Monitoring of the declining trend of Polychlorobifenyls concentration in milk of contaminated dairy cows

Filippo Rossi, Terenzo Bertuzzi, Antonio Vitali, Angelo Rubini, Francesco Masoero, Mauro Morlacchini, Gianfranco Piva
1Istituto di Scienze degli Alimenti e della Nutrizione, Università Cattolica del Sacro Cuore, Piacenza, Italy
2Dipartimento di Prevenzione Veterinaria, Bergamo, Italy
3Centro Ricerche per la Zootecnia e l’Ambiente, San Bonico (PC), Italy

Abstract

Six pregnant heifers, coming from a herd with a history of high concentration of Polychlorobifenyls (PCB) into the milk, were fed with a very low-PCB diet starting from the 6th month of pregnancy. After parturition cows were milked for at least 190 d with a maximum of 270 d. Diet was made of: corn silage (31.7% of DMI), dehydrated alfalfa (13.3% of DMI), grass hay (3.7% of DMI) and concentrate (51.3% of DMI). The average DMI was 23.12 kg/d. Milk production was recorded and samples of milk and blood were taken and analyzed for PCB (18, 28, 31, 52, 44, 101, 149, 118, 153, 156, 170, 180, 189, 191) isomers. The maximum PCB concentration (ppb) in milk fat is regressed against days in milk (DIM) the following significant equation was obtained:

\[
\log_{10} \text{PCB (ng/g of milk fat)} = 2.796 -0.00474 \text{DIM}; r^2 0.72; P<0.01
\]

The initial level of contamination is not the main factor affecting the time required in order for PCB to return to below the legal threshold of 100 ng/g fat, while daily milk yield significantly affects PCB excretion. Body condition and energy balance could be important factors affecting PCB excretion.

In the presence of high contamination, about 6 months of lactation are required in order to obtain milk with a sufficiently low PCB content.

Among the seven congeners considered by Italian legislation, PCB 101, PCB 118, PCB 138, PCB 153 constitute almost all the PCBs found in tissues and milk, with a much smaller presence of PCB 180, and the numbers of the congeners PCB 28 and PCB 52 are almost insignificant, probably because they accumulate little in the body.

The congeners PCB 138 and PCB 153 are those most frequently found. For PCB 138 the liver is the principal organ of accumulation, while for PCB 153 we found equal accumulations in the liver, kidneys and tail, but a low presence in milk. The PCBs 118 and 138 are those most abundant in milks.

Introduction

Polychlorobifenyls (PCB) are compounds constituted by two enjoined phenyllic rings, variously substituted with chlorine atoms (from 1 to 10 per molecule); of which there are 209 isomers. PCBs have been used as lubricants, insulators, heat conductors and fire-retardants; due to their elasticity they have also been widely used in varnishing. Currently their use is in marked decline because of their toxicity, as they are, in fact, endocrine disruptors, which induce alterations in the sexual cycle of mammals (including man), reduce spermatogenesis (Hays and Aylward, 2003) and damage thyroid (Langer et al., 2005). They can also cause dermatitis and are considered by the International Agency for Research on Cancer (IARC) to be human carcinogens (IARC, 1987; European Food Safety Authority, 2005).

Being lipophile molecules, they tend to accumulate in the adipose tissue of animals and then enter the human food chain through the ingestion of milk, eggs and meat, and fish have the highest levels of contamination, due to the accumulation of PCBs in marine sediments (Larsen, 1995; Duarte-Davidson and Jones, 1995). The contamination of foods of vegetable origin is also possible by root absorption and atmospheric deposition. In literature there are cases of intoxication due to accidental contamination in food production (Bernard et al., 1999). Recent cases of contamination by PCBs have occurred in Belgium and Denmark (Bernard et al., 2002).

Milk, which contains from 3.5 to 4% fat, can be contaminated by PCBs through ingestion of the molecule by animals, and for this and other reasons EU legislation has fixed limits for the quantities of PCBs present in foods (European Commission, 2006).

PCB excretion in milk is a process governed primarily by the lipophilicity of the congener and the lipid content of the carrier (McLachlan, 1994; Moser and McLachlan, 1999). Fox et al. (1994) observed that an increase in the degree of chlorination and the planarity of the molecule causes a slow transportation of the congener and consequently a reduction in its excretion through milk.

The aim of this work was to monitor the concentration of PCBs in primiparous cows’ milk accidentally exposed to contamination during pregnancy, to determine the time necessary for levels of contamination to return within legal limits and to monitor the excretion of individual congeners.

Materials and methods

Animals and diets

Six pregnant heifers (540±42 kg), coming from a herd with a history of PCB contamination through feeds, were fed with a low-PCB diet from the 6th month of pregnancy. After parturition, cows were milked for at least 190 d with a maximum of 270 d. The diets used for the last month of pregnancy and during lactation are reported in Table 1.
Table 1. Chemical composition and nutritional traits of the diets.

| Feeds                  | Dry period (kg/day) | Lactating period (kg/day) |
|------------------------|---------------------|---------------------------|
| Corn silage            | 6.0                 | 22.0                      |
| Alfalfa hay            | -                   | 3.5                       |
| Grass hay              | 9.0                 | 1.0                       |
| Concentrate            | 1.6*                | 12.0*                     |
| Mix corn (70%) and barley (30%) | -       | 1.5                       |
| Straw                  | 1.0                 | -                         |
| Dry matter intake, kg/d | 9.49               | 19.9                      |

Milk and blood samples

Milk production was recorded daily and individual milk samples (250 mL) and blood (50 mL) were taken every 15 days. Milk samples were taken from the two milkings and mixed in proportion to the respective production. Milk samples were analysed to determine the fat and protein content with Milkoscan, (Foss, Denmark). Blood samples were taken by venupuncture in Li-eparin Vacutainer (Becton Dickinson, Franklin Lakes, NJ, USA) and centrifuged at 3300 g for 10 min. Plasma was recovered and stored in a separate tube at -20°C before the PCB analysis.

All the milk produced was destroyed in accordance with the Italian Law.

Table 2. PCBs precursor and monitoring ion in MS-MS.

| PCBs sorted in order of retention time | Precursor ion | Monitoring ions |
|----------------------------------------|---------------|-----------------|
| PCB 18                                 | 258           | 186, 220        |
| PCB 28, 31                             | 258           | 186, 220        |
| PCB 52                                 | 292           | 222, 258        |
| PCB 44                                 | 292           | 222, 256        |
| PCB 101                                | 326           | 256, 291        |
| PCB 149                                | 326           | 254, 290        |
| PCB 118                                | 360           | 290, 325        |
| PCB 153                                | 360           | 290, 325        |
| PCB 138                                | 360           | 290, 325        |
| PCB 180                                | 394           | 324, 360        |
| PCB 194                                | 430           | 360, 395        |

Kidney and liver analysis

A 30 g homogenised kidney or liver was extracted with 100 mL hexane and shaken vigorously for 2 h; then the mixture was filtered through an anhydrous sodium sulphate layer. The solution was treated with sulphuric acid and purified by a Florisil column as described above.

Body fat analysis

An aliquot of 5 g homogenised fat was treated with 100 mL methanolic KOH 2N for 1 h at 80°C in a round-flask with bubble condenser. The PCBs were extracted with 100 mL of hexane three times in a separating funnel. Then the organic phase was treated as described above.

Data analysis

Statistical analyses were performed using the statistical package SAS (Release 8.0); PROC GLM was utilized for the analysis of congener pattern in milk and tissues. The comparison among regression’s equation of PCB contents was performed using the parallel test as described by Camussi et al. (1995).
Results and discussion

At the end of the trial the animals were slaughtered and internal organs were taken and analysed to determine levels of xenobiotic concentration. The PCB content of liver ranged from 0.35-1.2 ppb and was negatively correlated with average PCB blood levels (r=-0.84; P<0.05).

Rate of excretion of PCBs in milk

All the animals started lactation with very high levels of PCB in milk, ranging from 490 to 1388 ng/g fat, and the maximum PCB concentration of 100 ng/g of fat allowed by the Italian law was reached after 144-209 d of lactation (Table 3). After 204 days one animal had a PCB concentration of 102 ng/g fat.

In order to avoid painful biopsies for the animals, the initial PCB concentration in body fat was not determined, so it was not possible to correlate the total PCB excreted into the milk with PCB body burden.

The initial level of contamination is not the main factor affecting the time required for PCB levels in milk to return to below the legal threshold of 100 ng/g fat, actually the relationship between initial PCB concentration in milk and the time required to reach the legal threshold of 100 ng/g fat, is not significant: (r² = 0.423; P = 0.156). This could mean that the amount of PCB mobilized from body fat storage is not proportional to the initial amount of PCB.

Daily milk yield and days in milk (DIM) significantly affect PCB concentration; if the initial PCB concentration in any cow is assumed to be =100, the following equation can be obtained:

\[
\text{PCB in milk} = 107.87 - 1.678 \times \text{daily milk yield} - 0.299 \times \text{DIM}
\]

\(r^2 = 0.58; P<0.0001\)

This could be explained by the lipophilicity of PCB molecules, that mainly accumulate in body fat, the main route of excretion being through milk. After parturition feed intake does not match energy requirements for milk yield, so a strong fat mobilization occurs to support milk synthesis; this is believed to increase PCB excretion in milk. As lactation continues, feed intake increases, compensating for the energy balance of the animals and reducing the need for fat mobilization, which helps to lower PCB concentration in milk.

If log₁₀ of PCB concentration (ppb) in milk fat is regressed against days in milk (DIM), the following significant equation is obtained:

\[
\log_{10} \text{PCB (ng/g of milk fat)} = 2.796 - 0.00474 \times \text{DIM}
\]

\(r^2 = 0.72; P<0.01\)

Assuming the initial concentration of PCBs=100, the rate of excretion in milk was determined for each of PCBs and the following equations (significant at P<0.0001) were calculated:

- PCB 101 in milk fat:
  \[
  59.750 - 0.3037 \times \text{DIM}
  \]
  \(r^2 = 0.52\)

- PCB 118 in milk fat:
  \[
  61.926 - 0.3259 \times \text{DIM}
  \]
  \(r^2 = 0.52\)

- PCB 138 in milk fat:
  \[
  71.658 - 0.3281 \times \text{DIM}
  \]
  \(r^2 = 0.48\)

- PCB 153 in milk fat:
  \[
  62.364 - 0.2949 \times \text{DIM}
  \]
  \(r^2 = 0.39\)

The slopes of each equation were compared with the parallel test and found not different.

PCBs in tissues and organs

As already mentioned, PCBs are lipophilic substances and therefore are deposited in adipose tissue, which is confirmed in Table 4, where PCB levels are compared for the animals slaughtered in our study, and which indicates that it is in adipose tissue that PCBs are principally found. The concentration of PCBs in adipose tissue is around 30 times more than in the kidneys, 130 times more than in milk, 250 times more than in the liver and 300 times more than for haematic contamination. Considering that even with variations due to the nutritional and physiological state, adipose tissue represents around 15% of a cow's weight (Gibb et al., 1992), this confirms that for milk cows this is definitely where most PCBs are found (Thomas et al., 1999a).

Among the seven congeners considered by

Table 3. Initial PCB content and time required in order that PCB content become lower than maximum acceptable level.

|        | Cow 1 | Cow 2 | Cow 3 | Cow 4 | Cow 5 | Cow 6 |
|--------|-------|-------|-------|-------|-------|-------|
| Initial PCB content of milk, ng/g fat | 1388  | 1142  | 709   | 958   | 596   | 490   |
| Days to drop under 100, ng/g fat     | 167   | 165   | 209   | 144   | not reach | 189   |
| Average milk yield, kg/d             | 29.3  | 28.9  | 24.5  | 23.2  | 22.5  | 26.0  |

Table 4. PCB concentration (ng/g wet weight) in blood, milk and several tissues at the end of the experiment.

|        | Cow 1 | Cow 2 | Cow 3 | Cow 4 | Cow 5 | Cow 6 |
|--------|-------|-------|-------|-------|-------|-------|
| Body fat | 90.5  | 194.3 | 460.7 | 224.4 | 241.3 | 150.4 |
| Tail fat | 98.3  | 134.0 | 375.0 | 206.7 | 177.8 | 155.8 |
| Liver | 0.4   | 0.9   | 0.8   | 1.1   | 1.2   | 0.9   |
| Kidney | 2.3   | 5.5   | 10.2  | 6.0   | 8.7   | 7.9   |
| Blood | 0.5   | 0.6   | 1.0   | 0.5   | 0.7   | 0.6   |
| Milk | 1.1   | 1.5   | 1.6   | 1.3   | 1.9   | 1.6   |
Table 5. Variations of PCB congeners percentage in milk (on sum of PCB) during the trial.

|            | 28    | 52    | PCB congeners | 101 | 118 | 138 | 153 | 180 | SE   |
|------------|-------|-------|---------------|-----|-----|-----|-----|-----|------|
| First week | 0.61^a| 0.15^a| 14.55^b       | 34.91^b| 34.91 | 28.99 | 18.75| 4.04 | 0.592 |
| First month| 1.35^ab| 0.31^a| 14.13^b       | 35.64^b| 35.64 | 26.53 | 18.67| 3.34 | 0.326 |
| Second month| 3.02^b| 0.75^b| 12.79^b       | 30.61 | 30.61 | 32.63 | 19.63| 0.57 | 1.22 |
| Third month| 4.26^c| 1.22^b| 12.01^a       | 25.54 | 25.54 | 34.25 | 22.22| 0.53 | 1.32 |
| End trial  | 5.59^d| 1.32^c| 11.12^a       | 23.07 | 23.07 | 37.67 | 21.22| nd  | 1.42 |

SE: standard error of the means. ^a,b,c,d,e,f: P<0.05; ^A,B,C,D,E: P<0.01. *Under the detection level.

Table 6. Congener profiles of blood, milk and tissue (percentage of PCB's sum).

|            | 28   | 52   | PCB congeners | 101 | 118 | 138 | 153 | 180 | SEtissue |
|------------|------|------|---------------|-----|-----|-----|-----|-----|---------|
| Body fat   | 0.99 | 0.24 | 18.98         | 24.08 | 24.08 | 31.44 | 6.53 | 0.648 |
| Tail fat   | 0.04 | 0.25 | 8.67          | 22.89 | 22.89 | 31.80 | 6.68 | 1.099 |
| Liver      | 0.48 | 0.12 | 9.11          | 20.35 | 20.35 | 38.00 | 13.78 | 1.969 |
| Kidney     | 0.31 | 0.07 | 9.96          | 24.61 | 24.61 | 31.93 | 9.20 | 0.980 |
| Blood      | 0.67 | 0.33 | 4.44          | 34.92 | 34.92 | 24.75 | 2.54 | 3.205 |
| Milk       | 5.59 | 1.32 | 11.12         | 23.07 | 23.07 | 37.67 | 21.22| 1.575 |

SE: standard error of the means. ^a,b,c,d: P<0.05; ^A,B,C,D: P<0.01. *Under the detection level.

The persistence of PCB 153 was observed both in humans (Linderholm et al., 2007) and in sardines (Antunes et al., 2007). PCB 101 and 118 are found prevalently in milk or blood (Table 6), which is justifiable by the planarity of the molecules which facilitates mobilisation and excretion (Antunes et al., 2007).

PCBs in milk

The PCBs 118, 138, and 153 are those most abundant in milks (Table 5), confirming the results of Thomas et al. (1999a). The concentration of PCB 118 in milk is lower than in blood (Table 6), according to Thomas et al. (1999c), which can be explained by the fact that the congeners contained in blood are not necessarily totally amenable to being incorporated in tissues, perhaps because they are associated with haematic components, which could explain the discrepancy (Vrecl et al., 2005).

Further, after the first month of lactation the percentage of PCB 118 drops considerably, which is in itself strange, considering that this congener belongs to the group of those of evaluated persistence, but it must not be forgotten that among these PCB 118 is the one with the shortest period of permanence (Antunes et al., 2007).

The percentage of PCB 101 in milk observed in our study (11.12%) is clearly higher than the one reported in the data of Thomas et al. (1999c) (0.8 %), but it must be pointed out that in that study the percentage of PCB 101 deposited in bodies was also low compared to milk (0.5%). This behaviour is therefore analogous to the observations in our study, where greater corporeal accumulations of PCB 101 correspond to greater excretions in milk.

Decline of PCB content in cow's milk
the congeners 138, 153 and 180.

Although remaining low, the percentage of these congeners of the total number of PCBs tends to increase during lactation, probably due to the drop in the number of the more abundant congeners, but which are also excreted more rapidly due to a higher COR.

The low concentration PCB 180, markedly lower than congeners 138 and 153 particularly in milk, contrasts with the studies by Thomas et al. (1999a, 1999c) in which PCB 180 showed elevated persistence and constituted almost 10% of all PCBs in milk (Thomas et al., 1999c).

A low initial presence of this congener can be hypothesised, seeing that already after a week of lactation it constitutes only 4.04% of PCBs excreted in milk; the high value of COR reported by Thomas et al. (1999a, 1999c) indicates a consistent transfer of the congener into milk, exhausting the probably unimportant corporeal reserves.

The percentage of PCB 138 increases during lactation probably for two reasons: on one hand its elevated persistence and on the other one the elevated carry-over rate in milk (Thomas et al., 1999a, 1999c).

Conclusions

The initial level of contamination is not the main factor affecting the time required in order for PCB to return to below the legal threshold of 100 ng/g fat and milk yield did not affect the rate of PCB excretion ($r^2=0.35$; $P<0.22$). Body condition and energy balance could be the most important factors.

In the presence of high contamination, about 6 months of lactation are required in order to obtain milk with a sufficiently low PCB content.

There are differences in the excretion of different congeners, at the start of lactation PCB 118 is the most abundant, while at the end of the study PCB 138 was most present.

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