Long-term experiment on digestibility, accumulation and metabolisation of low-level dietary polychlorinated biphenyls in laying hens

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Introduction

Because of their lipophilic and persistent properties, polychlorinated biphenyls (PCBs) have become widespread environmental contaminants accumulating through food chains and causing permanent background contamination of foods[1]. PCBs are known to cause toxicological effects such as neurological and reproductive disorders, as well as dermatologic and hepatic abnormalities and increased cancer risk[2]. Animal exposure to PCBs is dominated by dietary intake. The presence of PCBs in animal tissues depends on their absorption efficiency through the gut, and further on the subsequent accumulation and metabolisation in the organism. To date only few studies regarding absorption and incorporation of PCBs in poultry have been conducted[3,4,5]. In a previous study from our laboratory, apparent faecal digestibility and incorporation of low-level dietary PCB amounts was investigated in broiler chickens[6]. Although the absorption of lipophilic PCBs from the aqueous environment of the gut lumen was believed to interfere with lipid assimilation via the incorporation into bile salt micelles, neither digestibility nor incorporation of PCBs was affected by dietary contents of supplemented fat ranging from 4% to 8%.

With regard to their bioaccumulation and toxicity, scientifically based standards concerning acceptable concentrations of PCBs in animal feeds and products should be determined. Therefore, insight in the accumulation of PCBs in the food chain is required. Until now, the maximally admitted PCB content in Belgian animal feeds and products, based on the sum of 7 reference PCB congeners, amounts to 200 ng/g fat[7].

The present study with laying hens was designed to examine the long-term effects of low-level dietary PCB contents on animal performance, egg quality, apparent PCB digestibility, PCB retention and PCB incorporation in egg yolk, abdominal adipose tissue, thigh and breast muscle tissues. Moreover, the effect of low dietary amounts of added fat, varying between 1.5% and 4.5%, on PCB digestibility and incorporation in laying hens was investigated. Also we addressed the question whether PCB incorporation in egg yolk as well as in adipose and muscle tissue would meet the current standard of 200 ng/g fat.

Methods and materials

Experimental design

As in practice, laying hens received two successive diets in order to meet the changing age-dependent nutrient requirements. Diets were based on corn, soybeans, soybean oil meal, alfalfa and peanut oil, and differed in amount of added fat (1.5%, 3% or 4.5%). Dietary contents of major amino acids, minerals and metabolisable energy were equal. For each level of added fat three PCB supplements were tested corresponding with 0 ng, 1.5 ng and 6 ng/g diet. PCBs were added to the diets as a mixture of 7 reference congeners (IUPAC no. 28, 52, 101, 118, 138, 153 and 180, Dr. Ehrenstorfer GmbH, Germany). The PCB contents in the diets were below the legally admitted amount in Belgian feeds.

The experiment was carried out with 36 laying hens (Isabrown) and lasted for 41 weeks. The animals were individually housed in metabolism cages. Room temperature was kept constant at 22°C and artificial light was on between 3 a.m. and 9 p.m. The animals had free access to water and feed. Ethical approval was given by the University Animal Ethics Committee (Catholic University of Leuven, Belgium).

The experiment started when the animals were 24 weeks old and showed regular egg production. Each test diet was fed to four laying hens. During the whole experiment, feed consumption was measured daily and corrected for spilling. Eggs were individually weighed every day. Feed conversion and percentage of egg production were determined weekly. Feed conversion was calculated as the ratio between feed intake and total weight of produced eggs. Egg shell thickness was measured every week.

Eggs were collected at regular times, in order to evaluate the time-related evolution of PCB content in the egg yolk. The first digestibility trial with each of the 9 experimental diets was carried out during the 9^{th} and 10^{th} week of the experiment. At that time PCB elimination via the egg yolk had reached a nearly constant level. All faeces and eggs were collected. In order to study the effect of age on PCB absorption, analogous digestibility trials were conducted during weeks 23 and 24 and weeks 40 and 41 of the experimental period. Apparent PCB digestibility was calculated as the difference between intake and faecal excretion, and expressed as percentage of intake. PCB retention values were calculated as the difference between apparent PCB digestibility and the PCB elimination via egg yolks. Following the third digestibility trial, animals were killed by decapitation at the age of 65 weeks. Samples were taken from abdominal adipose tissue and breast and thigh muscle tissues.

Analytical methods

Prior to fat extraction, abdominal adipose tissue was homogenised, while faeces, egg yolks and breast and thigh muscle tissues were dried at 50°C. Samples were extracted with acetone-hexane (1:1, v/v) using an accelerated solvent extractor (ASE-200) equipped with 22 ml cells (Dionex, Belgium). The extraction was carried out in 3 cycles of 3 min at 125°C and 1500 psi. Next, 10 ng of PCB 112 (Dr. Ehrenstorfer GmbH, Germany) was added as internal standard and the solvent was evaporated under nitrogen. Subsequently, the fat fraction was dried at 105°C for 1 h and weighed. Extraction of feed samples was performed in a similar way. For breast muscle samples 33 ml extraction cells were used in order to provide the required fat amount for PCB quantification.

Following fat determination, the lipid fraction was dissolved in 1 ml hexane pending clean-up according to a procedure based on the recommendations of the Belgian Bureau of Accreditation[8]. The clean-up was performed on a column containing from bottom to top, 6 g

acidified silica gel, 1 g deactivated alumina and 0.5 g anhydrous sodium sulphate. Following application of the lipid fraction onto the column, PCBs were eluted with 20 ml hexane. The eluate was evaporated to dryness and reconstituted into 1 ml iso-octane. Next, PCBs were quantified by means of a Thermo Finnigan gas chromatograph (model TRACE 2000, Interscience, Belgium), equipped with a split/splitless injection system, a capillary column (HT8, 25 m x 0.22 mm x 0.25 μm, SGE, Belgium) and an electron capture detector.

Statistical analysis

Data were subjected to analysis of variance using the General Linear Model (GLM) procedure of SAS (1988). Statistical significance of differences between means was examined with the Tukey test. Values were considered significantly different when P-values were less than 0.05. Experimental data are presented as means ± SD.

Results and discussion

During the whole experiment, both performance and egg quality parameters were normal. In general, they were not significantly influenced by dietary PCB or fat content. The rarely observed significant differences between treatment groups could be attributed to a temporary reduced performance of some animals. Daily feed intake during the whole experiment averaged 109.3 g and was nearly constant over time. Egg production decreased significantly from 100% in the first week of the experiment to 81% in the 41st week, while average egg weight increased significantly from 55.6 g to 62.5 g. Feed conversion was not significantly influenced by age of the laying hens and averaged 1.98 throughout the experiment. Egg shell thickness was constant at 0.38 mm. The finding that the ingested PCB amounts did not harm animals' health was in accordance with our initial objective, namely to simulate background PCB contamination of feeds for laying hens. Also in our previous experiments with broiler chickens, we found that performance was not affected when diets were fed containing up to 12 ng PCBs/g [6].

PCB incorporation in egg yolk was significantly influenced by dietary PCB content, while dietary fat content had no effect. Therefore, time-related data concerning PCB incorporation in the egg yolk could be averaged for each dietary PCB condition without taking into account the dietary fat content (Figure 1).

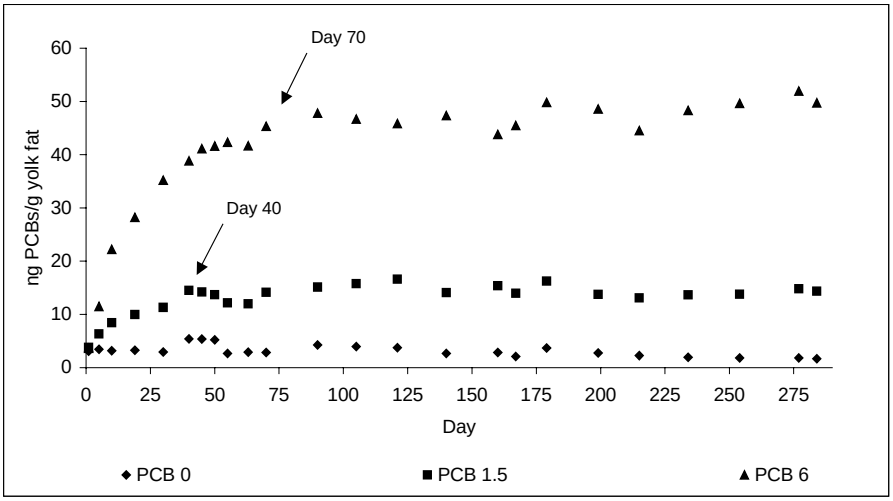

Figure 1. Time-related PCB incorporation in egg yolk. Laying hens consumed 0 (PCB 0), 1.5 (PCB 1.5) and 6 (PCB 6) ng PCBs/g diet. Each point represents the mean of 12 observations.

The lipid-based PCB concentration in the egg yolk of laying hens consuming 1.5 ng added PCBs/g diet reached a nearly constant level approximately 40 days after PCB consumption had started. Later on, the PCB concentration varied between 12.0 and 16.6 ng PCBs/g yolk fat. On the other hand, animals which received 6 ng added PCBs/g diet approached a steady state condition after approximately 70 days, whereafter the concentration varied between 43.8 and 51.9 ng/g yolk fat. Control animals showed a continuous low-level background contamination of PCBs in the yolk. These findings demonstrated that there is a positive correlation between the dietary PCB content and the time required to get constant PCB elimination via the egg yolk. Furthermore, it was obvious that the lipid-based PCB concentration in yolk from laying hens that had consumed the highest PCB amounts for 41 weeks never exceeded the current standard of 200 ng PCBs/g fat.

The apparent faecal PCB digestibility and PCB retention values obtained in digestibility trial 1 and 3 are presented in table 1. The results from the second digestibility trial were similar.

Table 1. Apparent faecal PCB digestibility and PCB retention in digestibility trial 1 and 3 (%)

Diet*	Trial 1		Trial 3	
	Digestibility (%)	Retention (%)	Digestibility (%)	Retention (%)
1.5 - 0	41.7 ± 6.8^{bc}	-11.3 ± 18.6^{b}	37.9 ± 7.6^{c}	-4.8 ± 6.0^{b}
3 - 0	47.6 ± 6.0^{b}	-7.9 ± 11.2^{b}	33.5 ± 3.7^{c}	-20.4 ± 11.0^{b}
4.5 - 0	36.4 ± 4.9^{c}	-25.3 ± 11.4^{b}	36.1 ± 6.4^{c}	-23.4 ± 12.4^{b}
1.5 - 1.5	78.3 ± 0.5^{a}	44.5 ± 5.6^{a}	83.3 ± 1.5^{ab}	49.3 ± 2.6^{a}
3 - 1.5	78.0 ± 2.0^{a}	43.6 ± 2.8^{a}	82.3 ± 1.8^{b}	42.7 ± 3.0^{a}
4.5 - 1.5	75.7 ± 4.0^{a}	41.4 ± 4.8^{a}	85.6 ± 0.6^{ab}	46.8 ± 5.2^{a}
1.5 - 6	83.5 ± 0.6^{a}	47.5 ± 4.5^{a}	86.7 ± 0.6^{ab}	52.1 ± 3.7^{a}
3 - 6	83.1 ± 1.0^{a}	48.9 ± 4.1^{a}	91.1 ± 1.2^{a}	52.7 ± 2.5^{a}
4.5 - 6	83.7 ± 2.8^{a}	52.1 ± 4.2^{a}	90.7 ± 1.6^{ab}	57.9 ± 0.7^{a}

Values are expressed as means ± SD, n = 4.
^{a,b,c} Values in a column without common letter are significantly different, $P<0.05$.
* The first and second figure refer to the content of added dietary fat (1.5%, 3%, 4.5%) and the amount of added PCBs (0 ng, 1.5 ng, 6 ng/g diet), respectively.

Although the amounts of added fat in the diets varied between 1.5% and 4.5%, there was no significant effect from dietary fat content on apparent faecal PCB digestibility nor on PCB retention in the three digestibility trials. On the other hand, apparent faecal PCB digestibility and PCB retention were significantly higher when diets were supplemented with PCBs. Negative retention values, as observed in the control groups without added PCBs, suggested a net excretion of PCBs from the body. However, interpretation of these data is complexed by the fact that PCB concentrations measured in control samples were of the same magnitude as the analytical detection limit. Within the groups fed the diets containing added PCBs, apparent PCB digestibility and PCB retention were elevated in laying hens fed the highest PCB amount, although these differences were not statistically significant. Based upon the present findings it could be concluded that, in accordance with the broiler experiments, dietary fat supplementation did not improve emulsification and absorption of small PCB quantities in the gut of laying hens. The data show that absorption of PCBs occurred more readily when they were added to the diet. As a result, PCBs do not experience major obstacles from feed cell structures in the digestion process. Another finding was that in experimental groups that were fed added PCBs, apparent faecal PCB digestibility significantly increased over time. On the other hand, the age effect on PCB retention was less clear and was only significant for the animals consuming 6 ng PCBs/g diet. From the differences between apparent faecal PCB digestibility and PCB retention, it could be concluded that following ingestion and absorption, approximately one third of dietary added PCBs arrived in the egg yolk. This value was independent of the dietary contamination level.

As measured at the end of the experiment, dietary fat content had no significant effect on the PCB content in the fat fraction of adipose and muscle tissues, whereas the effect from dietary PCB content was remarkable (Table 2).

Table 2. PCB concentration in abdominal fat, thigh and breast tissue measured at the end of the experiment (ng/g tissue fat)

Diet*	Abdominal fat	Thigh tissue	Breast tissue
1.5 - 0	2.9 ± 0.4^{c}	2.3 ± 0.5^{c}	1.8 ± 0.1^{c}
3 - 0	3.3 ± 0.2^{c}	2.3 ± 0.1^{c}	2.3 ± 0.4^{c}
4.5 - 0	3.4 ± 0.2^{c}	2.9 ± 0.2^{c}	3.8 ± 2.1^{c}
1.5 - 1.5	19.7 ± 2.3^{b}	18.3 ± 1.2^{b}	14.2 ± 1.6^{b}
3 - 1.5	19.5 ± 1.8^{b}	16.4 ± 2.5^{b}	11.5 ± 1.1^{b}
4.5 - 1.5	23.9 ± 3.2^{b}	18.2 ± 2.8^{b}	15.1 ± 4.0^{b}
1.5 - 6	66.6 ± 11.1^{a}	57.3 ± 4.9^{a}	43.2 ± 4.5^{a}
3 - 6	76.5 ± 9.3^{a}	65.8 ± 9.1^{a}	47.3 ± 5.8^{a}
4.5 - 6	76.6 ± 8.2^{a}	65.6 ± 13.4^{a}	48.0 ± 4.3^{a}

Legend as in Table 1.

PCB concentrations in the fat fraction of the three examined tissues showed a dose-dependent relationship with the dietary PCB contamination level and never exceeded the currently legal maximum of 200 ng PCBs/g fat. In fact, the present data show that the maximum allowable PCB content in tissue fat may be substantially reduced. Moreover, it is concluded that food safety legislation which is based on PCB amounts present in the fat fraction of edible products may be misleading. This follows from the finding that the fat fraction of the three examined tissues from treatment groups with equal PCB ingestion contained comparable PCB amounts, while the PCB amounts per g tissue were greatly different due to the differences in tissue fat contents. Fat content in adipose tissue varied between 89.7% and 97.3%, while fat content in thigh tissue ranged from 3.4% to 6.4% and in breast tissue from 0.8% to 1.8%.

The proportional distribution of the 7 reference PCB congeners in diet, faeces, egg yolk and abdominal adipose tissue of laying hens consuming 6 ng PCBs/g diet is presented in Figure 2.

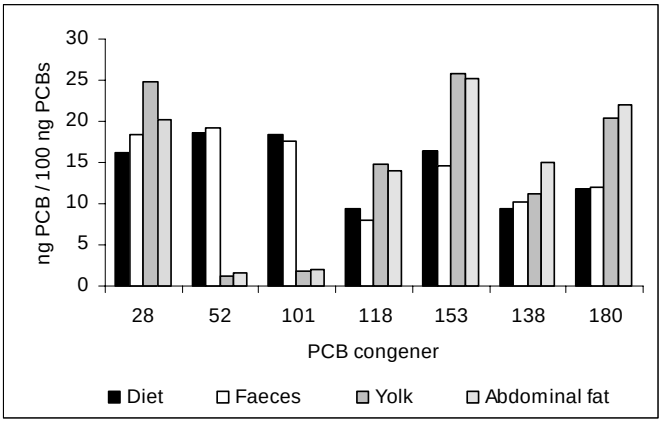

Figure 2. Profiles of 7 reference PCB congeners in the diet, faeces, egg yolk and abdominal fat tissue of laying hens consuming 6 ng PCBs/g diet.

Because the PCB profile in the diet was similar to that in the faeces it was obvious that the absorption of the 7 congeners in the gastro-intestinal tract occurred at comparable rates, notwithstanding their differences in chlorination level and thus in hydrophobicity. On the other hand, PCB profiles in egg yolk and in abdominal fat greatly differed from those in diet and faeces in a sense that only negligible amounts of PCB 52 and PCB 101 were found in yolk and fat tissue. As this was also true for muscle tissue it may be suggested that both congeners are metabolised in laying hens. Analogous conclusions were obtained from PCB analyses of total broiler carcasses[9].

Acknowledgements

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