DIOXIN AND PCB LEVELS IN FAT OF PIGS AND BROILERS FED WITH 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 150 liters of PCB oil, which contained high levels of in particular dibenzofurans. In chickens this resulted in a dramatic decrease of the hatching of eggs, and symptoms similar to chicken edema disease. Based on these effects, the feed producer, after ruling out other causes, decided to investigate the possible presence of dioxins in the feed. At the beginning of June, when the crisis became public, it was unclear which food items still contained elevated levels of dioxins and many of these products had to be checked. This 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, using a ratio of 50,000 between the sum of the indicator PCBs (# 28, 53, 101, 118. 138, 153 and 180) and dioxins (in TEQs). 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 in exposed animals would be constant over time, i.e. kinetics for the indicator PCBs and dioxins in animals would be identical. Although some studies have been performed in chickens on either dioxins or PCBs, no studies were available to support this assumption. Aim of the present study was to investigate the stability of this ratio in both broilers and pigs. 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 Federal Ministry of Agriculture in Belgium.

Animal studies

The contaminated feed was diluted ten-fold with clean feed. Fifteen broilers (3 weeks old) were fed with the diluted contaminated feed for 7 days, followed by a period of 0, 1 or 3 three weeks on clean feed. An additional five animals received clean feed for 7 days. At the end of the exposure period and after 1 or 3 weeks on clean feed five animals were slaugthered and abdominal fat collected. Prior to analysis, fat was heated in a micromave oven and filtered over anhydrous sodium carbonate.

Dioxin analysis
Dioxins, non-ortho and mon-ortho PCBs were analysed following clean-up of the fat by GPC, aluminium oxide and carbon as described by Tuinstra *et al*[1].
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% contaminated fat, was diluted ten-fold for this study. Levels of dioxins, non-ortho- and mono-ortho PCBs were respectively 45, 19 and 93 (total 157) ng TEQ/kg feed. Dioxins, non-orto PCBs and mono-ortho PCBs contributed for respectively 29, 12 and 59% to the total TEQ level. The level of indicator PCBs was 3.2 mg/kg and is in agreement with those of previous analysis of PCBs in the original feed showing levels of the 7 indicator PCBs of 32 mg/kg. Based on the fact that the highest contaminated original feed contained 4% contaminated fat, and the fact that the 7 indicator PCBs comprise about 30% of the total PCBs in Arochlor 1260, it can be calculated that the contaminated 60-80 tons of fat contained at least 160 kg of PCB-oil. In addition the feed contained high levels of dioxins and non-ortho PCBs, but in particular high levels of mono-ortho PCBs which exceed the dioxin levels two-fold. The ratio of the 7 indicator PCBs and the dioxins in the feed was 70,000, being close to the ratio of 50,000 which was the basis for the PCB residue limits set to control the crisis.

Broilers
During the exposure period the 15 broilers consumed on average 83 ± 17 gram of feed per day, as compared to 130 ± 8 g/day for the 5 broilers eating the control feed. As a consequence animals received a total dose of 26, 11 and 54 ng TEQ of respectively dioxins, non-ortho and mono-ortho PCBs, amounting to 91 ng total TEQ, in addition to 1.85 mg of the 7 indicator PCBs. Table 1 shows the level of the different dioxin and dioxin-like PCB congeners in the abdominal fat. With respect to the TEQ levels derived from dioxins in fat of broilers at the end of the treatment, 2,3,4,7,8-PeCDF was by far the most important congener (67%), followed by 2,3,7,8-TCDF (11%), 1,2,3,7,8-PeCDF (9%) and 1,2,3,4,7,8-HxCDF (6%). PCB 126 was the most important non-ortho PCB. Table 1 also shows the levels of the 7 indicator PCBs. Within the 3 week period on clean feed, PCB levels decreased from an initial 6.2 µg/g to 1.5 µg/g, which is very similar to the relative decrease in dioxin and non-ortho PCB levels. The major decrease in tissue levels

Table 1. Levels of dioxins, non-ortho and mono-PCBs (pg TEQ/g), and indicator PCBs (µg/g) in fat of broilers fed with contaminated feed for 1 week followed by clean feed for 3 weeks. Fat samples from 5 broilers per sampling point were pooled.

Week	dioxins	non-ortho PCBs	mono-ortho PCBs	total	Indicator PCBs	ratio ind PCBs/diox
0	102	84	ND	186	6.23	61,000
1	55	41	ND	96	3.17	58,000
3	26	22	ND	48	1.52	58,000

ND: not determined

appears to be due to the growth of the animals. Based on normal growth curves, body weight will increase from 800 at week 0, to respectively 1500 and 2500 grams at weeks 1 and 3. Fat levels will increase from 13 to 14 and 16% resulting in body fat levels of about 100, 200 and 400 gram. Dioxin and PCB levels decreased 2 and 4-fold during the period after the cessation of exposure.

Pigs

The five pigs consumed on average 1.2 kg of contaminated feed per day for 7 days. As a consequence animals received a total dose of 378 ng TEQ dioxins, 160 ng TEQ non-ortho PCBs and 781 ng TEQ mono-ortho PCBs amounting to 1319 ng total TEQ, in addition to 27 mg of the 7 indicator PCBs. Table 2 shows the levels of dioxins and dioxin-like PCBs in fat biopsy samples taken at different time periods after withdrawal of the contaminated feed. In terms of contribution to the TEQ levels in fat from animals sampled at the end of the treatment (week 0), 2,3,4,7,8-PeCDF was by far the most important dioxin congener (about 73% of the TEQ level caused by dioxins and 15% of the total TEQ), followed by 1,2,3,4,7,8-HxCDF (10%) and 1,2,3,7,8-PeCDD (9%). Remarkable were the relatively low levels of TCDF in the fat. PCB 126 was the only non-ortho PCB contributing significantly to the TEQ level. PCBs 156 and 118 were the most important mono-ortho PCBs, contributing respectively 44 and 34% to the TEQ level derived from this group of compounds, and 29 and 23% to the total TEQ level. As shown in Tabel 2, the mono-ortho PCBs were the most important group of dioxin-like compounds, contributing for 67% to the initial TEQ level but even increasing to 87% after 16 weeks on clean feed.

Levels of the 7 indicator PCBs decreased from an initial 6149 to 475 ng/g fat after 16 weeks on clean feed (Table 2), which is still above the limit of 200 ng/g used for pig meat in Belgium. As shown before, the levels of the lower chlorinated PCBs 28, 52, 101 and 118 decreased relatively rapid when compared with PCBs 138, 153 and 180 (Table 3). The ratio between the indicator PCBs and dioxins only tended to decrease initially but appeared to increase at longer withdrawal periods. It cannot be excluded that this is partly due to the low levels of dioxins being close to the limits of quantification. Remarkably, the initial ratio was about 3-fold higher than in the feed, indicating either a relatively poor absorption or faster elimination of dioxins, or possibly a diferent distribution in the body (e.g. storage of dioxins in liver). The ratio between indicator PCBs and the sum of dioxins and dioxin-like PCBs was much more stable over time.

Table 2. Levels of dioxins, non-ortho and mono-PCBs (pg TEQ/g), and the 7 indicator PCBs (μg/g) in fat of pigs fed with contaminated feed for 1 week followed by clean feed for 15 weeks. Biopsy samples were obtained from five pigs and pooled prior to analysis. Levels of the 7 indicator PCBs were determined in fat from individual pigs (n=5 per sampling point).

Week	dioxins	non-ortho PCBs	mono-ortho PCBs	total	indicator PCBs	ratio indicator PCBs/dioxins
0	26.1	15.3	81.9	123.3	6.15 ± 0.83	236,000
1	21.8	10.3	63.0	95.1	3.77 ± 0.40	173,000
2	15.0	6.4	48.1	69.5	2.50 ± 0.55	166,000
4	7.4	3.0	29.4	39.8	1.62 ± 0.23	218,000
8	3.3	1.3	17.7	22.3	0.88 ± 0.19	267,000
16	1.3	0.6	10.3	11.9	0.48 ± 0.05	376,000

Table 3. Relative contribution of different PCB congeners (%) to the total level of the 7 indicator PCBs in fat from pigs fed with contaminated feed for 1 week followed by clean feed for 15 weeks. Biopsy samples were taken at the end of week 0, 1, 2, 4, 8 or 16.

Congener	feed	week 0	week 1	week 2	Week 4	week 8	week 16
PCB 28	0.7	0.2	0.0	0.0	0.0	0.0	0.0
PCB 52	7.4	8.1	6.6	5.4	3.6	2.2	1.4
PCB 101	19.1	15.8	13.4	11.4	8.5	5.8	3.7
PCB 118	9.8	9.0	8.2	7.6	6.5	5.2	4.1
PCB 138	29.7	31.9	33.6	33.8	35.6	37.4	40.3
PCB 153	19.8	20.6	22.4	24.4	26.4	28.6	29.4
PCB 180	14.2	14.5	15.9	17.4	19.4	20.9	21.1

Pigs will normally grow from an initial 20 kg (11% fat) to 110 kg (28%) during the experimental period, resulting in an increase in body fat content of 2.2 to 30 kg. This 14 fold increase again reflects the decrease in PCB levels of about 13-fold. Dioxin-levels seem to decrease somewhat more rapidly (20-fold).

Conclusion

It can be concluded that the ratio between the 7 indicator PCBs and dioxins did not decrease to less than 50,000 or the ratio in the feed. Therefore, at least in the case of pigs the limit of 200 ng/g for the 7 indicator PCBs appears to have been too conservative for dioxins only but has prevented the consumption of meat containing too high levels of total TEQ, primarily due to a slower decrease in the mono-ortho PCB levels. In particular the mono-ortho PCBs appear to have contributed most to the exposure of consumers to dioxin-like compounds during the crisis.

References

1. Tuinstra L.G.M.Th., Traag W.A., Rhijn J.A. van and Spreng P.F. van de (1994) Chemosphere 29, 1859.