Low levels of polychlorinated biphenyls in diets for piglets: absorption and accumulation in body tissues

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Introduction

Due to their great stability and lipid solubility, polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants accumulating through food chains and causing permanent background contamination of foods.[1] PCBs are known to cause toxicologic effects such as neurologic 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 in the gut, and on accumulation and metabolism in the organism. In previous experiments performed in our laboratory, apparent faecal digestibility and incorporation of low dietary PCB amounts were investigated in broiler chickens and laying hens.[3,4] Up to now, only high dietary contamination levels have been studied with regard to PCB accumulation in pigs.[5,6] As a result, there are no data with regard to the transfer of dietary background PCBs into pig tissues. However, such data may provide insight in the storage of PCBs in the food chain and are needed to formulate scientifically based standards for maximum PCB contents in animal foods. Currently, the maximally admitted PCB content in Belgian animal feeds and products, based on the sum of 7 reference PCB congeners, is 200 ng/g fat; in dairy foods the limit is even set at 100 ng/g fat.[7]

The present experiments with piglets were conducted to investigate the effect of low dietary PCB levels on animal performance parameters, PCB digestibility and PCB accumulation in tissues. The lipid-based PCB contents in the test diets were below the legally admitted amount in Belgian feeds. Furthermore, the effect of dietary added fat content was studied on PCB digestibility and accumulation as absorption of lipophilic PCBs from the aqueous environment of the gut lumen may depend upon the presence of digestible fat.

Methods and materials

Experimental design

Two experiments were conducted with 12 crossbred (Belgian Landrace x Piétrain) castrated male piglets with an initial body weight of 10 ± 1 kg. The experiment lasted until the animals weighed 22 ± 1 kg. Feed and water were *ad libitum* available.Ethical approval for the experiment was given by the University Animal Ethics Committee (Catholic University of Leuven, Belgium).

All experimental diets were based on corn, soybeans, fine wheat middlings, whey powder and peanut oil. In the first experiment, diets contained 6 ng supplemented PCBs/g and varied in added fat amount (0.5% or 2% or 3.5%). In the second experiment, diets contained 2% added fat and varied in supplemented PCB amount (0 ng or 1.5 ng or 6 ng PCBs/g). PCBs were added to the diets as a mixture of the 7 reference congeners in peanut oil. The ratios between the contents of PCBs 28, 52, 101, 118, 138, 153 and 180 were 0.9/1.2/1.1/0.6/0.6/1.0/0.9. Each diet was fed to 4 piglets.

In both experiments, two digestibility trials were carried out when body weight ranged from 10 to 17 kg and from 17 to 22 kg, respectively. Throughout the whole experimental period, feed intake was measured daily and corrected for spilling. Faeces were quantitatively collected, weighed and 25% of the homogenised collection was frozen at -20°C. At the end of the digestibility trials, faeces samples were pooled per piglet, homogenised and dried at 50°C before fat and PCB analyses. Body weight gain and feed conversion were measured as well.

At the end of the experiments each piglet was euthanatised. Samples of subcutaneous adipose tissue were collected at the first sacral vertebra and at the first rib. Also liver and muscle tissue (M. *longissimusdorsi*) were

sampled. All samples were stored at -20°C until fat and PCB analyses.

Analytical methods

Prior to fat extraction, the adipose tissue was homogenised. Muscle and liver tissues were blended and dried for 48 h with celite at 50°C. Feed, faeces and tissue samples were extracted with acetone-hexane (1:1, v/v) using an accelerated solvent extractor (ASE-200, Dionex, Belgium). The clean-up of the lipid fractions of feed, adipose and muscle tissues was performed on a column with acidified silica gel, deactivated alumina and anhydrous sodium sulphate. As it was found that this clean-up was not sufficiently accurate for PCB determination in faeces and liver samples, the lipid fraction of these samples was previously purified by alkaline hydrolysis. The procedures applied for fat determination, alkaline hydrolysis and clean-up on acidified silica gel have been described in a recent publication from our laboratory.[8]

Statistical analysis

Unless otherwise specified, PCB data always refer to the sum of the 7 reference congeners. Data were subjected to one-way 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 mean values ± SD.

Results and discussion

Performance parameters of piglets were not affected by the experimental dietary fat and PCB contents. This was in accordance with the main objective of our experiment to test dietary PCB concentrations corresponding with the permanent background PCB contamination, so that the animals' health was not harmed. As a result, it was allowed to average performance data obtained from the 24 animals in order to evaluate the age effect. In the successive digestibility trials daily feed intake, daily growth and feed conversion significantly increased from 682 ± 79 g to 905 ± 131 g, from 429 ± 71 g to 512 ± 99 g and from 1.61 ± 0.21 to 1.79 ± 0.22, respectively (data not shown).

Throughout the experiment, apparent faecal PCB digestibility was not influenced by dietary added fat content. Thus, even the lowest dietary total fat level of 5.0% in our experimental conditions was sufficiently high in order to optimally emulsify and absorb small quantities of PCBs in the gut. Moreover, analysed dietary PCB contents ranging from 0.6 to 6.3 ng PCBs/g diet had no effect on the apparent faecal PCB digestibility. The high digestibility in piglets consuming control diets without added PCBs was in contrast with our previous findings in poultry.[3,4] Up to now, it has been assumed that supplemented PCBs are more readily absorbed in the gut as they do not experience major obstacles from feed cell structures in the digestion process.

Data from the 24 piglets in both experiments were averaged in order to evaluate the age effect on the apparent faecal PCB digestibility. This parameter averaged in the first and second trial 77.8 ± 4.4% and 79.2 ± 4.4%, respectively. It is worth mentioning that the experimental period in which animals grew from 10 kg to 22 kg only lasted for approximately four weeks. Probably, this period was too short to observe age effects with regard to PCB digestibility. In piglets consuming the highest contaminated diets in the first experiment, apparent faecal digestibility of the individual congeners ranged from 74.1 ± 2.6% for PCB 138 to 80.8 ± 2.3% for PCB 28. Despite the small significant differences, it may be concluded that there was no preferential absorption of particular PCB congeners in the gut (data not shown).

Table 1. PCB contents (ng/g tissue fat) in subcutaneous adipose tissue, muscle tissue and liver

Diet[1]	Adipose tissue			
	Sacral vertebra 1	Rib 1	Muscle tissue	Liver
Experiment 1				
0.5 - 6	27.1 ± 2.4 [a]	26.5 ± 2.8 [a]	19.6 ± 3.3 [a]	28.1 ± 1.9 [a]
2 - 6	30.2 ± 2.7 [a]	30.0 ± 3.9 [a]	21.7 ± 1.6 [a]	30.6 ± 3.1 [a]
3.5 - 6	27.4 ± 3.1 [a]	28.4 ± 3.8 [a]	18.5 ± 2.8 [a]	28.3 ± 2.3 [a]
Experiment 2				
2 - 0	3.6 ± 1.5 [c]	3.3 ± 1.3 [c]	3.0 ± 1.1 [c]	12.3 ± 3.5 [b]
2 - 1.5	9.6 ± 1.0 [b]	9.4 ± 0.8 [b]	6.3 ± 0.5 [b]	16.2 ± 0.9 [b]
2 - 6	30.2 ± 2.7 [a]	30.0 ± 3.9 [a]	21.7 ± 1.6 [a]	30.6 ± 3.1 [a]

[1] The first and second figure refer to the amounts of dietary added fat (0.5%, 2%, 3.5%) and PCBs (0 ng, 1.5 ng, 6 ng/g diet), respectively.

[a,b,c] Within a column and within each experiment values not sharing a superscript are significantly different, $P < 0.05$.

Similar to PCB digestibility, PCB contents in tissues from piglets were not affected by dietary fat content. On the other hand, lipid-based PCB contents in adipose and muscle tissues showed a dose-dependent relationship with dietary PCB level. Hepatic PCB contents were only significantly elevated in piglets consuming the most contaminated diets (Table 1). PCBs were equally distributed in the fat fraction of subcutaneous adipose tissue as there was no difference in PCB contents in samples taken at the first sacral vertebra and the first rib. The fat fraction of muscle tissue contained lower PCB levels, while liver had similar or higher lipid-based PCB levels. This indicated the preference of PCBs to accumulate in tissues with a high lipid affinity such as liver and adipose tissue. Moreover, it was evident that PCBs were not equally distributed in the total body fat. When PCB contents were expressed on a tissue weight basis, muscle and liver were much less contaminated compared to adipose tissue (data not shown). This was in accordance with tissue fat contents which ranged from 48.8% to 73.4% in subcutaneous adipose tissue, from 3.4% to 4.7% in liver and from 1.4% to 2.3% in muscle tissue. Based upon the present findings it is concluded that the current legislation based on PCB amounts present in the fat fraction of edible products may be misleading.

In our experimental conditions, PCB concentrations in the fat fraction of adipose tissue, muscle tissue and liver of piglets consuming PCB contaminated diets maximally amounted to 30.6 ng PCBs/g fat. This was far below the currently legal maximum of 200 ng PCBs/g fat. Consequently, it is advisable to lower the present standard.

With regard to the individual congeners, the dietary PCB pattern was altered in adipose tissue, muscle tissue and liver (Figure 1). Most obvious were the reduced relative contributions of PCB 28 in piglet tissues. PCB 28 represented about 17% of the sum of the 7 reference congeners in the highest contaminated diet. However, it only contributed between 4.5% and 7.2% to the total PCB amount in tissues of piglets consuming this diet. As PCB 28 showed no reduced apparent faecal digestibility in the gastro-intestinal tract, it may be suggested that this congener was metabolised in piglets. Moreover, relative contributions of PCBs 52, 101 and 118 in tissues were either slightly reduced or similar when compared to their contribution in the diet. Probably, these lower chlorinated PCB congeners were also metabolised, but at slower rates. The metabolism pattern in piglets clearly differed from the metabolism of PCBs 52 and 101 observed in poultry.[4,9] Furthermore, relative contributions of PCBs 138, 153 and 180 exceeded the dietary contributions, except for PCB 180 in liver. These trends were confirmed when PCB contents in body tissues were corrected for equal intake, i.e. 1 ng of each congener per g diet (data not shown). In general, intake-corrected contents of PCBs 28, 52, 101 and 118 were significantly lower compared to PCBs 138, 153 and 180, showing that the higher chlorinated congeners preferentially accumulated in piglet tissues. Nevertheless, liver showed a reduced affinity for the highest chlorinated PCB 180.

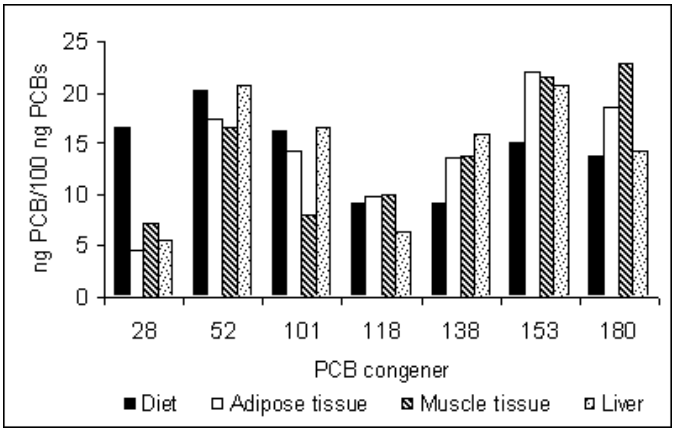

Figure 1. PCB patterns in the highest contaminated diet and in adipose tissue, muscle tissue and liver of piglets fed this diet (diet: n = 1; tissues: n = 4)

Acknowledgements

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