

PCDD/F and PCB transfer to milk in goats exposed to a long-term intake of contaminated hay

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Abstract

The purpose of this study was to determine the feed to milk transfer of 17 PCDD/Fs and 18 PCBs in goats exposed to a 10-week long-term intake of contaminated hay collected in the vicinity of a hazardous municipal waste incinerator. The sum of PCDD/Fs and PCBs WHO-I-TEQ was found to be higher than 3 ng kg⁻¹ of milk fat after the first experimental week. Carry-over rates (CORs) of PCDD/Fs and PCBs could be established at steady state conditions for each compound studied. For PCDD/Fs, 2,3,7,8-TCDD appeared as the compound having the highest COR (38.8%). Within dioxin-like-PCBs the highest COR were found at a similar level (higher than 80%) for PCBs 105, 118 and 157. Concerning indicator-PCBs, COR ranged from 5% (PCB 101) to more than 40% (PCBs 118, 153 and 180). The intensity of this transfer appeared to be a function of physico-chemical properties (chlorination or log *K*_{ow}) of the molecules and their metabolic behaviour.

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1. Introduction

Human activities produce polluting compounds such as the persistent organic pollutant group (POP), which may interact with agriculture. These compounds have raised concern about the risk of transfer through the food chain via animal products. POPs are characterised by a strong persistence in the environment. Moreover their lipophilicity leads to their accumulation in fat tissues. It is now well established that animal products are the main contributor to human POP body burdens (McLachlan et al., 1990). The lactating ruminant may be exposed to POPs when eating polluted roughage or soil during grazing. Thus, several authors have observed an increase of POP concentrations

in milk produced near potential POP emission sources (Rappe et al., 1987; Stevens and Gerbec, 1988; Eitzer, 1995; Hippelein et al., 1996; Ramos et al., 1997). The transfer of these pollutants in lactating ruminants has previously been carried out according to various modalities. For example, Slob et al. (1995) studied in laboratory conditions a single oral administration of polychlorodibenzo-*p*-dioxins (PCDDs), polychlorodibenzofurans (PCDFs) and polychlorobiphenyls (PCBs) in cows. Another modality to study the transfer was the use of selected compounds. In this way, Firestone et al. (1979) focused their studies on the highest chlorinated compounds. Vrecl et al. (2004) determined the individual excretion of PCBs 54, 80, 155 and 169 in milk and faeces in sheep after a single oral administration of the studied compounds. For other authors studies were oriented on steady state conditions for selected compounds. Thus, Ruoff (1995) observed that 2,3,4,7,8-PeCDF steady state was reached after a 2.5

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month administration in lactating cows, whereas it was obtained after two weeks for 2,3,7,8-TCDD by Arstila et al. (1981). Fries et al. (1973) studied milk and body fat contamination of cows exposed to a long-term PCB exposure (Aroclor 1254). In a field study, Slob et al. (1995) measured the specific bioavailability of the 17 PCDD/Fs and three co-planar PCB in lactating cows over a 60-day grazing period near a municipal waste incinerator (MWI). Thomas et al. (1999a) observed that with natural background contaminated diet, true steady state conditions were not attained at any time during a 14 week study in lactating cows.

According to the literature, the transfer of PCDD/Fs or PCBs from hay to milk has been studied in controlled conditions to allow calculations of feed to milk carry-over rates (COR) (Jilg et al., 1992; McLachlan and Richter, 1998; Fries et al., 1999; Winters et al., 2000; Lorber et al., 2000; Richter and McLachlan, 2001). However, both pollutants groups (PCDD/Fs and PCBs) transfer to milk have not been studied together from heavily contaminated hay in lactating goats. The availability of contaminated hay next to a hazardous waste incinerator (HWI) made it possible to establish in controlled conditions (i.e. feed intake and milk output) the COR of 17 PCDD/Fs and 18 PCBs from contaminated hay to milk at steady state.

2. Material and methods

2.1. Animals and feeding

The animal protocol was in accordance with the general Guidelines of the Council of European Communities (1986, No. 86/609/CEE) and the French Animal Care Guidelines. Three Alpine goats (mean body weight: 50 ± 5 kg) from the herd of the experimental station of the “Ecole Nationale Supérieure d’Agronomie et des Industries Alimentaires (ENSAIA) (Laneuvelotte, France)” were used. Ambient temperature was close to 22°C and natural light conditions prevailed. The lactating animals (third lactation, second month post-partum, average milk yield before the experiment of about 3 l) were milked mechanically twice a day. After a 10 day period to get adapted to the experimental facilities, the goats were fed daily with 800 g of contaminated hay (WHO-PCDD/F-TEQ: 2 ng kg^{-1} dry matter (DM); Indicators(I)-PCB: 1 ng g^{-1} DM; WHO-Dioxin-Like (DL)-PCB-TEQ: 0.38 ng kg^{-1} DM). This roughage was collected at Gilly-sur-Isère (France) next to a HWI. Water was administered ad libitum and animals received a daily concentrate ration composed of dehydrated sugar beet pulp (800 g), crushed maize (400 g), soybean meal (200 g) and mineral salt (10 g). Diet was established to meet requirements from maintenance and milk synthesis according to Jarrige (1988). Milk yield and hay intake were individually recorded daily. Milk samples of 500 ml were collected individually at the morning milking prior to the experiment and at days 7, 15, 22, 29, 36, 43, 57 and 71. Contaminated hay samples were collected every

week and pooled on a monthly basis for analysis. In this way, a representative sample of the concentrate ration was also checked for PCDD/F and PCB contamination.

2.2. Analyses

The PCDD/Fs and PCBs were analysed by gas chromatography coupled with a high-resolution mass spectrometry (GC-HRMS). These analyses were carried out in three stages: extraction, purification and quantification.

2.2.1. Extraction

Before extraction, ^{17}C -labelled PCDD/Fs and ^{18}C -labelled PCBs from Cambridge Isotope Laboratories and Wellington Laboratories were added to the various samples studied. After spiking, solid samples were freeze-dried or desiccated, powdered and transferred into Accelerated Solvent Extraction (ASE) cells. Pressure and temperature were set to 100 bar and 120°C respectively. The extraction solvent was a mixture composed of toluene (Picograde—1350 Promochem) and acetone (Picograde—1142 Promochem) at 70:30 (v/v), and three successive extraction cycles (5 min each) were performed. The extracts were redissolved in hexane for sample clean up after rotary evaporation.

For milk, the mixture of 35 ^{13}C -labelled internal standards (mimetic of the 17 PCDD/Fs, the 12 DL-PCBs and the 7 marker PCBs) was added to a 100 ml milk sample. Two ml of saturated sodium oxalate was added to precipitate the proteins followed by ethanol and diethyl ether and finally the fat was extracted twice with *n*-pentane. The extract was evaporated until it was dry, permitting the gravimetric determination of the fat content. Finally, 25 ml hexane (Picograde—1244 Promochem) was added before purification.

2.2.2. Purification

Purification included three chromatographic steps with successively silica, Florisil and carbon columns.

The first glass column (length 50 cm; ID 2.5 cm) was composed of successive layers including 5 g of anhydrous sodium sulfate (ASC, ISO, Merck Eurolab, Darmstadt, Germany), 5 g of pure silica (Fluka, Germany), 20 g of silica acidified with sulphuric acid (22%), 25 g of silica acidified with concentrated sulfuric acid (Merck) (44%) and 5 g of sodium sulphate. Dioxins and PCBs were eluted in the same flask with 150 ml hexane.

Florisil column (length 25 cm; ID 1.25 cm) was prepared by adding 6 g Florisil (Promochem) to 10 ml hexane. The samples purified on the previous column were reduced to a 1 ml volume and placed onto the Florisil column and rinsed with at least 9 ml hexane. Non-ortho, mono-ortho and di-ortho PCBs were eluted with 110 ml hexane prior elution of PCDD/Fs with 120 ml toluene. These two fractions were concentrated to an approximate volume of 1 ml and then evaporated until dry under a gentle stream of nitrogen, and dissolved again with 1 ml of hexane.

The fraction containing PCDD/Fs was transferred onto the carbon and celite column (length 20 cm; ID 1.0 cm) filled with 0.25 g of a Carbopack C and celite mixture 18/82 (w/w) previously washed with 10 ml toluene, followed by 5 ml of a toluene/methanol (Picograde—1263 Promochem)/dichloromethane (Picograde—1185 Promochem) mixture 5/20/75 (v/v/v), and then by a 5 ml dichloromethane/cyclohexane (Picograde—1179 Promochem) mixture 50/50 (v/v), and finally with 15 ml hexane.

The extract was rinsed with 2 ml hexane and 1 ml of toluene/methanol/dichloromethane 5/20/75. Elution was finally carried out with 30 ml toluene that was evaporated until dry after a supplementation with 25 pg of the internal standard (^{13}C 1,2,3,4-TCDD).

The fraction containing the PCBs was also transferred onto the carbon column (length 20 cm; ID 1.0 cm) but this time filled with 0.25 g of a Carbopack C and celite mixture 18/82 (w/w) previously activated at 130 °C overnight and 1 g of activated Florisil. Mono and di-ortho PCBs were eluted with 10 ml of hexane followed by 30 ml of toluene for non-ortho PCB elution. Both eluates were evaporated until dry after spiking with injection standard (^{13}C labelled 111-PCB) and reconstituted with 20 μl for non-ortho PCB fraction and 50 μl for the other with toluene.

2.2.3. Quantification and QA/QC

PCDD/F and PCB analyses were performed by GC-HRMS (gas chromatograph (HP-5890) from Hewlett Packard (Palo Alto, CA, USA). The mass spectrometer (JMS 700 D, Jeol, Tokyo, Japan) was set at a resolution of 10000, in electron ionisation mode (38 and 42 eV electron energy for PCDD/Fs and PCB respectively). Single Ion Monitoring (SIM) was used to record the two most abundant signals of the molecular ion (^{35}Cl and ^{37}Cl isotopic contribution). A DB5MS (30 m \times 0.25 mm \times 0.25 μm) capillary column from J&W was used in splitless mode. The GC temperature program for PCDD/F analysis was the following: 120 °C (3 min), 20 °C/min to 170 °C (0 min) and 3 °C/min to 275 °C (7 min). The GC program for PCB was 120 °C (3 min), 20 °C/min to 170 °C (0 min), 3 °C/min to 245 °C (0 min) and finally 20 °C/min to 275 °C (7 min). Signals were integrated by JEOL Diok V2 software. All these values were automatically corrected by taking into account the recovery rate of the ^{13}C labelled molecules. The dioxin detection limits in the different tissues analysed including all congeners were better than 30 fg g^{-1} of the matrix. To avoid any over estimation the feed samples and especially hay samples had been injected at the beginning of the experiment on a DB-Dioxin column from J&W (60 m \times 0.25 mm \times 0.15 μm) to ensure that 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF and 1,2,3,4,7,8-HxCDF were not over estimated. The resolution of the DB5MS column was efficient enough to quantify the congeners without co-elution of non 2,3,7,8-substituted congeners. All the procedures integrated the necessary quality assurance parameters to fulfil the requirements of the Commission Directive 2002/69/EC and 2002/70/EC of July 2002 laying

down the sampling methods and the methods of analysis for the official control of dioxins and the determination of DL-PCBs in foodstuffs and feedingstuffs respectively. Moreover, analyses were performed upon an accredited system ISO 17025. All the methods used have been validated and are accredited ISO 17025. Furthermore, this research project was conducted under a certified system ISO 9001 v. 2000 standard.

2.3. Calculations

Kinetics of milk concentration were modeled for each individual compound using the toolbox-curve fitting of Matlab software version 6.5 (The MathWorks, Natick, Massachusetts, USA) in order to estimate adequate parameters such as time needed to reach the steady state. In order to homogenize steady state conditions for all compounds, a plateau was considered as being obtained when the concentration reached 95% of $a + b$. This was possible with the following mathematical equation:

$$y = a + b(1 - e^{-cx}) \quad (1)$$

y is the concentration at given time (ng kg^{-1} milk fat); a is the initial concentration (ng kg^{-1} milk fat); $a + b$ is the concentration at plateau (ng kg^{-1} milk fat); c is the time constant rate; x is the time to reach steady state (days).

For all 35 studied compounds a COR could be evaluated and be calculated as follows:

$$\text{COR} = \frac{m * fy}{f * F} * 100 \quad (2)$$

COR is the carry-over rate (%); m is pollutant concentration in milk fat at steady state (ng kg^{-1}); fy is fat yield (g d^{-1}); f is pollutant concentration in diet (ng kg^{-1} DM); F is daily feed intake (g d^{-1}).

Like McLachlan and Richter (1998) we used COR as an ideal parameter to describe contaminant transfer in lactating ruminants. Indeed, the COR is not strongly influenced by lactation rate, body fat weight or the animal's diets.

2.4. Statistical analysis

The different COR values were treated with an analysis of variance model (ANOVA) in total randomization with one studied factor: compound (17 and 18 compounds for PCDD/Fs and PCBs respectively) and three repetitions (three goats) using the software StatBox version 6.5 (GrimmerSoft, Paris, France). Individual COR values were compared using a t -test according to Bonferroni ($p < 0.05$).

3. Results

3.1. PCDD/Fs and PCBs in feedstuffs

Table 1 indicates the mean levels of PCDD/Fs and PCBs for all the feeding stuffs that were analysed. It reveals that

Table 1
Mean concentrations (ng kg⁻¹ DM) of PCDD/Fs and PCBs in feedstuffs

PCDD/Fs	Control hay	Contaminated hay	Concentrate	PCBs	Control hay	Contaminated hay	Concentrate
2,3,7,8-TCDD	0.04	0.07	0.02	77	3.86	10.43	0.72
1,2,3,7,8-PeCDD	0.01	0.60	0.02	81	0.14	0.59	0.05
1,2,3,4,7,8-HxCDD	0.02	0.60	0.02	126	0.63	3.02	0.14
1,2,3,6,7,8-HxCDD	0.11	0.72	0.01	169	0.12	1.03	0.03
1,2,3,7,8,9-HxCDD	0.04	0.63	0.01	105	19.45	42.42	6.53
1,2,3,4,6,7,8-HpCDD	1.92	4.67	0.19	114	1.27	2.49	0.40
OCDD	6.71	10.56	0.82	118	63.69	126.70	18.94
2,3,7,8-TCDF	0.06	0.35	0.05	123	2.23	5.00	0.99
1,2,3,7,8-PeCDF	0.04	0.60	0.02	156	6.68	13.82	2.56
2,3,4,7,8-PeCDF	0.04	0.98	0.03	157	1.17	2.87	0.49
1,2,3,4,7,8-HxCDF	0.05	1.13	0.02	167	4.57	8.26	1.54
1,2,3,6,7,8-HxCDF	0.05	1.35	0.02	189	0.90	2.22	0.03
1,2,3,7,8,9-HxCDF	0.05	0.10	0.02	28	57.00	66.76	21.29
2,3,4,6,7,8-HxCDF	0.05	1.64	0.02	52	46.00	68.53	20.26
1,2,3,4,6,7,8-HpCDF	0.36	4.24	0.05	101	169.00	215.57	51.04
1,2,3,4,7,8,9-HpCDF	0.10	0.31	0.04	138	176.00	283.42	52.41
OCDF	0.40	1.35	0.06	153	319.00	457.98	93.02
				180	68.00	126.45	24.66

the contaminated hay collected in the vicinity of a HWI was the main source of PCDD/Fs and PCBs. Generally, PCDD/F concentrations in contaminated hay were much lower than PCB concentrations in the same matrix. When comparing the PCDD/F profile in control hay, contaminated hay and concentrate, it appeared that the concentration of nearly all compounds was extremely low in the control matrices. Only two compounds (1,2,3,4,6,7,8-HpCDD and OCDD) were quantitatively present in the control hay. For PCBs, the results show that significant amounts were also detected in control hay and in the concentrate. This was true for the I-PCBs and also for some DL-PCBs and particularly PCBs 77, 105, 114, 118, 123, 156, 167.

3.2. PCDD/F and PCB excretion in milk

The kinetics of the various compound concentrations in milk were similar and indicated that concentration increase until a plateau was reached. Table 2 indicates for each congener the mean concentrations of the pollutants studied and the time period (days after the beginning of contaminated hay intake) necessary to reach steady state conditions. They reached steady state concentration in milk fat progressively during or at the end of the experimental period which lasted 10 weeks. Regarding PCDD/Fs, these particular conditions were often obtained after at least 35 days of contaminated hay intake. For some minor compounds in hay (TCDF, 1,2,3,7,8-PeCDF), the plateau was reached

Table 2
Mean concentrations (ng kg⁻¹ milk fat) of PCDD/Fs and PCBs at steady state and time of reaching (days)

PCDD/Fs	Steady state mean concentration ($n = 3$)	Time to reach steady state (days)	PCBs	Steady state mean concentration ($n = 3$)	Time to reach steady state (days)
2,3,7,8-TCDD	0.48 ± 0.05	65	77	13.37 ± 1.34	8
1,2,3,7,8-PeCDD	2.81 ± 0.23	47	81	2.22 ± 0.10	8
1,2,3,4,7,8-HxCDD	1.97 ± 0.25	48	126	20.97 ± 0.46	15
1,2,3,6,7,8-HxCDD	2.20 ± 0.13	39	169	5.67 ± 0.15	15
1,2,3,7,8,9-HxCDD	1.19 ± 0.11	41	105	660.13 ± 7.93	15
1,2,3,4,6,7,8-HpCDD	3.28 ± 0.29	68	114	29.94 ± 1.20	15
OCDD	2.31 ± 0.34	72	123	15.01 ± 0.7	22
2,3,7,8-TCDF	0.52 ± 0.05	20	156	179.31 ± 9.25	15
1,2,3,7,8-PeCDF	1.17 ± 0.12	26	157	40.32 ± 1.94	15
2,3,4,7,8-PeCDF	3.83 ± 0.39	60	167	72.91 ± 3.25	15
1,2,3,4,7,8-HxCDF	3.34 ± 0.31	74	189	18.94 ± 0.64	15
1,2,3,6,7,8-HxCDF	3.20 ± 0.34	59	28	332.60 ± 7.19	15
1,2,3,7,8,9-HxCDF	0.08 ± 0.01	^a	52	140.00 ± 11.68	^a
2,3,4,6,7,8-HxCDF	2.81 ± 0.42	74	101	225.74 ± 13.45	^a
1,2,3,4,6,7,8-HpCDF	1.67 ± 0.15	57	118	1652.53 ± 52.18	15
1,2,3,4,7,8,9-HpCDF	0.16 ± 0.01	37	138	1890.00 ± 65.99	15
OCDF	0.16 ± 0.06	^a	153	3524.07 ± 85.21	15
			180	1191.76 ± 43.04	15

^a Plateau was reached immediately.

earlier. For PCBs, the steady state was found very quickly, the latest being 22 days after the beginning of the experiment.

PCDD/F compounds were detected at concentration levels ranging from 0.16 to 3.83 ng g⁻¹ milk fat. The lowest concentrations were found for several molecules hardly present in the contaminated hay (TCDD, TCDF, 1,2,3,7,8,9-HxCDF and 1,2,3,4,7,8,9-HpCDF). Congeners with high occurrence in milk were often the most present in the contaminated hay (PeCDD, HpCDD, OCDD, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF and 2,3,4,6,7,8-HxCDF). PCBs in milk fat (ranging from 2 to 3524 ng kg⁻¹) were dominated by the hexachlorinated congeners (153 > 138 > 118) and the pentachlorinated compounds (180). As expected, the concentration of I-PCBs in milk fat was higher in comparison with the other compounds (10–100 times higher). All other compounds were found at less than half of the concentrations of these congeners.

Table 3 presents the WHO-I-TEQ value kinetics in milk fat and reveals that two weeks after contaminated hay intake, the data were higher than the international limits for PCDD/Fs in milk of 3 ng kg⁻¹ milk fat. When pooling the PCDD/F and PCB values, the WHO-I-TEQ value was already above 3 ng kg⁻¹ after the first week.

3.3. Carry-over rates

Figs. 1 and 2 indicate mean COR values established at steady state conditions for each individual compound of PCDD/Fs and PCBs respectively. For PCDD/Fs, 2,3,7,8-TCDD appears as the congener having the highest COR (38.8%) and is immediately followed by 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF. The heaviest PCDD/F compounds (HpCDD, OCDD, 1,2,3,4,6,7,8-HxCDF, 1,2,3,4,7,8,9-HpCDF and OCDF) are transferred to milk to a limited extent, less than 10%. Generally, COR_{PCDD} seemed to be linked to the number of chlorine atoms, thus COR_{TCDD} > COR_{PeCDD} > COR_{HxCDD} > COR_{HpCDD} > COR_{OCDD}. For PCDFs the same tendency was observed except for three congeners whose COR was particularly reduced: TCDF, 1,2,3,7,8-PeCDF and 1,2,3,7,8,9-HxCDF. For DL-PCBs the highest COR were found at a similar level (higher than 80%) for PCBs 105, 118 and 157, while the lowest transfer rates (values below 20%) were obtained for PCBs 77 and 123. For I-PCBs, COR ranged from 5% (PCB 101) to more than 50% (PCB 180). A relation could be suggested between the transfer rate and the number of chlorine substituted atoms: the highest COR were found for PCBs

105, 118, 157, 156, 114, 189 whose chlorination degree was between 5 and 7. On the other hand, PCBs 28, 52, 77 and 101 with 3–5 chlorine atoms had the lowest transfer rate. Despite the higher concentration levels measured for I-PCBs when compared to DL-PCBs, it must be stated that the COR calculated were not significantly different for both PCB families.

4. Discussion

Lactating ruminants may contribute to the transfer of POPs into the human food chain by consuming large masses of herbage or silage. In the following paragraphs, several key points regarding the COR are discussed in order to better understand the particular behavior of the pollutants studied. From a general point of view Figs. 1 and 2 indicate that all studied PCDD/F and PCB compounds were found in milk and therefore transferred from the contaminated hay to the milk. The low standard deviations for both compound families demonstrated a quite similar behaviour and low interindividual effect. Furthermore, the WHO-I-TEQ value kinetics (Table 3) in milk fat reveal that the international limits for PCDD/Fs and PCBs in milk can be rapidly reached and surpassed. This result indicates the rapidity of PCDD/F and PCB transfer from feed to milk fat and consequently a risk regarding food safety.

4.1. Steady state conditions

Steady state conditions were reached within the experimental period for all the compounds studied (Table 2). Indeed, Arstila et al. (1981) obtained steady state conditions for TCDD after only two weeks when administering about 3000-fold more (200 ng) TCDD daily per goat compared to nearly 10 weeks in our study (70 pg TCDD daily per goat). Despite different ruminant species, similar steady state conditions for the 2,3,4,7,8-PeCDF were obtained by Ruoff (1995) on cows and our study with a plateau found between 2 and 2.5 months (in these two studies daily ingested amounts were in a range of 1–10). Regarding PCB steady state conditions, these were very quickly obtained (between 0 and 22 days) and were not simply due to the contaminated hay intake. Indeed, rather high PCB levels were detected in the control hay and in the concentrate, as shown in Table 1. In contrast, Thomas et al. (1999a) stressed the fact that with a natural background diet contaminated by PCBs (\sum PCBs 630 ng kg⁻¹ DM and 2050 ng kg⁻¹ DM for silage and concentrate respectively), steady state conditions were not attained at any time during a 14-week study in lactating cows. These authors also indicated that models that assume steady state conditions have to be used with care. Thus, rather high concentrations of PCDD/Fs and PCBs like those found in contaminated hay used in this trial are needed to reach steady state conditions.

Table 3
WHO-I-TEQ^a evolution in milk fat (ng kg⁻¹)

WHO-I-TEQ	Day 0	Day 8	Day 15	Day 71
PCDD/Fs	0.86	2.84	4.26	6.93
PCDD/Fs + PCBs	2.46	4.85	6.84	9.24

^a World Health Organization International Toxic Equivalent Quantities.

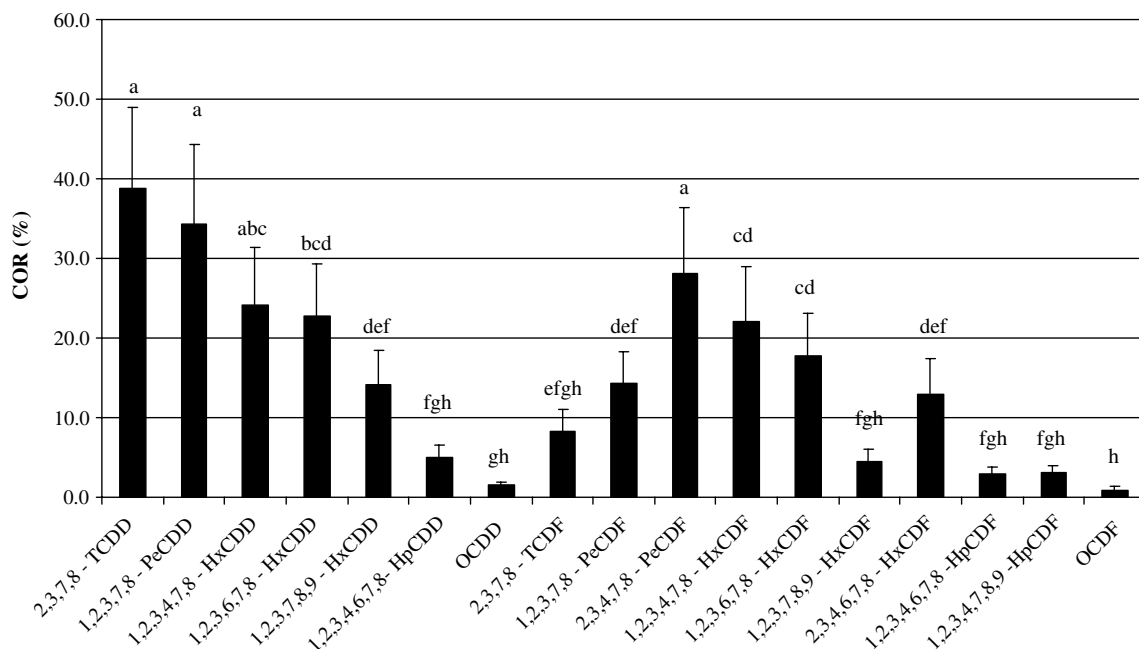


Fig. 1. Mean of COR_{PCDD/Fs} values (%) from feed to milk in goats. ^{a-h}Values without common letters are significantly different ($p \leq 0.05$).

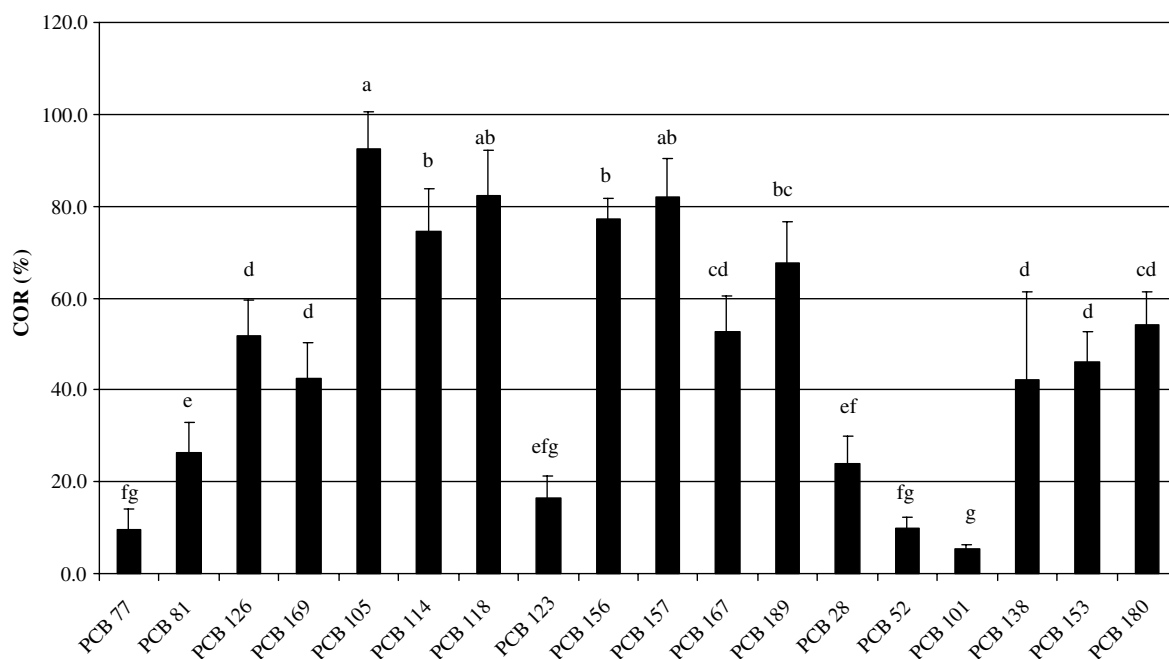


Fig. 2. Mean of COR_{PCBs} values (%) from feed to milk in goats. ^{a-g}Values without common letters are significantly different ($p \leq 0.05$).

4.2. Transfer of PCDD/Fs

To explain the very different COR between the compounds, several authors have pointed out the physical and chemical properties of the congeners (Firestone et al., 1979; McLachlan et al., 1990; Olling et al., 1991; Slob et al., 1995; McLachlan and Richter, 1998; Fries et al., 1999; Lorber et al., 2000). These authors demonstrated that

transfer of PCDD/Fs to milk generally decreases with increasing chlorination and some of these compounds appear poorly transferred from diet to milk due to metabolism. The low COR of OCDD/F and HpCDD/Fs from hay to milk found in this study suggests firstly a lower absorption by comparison with the other compounds, and secondly a greater metabolism of these compounds in the organism. A rapid decreasing absorption may be

the result of an increasing lipophilicity (i.e. K_{ow}). It seems to be the case of OCDD/F and HpCDD/Fs whose $\log K_{ow}$ is higher than for the other congeners. This is in concordance with data published by McLachlan (1993) who found that the absorption of persistent compounds was a function of K_{ow} and noticed a rapid decreasing absorption when $\log K_{ow}$ became higher than 6.5. The same tendencies concerning PCDD/F COR have been found by comparing results between this study with previous work (McLachlan and Richter, 1998; Fries et al., 1999; Lorber et al., 2000). For example, TCCD_{COR} was the highest value in all studies (36–41%) and OCDD_{COR} was found to be the lowest value (0.68–2%). It is interesting to emphasize that concentrations of these compounds in hay administered to goats were very much higher (10 to 1000 times according to the various compounds) than concentrations in feed administered at cows in the other studies. Thus, this study contributes to demonstrate that COR is not dose dependent. More, the feed to milk COR values seem not to be influenced by the ruminant species.

Fig. 1 did not make it possible to establish a linear relation between molecular weight or $\log K_{ow}$ and the COR calculated. The equation obtained failed to demonstrate significant correlation between COR and $\log K_{ow}$ for PCDD/Fs: $COR = -12 \log K_{ow} + 108$, $R^2 = 0.30$. Thus, the low regression coefficient seems to indicate that K_{ow} does not solely control the milk excretion route for PCDD/Fs. An important metabolism of certain compounds may explain the low values of R^2 . It is known for example that 2,3,7,8-TCDF and 1,2,3,7,8-PeCDF are strongly metabolised within the organism (McLachlan, 1997). McLachlan (1994) observed indeed that K_{ow} was a good predictor of COR for poorly metabolised compounds. Thus, compounds likely to be metabolised within the organism create a gap in the link between COR values and K_{ow} . Metabolism may occur within the digestive tract or within the animal after absorption into the bloodstream. After absorption, the pollutants combine with the lipid components (including cholesterol, tri-glycerides, lipoproteins) of the blood and circulate through the body. The liver contains most of the enzymes responsible for the metabolism of these organic pollutants.

4.3. Transfer of PCBs

Regarding COR_{PCBs}, some compounds appear to be transferred unchanged from hay to milk (e.g. I-PCBs as 138, 153, 180 or DL-PCBs as 105, 114, 118, 156, 157, 167 or 189) while others (e.g. I-PCBs as 52, 101 or DL-PCB as 77, 81, 123) are poorly transferred and thus represent a limited transfer risk for the human food chain. Sewart and Jones (1996) found the same dominant compounds in cow's milk. The chlorine pattern of the congeners found in milk are typically substituted at both para-positions (4 and 4'). In fact, McLachlan (1993) found that the 4 and 4' positions were the keys to PCB persistence in cows. Most COR_{PCBs} were found between 40% and 90% and indicate a

rather elevated feed to milk transfer of most PCBs. In an other study (Thomas et al., 1999a) which was conducted in lactating cows fed with natural background contaminated feed, CORs of the poorly metabolized PCBs as 118, 153, 180 and 183 were of 109%, 83%, 67% and 65% respectively. McLachlan (1993) also noted elevated CORs for the same compounds. In most published data, PCB 118 appeared as one of the highest excreted PCB congeners and thus could be considered as a reference compound for high transfer from feed to milk. These data also seem to indicate that the transfer ratio is not dose dependent since CORs were in a same range despite strong differences in contamination levels of compounds in feed. As it was observed for PCDD/F compounds, the K_{ow} cannot be considered as a satisfactory predictor of PCB transfer. Fig. 2 did not make it possible to establish a significant relation between molecular weight or $\log K_{ow}$ and the calculated COR ($COR = 27 \log K_{ow} - 135$, $R^2 = 0.30$). The low regression coefficient demonstrates that K_{ow} does not solely control the milk excretion route for PCB. An important metabolism of certain compounds may explain the low value for R^2 . Thomas et al. (1999b) found that PCBs 28, 52 and 101 may be largely metabolized, whereas PCBs 138, 153 and 180 appear resistant to biotransformation, suggesting clear differences for persistent and metabolised compounds, with a general increase depending on the degree of chlorination.

This observation can be related to our results where COR are rather low for PCBs 28, 52 and 101 and significantly higher for PCBs 138, 153 and 180 (Fig. 2). Vrecl et al. (2004) observed that compound planarity and lipophilicity may strongly influence the milk excretion of PCBs: they found that tetra-chlorobiphenyls (PCBs 54 and 80) are much less excreted in milk than hexa-chlorobiphenyls (PCBs 155 and 169). In our study the transfer of non-planar PCB compounds was higher than co-planar compounds. The lower transfer of co-planar PCBs suggests possible biotransformation in the case of the less chlorine-substituted congeners.

5. Conclusions

Following a long-term supply of PCDD/Fs and PCBs from highly contaminated hay in goats:

- (1) All compounds were transferred to milk at steady state.
- (2) The lactating ruminant exposure to contaminated hay resulted rapidly to high levels of PCDD/Fs and PCBs in milk. After only one week the international WHO-I-TEQ limit (3 ng kg^{-1}) was surpassed.
- (3) The number of chlorine atoms and the metabolism of these compounds seem to be the key factors of transfer intensity.
- (4) Feed to milk COR values seem not to be dose dependent and influenced by ruminant species.

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