PCDD/F AND DL-PCB TRANSFER TO MILK IN BUFFALOES EXPOSED TO CONTAMINATED FEED

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Introduction

Human activities produce environmental contaminants which may be transferred along the food chain via animal products. It is known that animal products are the main contributor to human exposure to persistent organic pollutants (POPs), such as polychlorodibenzo-*p*-dioxins (PCDDs), polychlorodibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs). Although the emissions of dioxins into the environment have been declined during the last decades, a number of dioxin incidents in feed and food may still occur. As a consequence, the withdrawal and destruction of thousands of tons of food and feed cause both huge economic damages and consumer distrust. Contaminated forages and pastures are considered the major pathways of PCDD/Fs and PCBs uptake in grazing animals. A better understanding of the pathways and processes by which PCDD/Fs and dioxin-like PCBs (DL-PCBs) are transferred from feed to animal is essential for developing and evaluating methods to reduce human exposure and associated health risk. Despite the number of studies already performed to evaluate the transfer of these contaminants in lactating ruminants, few data are available for buffaloes.

The aim of this study was to investigate the milk excretion of PCDD/Fs and DL-PCBs in buffaloes (*Bubalus bubalus*) exposed to contaminated feed under controlled conditions.

Materials and methods

Study design

Three lactating buffaloes (2-3 months post-partum), after a 30 day adaptation period to the experimental facilities, were fed daily with contaminated feed over a 100 day period. At the beginning of the investigation, all the animals were in good health and their weight ranged from 620 and 680 kg.

A known amount of 17 PCDD/F congeners (standard mixture purchased from Wellington Laboratories Inc., Ontario, Canada) and 12 DL-PCB congeners (standard mixture purchased from Dr. Ehrenstorfer, Augsburg, Germany) was added to about 20 g of complementary feed and a daily oral dose was administered to each buffalo. The dose was contaminated with PCDD/Fs at 1.5/3.75/7.5 ng for tetra/penta-hexa-hepta/octa chlorinated congeners respectively, and with DL-PCBs at 62.5/77/80.5/168.5/10062 ng for PCB 77-81-114-126-157-167-169-189/PCB-123/PCB-156/PCB-105/PCB-118.

Animals received a daily ration of about 16 kg of hay and 4 kg of complementary feed in order to ensure an adequate milk production over the study period. Everyday, the amount of PCDD/Fs and DL-PCBs ingested by animals in terms of toxic equivalents were 9.38 and 8.47 ng WHO-TEQ_{05} respectively.

Considering a daily diet of 20 kg, the concentrations of PCDD/Fs and DL-PCBs in feed were 0.47 and 0.42 ng WHO-TEQ_{05}/kg, respectively, close to the action levels set by the European Union (EU) for dioxins and DL-PCBs in feed materials of plant origin (Commission Regulation (EU) N. 277/2012).

Before starting the administration of contaminated feed, milk samples were analyzed for PCDD/Fs and DL-PCBs and background concentrations were measured (PCDD/Fs and DL-PCBs mean values were 0.08 and 0.29 pg WHO-TEQ_{05}/g fat, respectively). In addition, both samples of hay and complementary feed were analyzed before and during the investigation in order to check the contamination levels. The buffaloes were milked once a day with a portable milking machine, and milk production was recorded. Milk samples were collected from each buffalo and stored immediately at –20 °C until the chemical analyses were performed.

Chemical analysis

Samples were homogenized and analyzed by a validated method routinely used for PCDD/Fs and DL-PCBs analysis in food and feed. This method was also used to perform a number of proficiency tests with successful results.

Before analysis all samples were spiked with the specific PCDD/Fs and DL-PCBs standard solution, a mixture of $^{13}C_{12}$-labelled congeners (Wellington Laboratories, Ontario, Canada). The extraction and clean-up procedures as well as the analytical determination were carried out as previously reported[1].
Toxic equivalent (TEQ) values were calculated using the World Health Organization Toxic Equivalency Factors established by Van den Berg *et al*. in 2005. WHO-TEQs were expressed as upper bound concentrations assuming that all values of specific PCDD/F and DL-PCB congeners below the limit of determination (LOD) are equal to their respective LOD.

Modeling
Physiologically based pharmacokinetic (PBPK) model was applied to describe the concentrations of contaminants in milk fat during the exposure period. The following mathematical equation was used to estimate the time needed to achieve the steady state:

$$C(t) = a + b(1 - e^{-ct})$$

where a is the initial concentration (pg/g fat), $a+b$ is the concentration at plateau (pg/g fat), c is the time constant rate, t is the time to reach steady state (days), $C(t)$ is the concentration at a given time (pg/g fat). To homogenize steady state conditions for all congeners, a plateau was considered as being achieved when the concentration reached 95% of $a+b$.
Bioconcentration factors (BCFs) were calculated for each congener at steady state as the ratio of the concentration of a congener in milk (pg/g fat) to the concentration of the same congener in feed (pg/g).
Carry over rates (CORs) were also calculated for each congener at steady state as the average daily amount excreted via milk in relation to the average amount taken up daily via feed.
According to the following equations BCF and COR values were calculated:

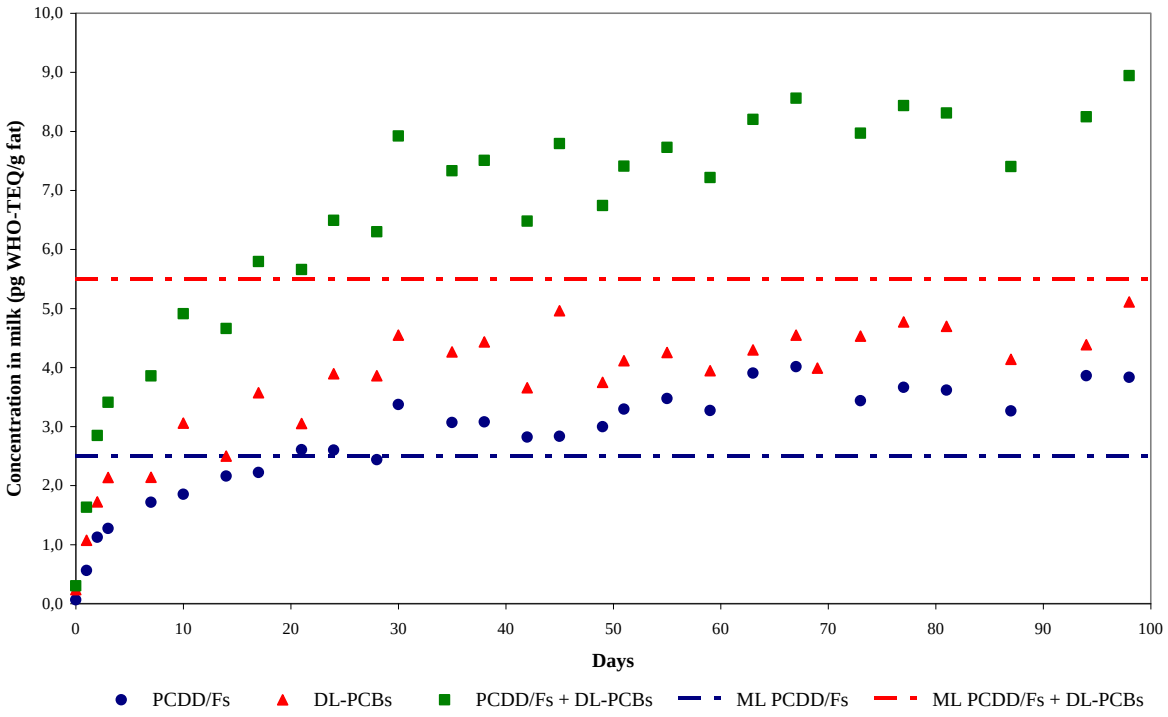
$$BCF = \frac{C_{milk}}{C_{feed}} \qquad COR = \frac{C_{milk}}{C_{feed}} \times \frac{P_{milk}}{F_{feed}}$$

where C_{milk} is the concentration of contaminant in milk at steady state (pg/g fat), C_{feed} is the concentration of contaminant in feed, P_{milk} is the fat yield per day (g/d), F_{feed} is the amount of feed ingested per day (g/d).

Results and discussion
Figure 1 depicts the trend of contamination of PCDD/Fs, DL-PCBs and PCDD/Fs + DL-PCBs as total TEQ during the entire period of the investigation. PCDD/F and DL-PCB congeners were readily transferred into the milk. Despite the levels of PCDD/Fs and sum of PCDD/Fs and DL-PCBs in feed were below the corresponding EU maximum levels, the concentrations in milk exceeded the EU maximum limits (MLs) set out in Commission Regulation (EU) 1259/2011 after 3 – 4 weeks of contaminated feed administration in the case of PCDD/Fs and 2 – 3 weeks for the sum of PCDD/Fs and DL-PCBs.
Results of kinetic descriptors are summarized in Table 1. Over the dosing period, the majority of compounds under investigation reached the steady state (range 5 – 153 days). For two congeners, OCDD and 2,3,7,8-TCDF, the mathematical model was not able to estimate the time to achieve a steady state because both compounds appeared to reach the plateau too quickly. Regarding dioxins, the time to reach steady state was quite variable and, 1,2,3,7,8,9-HxCDF was the first congener to reach the plateau (5 days), while 2,3,7,8-TCDD was the last one (153 days). On the contrary, DL-PCB congeners showed more homogeneous data with values ranging between 19 days for PCB 81 and 89 days for PCB 118. As reported by other authors, the low values found for 1,2,3,7,8,9-HxCDF, 1,2,3,7,8-PeCDF, PCB 77 and PCB 81, reflect their rapid metabolism in mammals.
From a toxicological point of view (TEQ values) the steady state was reached in 62 days for dioxins and 39 days for DL-PCBs. The levels of PCDD/F congeners at steady state ranged from 0.17 to 1.72 pg/g fat. Overall, the highest concentrations were recorded for lower chlorinated congeners, except for 1,2,3,7,8-PeCDF. The levels of DL-PCBs at steady state ranged from 1.94 to 7338 pg/g fat. The pentachlorinated and hexachlorinated congeners showed the highest concentrations in milk.
COR values of individual congeners varied largely with values ranging from 0.5 to 26 for dioxins and from 1.5 to 54 for DL-PCBs. Among PCDD/Fs, 2,3,7,8-TCDD recorded the highest value (25.9%) followed by 2,3,4,7,8-PeCDF (22.4%) and 1,2,3,7,8-PeCDD (20.4%), while highly chlorinated congeners such as 1,2,3,7,8,9-HxCDD,

1,2,3,4,6,7,8-HpCDD, OCDD, 1,2,3,7,8,9-HxCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF and OCDF, were poorly transferred to milk (COR values below 8%).
For PCDD congeners, CORs were inversely related to the number of chlorine. A similar relationship was also observed for PCDF congeners, except for TCDF, 1,2,3,7,8-PeCDF and 1,2,3,7,8,9-HxCDF whose COR values were below 4%.

Figure 1. Mean concentrations of PCDD/Fs, DL-PCBs and PCDD/Fs+DL-PCBs in milk of three buffaloes *versus* time. The blue and red dotted lines indicate the EU maximum level for PCDD/Fs and PCDD/Fs+DL-PCBs in milk.



As reported by other mass-balance studies in ruminants, the relationship between number of chlorine and COR suggests firstly a lower absorption of high chlorinated congeners in the gastrointestinal tract and, secondly a greater metabolism of these compounds in the organism[2,3]. The low absorption may be explained by the increasing lipophilicity (i.e. K_{ow}) of high chlorinated compounds. These results are consistent with those reported in previous studies carried out on cows[4,5].
On the contrary, the low CORs found for 2,3,7,8-TCDF, 1,2,3,7,8,9-HxCDF and 1,2,3,7,8-PeCDF can be due to metabolic processes occurring in the organism, as reported for other ruminants[6].
For DL-PCBs, higher CORs than those found for dioxins were calculated. The highest CORs were obtained for PCB 105, 114, 156 and 157 (values above 40%), while the lowest values were recorded for PCB 77 and 81 (values below 8%). The low CORs for tetra-chlorinated PCBs may suggest a possible biotransformation in the organism while the high values for penta and hexachlorinated PCBs indicate a resistant to metabolism of these congeners. According to McLachlan, the persistence of some PCB congeners is related to the chloro-substitution at both para positions (4, 4')[2]. These results are in concordance with those reported in studies carried out on bovines and goats[4,7].
Similar outcomes were found for BCFs. Values ranged from 0.2 to 11.6% for dioxins and from 0.7 to 23.7% for DL-PCBs. As reported for CORs, BCFs appear to be inversely related to the number of chlorine.
Taking into consideration that the present research was not performed in field conditions (use of "artificially contaminated" feed and consequent high bioavailability of congeners) the experimental data demonstrated that

buffaloes exposed to feed with contamination levels below the EU MLs, may determine concentrations of PCDD/Fs and DL-PCBs in milk above the EU MLs.

Table 1. Time to reach steady state, mean concentration of PCDD/Fs and DL-PCBs at steady state, COR and BCF values (%) from feed to milk. For COR and BCF the mean value ± standard deviation are reported.

Congener	Time to reach steady state (days)	Concentration at steady state (pg/g fat)	COR ± SD (%)	BCF ± SD (%)
2,3,7,8-TCDD	153	0.87	25.9 ± 2.7	11.6 ± 0.9
1,2,3,7,8-PeCDD	63	1.59	20.4 ± 2.3	8.9 ± 0.9
1,2,3,4,7,8-HxCDD	22	1.01	12.7 ± 1.3	5.6 ± 0.5
1,2,3,6,7,8-HxCDD	52	1.14	14.8 ± 2.3	6.4 ± 0.7
1,2,3,7,8,9-HxCDD	26	0.60	7.6 ± 1.4	3.3 ± 0.5
1,2,3,4,6,7,8-HpCDD	26	0.41	5.0 ± 1.5	2.2 ± 0.7
OCDD	---	---	1.8 ± 0.7	0.8 ± 0.2
2,3,7,8-TCDF	---	---	1.8 ± 0.1	0.8 ± 0.2
1,2,3,7,8-PeCDF	9	0.17	3.7 ± 1.8	1.6 ± 0.5
2,3,4,7,8-PeCDF	57	1.72	22.4 ± 1.8	9.7 ± 0.9
1,2,3,4,7,8-HxCDF	27	1.26	15.7 ± 2.1	7.0 ± 0.8
1,2,3,6,7,8-HxCDF	53	1.12	14.4 ± 1.3	6.2 ± 0.6
2,3,4,6,7,8-HxCDF	41	1.13	14.3 ± 1.7	6.2 ± 0.8
1,2,3,7,8,9-HxCDF	5	0.15	1.8 ± 0.5	0.8 ± 0.2
1,2,3,4,6,7,8-HpCDF	29	0.37	4.6 ± 0.9	2.1 ± 0.4
1,2,3,4,7,8,9-HpCDF	27	0.37	4.6 ± 0.8	2.1 ± 0.3
OCDF	26	0.08	0.5 ± 0.4	0.2 ± 0.2
PCB-77	21	1.94	1.5 ± 0.7	0.7 ± 0.4
PCB-81	19	9.71	7.4 ± 0.9	3.3 ± 0.5
PCB-126	33	28.54	21.8 ± 1.8	9.5 ± 0.9
PCB-169	58	38.79	30.4 ± 5.6	13.0 ± 2.8
PCB-105	30	162.48	45.9 ± 3.4	20.2 ± 1.0
PCB-114	65	58.22	44.8 ± 3.2	19.1 ± 1.6
PCB-118	89	7338	35.2 ± 2.3	15.1 ± 1.7
PCB-123	56	43.95	27.1 ± 2.5	11.8 ± 0.7
PCB-156	74	92.34	54.0 ± 5.8	23.7 ± 2.6
PCB-157	45	49.67	37.9 ± 3.0	16.2 ± 1.5
PCB-167	58	65.71	50.3 ± 5.1	21.5 ± 2.1
PCB-189	33	38.35	29.4 ± 3.1	12.8 ± 1.7

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