RESIDUES OF DIOXINS AND PCBs IN EGGS FOLLOWING SHORT-TERM EXPOSURE OF LAYING HENS TO FEED FROM THE BELGIAN CRISIS

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Introduction

In April 1999 RIKILT became involved in the Belgian dioxin crisis after detecting dioxin levels of respectively 781 ng TEQ/kg and 958 pg TEQ/g in chicken feed and chicken fat. Based on PCB levels determined later on, it became apparent that the fat used for the production of this feed in January had been contaminated by at least 100 liters of PCB oil, which contained high levels of in particular dibenzofurans. In this respect the incident was very similar to the Yu-Cho and Yu-Cheng incidents which occurred in 1968 and 1979, of course with the difference that in this case only animals were exposed. In the case of chickens this resulted in a dramatic decrease of the hatching of the eggs, and symptoms similar to chicken oedema disease. Based on these effects, the feed producer decided to investigate the possible presence of dioxins in the animal feed. At the beginning of June it was unclear which food items still contained elevated levels of dioxins and many of these products had to be checked. The latter could initially only be performed by the rather expensive and laborious GC/MS reference method, although in the Netherlands the use of the CALUX bioassay for screening was accepted. At the end of June it was decided to accept PCB analysis as an indicator for dioxins, assuming a ratio of 50,000 between the sum of the so-called indicator PCBs (PCBs 28, 53, 101, 118. 138, 153 and 180) and dioxins (expressed in TEQs). Based on this, PCB limits of 200 ng/g fat for animal and egg fat and 100 ng/g fat for milk fat were established. It was assumed that the ratio would be constant over time, i.e. kinetics for the indicator PCBs and dioxins in animals were assumed to be identical. Although some studies have been performed in chickens on either dioxins or PCBs, no studies were available to support this assumption. Last year we presented data on pigs and broilers[1], showing that the use of indicator PCBs did not result in an underestimation of dioxin levels. Aim of the present study was to investigate the stability of this ratio in eggs of laying hens. For this study the original Belgian feed was used but in order to avoid possible toxic effects, the original contaminated feed was diluted ten-fold.

Materials and methods

Chemicals

Chicken feed from the Belgian dioxin crisis in 1999, containing a mix of two chicken feeds with respectively 1 and 4% of the fat contaminated with PCB oil, was a kind gift of The Belgium Ministry of Agricultural.

Animal studies

The contaminated feed was diluted ten-fold with clean feed. Twenty laying hens were fed with the diluted contaminated feed for 7 days, followed by a period of 0, 1, 3, or 6 weeks on clean feed. An additional five animals received clean feed for 7 days. During the whole experiment eggs were collected. At the end of the exposure period and after 1, 3 or 6 weeks on clean feed five animals were slaughtered and abdominal fat and livers collected. Prior to analysis, fat was heated in a microwave oven and filtered over anhydrous sodium carbonate. Egg fat was extracted with pentane from egg yolk, following mixing with anhydrous sodium carbonate.

Dioxin analysis

Dioxins, non-ortho and mono-ortho PCBs were analysed following clean-up of the fat by GPC, aluminium oxide and carbon as described by Tuinstra *et al* 1994. The carbon-eluate containing the mono-ortho PCBs was analysed separately from the dioxins and non-rtho PCBs.

PCB analysis

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 via a Large Volume Injector.

Results and Discussion

The mixture of two original chicken feeds, containing 1 and 4% fat, was diluted ten-fold for this study. Levels of dioxins, non-ortho-, and mono-ortho and PCBs were respectively 61, 23, 116 ng TEQ/kg. In addition the feed contained 3.2 mg/kg of indicator PCBs, resulting in a ratio between the indicator PCBs and dioxins of 52,000. The feed used for the mixing was clean.

During the exposure period the 20 laying hens consumed on average 111 ± 12 gram of feed per day, as compared to 100 ± 20 g/day for the hens eating the control feed. As a consequence animals received a total dose of 47 ng TEQ dioxins, 18 ng TEQ non-ortho PCBs, 90 ng TEQ mono-ortho PCBs amounting to 155 ng TEQ, in addition to 2.5 mg of the 7 indicator PCBs. Exposure to the contaminated feed did not result in a decrease in egg production, which was on average 6.6 ± 0.8 per week for the 20 exposed animals as compared to 6.8 ± 0.4 for the 5 controls. Egg production in the 5 animals exposed for one week, followed by 6 weeks on clean feed were 6.8 ± 0.4, 6.2 ± 0.4, 6.6 ± 0.5, 6.6 ± 0.5, 7.0 ± 0.0 and 6.2 ± 0.8 in weeks 1, 2, 3, 4, 5 and 6 respectively (the last week contained only 5 days and were therefore excluded).

Initially eggs obtained during the last two days from each chicken, i.e. just before slaughter, were pooled and analysed for dioxins, non-ortho PCBs and indicator PCBs. As shown in Table 1, levels of dioxins and non-ortho PCBs determined at the end of the exposure period (days 6-7), were similar to those measured after one week on clean feed (days 13-14). Subsequently the dioxin levels started to decrease to about 40 and 30 % after 21 and 40 days on clean feed. Indicator PCB levels decreased already during the first week on clean feed. The ratio of indicator PCBs and dioxins was initially higher than in the feed but eventually decreased to a similar ratio.

Based on the first analysis, and the physiology of egg production requiring a 9-10 day period, eggs were pooled for a number of selected days and analysed for dioxins, non-ortho- and mono-ortho PCBs and indicator PCBs. As shown in figure 1, levels at day 7 were now higher than those at day 14, which can be explained by the fact that in the first series (Table 1), eggs from days 6 and 13 were mixed with those from days 7 and 14 respectively. Maximum levels were reached after 9 days, being 214, 92, and 370 pg TEQ/g fat for dioxins, non-ortho- and mono-ortho PCBs. Total levels amounted to 676 pg TEQ/g fat, meaning that one egg contained more than 3 ng TEQ.

It is evident that mono-ortho PCBs contribute most to the total TEQ levels in the eggs. Considering a 10-15 times dilution of the feed, eggs during the first weeks of the Belgian crisis could have contained 2-3 ng TEQ dioxins/g fat or a total amount of 30 to 45 ng TEQ. Actual levels of dioxins measured in the first egg were only 0.7 ng TEQ/g fat or 25 % of the estimated maximum, but it should

Table 1. Dioxin, non-ortho and indicator PCB levels in egg fat (first series). Chickens were exposed for 7 days, followed by 40 days on clean feed. Eggs were pooled over two days per chicken. Data present the mean and SD for the 5 chickens per group.

Time period Days	Dioxins	non-ortho PCBs (pg TEQ/g)	Total	indicator PCBs (µg /g)	Ratio (x1000)
6-7	127.8 ± 6.6	82.1 ± 4.1	209.9 ± 10.5	10.8 ± 0.6	84.5 ± 4.8
13-14	136.8 ± 7.7	83.7 ± 13.4	220.5 ± 20.7	8.2 ± 1.0	60.9 ± 10.3
27-28	57.2 ± 10.0	39.1 ± 6.6	96.3 ± 16.6	2.7 ± 0.5	47.8 ± 2.1
45-47	44.0 ± 11.5	31.0 ± 9.8	75.0 ± 21.3	2.2 ± 0.5	49.4 ± 4.4
Control	0.7 ± 0.7	1.1 ± 0.8	1.8 ± 1.4	0.01 ± 0.01	

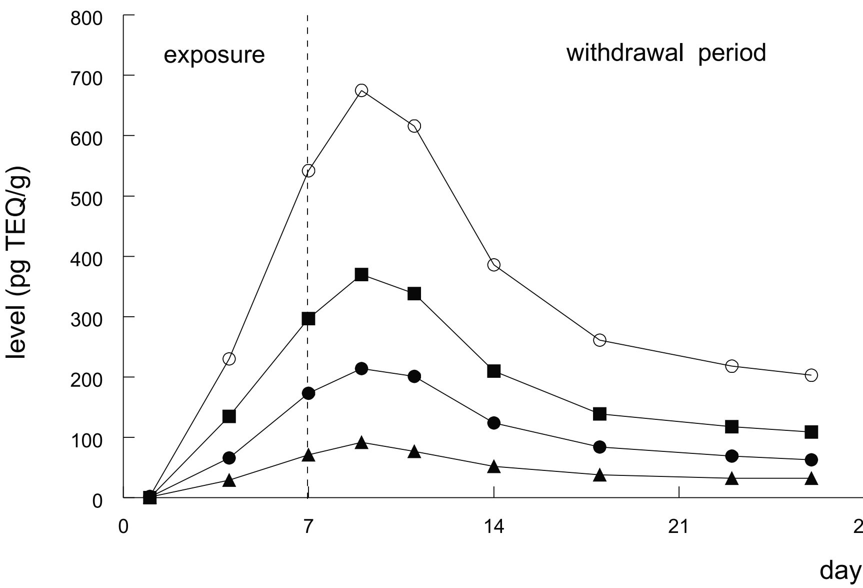

Figure 1. Levels of dioxins (closed circles), non-ortho (closed triangles) and mono-ortho (closed cubes) PCBs, as well as total TEQ levels (open circles) in chicken eggs. Chickens were exposed for 7 days and subsequently fed clean feed.

be pointed out that this egg was sampled two months after exposure of the chickens. In the present study, levels measured after 40 days on clean feed also contained only 25 % of the maximum levels. It is important, however, to realize that during the crisis the egg production was decreased and that linear extrapolation might not be completely correct.

As shown in Figure 1, the behaviour of dioxins and dioxin-like PCBs appears to be very similar over the exposure and depletion period, with relative contributions of dioxins to the total TEQ level of 29, 32, 32, 32, 32, 31% at days 4, 7, 9, 14, 18 and 25. As shown in Figure 2 and Table 1, indicator PCBs

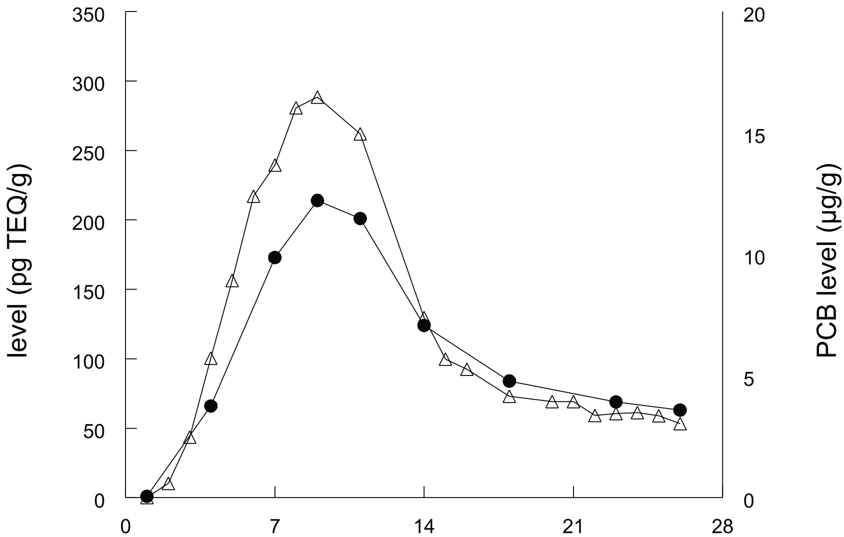

Figure 2. Levels of indicator PCBs (open triangles), as compared to dioxin levels in eggs of chicken exposed for 7 days, followed by clean feed.

appeared to be excreted into the eggs to a relatively higher extent during the exposure period, when most of the eggs of days 9, 10 and 11 were produced. As a result the ratio was initially higher than in the feed but gradually fell back to the same level. Based on this, it is concluded that the use of indicator PCBs appears to be a good alternative for dioxins in the case of co-exposure to both PCBs and dioxins.

Analysis of abdominal fat samples and liver show that a large part of the dioxins and PCBs are actually stored in these tissues and serve as a source during the depletion period (data not shown).

Reference

1. Traag, W.A., Kan, K., Bovee, T., Weg G. van der, Onstenk, C., Portier, L., and Hoogenboom R. (2001) Dioxin and PCB levels in fat of pigs and broilers fed with feed from the Belgian crisis. *Organohalogen Comp.* 51, 291-294.