Elimination and excretion of previously absorbed polychlorinated biphenyls in laying hens

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Introduction

Polychlorinated biphenyls (PCBs) are persistent organic pollutants which are ubiquitous environmental contaminants due to their great stability and lipid solubility. They are known to cause permanent background contamination of foods threatening the food chain.[1] Because of their toxicological effects, PCB body burden should be kept as low as possible. Dietary intake is the major way of PCB exposure of humans and animals. The accumulation of PCBs in animal tissues may be hampered by their metabolisation, faecal excretion and elimination via lipid-rich products such as milk and eggs.[2] In previous studies conducted in our laboratory, apparent faecal digestibility and incorporation of low dietary PCB amounts were investigated in broiler chickens and laying hens.[3,4] Apparent faecal PCB digestibilities generally varied between 80 and 90%. Moreover, it was found that PCB accumulation in tissues was related to the dietary contamination level. In laying hens, PCB accumulation in body tissues was retarded as approximately one third of the ingested PCBs were eliminated via the egg yolk. Based upon this phenomenon, it can be hypothesised that egg yolk provides an excretion pathway for previously absorbed PCBs resulting in a reduction of the body burden. Up to now, few experimental data are available with regard to the fate of absorbed PCBs in poultry when PCB feeding is stopped.[5]

The present study with laying hens was designed to investigate whether PCBs could be released from the body following a period of PCB ingestion. Therefore, faecal PCB excretion as well as PCB elimination via the egg yolk were followed as a function of time. Furthermore, the effect on PCB body burden was studied by measuring PCB contents in abdominal adipose tissue and liver. As Belgian legislation is based on the sum of 7 reference PCBs, the study was performed with regard to these particular congeners. PCB contents in the experimental diets were below 200 ng/g fat, which is the legally admitted content in Belgian feeds.

Methods and materials

Experimental design

Thirty-six laying hens (Isabrown) were randomly divided into three groups of 12 hens: the negative control group, the positive control group and the experimental group.The hens were 41 weeks old at the start of the experiment. The experiment was divided into 3 subperiods (day 1-40; day 41-112; day 113-133). Ethical approval for the experiment was given by the University Animal Ethics Committee (Catholic University of Leuven, Belgium).

Laying hens were fed two experimental diets based on corn, soybeans, soybean oil meal, alfalfa and peanut oil. Diets were supplemented with either 0 or 6 ng PCBs/g, respectively. PCBs were added as a mixture of 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. The added fat amount in both diets was 1.5%. During the entire experiment of 133 days, the negative and the positive control group received only the diet without and with added PCBs, respectively. The experimental group was fed the PCB supplemented diet during the first subperiod, followed by the dietwithout added PCBs (control diet) until the end of the experiment.

Each hen was provided *ad libitum* with feed and water during the whole experiment. However, in the third subperiod daily feed intake was limited to 80 g. Reduced feed intake was implemented to investigate whether increased mobilisation of body fat stimulates PCB excretion into the eggs. Feed intake was measured daily and corrected for spilling. Egg weight was recorded each day. Faeces production was only registered in subperiods 2 and 3. Eggs and excreta samples from all animals were taken periodically throughout the experiment. Egg yolks were stored at -20°C and excreta were dried at 50°C. A selection of the collected samples was analysed for fat and PCB content.

On days 40, 61 and 133 four laying hens out of each group were killed by decapitation. Samples were taken from liver and abdominal adipose tissue and contents of fat and PCBs were determined.

Analytical methods

The analytical procedures used to determine fat and PCB contents in diets, faeces, yolk and adipose tissue have been described in a recent report from our laboratory.[4] Before liver was analysed, it was homogenised by blending and dried for 48 h with celite at 50°C.

Statistical analysis

Unless otherwise specified, PCB contents in feed, yolk, faeces and tissues 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 means ± SD.

Results and discussion

In general, performance parameters of laying hens were not affected by dietary treatments throughout the experiment. A few significant differences suggested that the dietary PCB contamination of 6 ng PCBs/g adversely influenced daily feed consumption. However, the results were not consistent in the three subperiods. As in our previous experiment with laying hens feed intake was not affected by similar dietary PCB contaminations, the observed effects were rather attributed to temporarily differential behaviour of individual animals.[4] Moreover, egg mass, feed conversion and wet faeces production were normal and did not significantly differ between treatment groups. Thus, in general it could be concluded that consumption of the experimental diets was not hazardous in terms of animals' health. This was in accordance with the main objective of our research project.

In both control groups, faecal PCB contents remained relatively constant following the period of PCB ingestion. On the other hand, faecal PCB concentration in the experimental group rapidly declined after the transfer from the PCB contaminated diet to the control diet. On the first day of control diet consumption, faecal PCB content was only 37% of the value measured on the last day of PCB consumption. Two days after PCB consumption had ended, faecal PCB content was decreased to the level of the negative control group. Subsequently, no rise in faecal PCB concentration of the experimental group was observed until the end of the experiment.Data analysis regarding the individual PCB congeners revealed that the 7 PCB congeners behaved like their sum (data not shown). It was concluded that there was no meaningful faecal excretion of previously absorbed PCBs after switching from the PCB contaminated diet to the control diet. Consequently, faecal excretion is not a major pathway to reduce PCB body burden of layers.

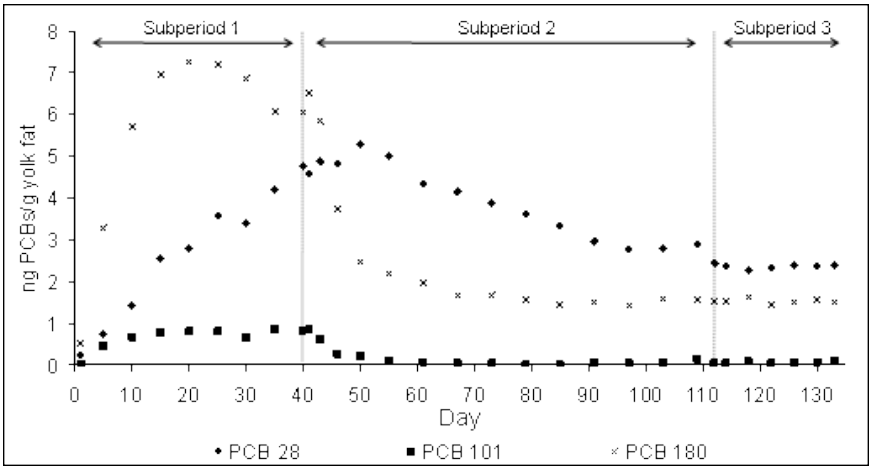

Figure 1. Lipid-based concentration of PCBs 28, 101 and 180 in egg yolks from the experimental group collected at

regular times. Each point represents the mean of four observations.

In contrast with the faecal excretion of PCBs, it was clear that elimination via egg yolk did not occur similarly for each individual congener (Figure 1). Although low PCB 101 quantities in egg yolk were measured, the content of this congener showed a small rise when PCB feeding was started and a fast decline following the transfer to the control diet. This was also true for PCB 52. The low levels of PCBs 52 and 101 were attributed to the preferential metabolism of these particular congeners in poultry.[4,6] During the first subperiod, PCB 180 accumulated faster in egg yolk as a function of time compared to PCB 28. PCBs 118, 138 and 153 showed an accumulation pattern in egg yolk that was comparable with PCB 180. When PCB feeding stopped, an immediate decline of the content of PCB 180 was observed in yolk. In contrast, PCB 28 content still increased until 10 days after dietary PCB contamination had ended. Furthermore, the decrease of PCB 28 content in egg yolk was much slower than the decrease of PCB 180. PCBs 118, 138 and 153 showed an immediate decline following the transfer to the control diet, although slower than PCB 180.

Total PCB content in yolks from the experimental group rapidly increased to 26.4 ng PCBs/g yolk fat at day 40 of PCB ingestion. Five days after switching to the control diet, it was significantly decreased by 28%. At the end of the second subperiod still 36% of the PCB content measured on day 40 was found in the egg yolk. These findings were in accordance with a previous experiment conducted with laying hens consuming larger PCB amounts.[5] Thus, it was obvious that following a PCB contamination eggs were still contaminated for a long period. During the next three weeks of subperiod 3, total PCB content did not further decrease in yolk, similarly to the contents of PCBs 28, 101 and 180 (Figure 1). Apparently, the expected further decrease in PCB content was compensated by the release of PCBs from the body stores towards the eggs due to the reduction in feed intake. This was confirmed by the stimulated increase of PCB content in yolks from the positive control group during the period of feed restriction. Egg yolks from the negative control group showed a constant background PCB contamination throughout the experiment (data not shown).

PCB concentrations in liver and abdominal adipose tissue from hens in the negative control group were hardly changed over time. On the other hand, in the positive control group a significant accumulation of PCBs was observed in both tissues. Switching to the control diet following a 40-day period of PCB ingestion resulted in a fast and significant decrease of the hepatic PCB concentration. The further reduction of the hepatic PCB concentration measured between day 61 and day 133 was not significant (Table 1). A similar pattern was found for each metabolically-stable individual congener (data not shown). PCB redistribution from an early depot such as liver to lipid-rich tissues such as skin and adipose tissue at least partially explained this phenomenon.[2] In abdominal adipose tissue only a strong trend towards decreased PCB concentration was found in the experimental group. This decrease occurred much slower than in liver, as 93 days after the transfer to the control diet only one third of the PCBs were removed from the abdominal adipose tissue (Table 1). The reduction was mainly caused by PCB 28 and to a smaller extent by PCB 118, which were the only metabolically-stable congeners that showed significantly decreasing contents in adipose tissue. The levels of PCBs 138, 153 and most obviously PCB 180 were not significantly changed following the transfer to the control diet.

Table 1. PCB contents (ng PCBs/g tissue fat) in liver and abdominal adipose tissue measured at day 40, day 61 and day 133 of the experiment

	Day 40	Day 61	Day 133
Liver tissue			
Negative control	1.8 ± 0.3 ^{k}	2.4 ± 0.8 ^{k}	2.7 ± 0.4 ^{k}
Positive control	22.3 ± 2.7 ^{l}	27.8 ± 4.7 ^{l}	41.6 ± 9.3 ^{k}
Experimental	20.0 ± 4.7 ^{k}	10.6 ± 1.0 ^{l}	7.0 ± 1.7 ^{l}
Abdominal adipose tissue			
Negative control	4.5 ± 0.1 ^{k}	4.6 ± 0.5 ^{k}	5.0 ± 0.4 ^{k}
Positive control	28.1 ± 5.2 ^{l}	38.0 ± 5.5 ^{l}	100.9 ± 12.0 ^{k}
Experimental	31.7 ± 7.8 ^{k}	27.9 ± 5.3 ^{k}	19.8 ± 4.8 ^{k}

^{k,l} Values in a row without a common superscript are significantly different, $P < 0.05$.

Based upon the present findings, it can be concluded that switching to a diet without supplemented PCBs following a period of PCB ingestion results in a small decrease of the PCB body burden of layers. The reduction is mainly due to PCB elimination via egg yolk. Moreover, PCB 28 appears to be a rather mobile congener, that is easily transferred

from liver to the adipose tissue and slowly eliminated via the egg yolk. When PCB feeding stops, PCB 28 is also readily mobilised from the adipose tissue to the yolk in order to re-establish the equilibrium of PCB contents in all body tissues. On the other hand, PCB 180 is a less mobile congener, which is initially more easily incorporated in the lipid depot of the egg yolk than in the adipose tissue. Furthermore, the migration of PCB 180 from the adipose tissue to the egg yolk is impaired, thus rapidly resulting in low levels in egg yolk when its supply from the diet is stopped.

Acknowledgements

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