	*	0.44	0.44	<0.25	<0.25	0.60	<0.25
1,2,3,4,7,8,9-HpCDF	< 0.25	< 0.25	< 0.25	*	0.44	0.47	0.43	0.48	0.38	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	0.48	<0.25
OCDF	< 0.50	< 0.50	< 0.50	0.50	0.80	<0.50	0.63	0.87	0.84	<0.50	<0.50	0.64	2.70	<0.50	<0.50	0.53	<0.50
2.3.7.8-TCDD	< 0.50	< 0.05	0.08	<0.05	0.25	0.29	0.40	0.34	0.46	0.49	0.54	0.23	0.33	0.47	0.22	0.42	0.22
1.2.3.7.8-PeCDD	< 1.00	< 0.10	0.21	0.51	0.72	0.88	1.11	1.08	1.09	1.06	1.01	0.73	0.51	0.90	0.52	1.11	0.44
1.2.3.4.7.8-HxCDD	< 0.25	< 0.10	0.28	0.86	*	1.07	1.16	1.11	1.00	0.75	0.65	0.43	0.27	0.68	0.43	1.22	0.28
1.2.3.6.7.8-HxCDD	< 0.25	0.15	0.33	0.71	*	0.91	1.13	1.08	0.96	0.71	0.73	0.39	0.27	0.62	0.40	1.08	0.28
1.2.3.7.8.9-HxCDD	< 0.25	< 0.10	0.28	0.66	0.76	0.94	0.94	0.89	0.79	0.47	0.51	0.28	0.19	0.46	0.25	0.96	0.16
1.2.3.4.6.7.8-HpCDD	0.73	0.62	0.78	1.13	1.17	1.07	1.10	1.11	1.28	0.68	1.07	1.11	1.71	0.44	0.44	1.13	0.39
OCDD	4.19	2.96	3.89	4.63	4.48	3.63	*	5.09	5.37	4.46	7.83	15.30	85.00	1.54	2.36	4.21	2.90
Total level [ub]	**0.34**	**0.38**	**0.75**	**1.34**	**1.78**	**2.31**	**2.75**	**2.69**	**2.71**	**2.41**	**2.50**	**1.62**	**1.30**	**2.26**	**1.24**	**2.81**	**1.07**
non-ortho PCBs																	
PCB 81	1.45	1.33	2.41	5.38	8.18	10.90	15.30	16.20	16.50	15.90	16.60	12.70	8.19	19.60	9.58	14.90	8.19
PCB 77	26.70	21.70	25.10	52.20	42.50	57.50	49.30	54.60	58.00	50.40	52.10	40.00	27.60	61.10	26.10	45.00	24.00
PCB 126	1.73	2.37	3.92	8.30	10.60	12.80	17.20	16.00	17.10	15.20	15.40	12.00	7.86	16.70	9.90	16.30	6.94
PCB 169	0.31	1.02	4.02	10.20	12.10	14.00	16.40	15.70	15.80	9.88	9.97	6.78	4.31	11.50	8.85	17.20	4.42
Total levels [ub]	**0.18**	**0.25**	**0.43**	**0.94**	**1.19**	**1.43**	**1.89**	**1.76**	**1.88**	**1.63**	**1.65**	**1.27**	**0.83**	**1.79**	**1.08**	**1.81**	**0.74**

							Eggs							abdominal fat		ovaries	
Day	**0**	**2**	**5**	**10**	**18**	**32**	**56**	**58**	**61**	**66**	**74**	**88**	**112**	**56**	**112**	**56**	**112**
mono-ortho PCBs																	
PCB 123	< 0.01	< 0.01	< 0.01	0.02	<0.01	0.02	0.03	0.03	0.03	0.03	0.03	0.02	0.01	0.03	<0.01	0.03	0.01
PCB 118	0.36	0.45	1.07	2.65	3.39	4.20	5.31	5.30	5.15	4.59	4.46	3.38	2.39	5.43	3.45	4.30	2.46
PCB 114	< 0.01	< 0.01	< 0.01	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.01	0.03	0.02	0.03	0.01
PCB 105	0.10	0.15	0.44	1.21	1.66	2.11	2.84	2.86	2.81	2.53	2.36	1.85	1.26	2.91	1.77	2.26	1.32
PCB 167	0.02	0.02	0.03	*	*	*	*	*	*	0.04	0.05	*	*	*	0.05	0.05	0.03
PCB 156	0.06	0.07	0.15	0.33	0.38	0.44	0.53	0.50	0.48	0.38	0.36	0.25	0.18	0.43	0.33	0.44	0.20
PCB 157	< 0.01	0.01	0.02	0.03	0.04	0.04	0.05	0.05	0.04	0.04	0.04	0.02	0.02	0.04	0.03	0.04	0.02
PCB 189	< 0.01	< 0.01	0.02	0.03	0.03	0.04	0.04	0.04	0.03	0.02	0.02	0.01	<0.01	0.03	0.02	0.04	0.01
Total level [ub]	**0.09**	**0.11**	**0.24**	**0.58**	**0.73**	**0.89**	**1.13**	**1.11**	**1.08**	**0.93**	**0.90**	**0.67**	**0.47**	**1.09**	**0.71**	**0.92**	**0.49**
Sum [ub]	**0.61**	**0.73**	**1.43**	**2.85**	**3.69**	**4.63**	**5.76**	**5.57**	**5.67**	**4.97**	**5.05**	**3.56**	**2.60**	**5.14**	**3.03**	**5.53**	**2.31**

Appendix 3D. Levels of dioxins. non-ortho and mono-ortho PCBs in eggs, abdominal fat and ovaries from hens fed with feed D. Individual levels expressed in pg/g fat (dioxins, non-ortho PCBs) and ng/g fat (mono-ortho PCBs). Total levels expressed as pg TEQ/g fat.

							Eggs							abdominal fat		ovaries	
Day	**0**	**2**	**5**	**10**	**18**	**32**	**56**	**58**	**61**	**66**	**74**	**88**	**112**	**56**	**112**	**56**	**112**
dioxins																	
2,3,7,8-TCDF	*	*	< 0.50	0.31	*	0.46	0.53	0.61	*	0.68	0.55	0.37	0.35	0.60	0.36	0.58	0.30
1,2,3,7,8-PeCDF	< 0.10	< 0.10	0.26	0.69	*	1.12	1.19	1.4	1.33	1.26	0.97	0.78	0.59	1.12	0.56	1.29	0.48
2,3,4,7,8-PeCDF	0.11	0.13	0.32	0.76	0.74	1.16	1.17	1.43	1.38	1.17	1.03	0.67	0.62	1.17	0.61	1.31	0.51
1,2,3,4,7,8-HxCDF	< 0.10	< 0.10	0.40	1.00	1.05	1.30	1.11	1.42	1.54	0.78	0.60	0.54	0.42	0.74	0.44	1.42	0.26
1,2,3,6,7,8-HxCDF	< 0.10	< 0.10	0.37	0.95	0.97	1.14	1.02	1.27	1.36	0.71	0.52	0.36	0.26	0.62	0.35	1.22	0.26
2,3,4,6,7,8-HxCDF	< 0.10	< 0.10	0.37	0.92	1.01	1.18	1.01	1.35	1.32	0.74	0.51	0.37	0.21	0.55	0.28	1.27	0.39
1,2,3,7,8,9-HxCDF	< 0.10	< 0.10	0.31	0.82	0.88	1.06	1.05	1.17	*	0.76	0.56	0.41	0.28	0.67	0.34	1.15	0.21
1,2,3,4,6,7,8-HpCDF	< 0.25	< 0.25	0.43	0.80	0.78	0.77	0.60	0.67	1.05	0.28	<0.25	0.40	0.36	0.28	<0.25	0.65	<0.25
1,2,3,4,7,8,9-HpCDF	< 0.25	< 0.25	< 0.25	0.56	0.54	0.58	0.40	0.57	0.51	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	0.54	<0.25
OCDF	< 0.50	< 0.50	< 0.50	0.98	0.87	0.56	0.59	0.84	0.88	<0.50	<0.50	0.61	0.54	<0.50	<0.50	<0.50	<0.50
2.3.7.8-TCDD	< 0.05	< 0.05	0.05	0.21	0.26	0.44	0.59	0.52	0.33	0.75	0.54	0.45	0.31	0.56	0.33	0.52	0.28
1.2.3.7.8-PeCDD	< 0.10	< 0.10	0.32	0.71	0.83	1.18	1.34	1.58	1.82	1.53	1.14	0.82	0.62	1.24	0.66	1.49	0.58
1.2.3.4.7.8-HxCDD	< 0.10	< 0.10	0.42	1.15	1.09	1.50	1.34	1.68	1.50	1.09	0.76	0.50	0.38	0.91	0.57	1.61	0.34
1.2.3.6.7.8-HxCDD	< 0.10	0.14	0.46	1.05	1.02	1.37	1.14	1.56	1.54	*	0.73	0.47	0.34	0.78	0.48	1.45	0.32
1.2.3.7.8.9-HxCDD	< 0.10	< 0.10	0.37	1.01	0.96	1.19	1.00	1.27	1.16	0.73	*	0.32	0.19	0.57	0.29	1.22	*
1.2.3.4.6.7.8-HpCDD	0.64	0.56	0.78	1.46	1.16	1.34	0.99	1.26	1.50	0.90	0.75	0.85	0.73	0.51	0.42	0.99	0.41
OCDD	3.55	2.50	3.42	5.68	4.70	4.54	4.97	4.87	4.94	5.65	5.56	11.10	7.81	1.79	2.04	3.16	3.11
Total level [ub]	**0.32**	**0.33**	**0.87**	**2.08**	**2.20**	**3.21**	**3.42**	**3.94**	**3.79**	**3.50**	**2.69**	**2.00**	**1.52**	**2.99**	**1.65**	**3.75**	**1.37**
non-ortho PCBs																	
PCB 81	0.92	1.44	3.46	8.78	10.20	15.10	21.50	22.20	20.80	22.50	18.70	14.40	11.90	22.30	12.60	17.70	9.79
PCB 77	16.10	19.50	30.60	69.90	41.80	60.20	61.10	57.00	59.10	57.80	49.80	39.10	34.00	65.60	30.20	53.20	26.70
PCB 126	1.80	2.31	4.76	11.10	13.10	18.00	21.80	24.80	23.80	21.80	17.90	14.60	11.70	21.10	12.80	21.50	9.29
PCB 169	0.30	0.92	5.90	14.70	15.50	19.80	18.70	22.90	23.90	15.00	11.40	8.96	6.85	16.80	11.80	23.10	5.86
Total levels [ub]	**0.18**	**0.24**	**0.54**	**1.26**	**1.47**	**2.01**	**2.38**	**2.72**	**2.63**	**2.34**	**1.91**	**1.55**	**1.24**	**2.29**	**1.40**	**2.39**	**0.99**

							Eggs							abdominal fat		ovaries	
Day	**0**	**2**	**5**	**10**	**18**	**32**	**56**	**58**	**61**	**66**	**74**	**88**	**112**	**56**	**112**	**56**	**112**
mono-ortho PCBs																	
PCB 123	< 0.01	< 0.01	0.01	0.02	0.02	0.04	0.04	0.04	0.04	0.04	0.03	0.02	0.02	0.04	0.02	0.04	0.02
PCB 118	0.36	0.50	1.50	3.53	4.36	6.07	7.20	8.02	7.82	6.20	5.72	4.62	3.73	7.09	4.51	6.37	3.28
PCB 114	< 0.01	< 0.01	0.01	0.02	0.02	0.03	0.03	0.03	0.04	0.03	0.03	0.02	0.02	0.03	0.02	0.03	0.02
PCB 105	0.10	0.16	0.66	1.63	2.13	3.17	3.90	4.28	4.26	3.76	3.29	2.54	2.02	3.86	2.34	3.35	1.75
PCB 167	0.02	0.02	0.04	*	*	*	*	*	*	0.06	0.05	*	*	*	0.06	0.10	0.03
PCB 156	0.05	0.07	0.20	0.44	0.47	0.62	0.63	0.76	0.74	0.52	0.45	0.33	0.27	0.56	0.43	0.66	0.26
PCB 157	< 0.01	0.01	0.02	0.05	0.04	0.06	0.06	0.07	0.07	0.05	0.04	0.03	0.02	0.06	0.04	0.07	0.03
PCB 189	< 0.01	< 0.01	0.02	0.04	0.04	0.05	0.04	0.06	0.06	0.03	0.02	0.02	0.01	0.03	0.03	0.06	0.01
Total level [ub]	**0.09**	**0.11**	**0.34**	**0.78**	**0.93**	**1.29**	**1.48**	**1.67**	**1.64**	**1.30**	**1.17**	**0.91**	**0.74**	**1.43**	**0.94**	**1.36**	**0.66**
Sum [ub]	**0.59**	**0.68**	**1.75**	**4.12**	**4.59**	**6.50**	**7.27**	**8.33**	**8.06**	**7.14**	**5.76**	**4.47**	**3.50**	**6.71**	**3.99**	**7.49**	**3.01**

Appendix 3E. Levels of dioxins. non-ortho and mono-ortho PCBs in eggs from hens fed with feed E. Individual levels expressed in pg/g fat (dioxins, non-ortho PCBs) and ng/g fat (mono-ortho PCBs). Total levels expressed as pg TEQ/g fat.

Day	**0**	**2**	**5**	**10**	**18**	**32**	**56**	**57 (n=16)**		**58**	**61**	**66**	**74**	**88**	**112**
dioxins								mean	SD						
2,3,7,8-TCDF	*	0.21	< 0.5	0.48	0.61	0.79	1.10	1.14	0.19	1.11	1.06	0.92	0.81	0.78	0.58
1,2,3,7,8-PeCDF	< 0.10	0.20	0.60	1.48	1.87	2.55	2.86	3.22	0.54	2.98	2.66	1.93	1.76	1.87	1.28
2,3,4,7,8-PeCDF	0.132	0.14	0.65	1.44	2.00	2.72	2.83	3.27	0.46	3.07	2.64	1.98	1.71	1.76	1.25
1,2,3,4,7,8-HxCDF	< 0.10	0.14	0.96	2.32	2.65	3.01	3.33	3.39	0.38	3.19	2.79	1.49	1.13	1.13	*
1,2,3,6,7,8-HxCDF	< 0.10	< 0.10	1.01	2.17	2.47	2.82	2.98	3.06	0.35	3.09	2.53	1.33	1.01	0.88	0.61
2,3,4,6,7,8-HxCDF	< 0.10	0.14	1.46	2.29	2.51	2.91	3.03	3.23	0.39	3.19	2.58	1.38	1.04	0.87	0.54
1,2,3,7,8,9-HxCDF	< 0.10	0.13	0.75	1.93	2.25	2.48	2.85	2.89	0.34	2.92	2.39	1.41	1.06	1.06	0.67
1,2,3,4,6,7,8-HpCDF	< 0.25	0.29	1.02	1.72	1.73	1.67	1.60	1.84	0.21	1.61	1.10	0.36	<0.25	0.35	0.37
1,2,3,4,7,8,9-HpCDF	< 0.25	< 0.25	0.67	1.28	1.38	1.51	1.44	1.54	0.22	1.45	1.08	*	<0.25	0.25	<0.25
OCDF	< 0.50	< 0.50	0.69	1.31	1.02	1.10	0.98	1.73	0.77	1.11	0.72	<0.50	<0.50	0.69	0.61
2.3.7.8-TCDD	< 0.05	0.18	0.18	0.33	0.69	1.00	1.31	1.36	0.27	1.32	1.22	1.14	0.95	0.98	0.69
1.2.3.7.8-PeCDD	*	< 0.25	0.57	1.78	2.01	2.81	3.39	3.66	0.60	3.93	3.13	2.39	2.15	2.05	1.46
1.2.3.4.7.8-HxCDD	< 0.10	< 0.10	1.21	2.79	3.04	3.44	3.80	3.93	0.43	3.97	3.43	1.77	1.31	1.17	0.85
1.2.3.6.7.8-HxCDD	0.103	0.23	1.04	2.31	2.62	3.06	3.45	3.44	0.43	3.38	2.92	1.68	1.22	1.06	0.72
1.2.3.7.8.9-HxCDD	< 0.10	< 0.10	0.99	2.25	2.57	2.75	2.87	3.13	0.37	2.96	2.52	1.21	0.94	0.75	0.54
1.2.3.4.6.7.8-HpCDD	0.583	0.55	1.31	2.21	2.30	2.18	2.15	2.60	0.44	2.40	1.53	0.85	0.63	1.00	1.03
OCDD	2.97	2.67	3.87	5.10	4.11	5.47	3.82	8.40	4.30	5.15	3.58	2.91	3.03	9.74	11.20
Total level [ub]	**0.33**	**0.64**	**1.92**	**4.61**	**5.72**	**7.48**	**8.65**	**9.29**	**1.37**	**9.37**	**7.86**	**5.75**	**4.91**	**4.78**	**3.32**
non-ortho PCBs															
PCB 81	1.87	1.63	6.98	15.60	23.70	33.30	46.70	46.91	10.06	46.40	46.60	40.60	36.80	35.20	26.80
PCB 77	35.20	17.60	42.40	68.50	71.40	97.20	112.00	135.13	23.15	117.00	116.00	105.00	90.20	83.30	71.10
PCB 126	1.99	2.86	10.30	23.90	30.70	40.30	54.10	53.54	10.05	56.60	49.40	40.00	35.60	33.90	25.20
PCB 169	0.33	2.12	14.90	36.30	41.50	48.60	55.40	56.28	7.68	57.20	45.80	28.10	21.70	20.30	15.10
Total levels [ub]	**0.21**	**0.31**	**1.18**	**2.76**	**3.49**	**4.53**	**5.98**	**5.93**	**1.08**	**6.25**	**5.41**	**4.30**	**3.79**	**3.60**	**2.68**

Day	**0**	**2**	**5**	**10**	**18**	**32**	**56**	**57 (n=16)**		**58**	**61**	**66**	**74**	**88**	**112**
dioxins								mean	SD						
mono-ortho PCBs															
PCB 123	< 0.01	< 0.01	0.02	0.04	0.06	0.07	0.10	0.09	0.02	0.10	0.09	0.07	0.07	0.05	0.02
PCB 118	0.40	0.71	3.26	7.93	9.85	13.70	17.10	17.31	3.64	18.30	16.90	13.70	12.10	10.50	7.93
PCB 114	< 0.01	< 0.01	0.02	0.04	0.05	0.06	0.07	0.08	0.02	0.08	0.07	0.06	0.05	0.05	0.04
PCB 105	0.11	0.27	1.50	3.85	4.98	7.31	9.36	9.51	2.08	10.00	9.19	7.94	6.79	6.01	4.42
PCB 167	0.02	0.03	0.06	*	*	*	0.20	0.11	0.02	0.22	0.18	0.07	0.11	*	*
PCB 156	0.06	0.10	0.44	1.00	1.17	1.39	1.75	1.66	0.29	1.84	1.55	1.04	0.90	0.73	0.55
PCB 157	0.01	0.01	0.05	0.10	0.11	0.13	0.15	0.15	0.03	0.16	0.14	0.10	0.08	0.07	0.05
PCB 189	< 0.01	0.01	0.04	0.10	0.11	0.12	0.14	0.13	0.01	0.14	0.11	0.05	0.04	0.03	0.02
Total level [ub]	**0.09**	**0.16**	**0.74**	**1.76**	**2.16**	**2.91**	**3.66**	**3.65**	**0.74**	**3.90**	**3.51**	**2.78**	**2.41**	**2.09**	**1.55**
Sum [ub]	**0.63**	**1.11**	**3.84**	**9.13**	**11.38**	**14.92**	**18.29**	**18.88**	**3.16**	**19.52**	**16.79**	**12.82**	**11.11**	**10.48**	**7.55**

Appendix 3F. Levels of dioxins. non-ortho and mono-ortho PCBs in abdominal fat and ovaries from hens fed with feed E. Individual levels expressed in pg/g fat (dioxins, non-ortho PCBs) and ng/g fat (mono-ortho PCBs). Total levels expressed as pg TEQ/g fat.

	abdominal fat								ovaries							
Day	**10**		**28**		**56**		**112**		**10**		**28**		**56**		**112**	
Dioxins	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
2.3.7.8-TCDF	0.51	0.14	0.85	0.33	1.06	0.14	0.70	0.11	0.43	0.16	0.85	0.13	1.33	0.39	0.53	0.09
1.2.3.7.8-PeCDF	0.71	0.22	2.64	0.57	2.27	0.30	1.37	0.16	1.46	0.22	2.64	0.39	3.75	1.12	1.03	0.23
2.3.4.7.8-PeCDF	0.77	0.24	2.70	0.58	2.24	0.32	1.38	0.16	1.41	0.37	2.70	0.33	3.54	1.09	1.14	0.25
1.2.3.4.7.8-HxCDF	0.49	0.17	3.26	0.37	1.45	0.23	1.09	0.26	1.95	0.46	3.26	0.50	3.75	1.27	0.62	0.15
1.2.3.6.7.8-HxCDF	0.40	0.14	2.89	0.34	1.23	0.21	0.92	0.14	1.67	0.37	2.89	0.34	3.38	1.21	0.55	0.07
2.3.4.6.7.8-HxCDF	0.36	0.13	2.87	0.26	1.11	0.20	0.79	0.17	1.75	0.43	2.87	0.43	3.29	1.24	0.52	0.15
1.2.3.7.8.9-HxCDF	0.41	0.17	2.64	0.37	1.35	0.20	0.80	0.10	1.47	0.34	2.64	0.32	3.32	1.19	0.52	0.13
1.2.3.4.6.7.8-HpCDF	0.50	0.39	1.91	0.21	0.35	0.08	0.44	0.21	1.62	0.63	1.91	0.82	1.73	0.64	<0.25	
1.2.3.4.7.8.9-HpCDF	< 0.25		1.47	0.05	0.33	0.06	0.30	0.04	1.04	0.25	1.47	0.33	1.63	0.66	<0.25	
OCDF	< 0.50		2.30	4.14	<0.50		0.62	0.18	9.07	11.24	2.30	0.92	0.99	0.36	<0.50	
2.3.7.8-TCDD	0.35	0.13	1.00	0.29	1.18	0.18	0.73	0.06	0.43	0.28	1.00	0.19	1.45	0.49	0.63	0.12
1.2.3.7.8-PeCDD	0.69	0.27	3.00	0.59	2.62	0.43	1.63	0.16	1.42	0.38	3.00	0.41	4.23	1.32	1.24	0.24
1.2.3.4.7.8-HxCDD	0.53	0.22	3.71	0.49	1.79	0.26	1.35	0.21	2.16	0.49	3.71	0.46	4.43	1.61	0.87	0.15
1.2.3.6.7.8-HxCDD	0.53	0.17	3.18	0.38	1.54	0.26	1.19	0.29	1.89	0.31	3.18	0.39	3.84	1.24	0.75	0.16
1.2.3.7.8.9-HxCDD	0.36	0.14	2.94	0.30	1.11	0.19	0.79	0.19	1.70	0.42	2.94	0.37	3.32	1.20	0.52	0.08
1.2.3.4.6.7.8-HpCDD	0.63	0.19	2.43	0.77	0.60	0.13	1.43	1.47	3.25	2.28	2.43	0.76	2.26	0.85	0.78	0.14
OCDD	2.88	1.15	10.13	8.03	2.25	0.74	6.09	8.57	22.19	25.73	10.13	3.45	5.05	1.41	5.89	1.47
Total level [ub]	**1.84**	**0.64**	**7.78**	**1.44**	**6.11**	**0.94**	**3.89**	**0.41**	**3.91**	**1.11**	**7.78**	**1.05**	**10.32**	**3.39**	**2.95**	**0.56**
non-ortho-PCBs																
PCB 81	14.72	4.91	34.50	11.83	46.66	6.34	30.39	2.79	13.57	3.32	34.50	5.69	55.12	17.10	24.33	4.03
PCB 77	53.40	14.63	90.08	27.26	106.40	9.50	63.78	5.17	61.92	17.92	90.08	13.65	123.54	36.78	54.61	7.34
PCB 126	14.01	4.61	41.08	11.04	43.18	6.23	29.23	3.03	19.70	5.24	41.08	6.04	58.82	18.24	20.49	3.59
PCB 169	7.97	3.55	59.00	8.27	32.68	6.72	25.93	4.22	27.72	6.44	59.00	6.63	65.14	20.64	13.64	2.88
Total level [ub]	**1.49**	**0.50**	**4.71**	**1.19**	**4.66**	**0.69**	**3.19**	**0.34**	**2.25**	**0.59**	**4.71**	**0.65**	**6.55**	**2.04**	**2.19**	**0.38**

	abdominal fat								ovaries							
Day	**10**		**28**		**56**		**112**		**10**		**28**		**56**		**112**	
mono-ortho-PCBs																
PCB 123	0.03	0.01	0.08	0.02	0.09	0.01	0.06	0.01	0.04	0.01	0.08	0.01	0.09	0.01	0.04	0.01
PCB 118	5.26	2.70	13.36	4.00	15.90	2.49	10.93	1.26	6.48	1.77	13.36	1.99	16.28	2.15	6.88	1.20
PCB 114	0.03	0.01	0.06	0.02	0.07	0.01	0.05	0.01	0.03	0.01	0.06	0.01	0.07	0.01	0.03	0.01
PCB 105	2.83	1.50	7.03	2.18	8.76	1.33	5.72	0.63	3.11	0.87	7.03	1.10	8.79	1.12	3.86	0.68
PCB 167	0.07	0.03	*		*		0.10	0.01	*		*		0.14	0.02	0.06	0.01
PCB 156	0.41	0.21	1.41	0.30	1.19	0.19	0.94	0.14	0.79	0.20	1.41	0.19	1.62	0.21	0.55	0.10
PCB 157	0.04	0.02	0.14	0.03	0.11	0.02	0.08	0.01	0.08	0.02	0.14	0.02	0.15	0.02	0.05	0.01
PCB 189	0.02	0.01	0.12	0.02	0.05	0.01	0.05	0.01	0.08	0.02	0.12	0.02	0.13	0.02	0.02	0.01
Total level [ub]	**1.06**	**0.54**	**2.87**	**0.79**	**3.16**	**0.49**	**2.21**	**0.26**	**1.42**	**0.38**	**2.87**	**0.42**	**3.45**	**0.44**	**1.40**	**0.24**
Sum [ub]	**4.38**	**1.66**	**15.35**	**3.43**	**13.94**	**2.12**	**9.29**	**0.86**	**7.59**	**2.07**	**15.35**	**2.10**	**20.33**	**5.54**	**6.55**	**1.14**

Appendix 3G. Levels of dioxins. non-ortho and mono-ortho PCBs in eggs, abdominal fat and ovaries from hens fed with feed F. Individual levels expressed in pg/g fat (dioxins, non-ortho PCBs) and ng/g fat (mono-ortho PCBs). Total levels expressed as pg TEQ/g fat.

							eggs							abdominal fat		ovaries	
Day	**0**	**2**	**5**	**10**	**18**	**32**	**56**	**58**	**61**	**66**	**74**	**88**	**112**	**56**	**112**	**56**	**112**
dioxins																	
2,3,7,8-TCDF	< 0.50	< 1.0	nd	0.79	1.22	1.53	2.33	2.78	2.28	1.94	1.84	1.79	1.21	2.90	1.28	3.30	0.99
1,2,3,7,8-PeCDF	< 0.25	< 0.25	nd	3.09	3.96	5.34	7.15	7.56	6.26	4.35	4.12	3.95	2.52	6.71	2.34	9.40	1.94
2,3,4,7,8-PeCDF	0.12	0.23	nd	3.03	3.90	5.33	6.76	7.37	5.91	4.17	4.24	3.97	2.58	6.60	2.41	9.36	2.20
1,2,3,4,7,8-HxCDF	< 0.10	< 0.10	nd	4.65	5.46	5.78	7.00	7.18	6.05	3.17	2.56	2.31	1.46	4.66	1.71	9.55	1.16
1,2,3,6,7,8-HxCDF	< 0.10	0.168	nd	4.31	4.92	5.26	6.44	6.49	5.23	2.84	2.25	1.88	1.19	4.01	1.36	8.80	0.99
2,3,4,6,7,8-HxCDF	< 0.10	< 0.10	nd	4.42	5.13	5.58	6.80	6.94	5.60	2.81	2.32	1.90	1.15	3.60	1.14	8.60	0.88
1,2,3,7,8,9-HxCDF	< 0.10	< 0.10	nd	3.75	4.41	5.03	6.28	6.23	4.99	3.08	2.67	1.99	1.15	4.23	1.23	8.38	0.97
1,2,3,4,6,7,8-HpCDF	< 0.25	< 0.25	nd	3.41	3.24	3.15	3.26	3.02	2.16	0.62	0.44	0.69	0.45	0.97	0.57	4.04	<0.25
1,2,3,4,7,8,9-HpCDF	< 0.25	< 0.25	nd	2.78	2.83	2.83	3.06	*	1.96	*	*	0.33	<0.25	1.08	0.48	3.94	<0.25
OCDF	< 0.50	< 0.50	nd	2.06	1.77	2.08	1.55	1.57	1.12	<0.50	<0.50	0.73	0.74	<0.50	<0.50	2.00	<0.50
2.3.7.8-TCDD	< 0.05	< 0.25	nd	0.88	1.28	2.25	3.26	3.65	2.73	2.22	2.39	2.05	1.45	3.24	1.25	3.79	1.14
1.2.3.7.8-PeCDD	< 0.10	< 0.50	nd	3.29	4.50	5.81	8.04	8.46	7.28	5.62	5.02	4.57	2.93	7.66	2.56	11.30	2.72
1.2.3.4.7.8-HxCDD	< 0.10	0.24	nd	5.47	6.26	6.72	8.38	8.73	7.05	3.49	3.23	2.81	1.91	5.85	2.34	11.70	1.65
1.2.3.6.7.8-HxCDD	< 0.10	0.25	nd	5.08	5.15	5.87	7.49	7.52	6.03	3.36	3.03	2.50	1.48	4.80	1.83	9.72	1.50
1.2.3.7.8.9-HxCDD	< 0.10	0.16	nd	4.28	5.09	5.43	6.49	6.62	5.16	2.55	2.18	1.81	0.92	3.67	1.10	8.61	0.96
1.2.3.4.6.7.8-HpCDD	0.60	0.65	nd	4.08	3.89	3.99	3.97	3.85	2.90	0.92	0.87	1.87	1.02	1.34	0.95	5.34	0.52
OCDD	2.89	2.87	nd	7.75	5.14	8.96	4.78	4.51	4.58	2.96	3.27	20.70	9.83	1.76	4.34	11.00	2.84
Total level [ub]	**0.35**	**1.10**		**9.21**	**11.79**	**15.21**	**20.26**	**21.49**	**17.59**	**12.48**	**11.76**	**10.53**	**6.86**	**17.94**	**6.35**	**27.24**	**5.98**
non-ortho PCBs																	
PCB 81	1.5	1.9	nd	28.7	48.2	72.0	102.0	121.0	105.0	86.4	85.6	82.4	55.1	130.0	56.3	144.0	46.5
PCB 77	23.6	19.5	nd	84.4	119.0	181.0	252.0	251.0	240.0	203.0	181.0	162.0	115.0	252.0	100.0	292.0	84.6
PCB 126	2.2	3.8	nd	45.3	62.1	81.6	123.0	140.0	110.0	85.5	84.6	81.4	56.1	124.0	53.8	153.0	39.5
PCB 169	< 0.25	2.8	nd	69.4	84.9	94.3	124.0	126.0	99.5	57.4	54.8	54.4	37.2	104.0	52.2	163.0	29.5
Total levels [ub]	**0.22**	**0.41**		**5.24**	**7.08**	**9.13**	**13.58**	**15.30**	**12.03**	**9.15**	**9.03**	**8.71**	**6.00**	**13.48**	**5.92**	**16.97**	**4.26**

							eggs							abdominal fat		ovaries	
Day	**0**	**2**	**5**	**10**	**18**	**32**	**56**	**58**	**61**	**66**	**74**	**88**	**112**	**56**	**112**	**56**	**112**
mono-ortho PCBs																	
PCB 123	< 0.01	< 0.01	nd	nd	0.12	0.14	0.22	0.24	0.20	0.16	0.15	0.13	0.08	0.23	0.10	0.26	0.07
PCB 118	0.40	0.92	nd	nd	20.50	28.20	40.60	44.80	38.00	29.00	29.30	24.90	17.00	41.00	19.20	41.00	13.70
PCB 114	< 0.01	< 0.01	nd	nd	0.08	0.12	0.16	0.18	0.16	0.13	0.12	0.10	0.07	0.18	0.08	0.21	0.06
PCB 105	0.11	0.38	nd	nd	10.60	15.10	23.00	25.00	21.40	17.30	16.50	14.00	9.23	23.00	10.20	23.90	7.74
PCB 167	0.02	0.03	nd	nd	*	*	0.41	0.40	0.35	0.23	0.21	*	*	0.16	0.18	0.39	0.10
PCB 156	0.06	0.13	nd	nd	2.39	2.74	4.03	4.28	3.41	2.27	2.17	1.82	1.25	3.37	1.79	4.45	1.13
PCB 157	0.01	0.02	nd	nd	0.22	0.25	0.39	0.38	0.31	0.21	0.19	0.17	0.10	0.27	0.14	0.43	0.11
PCB 189	< 0.01	0.01	nd	nd	0.22	0.22	0.29	0.30	0.23	0.11	0.10	0.08	0.05	0.16	0.11	0.33	0.05
Total level [ub]	**0.09**	**0.21**			**4.49**	**5.92**	**8.70**	**9.46**	**7.93**	**5.96**	**5.84**	**4.95**	**3.35**	**8.35**	**3.97**	**9.09**	**2.81**
Sum [ub]	**0.67**	**1.72**			**23.35**	**30.26**	**42.54**	**46.25**	**37.55**	**27.60**	**26.64**	**24.20**	**16.21**	**39.77**	**16.24**	**53.31**	**13.04**

Appendix 4A. Levels of indicator PCBs in eggs, abdominal fat and ovaries of hens fed with blank feed A. Individual levels in ng/g fat.

							eggs							abdominal fat		ovaries	
Day	**0**	**2**	**5**	**10**	**18**	**32**	**56**	**58**	**61**	**66**	**74**	**88**	**112**	**56**	**112**	**56**	**112**
PCB 28	<0.4	0.5	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	0.5	<0.4	<0.4	0.4	0.5	0.5	0.6
PCB 52	0.5	0.5	0.5	0.5	0.6	0.5	0.5	0.5	0.6	0.5	0.6	0.5	0.5	0.4	<0.4	0.5	<0.4
PCB 101	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
PCB 118	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
PCB 138	1.17	1.2	1.0	1.2	1.2	1.1	0.9	1.1	1.0	1.0	1.0	0.8	0.8	1.2	0.8	0.9	0.6
PCB 153	1.00	1.0	0.9	1.0	0.9	0.9	0.8	0.8	0.8	0.8	0.8	0.7	0.7	1.2	0.8	0.8	0.6
PCB 180	0.83	0.7	0.5	0.8	0.5	0.5	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	0.4	0.8	0.9	0.5	0.8
Total level [ub]	**3.5**	**3.8**	**2.9**	**3.5**	**3.2**	**2.9**	**2.2**	**2.4**	**2.4**	**2.3**	**2.9**	**2.0**	**2.4**	**3.9**	**3.7**	**3.1**	**3.4**

Appendix 4B. Levels of indicator PCBs in eggs, abdominal fat and ovaries of hens fed with feed B. Individual levels in ng/g fat.

							eggs							abdominal fat		ovaries	
Day	**0**	**2**	**5**	**10**	**18**	**32**	**56**	**58**	**61**	**66**	**74**	**88**	**112**	**56**	**112**	**56**	**112**
PCB 28	<0.4	0.4	<0.4	0.6	1.0	1.4	1.8	1.9	1.7	1.9	1.7	1.3	1.1	1.9	1.4	1.6	1.5
PCB 52	0.5	0.5	0.6	0.7	0.7	0.8	0.8	1.1	1.1	0.8	0.9	0.7	0.8	0.5	<0.4	0.6	<0.4
PCB 101	<0.4	<0.4	0.4	0.6	0.7	0.7	0.7	0.8	0.6	0.4	<0.4	<0.4	<0.4	0.6	<0.4	0.5	<0.4
PCB 118	<0.4	<0.4	0.7	1.3	1.6	2.0	2.6	2.6	2.4	2.1	1.8	1.6	1.4	2.4	2.0	2.1	1.5
PCB 138	1.1	1.2	2.1	3.7	4.4	4.9	5.5	5.8	5.4	4.1	3.6	3.1	2.8	5.0	4.8	5.0	3.0
PCB 153	1.0	1.0	2.0	3.8	4.4	5.0	5.8	5.8	5.3	3.8	3.2	2.8	2.4	4.9	4.9	5.0	2.8
PCB 180	0.6	1.2	1.8	4.3	3.8	4.6	4.5	5.5	4.7	2.6	2.7	1.7	1.9	2.9	3.4	4.2	1.7
Total level [ub]	**3.2**	**4.3**	**7.7**	**15.2**	**16.5**	**19.3**	**21.7**	**23.5**	**21.2**	**15.7**	**14.1**	**11.2**	**10.3**	**18.2**	**16.9**	**19.0**	**11.0**

Appendix 4C. Levels of indicator PCBs in eggs, abdominal fat and ovaries of hens fed with feed C. Individual levels in ng/g fat.

							eggs							abdominal fat		ovaries	
Day	**0**	**2**	**5**	**10**	**18**	**32**	**56**	**58**	**61**	**66**	**74**	**88**	**112**	**56**	**112**	**56**	**112**
PCB 28		0.5	0.6	1.2	1.5	2.3	3.1	3.0	3.3	3.4	3.6	2.2	1.6	3.4	2.1	3.0	1.8
PCB 52		<0.4	0.5	0.7	0.8	0.8	0.7	0.6	0.6	<0.4	<0.4	<0.4	<0.4	0.7	<0.4	0.8	<0.4
PCB 101		<0.4	0.5	1.0	0.9	1.0	1.0	1.0	0.7	<0.4	<0.4	<0.4	<0.4	1.1	<0.4	0.9	<0.4
PCB 118		0.4	0.9	2.2	2.5	3.4	4.5	4.5	4.4	4.2	4.6	2.7	1.9	4.8	3.2	4.5	2.3
PCB 138		1.3	2.8	6.3	6.8	8.4	10.6	10.7	9.8	8.3	8.2	5.3	3.6	9.4	7.0	10.8	4.5
PCB 153		1.1	2.9	6.9	7.2	8.9	10.7	11.0	9.7	7.7	8.4	4.9	3.4	9.2	7.2	10.6	4.2
PCB 180		0.8	2.6	6.2	6.3	7.5	8.5	8.4	6.9	4.0	4.5	2.4	1.7	4.7	4.8	8.6	2.5
Total level [ub]		**4.1**	**10.9**	**24.4**	**26.0**	**32.3**	**39.0**	**39.3**	**35.5**	**27.6**	**29.3**	**17.6**	**12.3**	**33.3**	**24.7**	**39.2**	**15.7**

Appendix 4D. Levels of indicator PCBs in eggs, abdominal fat and ovaries of hens fed with feed D. Individual levels in ng/g fat.

							eggs							abdominal fat		ovaries	
Day	**0**	**2**	**5**	**10**	**18**	**32**	**56**	**58**	**61**	**66**	**74**	**88**	**112**	**56**	**112**	**56**	**112**
PCB 28	<0.4	0.6	0.6	1.3	2.1	3.0	4.3	4.1	4.1	3.5	3.3	2.8	2.4	4.4	2.7	3.9	2.5
PCB 52	<0.4	<0.4	0.7	1.0	0.9	1.0	0.8	0.9	0.9	0.5	0.4	<0.4	0.5	0.9	<0.4	0.9	0.4
PCB 101	<0.4	<0.4	0.8	1.5	1.4	1.6	1.0	1.4	1.4	<0.4	<0.4	<0.4	<0.4	1.5	<0.4	1.3	<0.4
PCB 118	0.3	0.5	1.4	3.0	3.8	5.2	6.4	6.8	6.6	4.7	4.2	3.8	3.2	6.3	4.3	5.9	3.1
PCB 138	0.8	1.3	3.7	8.0	8.9	11.9	13.2	14.9	14.9	8.9	8.7	7.5	6.0	12.6	9.2	14.2	6.1
PCB 153	0.8	1.2	3.9	8.7	9.9	12.4	12.7	15.0	15.2	8.0	7.5	6.8	5.4	12.3	9.4	14.1	5.8
PCB 180	0.5	0.9	3.9	8.9	9.1	10.8	9.4	12.0	12.1	4.6	4.3	3.5	2.8	5.6	6.2	11.4	3.2
Total level [ub]	**2.4**	**4.3**	**15.0**	**32.5**	**36.1**	**45.8**	**47.8**	**55.1**	**55.2**	**30.2**	**28.3**	**24.4**	**20.3**	**43.6**	**32.2**	**51.6**	**21.2**

Appendix 4E. Levels of indicator PCBs in eggs of hens fed with feed E. Individual levels in ng/g fat.

	eggs												
Day	**0**	**2**	**5**	**10**	**18**	**32**	**56**	**58**	**61**	**66**	**74**	**88**	**112**
PCB 28	0.5	0.6	1.1	2.4	3.9	6.5	8.4	9.1	9.8	8.6	7.5	6.4	4.7
PCB 52	0.6	0.6	1.1	1.8	1.7	1.9	1.6	1.5	1.6	0.7	0.5	0.4	0.5
PCB 101	<0.4	0.4	1.9	3.0	3.2	3.4	3.5	3.6	2.6	0.8	0.5	<0.4	<0.4
PCB 118	<0.4	0.7	2.8	6.1	8.0	11.7	14.8	16.3	15.7	12.1	10.0	8.9	6.6
PCB 138	1.0	2.0	8.9	18.4	21.6	29.6	34.6	38.4	35.1	24.0	18.2	16.5	12.7
PCB 153	1.0	2.1	9.9	20.5	24.3	30.9	36.1	40.0	36.3	22.5	18.2	15.5	11.7
PCB 180	0.4	1.5	8.4	17.9	19.5	23.1	25.6	28.2	23.4	11.4	8.1	6.6	4.9
Total level [ub]	**3.4**	**7.9**	**34.2**	**70.1**	**82.2**	**106.9**	**124.7**	**137.1**	**124.5**	**80.0**	**63.1**	**54.3**	**41.1**

Appendix 4F. Levels of indicator PCBs in abdominal fat and ovaries of hens fed with feed E. Individual levels in ng/g fat.

	abdominal fat								ovaries							
Day	**10**		**28**		**56**		**112**		**10**		**28**		**56**		**112**	
	mean.	SD	mean	SD	mean	SD	mean	SD	mean.	SD	mean	SD	mean	SD	mean	SD
PCB 28	3.1	0.9	7.2	1.1	9.6	1.5	5.7	0.4	2.7	0.7	6.8	1.0	9.0	1.3	5.0	0.8
PCB 52	1.5	0.4	1.6	0.2	1.3	0.1	0.4	0.0	1.5	0.3	1.7	0.3	1.5	0.2	0.5	0.1
PCB 101	2.2	0.9	3.3	0.8	3.1	0.3	0.5	0.1	2.8	0.7	3.7	0.8	3.2	0.4	<0.4	
PCB 118	4.2	1.6	8.9	1.7	12.6	2.0	9.3	1.0	5.9	1.4	11.9	1.4	14.7	2.2	6.8	1.1
PCB 138	7.4	3.1	15.6	3.4	22.8	3.6	19.7	2.3	16.8	3.8	30.1	3.3	34.8	4.8	12.8	2.1
PCB 153	7.2	3.0	15.1	3.3	22.1	3.6	19.6	2.5	18.2	4.1	31.7	3.7	35.6	4.8	12.2	2.1
PCB 180	3.6	1.3	8.8	2.2	13.2	2.2	11.2	1.8	17.4	3.5	26.8	2.8	27.8	3.7	6.4	1.4
Total level [ub]	**29.3**	**11.1**	**60.5**	**12.5**	**84.8**	**12.9**	**66.2**	**7.8**	**65.1**	**14.4**	**112.7**	**13.1**	**126.6**	**16.5**	**43.8**	**6.7**

Appendix 4G. Levels of indicator PCBs in eggs, abdominal fat and ovaries of hens fed with feed F. Individual levels in ng/g fat.

							eggs							abdominal fat		ovaries	
Day	**0**	**2**	**5**	**10**	**18**	**32**	**56**	**58**	**61**	**66**	**74**	**88**	**112**	**56**	**112**	**56**	**112**
PCB 28	0.5	0.6	nd	4.5	8.2	14.2	20.7	24.0	21.5	17.4	17.4	14.2	9.5	25.3	10.3	23.5	9.5
PCB 52	<0.4	0.5	nd	2.6	2.6	2.4	2.9	3.2	2.2	0.7	<0.4	<0.4	<0.4	2.4	<0.4	2.8	0.4
PCB 101	<0.4	0.5	nd	5.9	6.3	6.1	7.5	8.6	5.1	1.3	0.7	<0.4	<0.4	6.6	<0.4	6.6	<0.4
PCB 118	<0.4	0.9	nd	12.2	17.8	25.9	38.5	43.7	36.5	25.9	26.1	23.2	15.9	36.0	18.0	38.4	14.9
PCB 138	1.0	2.6	nd	34.2	46.3	57.0	83.1	88.0	77.5	49.5	45.3	43.1	29.6	66.6	39.9	88.8	28.5
PCB 153	1.0	2.7	nd	39.1	50.8	61.1	86.2	93.2	77.8	46.9	45.5	41.1	28.3	65.4	40.4	90.3	27.5
PCB 180	0.5	2.1	nd	34.3	41.4	44.1	58.5	60.3	49.4	23.2	20.0	17.7	12.1	41.1	25.6	65.4	13.8
Total level [ub]	**3.0**	**9.8**	**nd**	**132.7**	**173.4**	**210.8**	**297.3**	**321.0**	**270.0**	**165.0**	**155.1**	**139.4**	**95.4**	**243.5**	**134.8**	**315.9**	**94.8**

Appendix 5. Levels of dioxins, non-ortho and mono-ortho PCBs in the different feeds used in study B. Results are expressed as the mean of 3 to 5 analyses, with the standard deviation and the variation coefficient. Individual levels in ng/kg. Total levels expressed in ng TEQ/kg.

	Soil A	Soil B	Feed E			Feed E + sand			10% soil A			10% soil B		
			mean	SD	%CV	mean	SD	%CV	mean	SD	%CV	mean	SD	%CV
Dioxins														
2,3,7,8-TCDF	1.1	0.45	0.11	0.00	4	0.10	0.01	6	0.12	0.03	24	0.09	0.02	22
1,2,3,7,8-PeCDF	1.1	0.53	0.24	0.02	7	0.23	0.02	7	0.11	0.04	33	0.10	0.03	30
2,3,4,7,8-PeCDF	1.5	0.72	0.26	0.01	3	0.24	0.01	3	0.18	0.04	20	0.15	0.02	16
1,2,3,4,7,8-HxCDF	1.7	1.1	0.29	0.01	5	0.25	0.01	2	0.17	0.04	23	0.13	0.03	19
1,2,3,6,7,8-HxCDF	1.4	0.78	0.26	0.01	4	0.24	0.01	3	0.14	0.04	27	0.11	0.03	26
2,3,4,6,7,8-HxCDF	2.3	1.2	0.23	0.05	20	0.23	0.01	6	0.21	0.05	23	0.13	0.02	15
1,2,3,7,8,9-HxCDF	0.41	0.34	0.24	0.02	10	0.22	0.02	7	0.07	0.04	49	0.08	0.02	22
1,2,3,4,6,7,8-HpCDF	43	17	0.99	0.48	49	0.53	0.04	8	3.59	0.47	13	1.22	0.08	7
1,2,3,4,7,8,9-HpCDF	0.86	0.55	<0.25			<0.25			0.10	0.04	37	0.08	0.03	33
OCDF	32	15	1.04	0.30	29	0.68	0.03	4	2.90	0.42	14	1.25	0.09	8
2,3,7,8-TCDD	0.15	0.065	0.10	0.01	9	0.09	0.01	8	0.04	0.03	68	0.04	0.01	33
1,2,3,7,8-PeCDD	0.76	0.37	0.26	0.02	9	0.24	0.01	4	0.09	0.04	44	0.07	0.04	58
1,2,3,4,7,8-HxCDD	0.86	0.28	0.29	0.02	6	0.27	0.02	8	0.12	0.04	37	0.08	0.03	43
1,2,3,6,7,8-HxCDD	3.6	0.76	0.29	0.03	11	0.23	0.01	4	0.34	0.05	13	0.11	0.03	27
1,2,3,7,8,9-HxCDD	1.8	1.5	0.30	0.02	6	0.26	0.00	1	0.18	0.04	25	0.10	0.03	34
1,2,3,4,6,7,8-HpCDD	52	10	1.07	0.57	54	0.47	0.02	4	4.06	0.47	12	0.91	0.06	6
OCDD	286	61	5.62	3.86	69	1.99	0.06	3	23.85	3.06	13	5.26	0.52	10
Total level [ub]	**4.0**	**1.7**	**0.73**	**0.03**	**5**	**0.65**	**0.02**	**4**	**0.44**	**0.12**	**26**	**0.29**	**0.09**	**30**
non-ortho-PCBs														
PCB 81	0.29	0.19	3.85	0.43	11	3.60	224	6	0.60	0.63	105	0.86	0.52	60
PCB 77	4.3	3.1	8.62	0.80	9	7.93	381	5	1.79	1.35	76	2.25	1.07	48
PCB 126	3.2	1.4	3.88	0.35	9	3.55	164	5	0.90	0.67	74	0.99	0.53	53
PCB 169	0.63	0.38	3.71	0.36	10	3.47	202	6	0.61	0.68	112	0.83	0.51	61
Total level [ub]	**0.32**	**0.15**	**0.43**	**0.04**	**9**	**0.39**	**0.02**	**5**	**0.10**	**0.07**	**76**	**0.11**	**0.06**	**54**

	Soil A	Soil B	Feed E			Feed E + sand			10% soil A			10% soil B		
			mean	SD	%CV	mean	SD	%CV	mean	SD	%CV	mean	SD	%CV
mono-ortho PCBs														
PCB 123	*	*	<10			<10			<10			<10		
PCB 118	170	52	1288	121	9	1183	68	6	193	231	119	296	169	57
PCB 114	<10	<10	<10			<10			<10			<10		
PCB 105	68	21	719	70	10	657	25	4	128	139	108	206	64	31
PCB 167	29	<10	<10			<10			<10			<10		
PCB 156	61	17	115	10	8	105	6	5	44	8	18	44	23	51
PCB 157	13	<10	11	0	2	<10			<10			<10		
PCB 189	<10	<10	<10			<10			<10			<10		
Total level [ub]	**0.07**	**0.03**	**0.27**	**0.02**	**9**	**0.25**	**0.01**	**5**	**0.05**	**0.05**	**85**	**0.08**	**0.05**	**59**
Sum [lb]	**4.4**	**1.9**	**1.41**			**1.28**			**0.53**			**0.37**		
Som [ub]	**4.4**	**1.9**	**1.42**	**0.09**	**7**	**1.29**	**0.05**	**4**	**0.64**	**0.17**	**27**	**0.54**	**0.13**	**23**

Appendix 6. Levels of dioxins, non-ortho and mono-ortho PCBs in eggs from hens fed with feed E, feed E mixed with 10% sand or the blank feed A mixed with 10% soil A or soil B. Individual levels expressed in pg/g fat (dioxins. non-ortho PCBs) and ng/g fat (mono-ortho PCBs). Total levels expressed as pg TEQ/g fat.

	feed E						feed E + 10% sand					feed A with 10% soil A					Feed A with 10% soil B				
Day	**t=0**	**5**	**10**	**14**	**22**	**32**	**5**	**10**	**14**	**22**	**32**	**5**	**10**	**14**	**22**	**32**	**5**	**10**	**14**	**22**	**32**
dioxins																					
2,3,7,8-TCDF	0.32	0.54	0.81	1.02	0.98	1.02	0.51	0.71	0.73	0.96	1.09	0.33	0.53	0.66	0.72	0.79	0.49	0.46	0.55	0.64	0.50
1,2,3,7,8-PeCDF	0.17	0.67	1.76	2.40	*	2.63	0.72	1.53	*	2.67	2.65	0.17	*	*	0.64	0.62	0.32	*	*	0.53	*
2,3,4,7,8-PeCDF	0.43	0.90	2.04	2.38	2.56	2.41	0.90	1.57	1.72	2.41	2.47	0.43	0.88	0.88	1.09	0.85	0.55	0.71	0.68	0.81	0.84
1,2,3,4,7,8-HxCDF	0.29	0.88	2.33	3.13	2.87	2.84	0.84	2.10	2.32	2.76	3.10	0.28	1.00	1.22	1.29	1.25	0.54	0.67	0.86	0.93	0.88
1,2,3,6,7,8-HxCDF	0.11	0.71	2.45	2.89	2.59	2.66	0.71	1.91	2.03	2.61	2.72	*	0.71	0.96	1.00	1.01	0.33	0.46	0.59	0.64	0.63
2,3,4,6,7,8-HxCDF	*	0.66	2.61	2.71	2.60	3.22	0.70	1.83	2.42	2.52	1.33	0.26	0.97	1.54	1.30	1.58	*	0.59	0.91	0.78	1.00
1,2,3,7,8,9-HxCDF	<0.10	0.59	1.91	2.51	2.38	2.48	0.62	1.71	1.80	2.37	2.57	<0.10	<0.10	0.25	<0.10	0.16	<0.10	<0.10	0.10	<0.10	0.11
1,2,3,4,6,7,8-HpCDF	<0.25	0.72	1.43	1.74	1.45	1.46	0.62	1.25	1.29	1.54	1.47	*	10.90	11.80	12.70	10.50	*	4.26	5.21	4.82	3.70
1,2,3,4,7,8,9-HpCDF	<0.25	0.46	1.32	1.51	1.33	1.22	0.45	1.11	1.11	1.30	1.40	<0.25	0.26	0.30	0.32	0.30	<0.25	<0.25	<0.25	<0.25	<0.25
OCDF	<0.50	0.52	0.95	1.08	0.95	0.75	0.59	0.80	0.77	0.85	1.04	*	3.40	3.52	3.81	*	1.44	1.73	2.02	1.87	1.43
2.3.7.8-TCDD	0.10	0.13	0.55	0.76	0.86	0.94	0.15	0.39	0.56	0.80	1.19	<0.05	<0.05	0.19	0.08	0.08	<0.05	0.07	0.05	<0.05	0.08
1.2.3.7.8-PeCDD	0.11	0.47	1.86	2.69	2.64	2.99	0.48	1.54	1.82	2.57	3.11	<0.10	0.26	0.42	0.39	0.53	0.13	<0.10	0.14	0.17	0.13
1.2.3.4.7.8-HxCDD	<0.10	0.71	2.69	3.56	3.37	3.36	0.67	2.25	2.64	3.17	3.51	<0.10	0.51	0.67	0.71	0.69	*	0.18	*	0.28	*
1.2.3.6.7.8-HxCDD	0.16	0.76	2.42	3.07	2.84	3.10	0.70	2.13	2.32	2.71	2.99	0.24	2.72	3.38	3.65	3.47	0.82	0.64	0.78	0.84	0.80
1.2.3.7.8.9-HxCDD	<0.10	0.69	2.25	2.85	2.73	2.59	0.62	1.93	2.05	2.61	2.68	0.13	0.88	0.97	1.20	1.11	*	0.33	0.49	0.46	0.43
1.2.3.4.6.7.8-HpCDD	1.32	1.07	1.85	2.25	1.82	1.95	1.01	1.57	1.63	1.80	1.90	1.57	18.90	20.30	23.30	18.60	5.36	3.83	5.58	4.93	4.18
OCDD	14.76	3.61	4.55	4.89	3.66	4.18	3.07	3.12	3.37	3.51	3.83	5.62	75.20	71.10	94.00	66.90	20.10	12.10	15.40	18.20	11.40
Total level [ub]	**0.55**	**1.66**	**5.31**	**6.99**	**6.87**	**7.44**	**1.67**	**4.29**	**4.92**	**6.73**	**7.71**	**0.55**	**1.80**	**2.35**	**2.41**	**2.37**	**0.78**	**0.96**	**1.08**	**1.22**	**1.16**
non-ortho PCBs																					
PCB 81	0.6	4.5	18.4	27.2	31.8	40.3	4.0	15.7	19.1	29.4	36.3	0.4	0.6	1.3	0.6	0.7	0.5	0.5	0.6	0.5	0.7
PCB 77	11.0	13.6	41.6	69.5	70.6	95.2	13.0	36.6	51.6	65.5	91.0	4.6	6.2	15.4	6.8	16.2	*	5.9	11.8	6.2	12.4
PCB 126	1.4	8.0	27.3	37.8	39.2	47.4	7.2	22.8	26.3	37.1	44.2	1.6	2.9	3.7	3.6	4.0	1.9	2.0	2.2	2.4	2.3
PCB 169	0.3	10.8	39.1	51.7	48.0	50.8	9.5	32.6	37.2	46.0	48.8	0.2	0.8	1.3	1.0	*	*	0.5	0.7	0.6	0.8
Total levels [ub]	**0.15**	**0.91**	**3.13**	**4.31**	**4.41**	**5.26**	**0.81**	**2.61**	**3.01**	**4.18**	**4.92**	**0.16**	**0.30**	**0.38**	**0.37**	**0.40**	**0.19**	**0.21**	**0.23**	**0.25**	**0.24**

		feed E					feed E + 10% sand					feed A with 10% soil A					Feed A with 10% soil B				
Day	**t=0**	**5**	**10**	**14**	**22**	**32**	**5**	**10**	**14**	**22**	**32**	**5**	**10**	**14**	**22**	**32**	**5**	**10**	**14**	**22**	**32**
mono-ortho PCBs																					
PCB 123	<0.01	0.02	0.05	0.07	0.07	0.08	0.11	0.04	0.05	0.07	0.08	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	*	*	*	*
PCB 118	0.25	2.36	8.53	12.00	12.40	14.70	2.24	7.10	8.60	12.30	13.90	0.27	0.33	0.38	0.34	0.39	0.33	0.27	0.29	0.26	0.29
PCB 114	0.01	0.01	0.04	0.05	0.05	0.06	0.10	0.03	0.03	0.05	0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PCB 105	0.07	1.09	4.19	6.24	6.38	8.14	1.06	3.52	4.54	6.41	7.72	0.08	0.10	0.12	0.11	0.13	0.09	0.08	0.09	0.08	0.09
PCB 167	0.03	0.06	0.12	0.16	0.14	0.15	0.12	0.11	0.13	0.14	0.15	0.03	0.05	0.04	0.05	0.04	0.05	0.03	0.03	0.03	0.03
PCB 156	0.07	0.34	1.06	1.40	1.34	1.48	0.37	0.90	1.03	1.31	1.40	0.06	0.11	0.12	0.11	0.11	0.10	0.07	0.07	0.07	0.07
PCB 157	<0.01	0.03	0.10	0.12	0.12	0.12	0.07	0.09	0.09	0.13	0.13	<0.01	*	0.02	0.02	0.02	0.01	<0.01	0.01	0.01	0.01
PCB 189	0.02	0.04	0.11	0.13	0.12	0.12	0.06	0.09	0.10	0.12	0.12	0.01	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.01	*
Total level [ub]	**0.08**	**0.54**	**1.89**	**2.63**	**2.65**	**3.14**	**0.62**	**1.59**	**1.91**	**2.63**	**2.98**	**0.08**	**0.11**	**0.12**	**0.12**	**0.12**	**0.11**	**0.08**	**0.09**	**0.08**	**0.08**
Sum [ub]	**0.77**	**3.11**	**10.32**	**13.93**	**13.93**	**15.84**	**3.10**	**8.48**	**9.84**	**13.54**	**15.61**	**0.79**	**2.20**	**2.85**	**2.90**	**2.90**	**1.08**	**1.25**	**1.40**	**1.55**	**1.48**

Appendix 7. Levels of indicator PCBs in eggs from hens fed with feed E, feed E mixed with 10% sand or the blank feed A mixed with 10% soil A or soil B. Individual levels expressed in ng/g fat.

	t=0	feed E					feed E + 10% sand					feed A with 10% soil A					feed A with 10% soil B				
Day	**0**	**5**	**10**	**14**	**22**	**32**	**5**	**10**	**14**	**22**	**32**	**5**	**10**	**14**	**22**	**32**	**5**	**10**	**14**	**22**	**32**
PCB 28	0.97	1.49	3.71	5.29	7.35	9.23	1.18	3.26	3.78	6.48	7.80	0.96	0.86	0.56	0.90	0.43	1.06	0.58	<0.4	0.72	<0.4
PCB 52	<0.40	1.27	1.95	2.08	2.01	2.07	0.82	1.82	1.91	2.59	2.08	0.49	0.41	0.55	0.45	0.52	0.78	0.45	0.41	0.61	<0.4
PCB 101	0.55	2.52	4.56	5.64	4.94	5.60	1.84	4.72	4.84	5.23	5.88	0.75	0.64	1.09	0.57	0.99	1.15	0.64	0.84	0.65	0.74
PCB 118	0.55	2.93	7.79	11.07	12.23	15.31	2.13	7.00	8.04	10.76	13.18	0.76	0.65	0.95	0.61	0.92	1.06	0.68	0.77	0.69	0.72
PCB 138	1.94	8.46	20.28	26.56	28.41	34.04	6.53	19.23	20.25	26.26	30.12	2.41	2.02	3.13	2.05	3.15	3.80	2.66	2.34	2.86	2.08
PCB 153	1.40	8.76	23.23	30.98	32.39	36.74	6.77	21.25	22.49	28.12	32.44	1.72	1.49	2.35	1.49	2.25	2.56	2.03	1.57	2.11	1.58
PCB 180	0.76	6.89	20.52	27.83	26.68	28.64	6.02	19.25	20.66	22.53	25.62	0.91	0.64	1.82	0.75	1.65	1.23	1.19	1.27	1.10	1.29
Total level [ub]	**6.52**	**32.32**	**82.04**	**109.45**	**114.02**	**131.62**	**25.29**	**76.54**	**81.97**	**101.96**	**117.12**	**8.00**	**6.71**	**10.45**	**6.83**	**9.90**	**11.64**	**8.22**	**7.51**	**8.73**	**7.16**

Appendix 8. Levels of dioxins, non-ortho and mono-ortho PCBs in abdominal fat from hens fed with feed E, feed E mixed with 10% sand or the blank feed A mixed with 10% soil A or soil B for 32 days. Individual levels expressed in pg/g fat (dioxins, non-ortho PCBs) and ng/g fat (mono-ortho PCBs). Total levels expressed as pg TEQ/g fat.

	feed E		feed E + 10% sand		feed A with 10% soil A		feed A with 10% soil B	
Day	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
Dioxins								
2,3,7,8-TCDF	1.13	0.16	1.08	0.17	0.81	0.12	0.59	0.06
1,2,3,7,8-PeCDF	2.31	0.41	2.08	0.37	0.50	0.04	0.43	0.06
2,3,4,7,8-PeCDF	2.36	0.37	2.14	0.35	0.90	0.07	0.76	0.10
1,2,3,4,7,8-HxCDF	1.46	0.26	1.31	0.25	0.59	0.06	0.47	0.11
1,2,3,6,7,8-HxCDF	1.36	0.29	1.16	0.18	0.44	0.07	0.36	0.06
2,3,4,6,7,8-HxCDF	1.46	0.38	1.38	0.46	0.82	0.18	0.47	0.12
1,2,3,7,8,9-HxCDF	1.38	0.27	1.20	0.23	<0.10		<0.10	
1,2,3,4,6,7,8-HpCDF	0.37	0.06	0.33	0.08	1.87	0.30	0.82	0.22
1,2,3,4,7,8,9-HpCDF	0.33	0.06	0.30	0.05	<0.25		<0.25	
OCDF	<0.50		0.87	0.48	0.62	0.10	<0.5	
2.3.7.8-TCDD	1.21	0.22	1.15	0.24	0.11	0.03	0.08	0.01
1.2.3.7.8-PeCDD	2.58	0.50	2.30	0.49	0.32	0.04	0.14	0.05
1.2.3.4.7.8-HxCDD	1.76	0.16	1.70	0.41	0.37	0.07	0.17	0.03
1.2.3.6.7.8-HxCDD	1.65	0.39	1.46	0.32	1.65	0.23	0.44	0.10
1.2.3.7.8.9-HxCDD	1.25	0.25	1.12	0.26	0.49	0.07	0.21	0.03
1.2.3.4.6.7.8-HpCDD	0.47	0.08	0.40	0.09	3.02	0.45	0.90	0.21
OCDD	1.37	0.45	1.09	0.14	3.32	0.36	1.76	0.35
Total level [ub]	6.21	1.05	5.68	1.07	1.48	0.13	0.88	0.15
non-ortho PCBs								
PCB 81	46.8	6.3	42.3	8.3	0.7	0.1	0.54	0.15
PCB 77	94.6	11.7	87.0	15.7	8.9	1.0	6.13	0.58
PCB 126	41.4	7.0	37.6	7.4	3.4	0.5	2.27	0.67
PCB 169	29.8	6.3	26.9	6.6	0.6	0.1	0.44	0.14
Total levels [ub]	4.45	0.76	4.04	0.81	0.32	0.05	0.23	0.07

	feed E		feed E + 10% sand		feed A with 10% soil A		feed A with 10% soil B	
Day	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
mono-ortho PCBs								
PCB 123	79	14	61	18	371	96	228	
PCB 118	15040	2682	13040	2490	<10		263	84
PCB 114	64	19	54	12	124	30	<10	
PCB 105	8620	1308	7372	1318	37	8	69	35
PCB 167	107	15	100	26	97	19	31	18
PCB 156	1112	218	979	229	14	2	60	14
PCB 157	89	19	88	20	16	2	11	
PCB 189	47	10	43	12	371	96	<10	
Total level [ub]	3.01	0.53	2.60	0.52	0.10	0.02	0.08	0.02
Sum [ub]	13.67	2.32	12.32	2.39	1.91	0.20	1.19	0.23

Appendix 9. Levels of dioxins, non-ortho and mono-ortho PCBs in eggs from hens kept at the two farms where the soil was sampled in 2002, and which had been sampled previously in 2001. Individual levels expressed in pg/g fat (dioxins. non-ortho PCBs) and ng/g fat (mono-ortho PCBs). Total levels expressed as pg TEQ/g fat.

	Eggs farm A		**Eggs farm B**	
	2001	**2002**	**2001**	**2002**
dioxins				
2,3,7,8-TCDF	2.84	2.3	0.63	0.72
1,2,3,7,8-PeCDF	2.46	2.3	0.28	0.54
2,3,4,7,8-PeCDF	3.56	3.1	0.44	0.66
1,2,3,4,7,8-HxCDF	3.78	3.5	0.35	0.52
1,2,3,6,7,8-HxCDF	2.88	2.8	0.26	0.40
2,3,4,6,7,8-HxCDF	3.98	3.8	0.36	0.43
1,2,3,7,8,9-HxCDF	0.15	0.16	< 0.10	< 0.10
1,2,3,4,6,7,8-HpCDF	27.7	21.6	1.58	1.2
1,2,3,4,7,8,9-HpCDF	0.62	0.54	< 0.25	< 0.25
OCDF	6.62	4.9	0.68	< 0.50
2.3.7.8-TCDD	0.55	0.34	0.07	0.09
1.2.3.7.8-PeCDD	2.00	1.9	0.12	0.31
1.2.3.4.7.8-HxCDD	2.64	2.6	0.16	0.28
1.2.3.6.7.8-HxCDD	11.5	10	0.39	0.64
1.2.3.7.8.9-HxCDD	3.15	3.1	0.20	0.27
1.2.3.4.6.7.8-HpCDD	40.9	37	2.19	1.9
OCDD	74.5	54	8.51	3.8
Total level [ub]	**8.25**	**7.29**	**0.70**	**1.13**
non-ortho PCBs				
PCB 81	1.73	< 0.05	1.18	8.7
PCB 77	21.7	28	16.1	148
PCB 126	15.9	16	44.6	13
PCB 169	2.82	3.0	6.24	2.7
Total levels [ub]	**1.62**	**1.63**	**4.53**	**1.37**
mono-ortho PCBs				
PCB 123	0.02	0.02	<0.01	0.03
PCB 118	1.09	1.1	0.85	1.2
PCB 114	0.01	0.01	<0.01	0.04
PCB 105	0.31	0.36	0.16	0.52
PCB 167	0.24	0.20	0.28	0.12
PCB 156	0.44	0.38	0.43	0.19
PCB 157	0.07	0.06	0.07	0.03
PCB 189	0.07	0.06	0.07	0.05
Total level [ub]	**0.41**	**0.38**	**0.36**	**0.31**
Sum [ub]	**9.87**	**9.31**	**5.22**	**2.81**

Appendix 10. Levels of dioxins, non-ortho and mono-ortho PCBs in the different feeds used in study C. Results are expressed as the mean of 3 to 5 analyses, with the standard deviation and the variation coefficient. Individual levels in ng/kg. Total levels expressed in ng TEQ/kg.

	feed E			Exal H			MycoAd Z			Humac			Klinofeed		
	mean	**SD**	**%CV**	**mean**	**SD**	**%CV**	**mean**	**SD**	**%CV**	**mean**	**SD**	**%CV**	**mean**	**SD**	**%CV**
Dioxins															
2,3,7,8-TCDF	0.11	0.01	4	0.11	0.01	8	0.11	0.00	4	0.11	0.01	9	0.11	0.01	10
1,2,3,7,8-PeCDF	0.24	0.02	7	0.29	0.00	1	0.29	0.01	3	0.30	0.01	2	0.28	0.01	4
2,3,4,7,8-PeCDF	0.26	0.01	3	0.28	0.01	2	0.30	0.01	4	0.29	0.01	3	0.28	0.01	2
1,2,3,4,7,8-HxCDF	0.29	0.01	5	0.32	0.01	4	0.33	0.02	5	0.31	0.01	3	0.31	0.01	4
1,2,3,6,7,8-HxCDF	0.26	0.01	4	0.29	0.01	3	0.30	0.01	4	0.29	0.01	2	0.28	0.01	3
2,3,4,6,7,8-HxCDF	0.22	0.05	20	0.33	0.01	2	0.33	0.01	4	0.31	0.01	5	0.30	0.01	2
1,2,3,7,8,9-HxCDF	0.24	0.02	10	0.30	0.00	1	0.30	0.01	3	0.29	0.02	6	0.29	0.01	3
1,2,3,4,6,7,8-HpCDF	0.99	0.48	49	0.39	0.02	4	0.39	0.01	3	0.38	0.01	2	0.38	0.01	4
1,2,3,4,7,8,9-HpCDF	<0.25			<0.25			<0.25			<0.25			<0.25		
OCDF	1.04	0.30	29	0.62	0.07	11	0.63	0.05	8	0.58	0.02	3	0.57	0.02	3
2,3,7,8-TCDD	0.10	0.01	9	0.13	0.00	3	0.13	0.01	5	0.12	0.01	8	0.13	0.02	14
1,2,3,7,8-PeCDD	0.26	0.02	9	0.35	0.03	8	0.38	0.05	13	0.32	0.02	7	0.32	0.01	3
1,2,3,4,7,8-HxCDD	0.29	0.02	6	0.35	0.01	3	0.36	0.02	4	0.36	0.01	4	0.35	0.01	4
1,2,3,6,7,8-HxCDD	0.29	0.03	11	0.29	0.01	3	0.30	0.02	5	0.29	0.01	3	0.29	0.01	3
1,2,3,7,8,9-HxCDD	0.30	0.02	6	0.33	0.01	2	0.34	0.01	4	0.33	0.01	2	0.34	0.01	4
1,2,3,4,6,7,8-HpCDD	1.07	0.57	54	0.42	0.07	17	0.40	0.02	5	0.39	0.01	3	0.38	0.02	5
OCDD	5.62	3.86	69	1.76	1.28	73	1.19	0.09	7	1.20	0.15	12	1.14	0.14	13
Total level [ub]	**0.73**	**0.03**	**5**	**0.87**	**0.03**	**4**	**0.93**	**0.06**	**7**	**0.84**	**0.04**	**5**	**0.84**	**0.03**	**4**
non-ortho-PCBs															
PCB 81	3.85	0.43	11	4.79	0.13	3	4.96	0.11	2	4.87	0.09	2	4.62	0.07	1
PCB 77	8.62	0.80	9	10.49	0.34	3	11.00	0.29	3	11.36	0.21	2	10.36	0.11	1
PCB 126	3.88	0.35	9	4.78	0.10	2	4.94	0.16	3	4.81	0.10	2	4.67	0.11	2
PCB 169	3.71	0.36	10	4.76	0.09	2	4.89	0.17	3	4.63	0.15	3	4.53	0.10	2
Total level [ub]	**0.43**	**0.04**	**9**	**0.53**	**0.01**	**2**	**0.55**	**0.02**	**3**	**0.53**	**0.01**	**2**	**0.51**	**0.01**	**2**

	feed E			Exal H			MycoAd Z			Humac			Klinofeed		
	mean	**SD**	**%CV**	**mean**	**SD**	**%CV**	**mean**	**SD**	**%CV**	**mean**	**SD**	**%CV**	**mean**	**SD**	**%CV**
mono-ortho PCBs															
PCB 123	<0.01			<0.01			<0.01			<0.01			<0.01		
PCB 118	1.29	0.12	9	1.57	0.04	3	1.62	0.06	3	1.63	0.02	1	1.58	0.06	4
PCB 114	<0.01			<0.01			<0.01			<0.01			<0.01		
PCB 105	0.72	0.07	10	0.89	0.02	2	0.91	0.03	3	0.92	0.01	1	0.90	0.03	3
PCB 167	<0.01			0.01	0.00	1	0.01	0.00	3	0.01	0.00	1	0.02	0.01	63
PCB 156	0.12	0.01	8	0.14	0.00	2	0.15	0.01	3	0.15	0.00	1	0.15	0.01	9
PCB 157	0.01	0.00	2	0.01	0.00	5	0.01	0.00	5	0.01	0.00	5	0.02	0.01	65
PCB 189	<0.01			<0.01			<0.01			<0.01			<0.01		
Total level [ub]	**0.27**	**0.02**	**9**	**0.33**	**0.01**	**2**	**0.34**	**0.01**	**3**	**0.34**	**0.00**	**1**	**0.34**	**0.02**	**6**
Som [ub]	**1.42**	**0.09**	**7**	**1.73**	**0.04**	**2**	**1.81**	**0.08**	**5**	**1.71**	**0.05**	**3**	**1.70**	**0.05**	**3**

Appendix 11. Levels of dioxins, non-ortho and mono-ortho PCBs in eggs (day14,32)and abdominal fat from hens fed with feed E containing 4 different binders. Individual levels expressed in pg/g fat (dioxins. non-ortho PCBs) and ng/g fat (mono-ortho PCBs). Total levels expressed as pg TEQ/g fat.

	feed E			Exal H			MycoAd Z			Humac			Klinofeed		
Day	**14**	**32**	**fat**	**14**	**32**	**fat**	**14**	**32**	**fat**	**14**	**32**	**fat**	**14**	**32**	**fat**
dioxins															
2,3,7,8-TCDF	1.02	1.02	1.13	0.74	1.08	1.20	0.64	0.98	1.08	0.84	1.19	1.22	0.66	*	0.98
1,2,3,7,8-PeCDF	2.40	2.63	2.31	*	2.57	2.27	1.44	*	1.92	1.95	*	2.42	*	2.44	1.66
2,3,4,7,8-PeCDF	2.38	2.41	2.36	1.75	2.65	2.23	1.42	2.26	2.06	1.81	3.07	2.46	1.76	2.40	1.87
1,2,3,4,7,8-HxCDF	3.13	2.84	1.46	2.26	2.70	1.39	1.86	2.34	1.21	2.56	3.10	1.57	2.21	2.50	1.13
1,2,3,6,7,8-HxCDF	2.89	2.66	1.36	2.03	2.43	1.16	1.72	2.05	1.02	2.22	2.90	1.30	1.93	2.23	0.94
2,3,4,6,7,8-HxCDF	2.71	3.22	1.46	2.58	1.99	0.99	1.20	2.72	0.84	2.70	3.42	0.93	2.44	2.64	0.84
1,2,3,7,8,9-HxCDF	2.51	2.48	1.38	1.85	2.30	1.26	1.37	1.72	0.93	1.98	2.73	1.40	1.73	2.12	1.05
1,2,3,4,6,7,8-HpCDF	1.74	1.46	0.37	1.33	1.41	0.34	*	1.10	0.28	1.54	1.71	0.29	1.22	1.22	0.29
1,2,3,4,7,8,9-HpCDF	1.51	1.22	0.33	1.09	1.19	0.35	0.85	0.94	0.31	1.34	1.35	0.35	1.02	1.01	0.27
OCDF	1.08	0.75	<0.50	0.77	0.80	<0.50	0.66	0.67	<0.50	0.89	0.96	<0.50	0.72	0.74	<0.50
2.3.7.8-TCDD	0.76	0.94	1.21	0.58	1.01	1.10	0.52	0.86	1.06	0.65	1.22	1.32	0.52	0.94	0.92
1.2.3.7.8-PeCDD	2.69	2.99	2.58	1.97	2.97	2.36	1.59	2.46	2.26	2.02	3.32	2.73	1.77	2.76	1.98
1.2.3.4.7.8-HxCDD	3.56	3.36	1.76	2.48	3.14	1.81	2.33	2.77	1.71	2.82	3.64	2.09	2.44	2.90	1.42
1.2.3.6.7.8-HxCDD	3.07	3.10	1.65	2.28	2.73	1.55	2.07	2.50	1.43	2.52	3.39	1.67	2.15	2.69	1.25
1.2.3.7.8.9-HxCDD	2.85	2.59	1.25	1.98	2.47	1.22	1.59	1.91	1.01	2.27	2.91	1.35	2.00	2.29	0.97
1.2.3.4.6.7.8-HpCDD	2.25	1.95	0.47	1.79	1.75	0.44	1.39	1.63	0.43	2.07	2.22	0.47	1.60	1.75	0.39
OCDD	4.89	4.18	1.37	3.65	3.70	0.87	3.04	4.28	1.26	4.50	4.96	0.79	3.62	3.56	0.77
Total level [ub]	**6.99**	**7.44**	**6.21**	**5.10**	**7.36**	**5.71**	**4.20**	**6.19**	**5.37**	**5.51**	**8.46**	**6.53**	**4.77**	**6.81**	**4.75**
non-ortho PCBs															
PCB 81	27.2	40.3	46.8	21.3	38.5	41.7	17.6	34.9	39.3	22.0	44.9	48.4	18.3	33.5	34.8
PCB 77	69.5	95.2	94.6	60.6	94.8	85.2	47.2	92.5	82.0	62.5	109.0	99.2	49.4	82.3	73.2
PCB 126	37.8	47.4	41.4	26.7	42.3	39.7	23.8	38.9	36.8	29.0	48.9	44.5	24.8	38.5	31.6
PCB 169	51.7	50.8	29.8	35.4	45.2	28.6	31.6	41.7	26.2	40.2	55.6	32.2	35.4	44.0	22.7
Total levels [ub]	**4.31**	**5.26**	**4.45**	**3.03**	**4.70**	**4.27**	**2.70**	**4.32**	**3.96**	**3.31**	**5.46**	**4.79**	**2.84**	**4.30**	**3.40**

	feed E			Exal H			MycoAd Z			Humac			Klinofeed		
Day	**14**	**32**	**fat**	**14**	**32**	**fat**	**14**	**32**	**fat**	**14**	**32**	**fat**	**14**	**32**	**fat**
mono-ortho PCBs															
PCB 123	0.07	0.08	0.08	0.05	0.08	0.07	0.04	0.07	0.07	0.06	0.09	0.08	0.05	0.07	0.06
PCB 118	12.00	14.70	15.04	8.50	13.90	14.18	7.36	12.00	12.73	9.06	15.90	15.82	7.52	11.90	11.39
PCB 114	0.05	0.06	0.06	0.04	0.06	0.06	0.04	0.05	0.05	0.04	0.07	0.07	0.03	0.05	0.05
PCB 105	6.24	8.14	8.62	4.46	7.67	8.14	3.85	6.53	7.21	4.77	8.69	9.05	3.89	6.52	6.48
PCB 167	0.16	0.15	0.11	0.12	0.14	0.11	0.10	0.12	0.10	0.13	0.16	0.12	0.11	0.13	0.09
PCB 156	1.40	1.48	1.11	0.99	1.34	1.11	0.88	1.21	0.98	1.09	1.59	1.16	0.91	1.22	0.83
PCB 157	0.12	0.12	0.09	0.08	0.11	0.09	0.08	0.11	0.08	0.10	0.14	0.10	0.09	0.11	0.07
PCB 189	0.13	0.12	0.05	0.09	0.11	0.05	0.09	0.10	0.05	0.10	0.13	0.05	0.09	0.10	0.04
Total level [ub]	**2.63**	**3.14**	**3.01**	**1.87**	**2.93**	**2.87**	**1.63**	**2.55**	**2.56**	**2.01**	**3.38**	**3.17**	**1.67**	**2.55**	**2.27**
Sum [ub]	**13.93**	**15.84**	**13.67**	**10.00**	**14.99**	**12.86**	**8.53**	**13.06**	**11.89**	**10.83**	**17.30**	**14.48**	**9.28**	**13.66**	**10.42**

Appendix 12. Levels of indicator-PCBs in eggs (day 14,32) and abdominal fat from hens fed with feed 750 containing 4 different binders. Individual levels expressed in ng/g fat

	feed E sand			Exal H			MycoAd Z			Humac			Klinofeed		
Day	**14**	**32**	**fat**	**14**	**32**	**fat**	**14**	**32**	**fat**	**14**	**32**	**fat**	**14**	**32**	**fat**
PCB 28	3.8	7.8	9.2	3.9	7.9	9.5	3.4	6.8	8.7	4.4	8.8	8.7	3.4	6.6	7.8
PCB 52	1.9	2.1	2.4	1.6	1.7	1.8	1.5	1.5	1.8	2.1	2.1	2.6	1.4	1.5	1.6
PCB 101	4.8	5.9	5.2	4.3	4.5	4.2	4.3	4.5	4.3	5.2	5.6	7.0	3.7	4.1	3.7
PCB 118	8.0	13.2	11.8	8.0	13.0	12.5	7.2	11.0	11.3	8.9	14.5	16.6	7.3	11.3	10.1
PCB 138	20.3	30.1	23.5	19.4	28.4	25.3	18.1	25.1	23.0	23.1	32.9	26.8	18.9	26.0	20.1
PCB 153	22.5	32.4	21.9	21.6	30.1	23.9	20.0	26.2	21.5	24.4	34.5	22.7	20.8	27.6	18.7
PCB 180	20.7	25.6	10.0	19.3	23.4	11.6	18.4	21.4	10.1	22.8	27.5	11.9	19.4	22.6	8.8
Total level [ub]	**82.0**	**117.1**	**83.9**	**78.0**	**109.0**	**88.7**	**73.0**	**96.5**	**80.6**	**90.7**	**125.9**	**95.6**	**74.8**	**99.7**	**70.8**

Appendix 13. Carry-over rates of the different dioxin and PCB congeners as determined at day 42 for the two feeds containing the highest levels

	feed E	feed F
Dioxins		
2,3,7,8-TCDF	40	40
1,2,3,7,8-PeCDF	39	48
2,3,4,7,8-PeCDF	38	44
1,2,3,4,7,8-HxCDF	43	44
1,2,3,6,7,8-HxCDF	40	42
2,3,4,6,7,8-HxCDF	31	35
1,2,3,7,8,9-HxCDF	39	41
1,2,3,4,6,7,8-HpCDF	18	18
1,2,3,4,7,8,9-HpCDF	20	21
OCDF	7	5
2,3,7,8-TCDD	41	47
1,2,3,7,8-PeCDD	41	45
1,2,3,4,7,8-HxCDD	42	44
1,2,3,6,7,8-HxCDD	44	44
1,2,3,7,8,9-HxCDD	32	34
1,2,3,4,6,7,8-HpCDD	22	21
OCDD	13	8
non-ortho-PCBs		
PCB 81	40	41
PCB 77	45	50
PCB 126	48	50
PCB 169	50	52
mono-ortho PCBs		
PCB 123	78	70
PCB 118	48	52
PCB 114	52	51
PCB 105	46	52
PCB 167	84	76
PCB 156	55	58
PCB 157	51	65
PCB 189	61	61
Indicator-PCBs		
PCB 28	38	43
PCB 52	5	4
PCB 101	5	5
PCB 118	47	55
PCB 138	51	54
PCB 153	57	61
PCB 180	59	41

Appendix 14 Technical information Mathematical model development

The kinetics of dioxin-like substances in eggs contains three main aspects: absorption of the ingested contaminants over the gut wall, distribution of the contaminants over the different tissues and developing yolks, and elimination from the body by eggs and liver metabolism. Each different congener may have its own characteristic properties, such as its fraction absorbed, its tissue:blood partition coefficients, its metabolic clearance and its transfer from blood to the developing yolks. This may well implicate that each congener has its own typical kinetics which would force a different parameterisation of the model parameters for each different congener. In a first approach it was tried to model the mix of administrated congeners by considering the mix as if one substance only, quantitatively represented by its amount of total Toxic EQuivalents (TEQs).

Absorption

Because of the high lipophilicity of the dioxin-like substances (PCDDs, PCDFs and PCBs), it may be assumed that in the gut they are mainly dissolved in the lipid fraction of food. Consequently, the absorption of the contaminants over the gut wall is correlated to the efficiency of lipid uptake. As the hens lay eggs with a mean fat weight of 6 (g/egg) and with an efficiency of about 85%, the daily loss of fat of a hen is 5.1 (g/day). From the experiments, it appears that the mean hens food intake was 116 (g/day) with a feed lipid fraction that was estimated to range from 3 to 5%, which amounts to a lipid intake in the range of about 3.5-5.8 (g/day). Thus, lipid absorption, and so absorption of ingested contaminants, is expected to be efficient and to range from 90 to 100%.

Pirard and De Pauw (2002) found absorption percentages for PCDDs and PCDFs in a range from 90-100% for log Kow values up to 7.8, with an exception for a number of PCDFs in the log Kow range of 7.1-7.8. For still higher log Kow values, absorption declined to about 30%. However, these high chlorinated congeners tend to have Toxic Equivalent Factors (TEFs) ranging from 0.01 to 0.0001, so they do not substantially contribute to the total of administrated TEQs. For non-ortho PCBs Pirard and De Pauw found absorption percentages up to 96%, independent of the degree of chlorination.

Distribution

Partition of the contaminants between different tissues is determined by solution of the contaminants in the tissues lipid fraction and by binding to tissue proteins, mainly to (plasma) albumin. In the liver dioxins can possibly bind additionally to specific liver enzymes. Because of the lipid solubility, partition is determined by the tissues lipid content and the tissues lipid composition, i.e., the subdivision of the lipid fraction in phospholipids and neutral lipids (Poulin and Krishnan, 1995a, 1995b).
The neutral lipid content of adipose tissue is almost 100% in every mammal (*ibid*.), while in rats blood lipid is composed of 51% of phospholipids and 49% of neutral lipids, liver lipid is composed of 42% of phospholipids and 58% of neutral lipids, and muscle of 54% of phospholipids and 46% of neutral lipids (Poulin and Krishnan, 1995a). In humans, these percentages are 32% and 68% for blood, 42% and 58% for liver and 23% and 77% for muscle, respectively. So, the interspecies differences can be considerable.

Germs (1985) found lipid contents of a number of tissues but did not consider the composition of the lipid fraction of tissues. Based on these data, only crude approximations to the tissue:blood partition coefficients can be made. E.g., the lipid content of blood is about 1%. When 51% consists of phospholipids, like in rats, then the adipose tissue:blood partition coefficient is about 150, when 32% consists of phospholipids, like in humans, the partition coefficient is about 120, while when all blood lipid would be neutral lipid, then the partition would be 95. These estimations are obtained by assuming that also in hens adipose tissue consists of about 100% of neutral lipids.

Likewise, ranges for partition coefficients in other tissues can be found. Generally, from PBPK models it is found that partition coefficients of dioxin like substances for tissues other than adipose tissue range from about 5-10% of the partition coefficient for adipose tissue. However, in rats liver enzymes are induced which not only causes augmented metabolism rate, but also a considerable additional binding storage such that the liver:blood partition exceeds the adipose tissue:blood partition by several factors (Abraham *et al*., 1988; Zeilmaker and van Eijkeren, 1997, Zeilmaker *et al*., 1999). In cattle, also such a phenomenon is noticed but not to such an extreme level (Thomas *et al*., 1999).

The only way different congeners may possess substantially different tissue:blood partition coefficients is by their chemical property of binding to proteins. If such binding is negligible, the partition coefficient values based on lipid content and lipid composition only would be almost the same for all congeners.

Metabolism

Elimination by metabolism mainly takes place in the liver. Metabolism may be enhanced by induction of metabolising enzymes, just like binding storage capacity. In rats induction of enzymes takes place to such extend that both metabolism rate and storage capacity are augmented considerably (Abraham, 1988; Zeilmaker and van Eijkeren, 1997, Zeilmaker *et al*., 1999). In cattle, such an extreme induction effect is not noticed. In lactating subjects, excretion via milk fat dominates elimination by metabolism. In a first approach, it will be assumed that, likewise, in laying hens elimination by metabolism is dominated by excretion via egg yolk fat.

Excretion

In a series of papers by Donoghue and co-workers (Donoghue *et al*. 1996,1997[a] , 1997[b], 1999, 2000, 2001) investigate residue uptake by eggs of different drugs and the pesticide lindane. They clearly distinct the uptake by the developing yolks and by egg albumin. The former takes place during the development of the yolks during several days, while the latter takes place when the ovulated yolk is surrounded by the egg white in the oviduct. Because of the high lipophilicity of the contaminants, it is assumed that from the start of egg white formation there is no further transport of contaminants from blood to the egg yolk, while the contamination of egg white is considered to be negligible.

Donoghue and co-workers showed a high correlation between uptake of a single administration of drug into egg yolks and the egg yolks growth phase. Based on these observations they developed a model to predict the pattern of residues, i.e., relative levels, after a number of days of administration, but not to predict the absolute residue levels themselves (Donoghue *et al*. 1996, 1997[b], 2001). From these results it is assumed that uptake of contaminants in the developing yolks is proportional to the product of the contaminant concentration in blood and the daily growth rate of each yolk. Moreover, based on an experiment with magnevist (Donoghue and Meyers, 2000), it was assumed that incorporation of the contaminant in egg yolk is irreversible. This approach needs information about yolk development.

Yolk development and egg yolk excretion model

Gilbert (1972) presents data on ovum weight from about 8 days before ovulation to 1 day before. Donoghue *et al*. (1996) present data on daily change in yolk weight. Based on these data, a model was chosen that allowed for virtually no yolk development until 10 days before ovulation, maximum growth about 3 days before ovulation and symmetry of development during the periods before and after the time of maximum growth. Taking the time of ovulation, for reason to follow denoted by s instead of t, as $s = 0$, the model used is

$$w(s) = w_{min} + (w_{max} - w_{min})\tfrac{1}{2}(\tanh(\dot{\omega}(\tau - s)) + 1) \qquad (1)$$

Here, w_{min} is the weight of the smallest yolks, w_{max} is a virtual yolk weight that would be attained if yolk development was not stopped by ovulation and $\dot{\omega}$ determines both maximum growth rate and the time interval during with growth is essentially non zero, say from ten days before ovulation until ovulation. Note, that at the time of ovulation, yolk weight is

$$w(0) = w_0 = w_{min} + (w_{max} - w_{min})\tfrac{1}{2}(\tanh(\dot{\omega} \cdot \tau) + 1) \qquad (2)$$

A slight complication enters the yolk development model by the definition of zero time being time of ovulation: every yolk has its own time of ovulation and consequently its own subjective time. Another, objective, time measures time evolution during the experiment. If the start of the experiment is taken to be the objective time $t = 0$, then a yolk that will be ovulated at time *s* has as its growth curve

$$w(t,s) = w(t-s) = w_{min} + (w_{max} - w_{min})\tfrac{1}{2}(\tanh(\dot{\omega}(t + \tau - s)) + 1) \qquad (3)$$

Using the data in Gilbert (1972) and Donoghue *et al.* (1996) and estimating the egg yolk volume to be 1/3 of the egg weight (Kans Yellow Rule, Kan, 2002), the parameterisation of the model in equation (3) is

$$w_{min} = 0.09 \text{ (g)};\ w_{max} = 20.0 \text{ (g)};\ \dot{\omega} = 0.5 \text{ (-/day)};\ \tau = 3.3 \text{ (day)} \qquad (4)$$

This parameterisation resulted in a egg yolk weight of $w_0 = 18.1$ (g).

So, in the laying hen model, an array of developing yolks is modelled: time *s* is discrete because of the discrete time points of ovulation and to each position in the array a corresponding time of ovulation is associated. When the objective time has surpassed the time of ovulation of a yolk, its corresponding array position is ignored in the progressing calculations, otherwise it will still develop following equation (3) and contribute to the excretion by adding a term

$$\left(\frac{dA_b(t)}{dt}\right)_{\substack{\text{excretion to} \\ \text{i-th follikel}}} = -\gamma \dot{w}(t - s_i) C_b(t) \qquad (5)$$

where $A_b(t)$ and $C_b(t)$ denote the amount and concentration of TEQs in blood at time *t*, $\dot{w}(t - s_i)$ is the growth rate at time *t* of yolk *i*, to be ovulated at time s_i and γ is a factor determining the efficiency of transfer of the contaminants from blood the yolks.

Laying hen physiological model

Following the standard Physiologically Based PharmacoKinetic (PBPK) modelling approach, the laying hen was subdivided in five compartments: blood (for transport), fat (for storage of the highly lipophilic contaminants), liver (for metabolism) and the remainder subdivided in slowly and richly perfused tissues. This compartimentation was based on the laying hens body composition as reported by Germs (1985) and Uijttenboogaart (1985). Although the model provided a reasonably fair description of the experimental data, there is a great number of unknown or only poorly known parameters, such as compartmental blood flows, tissue:blood compartment partition coefficients, liver specific intrinsic

clearance and the efficiency of contaminant incorporation into egg yolk. Fitting all these parameters is not sensible, and fitting only a subset yields results that explicitly depend on the values attributed to the parameters of the other subset that is not fitted. Sensitivity analysis of the individual parameters is not always helpful when varying sensitive parameters which are correlated.

As a consequence, it was decided to reduce the number of compartments. Sensible lumping of compartments highly depends on the kinetics of the substance of interest. In the case of lipophilic contaminants, the fat compartment not only serves as a high storage distribution volume but also, because of its relative poor blood perfusion, as a compartment with a relative long characteristic time of contaminant concentration equilibration with the blood compartment. From PBPK models for TCDD in rats, cattle and humans, it appears that these times are at least an order of magnitude longer then the next characteristic times for equilibration of blood with slowly perfused tissue. For hydrophilic drugs, e.g., this can be quite different.

These considerations lead to the decision to model two compartments only: the adipose tissue compartment and the rest of the body. The blood concentration, necessary to determine excretion of the contaminants by eggs, is determined by the distribution volume of the rest compartment

$$V_D = P_r V_r = \sum_{compartments} P_c V_c \tag{6}$$

determined by the sum of the distribution volumes of the other compartments. This model implies that the contaminant concentrations of all the tissues contained in the rest compartment are in instantaneous equilibrium. This alternative compartmentation is corroborated by considerations of parameter identification as will be shown in the next section: the data available do not allow for a more refined compartmentation when corresponding introduced parameters are unknown.

Mathematical model formulation

Physiologically based model

The kinetics of dioxin-TEQs in laying hens is modelled by the next mass balances

$$\begin{aligned}
\frac{dA_r}{dt} &= -Q_f(C_r/P_r - C_f/P_f) - CL_l C_r/P_r - \sum_i \gamma \dot{w}(t-s_i) C_r/P_r + F_{abs}\dot{D} \\
\frac{dA_f}{dt} &= Q_f(C_r/P_r - C_f/P_f) \\
\frac{dA_i}{dt} &= \gamma \dot{w}(t-s_i) C_r/P_r
\end{aligned} \tag{7}$$

The first line shows the rate of change of the amount of TEQs in the rest compartment (left hand side) because of loss to (or gain from) the fat compartment (first term right hand side), loss by metabolic clearance in the liver (second term r.h.s), residue excretion to the developing yolks (third term r.h.s) and because of gain by absorption of the daily dose of contaminants over the gut wall (fourth term r.h.s). The second line shows the rate of change of the amount of TEQs in the fat compartment (l.h.s) because of gain from (or loss to) the rest compartment (r.h.s). The last line shows the incorporation of the contaminants in the yolks. The concentrations in equation (7) are derived by dividing the amounts by the corresponding compartment volumes.

Classical compartmental model

The model in equation (7) can be reformulated in terms of the amounts of contaminant in the compartments only. When the following quantities

$$q_r = \frac{Q_f}{P_r V_r}, \quad q_f = \frac{Q_f}{P_f V_f}, \quad k = \frac{CL_l}{P_r V_r}, \quad Y = \frac{\gamma}{P_r V_r} \tag{8}$$

are defined, then equation (8) can be reformulated as:

$$\begin{aligned} \frac{dA_r}{dt} &= -\left(q_r + k + \sum_i Y\dot{w}(t - s_i) \right) A_r + q_f A_f + F_{abs}\dot{D} \\ \frac{dA_f}{dt} &= q_r A_r - q_f A_f \\ \frac{dA_i}{dt} &= Y\dot{w}(t - s_i) A_r \end{aligned} \tag{9}$$

Note that the daily dose in equation (7) and (9) is just the mean contaminant intake per day, i.e., the actual intake at any moment of the day integrated over one day (and divided by 1 (one day)). Likewise, one could integrate the growth over one day. Summing that result over all the yolks, one arrives at the total yolk growth per day

$$\int_t^{\infty} \dot{w}(t - s)ds = \int_{-\infty}^{0} \dot{w}(\sigma)d\sigma = w_0 - w_{min} \tag{10}$$

and a new parameter

$$y = Y(w_0 - w_{min}) \tag{11}$$

is defined accordingly. Note that sometimes equation (1) is interpreted as a weight, and sometimes as a weight per day. The latter assume division by 1 (one day). This is the usual confusion introduced when multiplying or dividing by one unit.

Proceeding this way, the first line in equation (9) can be reformulated as

$$\frac{dA_r}{dt} = -\left(q_r + k + y \right) A_r + q_f A_f + F_{abs}\dot{D} \tag{12}$$

The first two lines in equation (9) constitute an autonomous system of equations, that can be solved independently of the third line. Though these equations were derived from the physiological model in equation (7), they provide a possible explanation of the data even without this physiological interpretation. Then the equations just state that the amount of contaminants administered redistributes over the hens body by contaminant transfer between two compartments and contaminant loss by two routes of elimination of which one is excretion by egg yolk. Such kind of modelling is the typical classical compartmental modelling approach. The classical model of equation (9) and (12), can easily be solved and analysed for parameter identification.

Classical kinetic model

Assuming that initially there is no contamination with dioxins at all, the analytical solution to the first two lines of equation (9) is

$$\begin{aligned} A_r(t) &= \frac{F_{abs}\dot{D}}{y+k}\cdot\left(1-\frac{(\lambda_1+y+k)e^{t\lambda_2}-(\lambda_2+y+k)e^{t\lambda_1}}{\lambda_1-\lambda_2}\right) \\ A_f(t) &= \frac{q_r}{q_f}\frac{F_{abs}\dot{D}}{y+k}\cdot\left(1-\frac{\lambda_1 e^{t\lambda_2}-\lambda_2 e^{t\lambda_1}}{\lambda_1-\lambda_2}\right) \end{aligned} \qquad (13)$$

where the exponential rates are given by

$$\begin{aligned} \lambda_1 &= -\tfrac{1}{2}\left(q_f+q_r+y+k+\sqrt{(q_f+q_r+y+k)^2-4q_f(y+k)}\right) \\ \lambda_2 &= -\tfrac{1}{2}\left(q_f+q_r+y+k-\sqrt{(q_f+q_r+y+k)^2-4q_f(y+k)}\right) \end{aligned} \qquad (14)$$

A purely descriptive analogue of equation (13) would be

$$\begin{aligned} A_r(t) &= A_{r,\infty}\cdot\left(1-a_{r,2}e^{t\lambda_2}-a_{r,1}e^{t\lambda_1}\right) \\ A_f(t) &= A_{f,\infty}\cdot\left(1-a_{f,2}e^{t\lambda_2}-a_{f,1}e^{t\lambda_1}\right) \end{aligned} \qquad (15)$$

which is the typical kind of approach in classical kinetic modelling. Note that this approach can be fruitfully applied for data interpolation even without the classical compartmental modelling interpretation. Nevertheless, if initial amounts are to be zero, then one should be aware that $a_{r,1}+a_{r,2}=a_{f,1}+a_{f,2}=1$. Moreover, if the classical kinetic model is to be interpreted in terms of classical compartmental modelling, the exponential coefficients for the fat compartment cannot be chosen freely at all: they should be the coefficients in equation (13).

Compartmental system identification analysis

Identification of model parameters by fitting them to experimental data can only be successful when the experimental set-up is devised as to allow for that purpose, i.e., it depends on how and where the forcing is applied (e.g., intravenous or subcutaneous or inhalatory or oral or whatsoever combination of these, bolus injection, discontinuous infusion or continuous administration) and what is sampled (e.g., concentration in blood, fat, egg yolk or whatsoever).

These experiments concern the research of egg contamination as a result of oral intake of environmental contaminants, so, the forcing is established as is the monitoring of egg yolk contamination. Moreover, the choice was made to sample abdominal fat. This choice was made in order to check the quite interesting phenomenon that seems to be observed occasionally, that eggs have a greater concentration of this kind of contaminants per gram of egg yolk than adipose tissue per gram of fat.

If concentrations in abdominal adipose tissue are representative for the concentration in the fat compartment as defined in the physiological model, which compartment includes (sub)-cutaneous fat, inter-muscular fat and bone marrow fat at least, then, in terms of the model, the sampled concentrations can be described by $A_f(t)/V_f$. The egg yolk concentrations in the model, do not precisely represent

$A_r(t)$, but a weighted integral of this quantity over the past. The weights are the product of the contaminant incorporation efficiency and the daily yolk growth rates which are known. However, for simplicity of identification analysis, it will be assumed that the egg ovulated at time *t* just contains precisely the concentration $yA_r(t)/V_{y,f}$ TEQ per gram of egg yolk, instead of an integral over the past. $V_{y,f}$ is the egg yolk volume. Actually, in the following it will be seen that this approach yields a far better description of the data. The volume of egg yolk is estimated to be 1/3 of the volume of egg yolk itself (Kans Yellow Rule once more). Under this assumption, the identification analysis proceeds as follows. From the analytical solution in equation (13), one can see that from the experimental set-up the following observable parameters can be estimated from the data:

Terminal, slow, exponential rate λ_2

Initial, fast, exponential rate λ_1

Factor of the slow rate exponent $(\lambda_1 + y + k)/(\lambda_1 - \lambda_2)$, or, equivalently of the fast rate exponent $(\lambda_2 + y + k)/(\lambda_1 - \lambda_2)$.

Maximum level of egg yolk fat concentration $\dfrac{yF_{abs}\dot{D}/V_{y,f}}{y+k}$

Maximum level of adipose tissue concentration $\dfrac{q_r}{q_f}\dfrac{F_{abs}\dot{D}/V_f}{y+k}$

In the following, parameters or combinations of them, which are known or identified will get an asterisk as superscript. From the exponential rates, one identifies from their sum (see equation (14) the sum $q_f + q_r + y + k = -(\lambda_1^* + \lambda_2^*)$, and from their product the product $q_f(y+k) = \lambda_1^* \cdot \lambda_2^*$.

From the third observable, one identifies the total elimination:
$y + k = (\lambda_1^* - \lambda_2^*)\left((\lambda_1 + y + k)/(\lambda_1 - \lambda_2)\right)^* - \lambda_1^*$. And so the parameter
$q_f = \lambda_1^* \cdot \lambda_2^* / (y+k)^*$ i.e., the rate of transfer of contaminants from the fat compartment to the rest compartment is identified, while the reverse transfer rate $q_r = -(\lambda_1^* + \lambda_2^* + q_f^* + (y+k)^*)$ is identified too.

All these expressions of compartmental model parameters contain the sum $y+k$ of both elimination rates, as does the fifth observable. The only way elimination by excretion or metabolism can be identified separately is by using the fourth observable. However, this observable contains the unknown absorption fraction F_{abs}. This parameter could be identified from the fifth observable, but that implies that the volume of what is considered as the fat compartment (and which may include, additional to abdominal fat and (sub) cutaneous, inter-muscular fat, fat contained in bone marrow and fat contained in feathers) must be known. Uncertainty in this parameter could well dominate the uncertainty in the absorption fraction for which there is a reasonable estimation that it lies between 90 and 100%.

First, assume that the absorption of the contaminants is almost complete, i.e., $F_{abs} = 1$, then *y* can be identified from $y = \left(\dfrac{yF_{abs}\dot{D}/V_{y,f}}{y+k}\right)^* \cdot \dfrac{(y+k)^*}{\dot{D}^*/V_{y,f}^*}$ Once this parameter is identified, the metabolism elimination rate can be identified too: $k = (y+k)^* - y^*$. Note that this way all the compartmental system parameters in equation (8) can be identified. The fifth observable parameter allows for the additional identification of the fat compartment volume:

$$V_f = \frac{q_r^*}{q_f^*}\frac{\dot{D}^*}{(y+k)^*} \Big/ \left(\frac{q_r}{q_f}\frac{F_{abs}\dot{D}/V_f}{y+k}\right)^*$$

On the other hand, there are no data about the formation of metabolites and any value found for this parameter cannot be validated. So, as another extreme, assume that there is no elimination by metabolism at all, i.e., $k = 0$. This assumption leads to the determination of the absorbed fraction $F_{abs} = V_{y,f}{}^* \left(F_{abs}\dot{D}/V_{y,f}\right)^* / \dot{D}^*$. And again all the compartmental system parameters in equation (8) can be identified as well as the fat compartment volume.

Straightforward calculation shows that the values of the absorption fraction and the metabolism rate are related by

$$\frac{1}{F_{abs}} = \left(\left(\frac{yF_{abs}}{y+k}\right)^*\right)^{-1} \cdot \left(1 - \frac{k}{(y+k)^*}\right) \qquad (16)$$

where $\left(\frac{yF_{abs}}{y+k}\right)^* = \left(\frac{yF_{abs}\dot{D}/V_{y,f}}{y+k}\right)^* / \left(\dot{D}^*/V_{y,f}{}^*\right)$. So, when $k = 0$, $F_{abs} = \left(\frac{yF_{abs}}{y+k}\right)^*$, and when $F_{abs} = 1$, $k = \left(1 - \left(\frac{yF_{abs}}{y+k}\right)^*\right)(y+k)^*$

As a conclusion of this analysis it can be stated that the experimental data are insufficient for a complete identification. Complete identification is only possible if the absorbed fraction can be estimated from sampling faeces, or if the metabolism rate can be determined, which requires the detection of the total of metabolites. The latter option seems to be prohibited by the enormous effort it would take analytically. Note, that it would not help at all if the concentration in blood ($= C_r / P_r$) was sampled additionally to the concentration in egg yolk: its analytical expression contains the sum $y + k$ only (see equation (7), $C_r = A_r / V_r$). So, if for instance the concentration in blood was sampled instead of the concentration in egg yolk, identification would not have been possible at all.

Physiological model identification analysis

Once the compartmental system parameters have been identified, one should consider whether this identification leads to identification of the physiological model parameters. Note that the compartmental model parameters depend on the physiological parameters as in equation (8). Depending on the choice $F_{abs} = 1$ or $k = 0$, five other compartmental model parameters can be identified, so including the assumption on absorption or metabolism rate to the set of identified parameters, six model parameters are known.

Besides the fraction absorbed, the physiological model counts seven other unknown parameters: $Q_f, P_r, P_f, \gamma, CL_l, V_f$ and V_r. However, as the total body weight is known, the last parameter can be identified once the fat compartment volume has been identified, so, there remain only seven unknown parameters in the physiological model, one more than can be identified from the compartmental model. So, complete identification of the physiological model is never possible without additional knowledge of the value of one of its parameters Q_f, P_r, P_f, γ or CL_l. Once one of these parameter values is known, e.g., the regional fat compartment blood flow, the other ones can be derived from the compartmental model parameters.

Experimental data

Laying hen body weights of 85 hens were measured with a mean of 1840 (g), population standard deviation of 260 (g) and a median of 1810 (g).

The hens abdominal fat was weighed and an estimation of the weight of the remaining abdominal fat was made. From these two data the total abdominal fat weight was estimated. There appeared to be a strong correlation between relative abdominal fat weight and body weight. The abdominal fat weight of a hen of 1840 (g) was estimated to be 86.5 (g). Uijttenboogaard (1985) reports a relative skin weight of laying hens of about 9% and Germs (1985) reports relative fat percentages of about 50%, which amounts to another 4.5% of relative fat weight, i.e., 82.8 (g). Another slowly perfused storage compartment is bone marrow, from data presented by Germs (1985) to be about 1.4% of body weight, i.e., 25.8 (g). Yet another storage compartment in this respect are the hens feathers with about 5% of body weight (Uijttenboogaard, 1985) and 4% of lipid content, i.e., another 4 grams contributing. All these contributions would amount to a total fat compartment volume of about 200 (g).

Weights of 76 eggs were measured with a mean weight of 54.4 (g), population standard deviation of 1.7 (g) and median of 54.5 (g). Application of Kans Yellow Rule gives an estimate of egg yolk weight of 18.2 (g) and application of the rule once more results in an estimated egg yolk fat weight of 6.1 (g).

The experimental data are the concentration in abdominal fat and the concentration in egg yolk fat of the congeners and the total concentration in TEQs. Note that from the identification analysis above it appears that the egg yolk data are, within the uncertainty with respect to the fraction absorbed and the metabolic clearance rate, sufficient for identification of the classical compartmental model parameters. The additional data on the concentration in abdominal fat will be used for an estimate of the fat compartment volume.

Practical model identification: classical compartmental model

The model, as described by the equation (9) together with the modification as described in equation (12) has been implemented in the versatile modelling package ACSL-model (Advanced Continuous Simulation Language; AEgis Technologies Group, Inc., Huntsville, AL, USA). The packages ACSL-Math and ACSL-Optimize were used to fit the model parameters to all the egg yolk and abdominal fat data for all the five different dose levels simultaneously. The heteroscedasticity parameter of the error model was fitted too. When this parameter value is zero, errors are purely absolute, and when it has the value 2, errors are purely relative. Both for the data in egg yolk and fat, values in the vicinity of 1 were found.

Besides the parameters in the foregoing discussion, one more parameter was fitted. This parameter can be identified independently of the others and regards the following problem. At the start of the experiment, i.e., the time of first administration of contaminated feed, some of the hens have just ovulated, others will have their ovulation one hour later, etc. Eggs are collected and to avoid noise by inter-individual variation as much as possible, mixed yolks are analysed. In the model an off-set time is modelled, representing the mean (median) time between start of the experiment and time of first ovulation. Incidentally, the yolk ovulated at some time, is laid one day later.

One other slight complication is that not intentionally contaminated feed is still contaminated by background contamination. This contamination results in an initial background contamination of egg yolk and the hens tissues. Experimental data of background egg concentrations were available and an the estimated initial concentration in egg yolk is 0.52 pg TEQ/g egg yolk fat. Assuming that background contamination is steady state, the initial concentrations in the compartments could be estimated.

Although the model fits resulted in a reasonably fair description of the data (see figure 1), it was found that the egg yolk concentrations of the first days of sampling (notably day 2 and 5) were

systematically under estimated. As the concentration-time data show a clear bi-exponential behaviour, it was decided to try to model the data by the classical kinetic model.

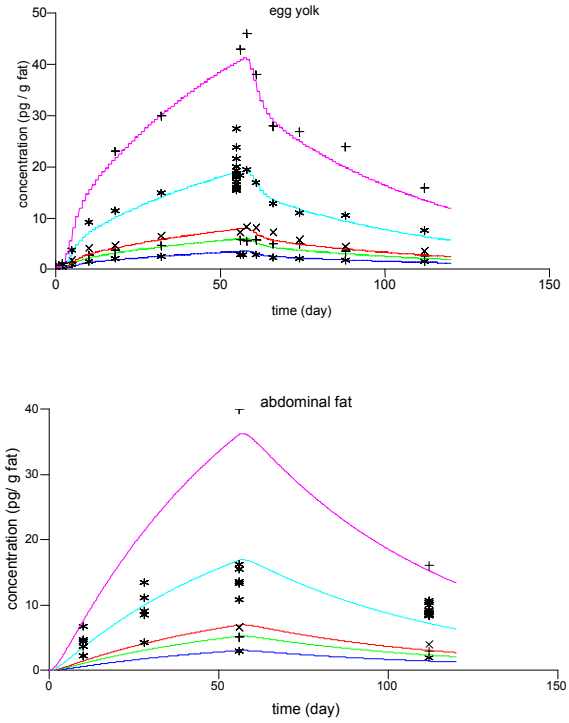

Figure 1. Classical compartmental model. Concentration in egg yolk (left panel) and abdominal fat (right panel). The lines show the calculated concentration-time curves, the symbols denote experimental data. Five contamination levels: 0.34 ng / kg feed (lower line, *), 0.58 ng / kg feed (next lower line, +), 0.76 ng / kg feed (middle line, x), 1.85 ng / kg feed (next upper line, *) and 3.95 ng / kg feed (upper line, +).

Practical model identification: classical kinetic model

In order to fit the classical kinetic model, which describes concentrations in the rest and the fat compartment, a relation between the concentration in the rest compartment and egg yolk has to be imposed. As the use of this model is to check whether the egg yolk concentrations are better approached with a bi-exponential model, the most obvious relation is proportionality between egg yolk concentration and the concentration in the rest compartment. This way a yolk development model, together with contaminant incorporation in yolk as outlined above, is abandoned.

Ignoring the initial concentrations caused by background contamination, the same fit procedure as in the preceding section was carried out. Again, the fits show a fair description of the data (see figure 2), but now also the concentrations in egg yolk at first stage of the experiment were far better represented by the calculated concentration-time curves. Moreover, the concentration in abdominal fat seems to be represented better by this model. As an objective criterion for goodness of fit, the loglikelihood is maximised. While the loglikelihood for the classical compartmental approach was found to be – 192, the likelihood of this approach was far greater: - 179.

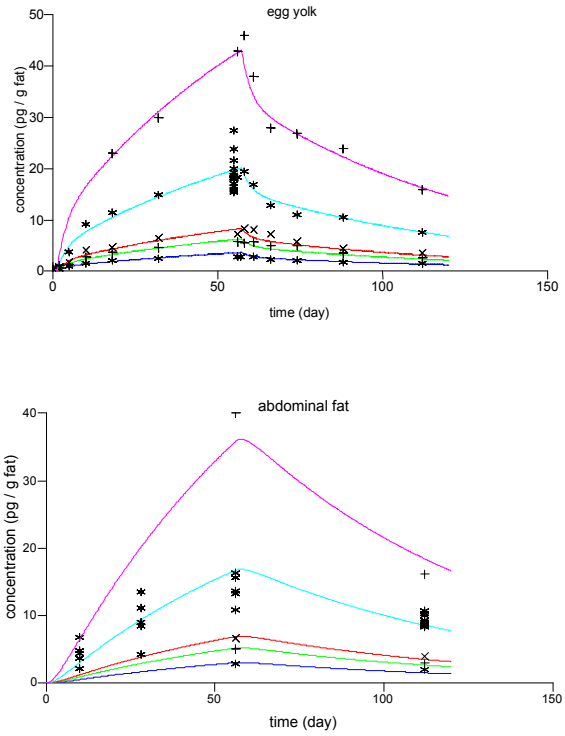

Figure 2. Classical kinetic model. Concentration in egg yolk (left panel) and abdominal fat (right panel). The lines show the calculated concentration-time curves, the symbols denote experimental data. Five contamination levels: 0.34 ng / kg feed (lower line, *), 0.58 ng / kg feed (next lower line, +), 0.76 ng / kg feed (middle line, x), 1.85 ng / kg feed (next upper line, *) and 3.95 ng / kg feed (upper line, +).

This result indicates that, while for drugs Donoghue and co-workers could develop a model for drug residues in egg yolk that was based on the stages of development of the yolks, a simple partitioning model offers a far better approach for highly lipophilic contaminants like dioxins. So, it was decided to abandon the submodel for incorporating contaminants proportional to the yolk growth rate and replace it by a model where concentration in egg yolk fat is proportional to the concentration in blood:

$$y = P_{y,f} V_{y,f} \text{ [-/day]} \qquad (17)$$

instead of the expression in equation (11). Here, $P_{y,f}$ is the egg yolk fat:blood partition coefficient.

However, before proceeding this way, one more interesting result of the fit should be reported. The fit procedure fits, a.o., the steady state concentrations in egg yolk fat and in abdominal fat. It appears that the estimates for these parameters coincide within 4%, and one might well conclude that at steady state, the yolk egg fat concentration equals the abdominal fat concentration. There have been observations of egg yolk fat concentration of TEQs exceeding abdominal fat concentration. This could possibly have been explained, e.g., by irreversible incorporation of these kind of contaminants in egg yolk, i.e., using the original modelling approach. From our results one might as well assume that this may be more of a kinetic phenomenon. In the classical kinetic model, the concentration in egg yolk fat, when increasing to steady state, will exceed the concentration in abdominal fat. However, when decreasing from a high steady state concentration level to a low one, abdominal fat concentration will exceed egg yolk fat concentration. See also figure 4. The consequence of this obsevation is that the egg yolk fat :blood partition coefficient equals the fat:blood partition coefficient, and so

$$y = P_f V_{y,f} \text{ [-/day]} \qquad (18)$$

Practical model identification: classical compartmental model with partitioning between concentration in blood end in egg yolk fat

The same fitting procedure as in the preceding sections was carried out. Of course, now again initial concentration were accounted for. This fit resulted in a still higher maximum likelihood of – 167.4, which is mainly due to the fact that background levels are accounted for, in contrast to the classical kinetic model. For the result, see figure 3.

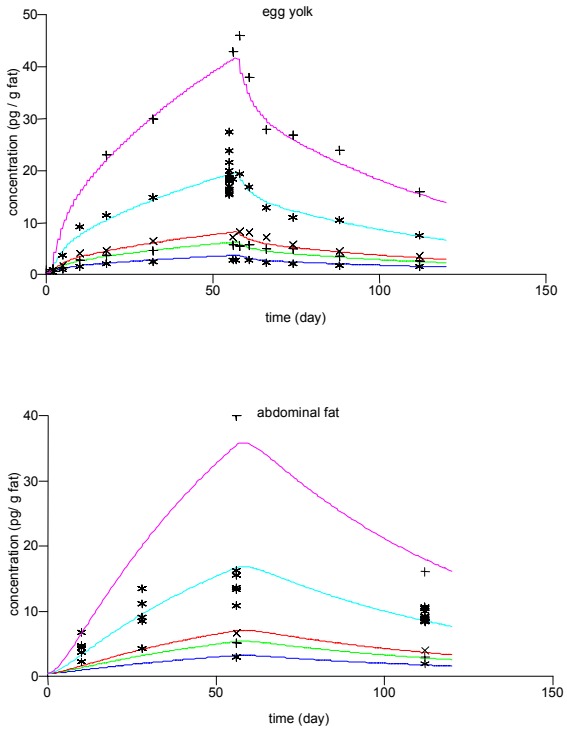

Figure 3. Classical compartmental model with partitioning instead of developing yolk incorporation model. Concentration in egg yolk (left panel) and abdominal fat (right panel). The lines show the calculated concentration-time curves, the symbols denote experimental data. Five contamination levels: 0.34 ng / kg feed (lower line, *), 0.58 ng / kg feed (next lower line, +), 0.76 ng / kg feed (middle line, x), 1.85 ng / kg feed (next upper line, *) and 3.95 ng / kg feed (upper line, +).

Up to here, fits have been carried out by setting the value of the fraction absorbed to 100%. The fitted metabolism rate appears to be 12% of the rate of excretion by egg yolk. If instead the metabolism rate is set to the value 0, then the corresponding absorption fraction is fitted to be 90%, and the same value for the loglikelihood is obtained. Now, the value of total elimination, i.e., $y+k$ in the identification analysis section, should be the same for both fits. The sum of the rates of the fit taking $F_{abs}=1$ equals the value 0.0514/day, while the value for the excretion rate found taking $k=0$ is 0.0518 which is within 0.8% of 0.0514. Also, from the identification analysis one finds that the fat compartment volume is directly proportional to the fraction absorbed, so the volume found in the last fit should be 90% of the value found in the first one: it is within 0.6%.

The corresponding initial exponential rate is $\lambda_1 = 0.3$ (-/day) and the terminal exponential rate is $\lambda_2 = 0.0135$ (-/day). So, the corresponding half life times are about 2.3 and 50 days. If the time to reach steady state is taken to be 5 terminal half life times, then after 250 days, say after 8 months, steady state will be reached. Note that the experimental periods of feeding contaminated food (charging) and clean food (discharging) are about one terminal half life time.

The volume of the fat compartment ranges from 265 to 295 (mL) proportional to range for the fraction absorbed, 90 – 100%. The corresponding weight ranges from 245 – 270 (g). Its weight was

estimated from experimental data and data presented in Uijttenboogaard and Germs to be about 200 (g), which is 22.5 – 35 % less.

As for the classical kinetic model, it appears hat the steady state concentrations in egg yolk fat and abdominal fat are almost equal.

In the next figure 4, the concentration-time profiles in egg yolk fat and abdominal fat for the dose of 1.85 ng/ kg feed are shown, together with the experimental data. Note that during the period of charging, the concentration in egg yolk exceeds the concentration in abdominal fat, while soon after the start of the period of discharging abdominal fat concentration exceeds egg yolk fat concentration.

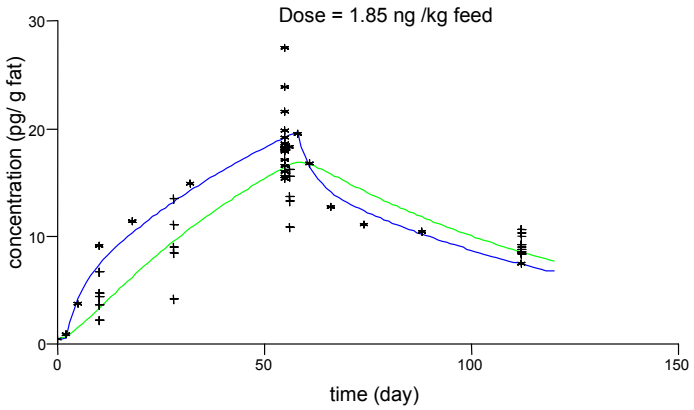

Figure 4. Classical compartmental model with partitioning instead of developing yolk incorporation model. Concentration in egg yolk and abdominal fat. Administered dose = 1.85 ng / kg feed. The lines show the calculated concentration-time curves, the symbols denote experimental data: egg yolk initially exceeding (*), abdominal fat terminally exceeding (+). At $t = 55$ day individual, rather a number of than mixed egg yolks were sampled and analysed: note the quite large inter-individual variation. Note also the inter-individual variation of the abdominal fat data. Abdominal fat data in the preceding figures for the other dose levels were sampled from mixed individuals.

The classical compartment parameters found by fitting are

$$q_f = 0.0784 \text{ (-/day)}, \quad q_r = 0.182 \text{ (-/day)}, \quad t_{offset} = 0.18 \text{ (day)}$$
$$\text{and} \quad F_{abs} = 1, \quad k = 0.00532 \text{ (-/day)}, \quad y = 0.0461 \text{ (-/day)}, \quad V_f = 295 \text{ (mL)}$$
$$\text{or} \quad F_{abs} = 0.9, \quad k = 0.0 \text{ (-/day)}, \quad y = 0.0518 \text{ (-/day)}, \quad V_f = 267 \text{ (mL)}$$

Here, t_{offset} is the mean time of first ovulation after the start of the experiment, about $4\frac{1}{2}$ hours.

Practical model identification: physiologically based model

As outlined in the section on parameter identification, the parameters of the physiological based model can only be derived from the classical compartmental model if some additional parameter value, notably the blood flow through the fat compartment, is known. However, such additional information is not available. Though, a reasonable set of parameters could still be derived if, e.g., the fat:blood partition coefficient could be estimated.

Suppose that the lipid content of laying hen blood, which contains about 1% of lipids (Germs), is composed of 50% of phospholipids and 50% of neutral lipids (like in rats). Then, based on an algorithm proposed by Poulin & Krishnan (1995[a,b]), under neglecting of possible binding to plasma proteins, the partition coefficient is about 150. From this value, one may derive

$$Q_f = q_f P_f V_f \approx 3.0 - 3.4 \text{ (L/day)} \qquad (19)$$

In adult women, with a relative body fat content of 30% the relative adipose tissue blood flow is about 8.5%. Laying hens, assuming a relative body fat content of about, say, 15% (= 100 × .25 (fat weight)/1.84 (body weight)) would correspondingly lead to a relative adipose tissue blood flow of about 4%. So, the total blood flow would range from 75 – 85 (L/day). Given a relative volume of blood of about 2.7% body weight (Uijttenboogaard, 1985), i.e., about 50 mL, it follows that a laying would pump its total blood volume around 1 – 1.2 times per minute. This number is comparable to the same number for humans.

Given the volume of the rest compartment $V_r \approx BW - V_f \approx 1.56$ (L), the value for the partition coefficient of the rest compartment $P_r = Q_f / q_r V_r$ would range in an interval of about 10 - 12.
Correspondingly, the parameter values for the excretion by egg yolk and metabolism

$$\begin{aligned} Y &= y \cdot P_r V_r \approx 0.75 \text{ - } 1 \text{ (L/day)} \\ CL_l &= k \cdot P_r V_r \approx 0 \text{ - } 0.1 \text{ (L/day)} \end{aligned} \qquad (20)$$

From the other hand, the interpretation of the excretion rate in terms of egg yolk flow and laying efficiency is

$$Y = \varepsilon P_f V_{y,f} = 0.85 \times 150 \times 0.006 = 0.765 \text{ (L/day)} \qquad (21)$$

Here a laying efficiency ε of about 85% per day, i.e., 100 hens lay 85 eggs per day or one hen lays 85 eggs during a period of 100 days, was observed.
These estimated parameter values have been used as initial parameters when fitting the physiological model parameters to the data. This way, a consistent set of physiological parameters could be estimated (See figure 5). However, it appears that the initial estimate for the fat compartment blood flow of 3 (L/day) was hardly changed. Equation (8) learns that the estimations of the fat:blood and rest:blood partition coefficients as well as the estimations of the metabolism rate and the egg yolk excretion rate depend linearly on the value of the fat compartment blood flow. So, taking all these parameters 10 times as high or as low as the parameters found by fitting, would result in the same concentration-time curves. This is confirmed by calculations.
While from the one hand this is a disappointing result, from the other hand at least the mutual ratios of these parameters is well identified:

$F_{abs} = 1,$
$Q_f : V_f : P_f : P_r : CL = 3 \text{ (L/day)} : 292 \text{ (mL)}:133 \text{ (-)}:11.3 \text{ (-)}:77 \text{ (mL/day)}$
$F_{abs} = 0.91,$
$Q_f : V_f : P_f : P_r : CL = 3 \text{ (L/day)} : 267 \text{ (mL)}:144 \text{ (-)}:10.9 \text{ (-)}:0 \text{ (mL/day)}$

i.e., given an absorption fraction of 1 and the fat compartment blood flow to be 2 (L/day), the corresponding value for the fat:blood partition coefficient would be 2/3×133 = 88.7., etc.

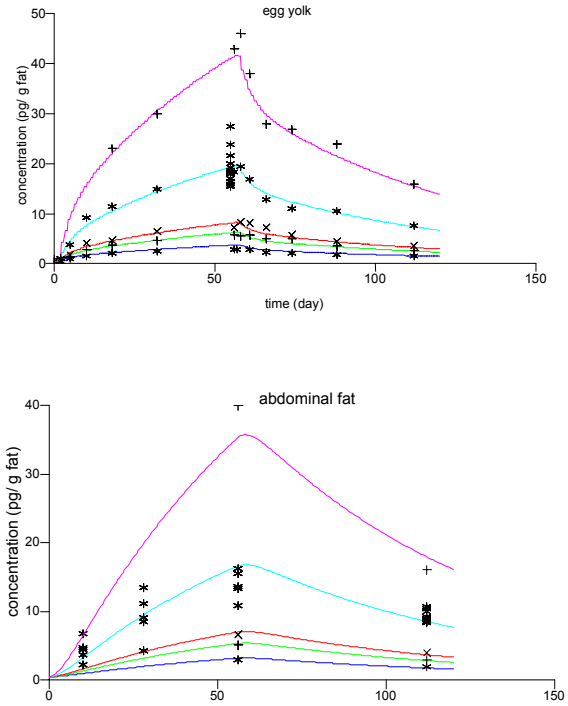

Figure 5. Physiologically based model with partitioning instead of developing yolk incorporation model. Concentration in egg yolk (left panel) and abdominal fat (right panel). The lines show the calculated concentration-time curves, the symbols denote experimental data. Five contamination levels: 0.34 ng / kg feed (lower line, *), 0.58 pg / kg feed (next lower line, +), 0.76 ng / kg feed (middle line, x), 1.85 ng / kg feed (next upper line, *) and 3.95 ng / kg feed (upper line, +).

The last figure shows the difference in the calculated concentration-time curve for the dose level of 1.85 (ng/ kg feed).

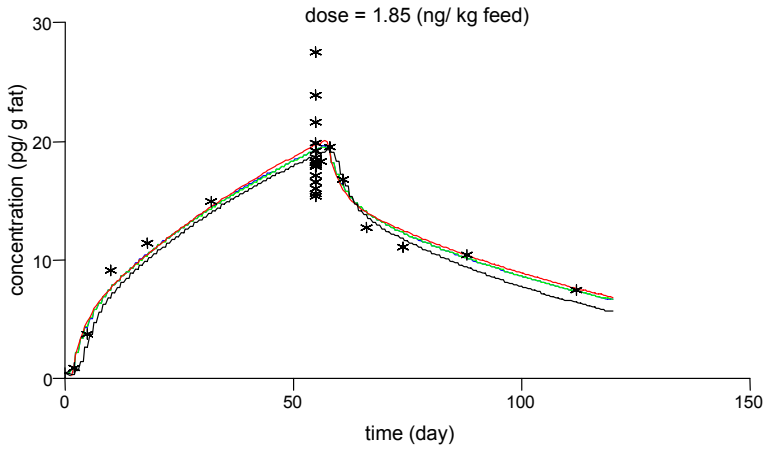

Figure 6. The line which is initially and finally the lower line shows the egg yolk fat concentration calculated with the classical compartmental model together with incorporation proportional to yolk development; the line which is initially and finally the upper line shows the egg yolk fat concentration calculated with the classical kinetic model assuming egg yolk fat:blood partition, it is hardly discerned

from the two lines that cannot be discerned at all, showing the egg yolk fat concentration calculated with the classical compartmental model and the physiologically based model ($Q_f = 3$ (L/day)) assuming egg yolk fat:blood partition.

Discussion

Although the different congeners administrated will show different kinetics, it was tried to model the total of contaminants represented by their sum of toxic equivalents (TEQs). Besides the fact that such an approach is less laborious, it has the practical advantage that usually the contamination with dioxin-like substances concerns a mix of different congeners, which is most easily represented by its TEQ value.

Based on the lipid solubility of the contaminants in feed and lipid uptake of laying hens from feed, it was estimated, partially in accordance with Pirard and De Pauw, that absorption would be fairly complete. This was confirmed by the estimation of the range of the fraction absorbed: 90 to 100%. Higher chlorinated congeners that show a reduced absorption, contribute to a small extend to the total of TEQs.

Although the data do not allow for an absolute value of the fat:blood partition coefficient, an *ad hoc* estimation for this parameter, based on lipid contents (Uijttenboogaard, 1985) and lipid composition of these tissues, was made (Poulin and Krishnan, 1995[a,b]. This estimation resulted in an estimation of laying hens cardiac output of about one total blood volume per minute, comparable to humans. The corresponding estimation of the value of partition coefficient of the rest compartment appeared to be about 7% of the fat:blood partition coefficient, which is consistent with data in the literature. The high lipophilicity of all the congeners leads to the same lipid solubility based fat:blood partition coefficient. The only difference with respect to partition coefficient values between congeners is their chemical property of binding to proteins.

The rate of elimination by egg yolk excretion appeared to dominate (at least a factor of 10) the rate of elimination by metabolism. So, differences in metabolism rate of different congeners do not substantially contribute to difference in their elimination. It appeared that excretion by egg yolk is likely to based on egg yolk fat:blood partition. This is in contrast to the findings of Donoghue and co-workers (Donoghue *et al*. 1996,1997[a] , 1997[b], 1999, 2000, 2001). They found that for drugs as well as lindane egg yolk development plays a major role in their egg yolk excretion: data showed a strong correlation between yolk growth rate and drug incorporation in egg yolk.

From a number models of descending and again increasing complexity, ultimately a physiologically based kinetic model with two compartments was formulated to describe the kinetics of dioxin TEQs in laying hens. The advantage of physiologically based compartmental modelling is that it is mechanistically based and formulated in terms of tissue volumes, tissue blood flows, partition of substances between tissues and intrinsic elimination rates. Such a formulation leads to a better understanding of processes as absorption, distribution and elimination, as well as their interaction. Such an understanding cannot be obtained from the equivalent classical compartmental model, which is based on more abstract notions of contaminant transfer between compartments and system elimination rates. E.g., the rate of transfer from the fat compartment to the rest compartment is determined by fat compartment volume, blood flow and the fat:blood partition coefficient (see equation (8)). Nevertheless, the classical compartmental model as well as the classical kinetic model were very helpful for the identification of the physiologically based model parameters.

Dioxin-like contaminants are known for their persistence. This is confirmed by the kinetics of total TEQ in laying hens. The terminal half-life time was estimated to be about 1.7 months. Without laying, half life time would be about 14 months.