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

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Abstract

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 56 days on clean feed. Dioxin levels in feed varied from background levels to three times the current EU tolerance limit of 0.75ng TEQ/kg. At all dose levels a rapid increase was observed in the dioxin levels in eggs. There was a clear linear dose–response relationship between the dioxin levels in eggs and feed. The feed containing 0.4ng TEQ dioxins per kg resulted in egg levels just above the EU limit of 3pg TEQ/g fat. Dioxin-like and indicator PCB residues followed a pattern very similar to that of dioxins. Exposure to the highest indicator PCB level of 32µg/kg resulted in egg levels around 300ng/g fat. Exposure to dioxins through contaminated soil, mixed at 10% into the feed, resulted in a similar carry-over as from feed. Mycotoxin binders, mixed at 0.5% into the feed, had little effect on the carry-over of dioxins from the feed to the egg. It can be concluded that consumption of feed or soil with even moderate levels of dioxins and dioxin-like PCBs rapidly results in increased levels in eggs. The current EU dioxin limit for feed cannot guarantee egg dioxin levels below the EU-limit.

Keywords: Dioxins, PCBs, carry-ver, chickens, eggs, feed, soil.

Introduction

Incidents with residues of PCBs and dioxins have shown that these compounds pose a major threat to the quality of edible products derived from food producing animals (Hoogenboom 2005). As the exposure of part of the populations in Western countries still exceeds the exposure limits set by, e.g., the Scientific Committee on Food (SCF 2001), the EU has developed a strategy for further reducing the exposure to dioxins and dioxin-like PCBs. This includes the establishment of residue limits for dioxins in food (EC 2001) and feed (EC 2002).

Since the dioxin crisis in 1999 in Belgium, the Dutch government established a number of monitoring programmes for dioxins aimed at the early detection of contaminated feed and food and subsequently the revelation and elimination of novel sources of dioxins. In 2001, over 300 samples of animal origin were screened in the DR CALUX-assay and if suspected positive for dioxins, analysed

by HRGC/HRMS. In one case, eggs obtained from a small farm with free-range hens were shown to contain dioxin levels above the then existing Dutch limit of 5pg TEQ/g fat (Traag et al. 2002). This initial observation was followed by a focussed action on these types of eggs and showed a clear elevation in dioxin levels in eggs from free-range hens, in particular those from farms producing eggs according to strict organic standards. This was confirmed in a survey by The Dutch Food Inspection Service, showing that eggs from six out of 68 farms would exceed the new EU dioxin limit for eggs of 3pg TEQ/g (as of July 2003), and three exceeded the then existing limit of 5pg TEQ/g (De Vries 2002). In addition some of the samples contained high levels of dioxin-like PCBs, with the highest total TEQ level at 15pg TEQ/g fat. Follow-up studies were performed during the winter at the first farm and the levels in eggs from hens that were kept inside, strongly suggested that the source of the contamination was not the feed, but the outdoor environment (unpublished). Similar has been observed in other studies but in these cases the soil was clearly contaminated with dioxins (Stephens et al. 1990, 1994, 1995, Schuler et al. 1997, Lovett et al. 1998, Harnly et al. 2000, Petreas et al. 1991, Air et al. 2002, Pussemier et al. 2004). Another study was performed in the autumn of 2003, when a large number of farms producing organic eggs were visited and investigated for possible sources of dioxins and factors that may contribute to the exposure of hens to dioxins (Brandsma et al. 2004). This study again showed that most eggs comply with the current dioxin limit of 3pg TEQ/g fat but 13% of the eggs, produced on 26% of the farms would not be in compliance with this limit.

In July 2003, new EU limits for dioxins in food and feed became official, including limits for eggs and feed, at 3pg TEQ/g fat and 0.75ng TEQ/kg feed respectively (EC 2001, 2002). Eggs from free-ranging hens were excluded from these limits until 1 January 2004, and then later to 1 January 2005. At the same time, studies were started to investigate the carry-over of dioxins and PCBs from feed and soil to eggs at relatively low levels, and to investigate possible ways for reducing the exposure and carry-over. The current paper describes a controlled study on the relationship between dioxin and PCB levels in eggs and feed (Study I), as well as soil (Study II). Contrary to previous studies, six different levels in feed were used in a range around the current and proposed EU-limits for dioxins and dioxin-like PCBs. In addition we studied the depletion of these contaminants, which is relevant for risk management purposes in cases of crisis situations. As a potential tool to decrease the carry-over to eggs we also tested the effects of so-called mycotoxin binders on the carry-over of dioxins and PCBs (Study III). The results of the studies were used to develop a mathematical model based on the physiology of the laying hen, which can be used for predicting the levels in eggs resulting from a certain feed contamination, and the effect of certain variables on this relationship (van Eijkeren et al. 2006).

Materials and methods

Preparation of feed

Preparation of feed for Study I: Relation between levels in feed and eggs

Feed was prepared with soy oil spiked with different dioxin and PCB congeners. The composition of the feed is given in Table I. 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 solution of contaminated oil was prepared with about 7500pg TEQ/g of dioxins and dioxin-like PCBs. A 200g aliquot of oil was mixed with the following PCB stock solutions: 7.5ml PCB 105 at 100ng/ml, 15ml PCB 118 at 100ng/ml, 1.2ml PCB 156 at

100ng/ml, 7.5ml PCB 28 at 100ng/ml, 15ml PCB 52 at 100ng/ml, 30ml PCB 101 at 100ng/ml, 30ml PCB 138 at 100ng/ml, 30ml PCB 153 at 100ng/ml and 15ml PCB 180 at 100ng/ml. The organic solvent (nonane) was removed under vacuum. Subsequently the following standard mixtures were added: 44µl CIL EDF-4096 dioxins (tetra's 2.5ng/µl, penta's, hexa's and hepta's 6.25ng/µl and octa's 12.5ng/µl), 408µl CIL EC-4986 non-ortho PCBs (10µg/ml) and 785µl CIL EC-4987 mono-ortho PCBs (10µg/ml). The organic solvent was again removed under vacuum (1h, 63°C).

Table I. *The ingredients of the feed, Topline extra.*

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

The 200g oil was first diluted to 1500g, and subsequently diluted for incorporation into the different feeds, as follows: 30g+1470g blank oil (b used for feed B), 60g+1440g blank oil (c used for feed C), 265g+3237g blank oil (d used for feed D), 685g + 3882g blank oil (e used for feed E), and 420g + 980g blank oil (f used for feed F).

Analysis of oil (b) by GC/MS showed a total TEQ content of 20pg TEQ/g, with a contribution of 11pg TEQ/g for dioxins, 5.3pg TEQ/g for non-ortho PCBs and 3.7pg TEQ/g for mono-ortho PCBs. The blank oil contained a total TEQ content of 0.4pg 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 650kg blank feed (prepared in 2

batches), 110kg of feeds B, C, D and F, and 400kg of feed E. Table II shows the analysed dioxin content of the different feeds.

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

Feed	Dioxins (ng TEQ/kg)	no-PCBs (ng TEQ/kg)	mo-PCBs (ng TEQ/kg)	Total TEQ (ng TEQ/kg)	Indicator PCBs (µg/kg)
*Only in the case of feed A, lowerbound differed from upperbound levels, being respectively 0.01 and 0.02ng TEQ/kg for dioxins and total TEQ.					
A	0.04 (10)	0.00 (16)	0.00 (3)	0.04 (10)	0.2 (14)
B	0.20 (18)	0.09 (4)	0.06 (4)	0.34 (12)	2.3 (1)
C	0.30 (2)	0.17 (3)	0.11 (1)	0.58 (2)	4.3 (7)
D	0.40 (2)	0.22 (2)	0.14 (1)	0.76 (1)	6.0 (4)
E	0.97 (1)	0.55 (2)	0.33 (1)	1.85 (1)	14.2 (2)
F	2.04 (2)	1.19 (1)	0.72 (2)	3.95 (1)	31.7 (3)

Preparation of feed for Study II: Relation between levels in soil and eggs

The 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 dioxin-like PCB levels in eggs. The soils had been analysed previously showing dioxin levels of 5.88ng TEQ/kg (soil A) and 1.94ng 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 7.3pg TEQ/g fat. An additional feed was prepared as a control, by mixing feed E (Study I) with 10% of clean sand.

Preparation of feed for Study III: Effect of binders on carry-over from feed to eggs

The aim of this study was to assess whether or not binders would influence the bioavailability of dioxins and PCBs in feed. A number of different binders were obtained for this study 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 DR CALUX® and analysed by GC/MS, and shown not to contain elevated dioxins and PCBs. The following feeds were prepared: feed E mixed with 0.5% Exal H (Tessenderlo, Madrid, Spain), feed E mixed with 0.5% MycoAd A-Z (Jadis, Schiedam, The Netherlands), and feed E mixed with 0.5% Klinofeed (Unipoint, Truttikon, Switzerland). The level of the binders used was based on the recommendations of the providers.

Animal studies

Study I

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–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.

Study II and III

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 five animals followed an adaptation period of one week on clean feed, followed by 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.

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 pre-treatment, ^{13}C labeled dioxins and dioxin-like PCBs were added to the fat phase of the samples. Feed samples were extracted with ethyl acetate or, when minerals or soil was added to the feed (Study II and III), 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 12.5ml of sample solution, and a fraction collector (Gilson, model 202) adapted to collect 300ml fractions using 500ml glass collection flasks. The glass GPC column (Spectrum) (60×2.5cm) was packed with Biobeads SX3. 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 1g deactivated alumina (7% water) shortly before use. Porous graphitized carbon clean-up was performed using an 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 5ml loop and a fraction collector (Gilson, model 202) adapted to collect 100ml fractions. The column used was Hypercarb (100×4.6mm) (Shandon). The final extract with planar compounds was concentrated to 10µl and the extract containing the non-planar PCBs to 200µl. Both extracts were analysed with gas chromatography-high-resolution-mass spectrometry (HRGC-HRMS) (Autospec, Micromass). The mass spectrometric method to determine the tetra through octa dioxins is based on US 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. Non-detectable congeners were quantified at the limit of detection (upperbound). Absolute levels were transfered to TEQ levels using the TEF values described by Van den Berg et al. (1998).

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, 200mg of the homogenized fat sample is mixed with 800µl of the internal standard solution of ^{13}C PCB 118 and PCB 198 (6.25ng/ml). Subsequently, 50µl 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 3ml dichloromethane. The PCB-containing fraction (approximately 1ml) 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 10m, which ends up in a retaining column of 1-m 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 PCBs are retained on the 1-m 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 conducted by mass-spectrometry with a bench top system in Selected Ion Mode (SIM).

DR CALUX® analysis

The DR CALUX® test was carried out as described earlier by Bovee et al. (1998). An aliquot of 0.5g 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 6pg TEQ/g fat were included. The response obtained with the butter fat sample of 3pg TEQ/g, containing 1.5pg TEQ/g dioxins and 1.5pg 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 15ml methanol/water 85/15 (v/v) and extracted twice with 20ml hexane/diethyl ether 97/3 (v/v). The extract was reduced to 5ml and purified over acid silica columns as described above. Feed samples were included as control samples.

Results and discussion

Carry-over from feed to eggs (Study I)

Dioxin and PCB levels in the feeds. Table II shows the total levels of dioxins, dioxin-like PCBs and indicator PCBs in the various feeds. Each feed was analysed 3–5 times, corresponding to the number of samples taken from the different bags. The variation coefficients were in general below 5%, demonstrating the good homogeneity of the feeds. The relative contribution of dioxins, non-ortho and mono-PCBs to the total TEQ level was as intended, at 50, 30 and 20% respectively. Table III shows the levels of the various congeners in the two highest contaminated feeds. The overall levels were higher than intended, which cannot be explained by the background levels in the feed ingredients, as shown by the level in the blank feed (A). However, since a rather broad range of different concentrations in feed was used, this deviation had no consequences for the aim of the study.

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

	Feed E	Eggs E day 56	Carry-over (%)	Feed F	Eggs F day 56	Carry-over (%)
Dioxins	ng/kg	pg/g fat		ng/kg	pg/g fat	
2,3,7,8-TCDF	0.12	1.10	40	0.25	2.33	40
1,2,3,7,8-PeCDF	0.33	2.86	39	0.66	7.15	48
2,3,4,7,8-PeCDF	0.33	2.83	38	0.67	6.76	44
1,2,3,4,7,8-HxCDF	0.34	3.33	43	0.70	7.00	44
1,2,3,6,7,8-HxCDF	0.33	2.98	40	0.68	6.44	42
2,3,4,6,7,8-HxCDF	0.43	3.03	31	0.84	6.80	35
1,2,3,7,8,9-HxCDF	0.32	2.85	39	0.67	6.28	41
1,2,3,4,6,7,8-HpCDF	0.40	1.60	18	0.82	3.26	18
1,2,3,4,7,8,9-HpCDF	0.32	1.44	20	0.66	3.06	21
OCDF	0.63	0.98	7	1.39	1.55	5
2,3,7,8-TCDD	0.14	1.31	41	0.30	3.26	47
1,2,3,7,8-PeCDD	0.37	3.39	41	0.79	8.04	45
1,2,3,4,7,8-HxCDD	0.40	3.80	42	0.84	8.38	44
1,2,3,6,7,8-HxCDD	0.35	3.45	44	0.74	7.49	44
1,2,3,7,8,9-HxCDD	0.40	2.87	32	0.84	6.49	34
1,2,3,4,6,7,8-HpCDD	0.42	2.15	22	0.84	3.97	21
OCDD	1.34	3.82	13	2.57	4.78	8
Non-ortho-PCBs	ng/kg	pg/g fat		ng/kg	pg/g fat	
PCB 81	5.12	46.70	40	10.86	102.0	41
PCB 77	10.85	112.00	45	22.06	252.0	50
PCB 126	4.98	54.10	48	10.86	123.0	50
PCB 169	4.89	55.40	50	10.55	124.0	52
Mono-ortho PCBs	ng/kg	ng/g fat		ng/kg	ng/g fat	
PCB 123	5	0.10	78	14	0.22	70
PCB 118	1576	17.10	48	3454	40.60	52
PCB 114	6	0.07	52	14	0.16	51
PCB 105	894	9.36	46	1940	23.00	52

PCB 167	10	0.20	84	24	0.41	76
PCB 156	139	1.75	55	307	4.03	58
PCB 157	13	0.15	51	26	0.39	65
PCB 189	10	0.14	61	21	0.29	61
Indicator-PCBs	µg/kg	ng/g fat		µg/kg	ng/g fat	
PCB 28	0.98	8.4	38	2.13	20.7	43
PCB 52	1.30	1.6	5	2.91	2.9	4
PCB 101	2.88	3.5	5	6.33	7.5	5
PCB 138	2.99	34.6	51	6.72	83.1	54
PCB 153	2.79	36.1	57	6.27	86.2	61
PCB 180	1.89	25.6	59	4.27	58.5	41

Levels in eggs

Table IV shows the average feed consumption and the productivity of the hens, both during and after the exposure period. The treatment did not have an effect 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 48–51g per egg (without shell). There is no explanation for this decrease, or possible consequences for the residue levels in the eggs.

Table IV. *Feed consumption and productivity of the hens during and after the exposure period. Data are based on the overall feed consumption and egg production over the whole exposure or withdrawal period.*

	Exposure period			**Withdrawal period**		
Feed	**Feed consumption (g/day)**	**Egg production (%)**	**Egg weight* (g)**	**Feed consumption (g/day)**	**Egg production (%)**	**Egg weight* (g)**
*Average weight per egg without eggshell.						
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

Figure 1 presents the time-related levels of dioxins (A), non-ortho PCBs (B), mono-ortho PCBs (C) and total TEQ (D) in the eggs from the different exposure groups. These data show that even after 56 days on contaminated feed, levels in eggs continued to increase. In a similar study, Pirard and De Pauw (2005) exposed laying hens to a dioxin level ten-fold higher than our highest

concentration. They observed a continuous increase in the levels for 4 weeks, at which time point the animals stopped laying eggs.

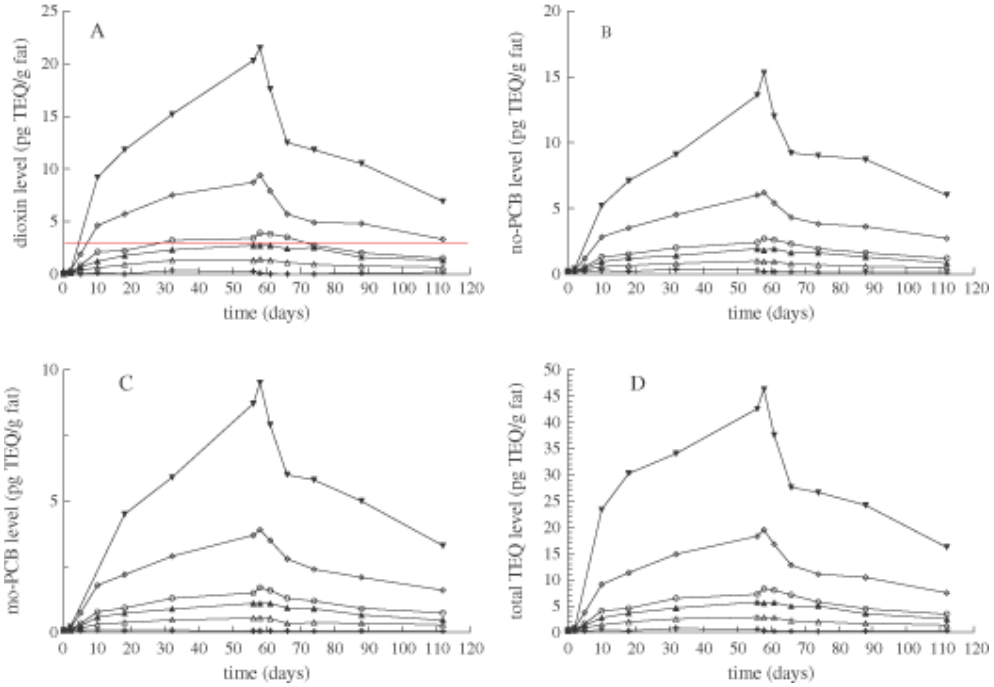

Figure 1. Levels of dioxins (A), non-ortho PCBs (B), mono-ortho PCBs (C) and total TEQ (D) in egg fat from hens fed with feeds containing 0.04 (A,+), 0.34 (B, △), 0.58 (C, ▲), 0.76 (D, ○), 1.85 (E, ◇) and 3.95 (F, ▼) ng TEQ/kg of dioxins and PCBs. Results from single analysis.

As shown in Figure 1, after cessation of the exposure, there was initially a small increase in the residues, then a rapid decline and subsequently a much slower decrease. A similar profile was observed previously in a study with much higher dioxin and PCB levels (Hoogenboom et al. 2002). The explanation lies in the fact that the production of a full-grown yolk requires about 10 days. Thus yolks of eggs laid during the first days on clean feed were largely formed and contaminated 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 1A also shows that the current limit for eggs of 3pg TEQ/g fat was rapidly exceeded, when exposed to feed containing dioxins just above the current limit for feed of 0.75ng TEQ/kg.

Figure 2 shows the levels of the 7 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. Again, data indicate that egg levels continued to increase even after 56 days. At similar dose levels (1.8 or 6 μg/kg feed), De Vos et al. (2005) observed steady-state levels after respectively 40 or 70 days. The highest level obtained at 56 days of exposure via the most contaminated feed was around 325ng/g fat. Based on 4% fat in the feed, the feeds E and F would contain 350 and 800ng/g fat respectively, thereby exceeding, e.g., the Belgium limit for feed of 200ng/g. Only eggs from hens fed the highest contaminated feed F would actually exceed the Belgium limit for indicator PCBs of 200ng/g fat, although it cannot be excluded that after longer exposure eggs from hens fed with feed E might have approached the limit as well.

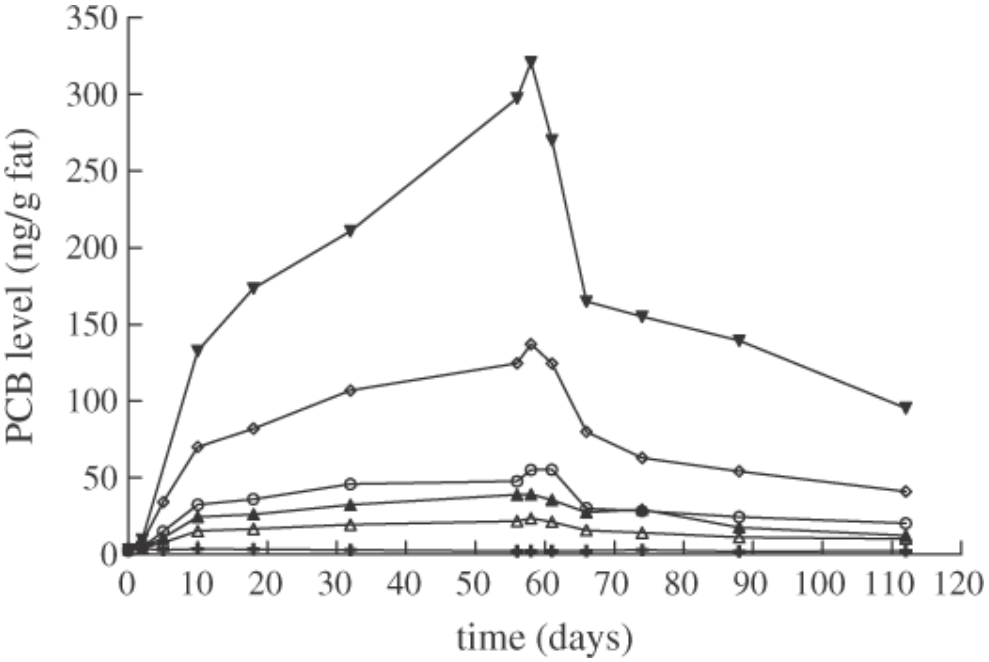

Figure 2. Levels of indicator PCBs in egg fat from hens fed with feeds containing 0.2 (A,+), 2.3 (B, △), 4.3 (C, ▲), 6.0 (D, ○), 14.2 (E, ◇) and 31.7 (F, ▼) μg/kg feed of indicator PCBs. Results from single analysis.

The relationship between the dioxin levels in feed and eggs after different periods of exposure is graphically presented in Figure 3. 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 0.75ng TEQ/kg, the limit for eggs was exceeded.

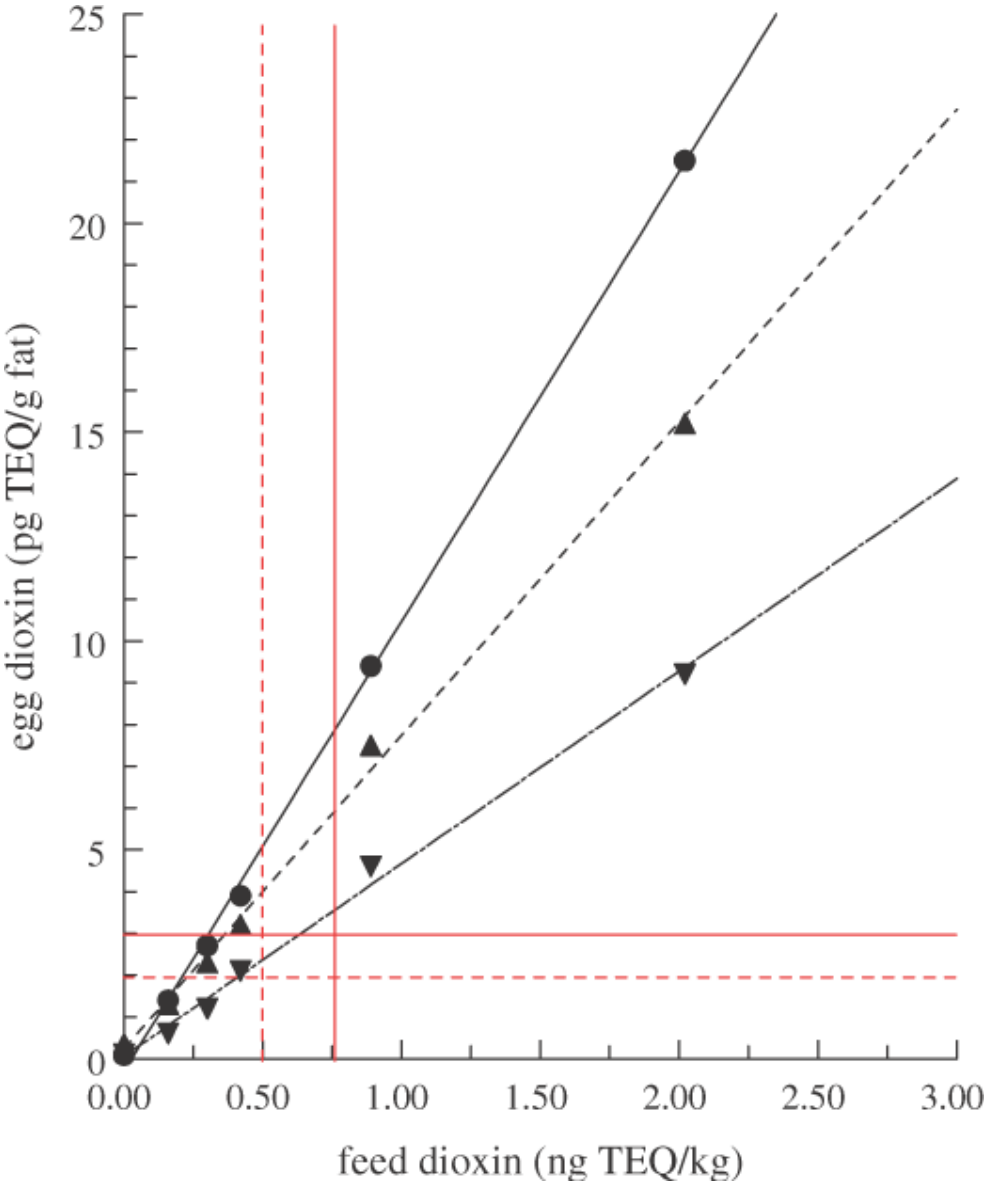

Figure 3. Relation between dioxin levels in feed and egg fat after 10 (▼), 32 (▲) and 58 (•) days of feeding contaminated feed. Solid lines represent the current limits in feed and eggs, dashed lines the action limits in the EU.

Levels in abdominal and ovary fat

The time-related levels of the sum of dioxins and dioxin-like PCBs (A), as well as the 7 indicator PCBs (B) in egg, abdominal and ovary fat sampled from the chickens fed with feed E are shown in Figure 4.

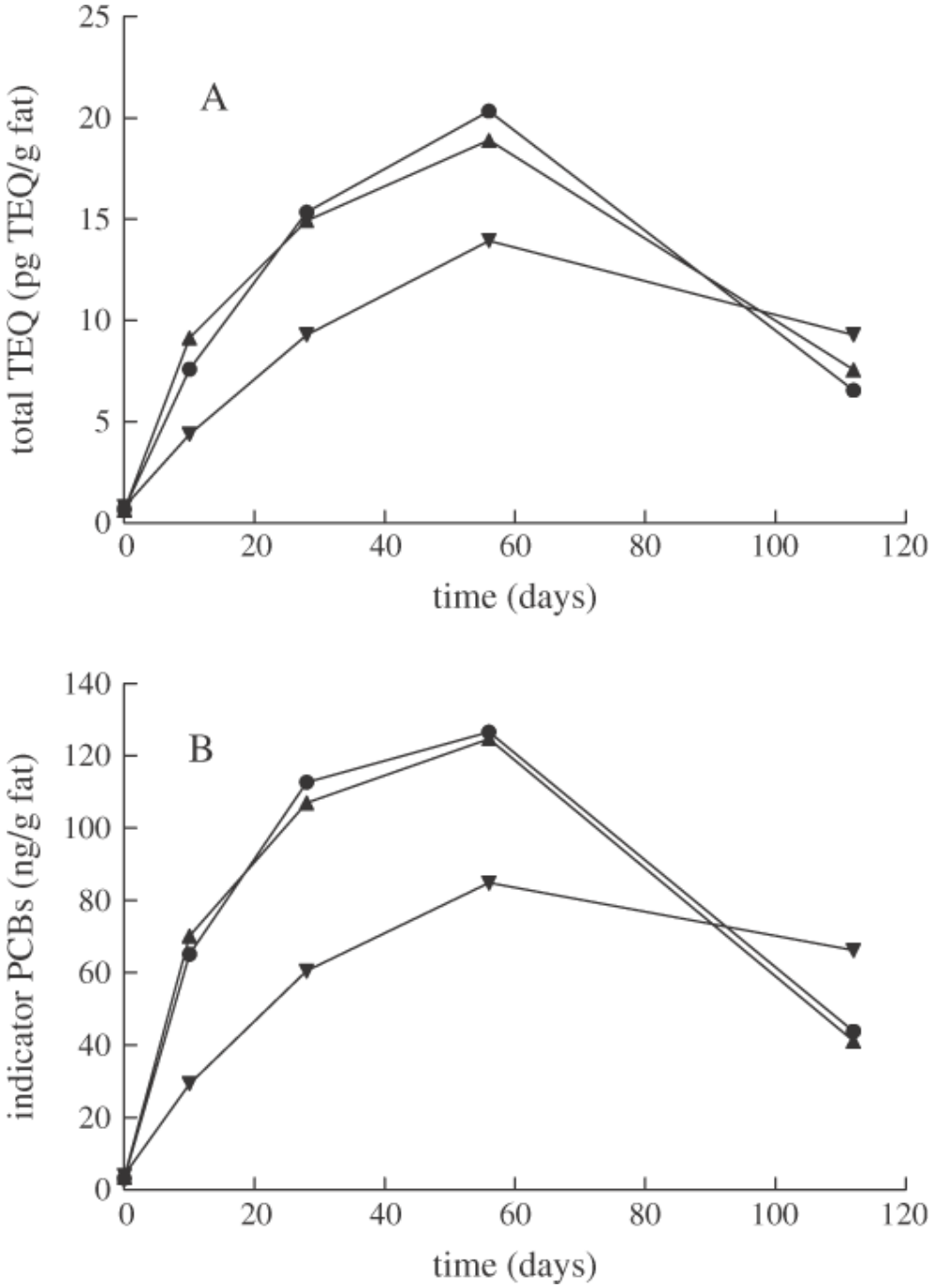

Figure 4. Levels of dioxins and dioxin-like PCBs (A), and indicator PCBs (B) in egg (▲), abdominal (▼) and ovary fat (•) from chickens fed with feed E. Results from single analysis.

During the 56-day exposure period, levels in eggs and ovary fat were comparable and generally higher than in the abdominal fat. This was particularly true for the indicator PCBs. This difference was also clear for either dioxins or dl-PCBs (data not shown). After another 56 days on clean feed, levels of dioxin-like compounds were very similar in ovary and egg fat and slightly lower than in the abdominal fat. However, levels of the indicator PCBs were clearly elevated in the abdominal fat. The combined results indicate that the decrease of dioxins and PCBs is much slower in the body fat than in the eggs and related ovaries. From

the model for carry-over (van Eijkeren et al.) it appears that the levels in eggs possess a clearly bi-phasic nature, an initial phase with fast rising or, after cessation of contamination, fast declining levels, followed by a terminal phase with slowly increasing or decreasing levels. On the other hand, in abdominal fat, which acts as a great capacitor for these highly lipophilic compounds, the relative contribution of the fast rising phase is very low and the kinetics of contamination is almost mono-exponential with the rate of the slow terminal phase.

Carry-over rates of individual congeners from feed to egg

To compare the carry-over of individual congeners from feed to eggs, it is possible to calculate the so-called Carry-Over Rate (COR), defined as the fraction of a certain congener excreted in the eggs, in relation to the amount ingested from the feed. Ideally, this should be done under steady-state conditions, but this was not achieved within the current study period. Therefore, the COR values should be regarded as minimum values. Based on the daily egg fat production of 5.1g and daily feed intake of 116g, CORs were calculated for feeds E and F in I (Table III). 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. This is in line with the observation by Pirard and De Pauw (2005) who also showed a much higher excretion of the higher chlorinated PCDD/Fs into the feces. 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 group of the indicator PCBs, the lower chlorinated PCBs 53 and 101 showed very low carry-over rates, contrary to PCB 28. Similar results have been observed previously in laying hens (De Vos et al. 2005) and broilers (Hoogenboom et al. 2004, Maervoet et al. 2004). Based on excretion in the feces, De Vos et al. (2005) showed that active metabolism of these congeners must underly this observation.

Before steady state conditions are reached, part of the absorbed compounds are still accumulated in the body fat. Based on the mathematical modelling it is expected that after reaching steady-state, COR values will be doubled, implying that the amounts excreted in the eggs will be close to the amounts ingested (van Eijkeren et al. submitted). As for total TEQ levels, the absorbed fraction ranges between 90% and 100%, the COR for total TEQs at steady state is expected to range between the same limits.

Carry-over from soil to eggs (Study II)

Table V 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. The two soils were reanalysed and shown to contain 4.0 and 1.7ng TEQ dioxins per kg respectively and only minor amounts of dioxin-like PCBs. The level in the feed containing soil A is in agreement with the dilution, whilst the level in the feed with soil B is relatively high and can best be explained by the application of the upperbound principle. Lower bound levels (i.e., levels of non-detects set at 0) in these two feeds were 0.40 and 0.19ng TEQ/kg respectively, reflecting the differences observed in the soil.

Table V. *Levels of dioxins, non-ortho and mono-ortho PCBs in the different feeds used in studies II and III, as determined by GC/HRMS (mean of n=5, upperbound levels, lowerbound between brackets where different from upperbound).*

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

Figure 5 shows the dioxin and dioxin-like PCB levels in the eggs from the hens obtaining these feeds. Time-related levels in the eggs from the hens fed with feed E, either without or with 10% sand were very similar to the results obtained in Study I.

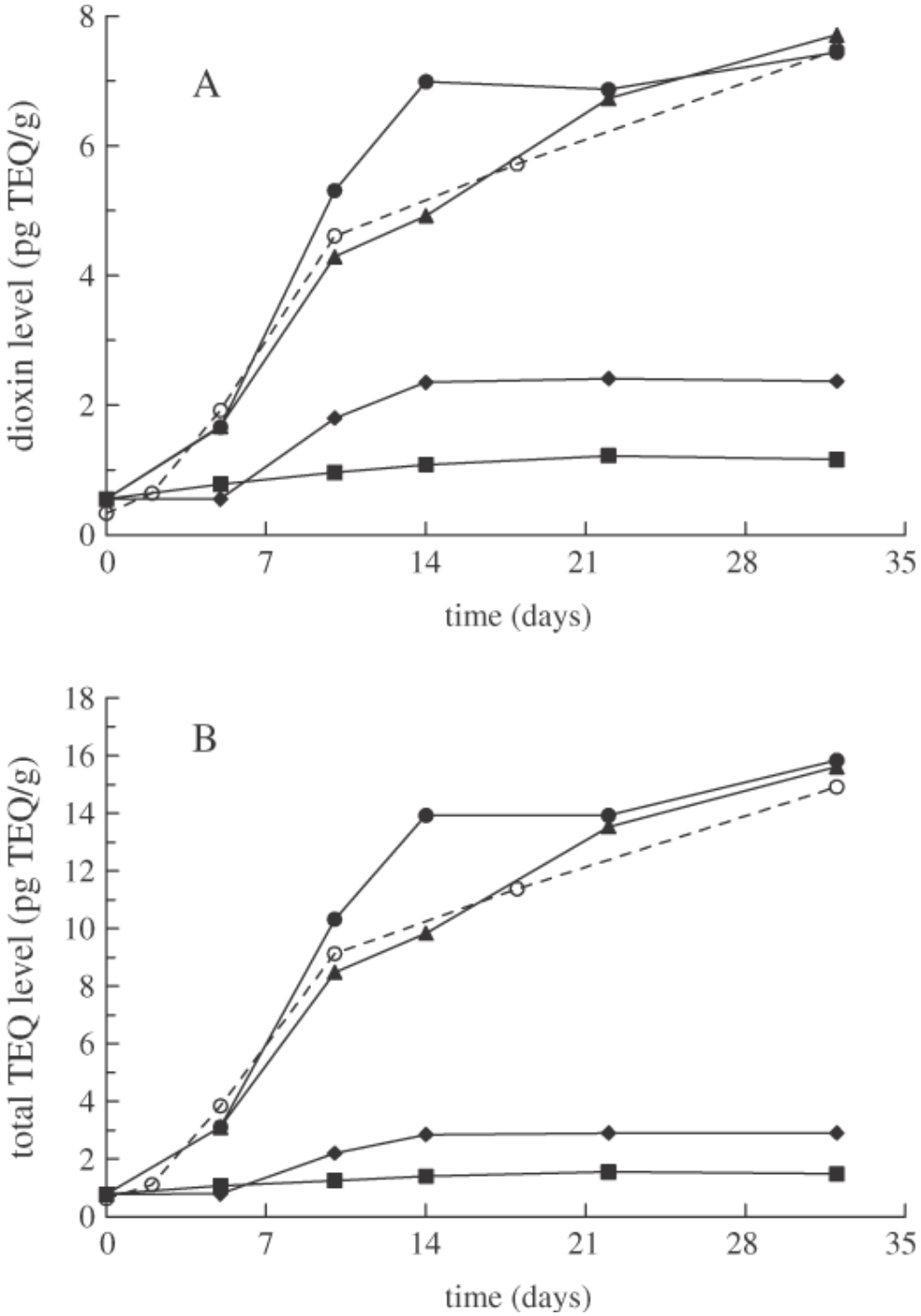

Figure 5. Levels of dioxins (A) and total TEQ (B) in egg fat from hens fed with feed E (•), feed E mixed with 10% sand (▲), and blank feed A mixed with 10% soil A (♦) or soil B (■). For comparison the results obtained with feed E in the first study are included (○). Results from single analysis.

The dilution of contaminated feed with 10% 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. A plateau level appeared to be reached in this study 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 2.9pg TEQ/g fat. In the case of soil B, highest levels of 1.2 and 1.6pg TEQ/g were observed. Again, levels in abdominal fat were lower than those for egg fat (data not shown). For comparison, levels determined in pooled egg samples from the farms where the soil was sampled were 7.3 and 1.0pg TEQ/g fat.

Effect of binders (Study III)

Table V includes the levels of dioxins and PCBs in the four feeds supplemented with the different binders. Levels in egg and abdominal fat of the hens fed with these feeds showed that the binders, at least at the levels used in this study, had little or no effect on the residue levels of dioxins and PCBs in the eggs (Table VI), although in the case of MycoAd AZ and especially Klinofeed, both aluminium silicates, there appeared to be a slight decrease in the levels of the different contaminants in both the eggs and the abdominal fat. It seems worthwhile to investigate if higher levels may have a more profound effect, and whether this also works with the absorption of dioxins and PCBs from soil.

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

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

Conclusions

- Laying hens are sensitive indicators for dioxin exposure, meaning that relatively low intake levels result in high levels in the egg yolk. This is not only true for contaminated feed but also for contaminated soil.

- The current EU limit for feed of 0.75ng TEQ/kg, established in 2002, is insufficient to guarantee that levels in eggs will not exceed the limit of 3ng TEQ/kg fat. A further decrease in the feed limit by a factor 4 is required to achieve this goal. The alternative is to increase the limit in eggs, but this is not in line with the further reduction in the exposure aimed at by the EU authorities.
- There are currently no Dutch or European limits for indicator PCBs in feed or eggs. However, the Belgium limit of 200ng/g fat for feed does ensure that the limit for eggs of 200ng/g fat is not exceeded.
- Addition of binders at the tested levels has minimal effects on the resulting residues of dioxins and PCBs in hens.

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