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Polychlorinated Biphenyl (PCB) Decontamination Kinetics in Lactating Goats (*Capra hircus*) Following a Contaminated Corn Silage Exposure

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Supporting Information

ABSTRACT: This study aimed to determine the kinetics of contamination and decontamination of PCBs and PCDD/Fs in milk of lactating goats. Four goats were fed during 39 days with corn silage collected in an area accidentally contaminated and then with uncontaminated silage during 20 days. Concentrations of DL-PCBs + PCDD/Fs in milk exceeded rapidly (<15 days) the European limit value and approached steady state after 5 weeks. The decontamination kinetics in milk included first a rapid elimination phase (<10 days) followed by a slower elimination phase of 33, 51, and 59 days for DL-PCBs, NDL-PCBs, and PCDD/Fs, respectively. Therefore, in lactating goats, PCBs and PCDD/Fs contaminated forage raises concerns in terms of food safety. The study also indicates that a decontamination process of lactating animals remains feasible; 20 days was considered to be sufficient to obtain a DL-PCBs + PCDD/Fs level in milk below the regulatory value.

KEYWORDS: *PCBs, goat, transfer, milk, half-life*

INTRODUCTION

In the past few decades, many cases of contaminated food have been reported due to exposure of farm animals to contaminated feed. In fact, the European Union has set maximum levels for polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans (PCDD/Fs), dioxin-like polychlorinated biphenyls (DL-PCBs), and non-dioxin-like polychlorinated biphenyls (NDL-PCBs) in food and animal feed.[1,2] However, the contamination pathway to the largest livestock appears to be related to the ingestion of contaminated environmental matrices such as soil and fodder.[3−5]

During the process of contamination and decontamination of livestock, persistent organic pollutants (POPs) undergo four main different steps including (i) an initial phase of bioaccumulation in the tissues and organs, (ii) an equilibrium phase when POP concentrations in tissues, organs, or excretion products such as milk do not increase any more (plateau or steady state), (iii) after the end of exposure, a decontamination phase characterized by a rapid dissemination of the pollutants from tissues and organs to peripheral blood and excreta, and (iv) finally, a last phase during which these exchanges are slower.[6−10] The contamination phase of ruminants has been thoroughly investigated by several authors. In dairy cows exposed to PCBs, several authors[6,9,11,12] observed, for example, a rapid increase of PCB concentration in milk during the 15 days following exposure to contaminated matrices. In lactating goats, two studies[13,14] noted that the concentration of most PCB congeners in milk was highest 15 days after the beginning of the PCB exposure, indicating that the plateau or steady state was nearly reached and that a balance between entrance of PCBs in the organism and output in milk was obtained.

Decontamination kinetics of POPs in ruminants have not yet been extensively described. Preliminary available data[6,8,10,15,16] indicate a potential for accidentally contaminated animals to be decontaminated and reach acceptable levels in animal products. Fries et al.[6] showed, for example, a 50% decrease in the concentration of PCBs in milk of cows 2 weeks after an exposure period of 60 days to Aroclor 1254. After this first phase of decontamination, the PCB concentrations declined more slowly. In a study conducted by Chamberland et al.,[16] the PCB concentration in fat tissue of contaminated heifers declined by >80% over 200 days after the end of exposure. According to these authors, this effect was mainly due to the allometric increased volume of adipose tissue of the animals during their growth. A similar study[15] shows that a 13 month period was necessary to reduce the PCB concentration by 83% in young ruminants which were contaminated during the suckling period. These available data are of real interest because they indicate the potential for contaminated cattle to be decontaminated.

However, several aspects of the decontamination kinetics were not fully described by these previous studies. For example, the specific kinetics of decontamination of each individual PCB congener in milk or fat tissue remains unclear. Given the different physicochemical properties of molecules, as log K_{ow} (between 5.7 and 7.2 for NDL-PCB),[17] and their different toxico-dynamics in the organism, it appears essential to

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characterize the kinetics of decontamination of each compound and its corresponding half-life in the organism. In addition, the new regulation distinguishes DL-PCBs and NDL-PCBs and for the first time indicates a regulatory limit value for the latter. Other limiting factors concerned (i) the reduced number of experimental animals[8,9] and/or (ii) the number of experimental points, which often did not allow the parameters of the kinetics to be characterized.[10,16] Furthermore, in most studies, the animals were orally challenged by an artificial mixture of high dosage of pollutants, which does not reflect the exposure via conventional contaminated matrices such as fodder or soil.

Thus, the aim of this experiment was to precisely characterize the parameters of the contamination and decontamination kinetics in lactating goats primarily exposed to corn silage accidentally polluted by PCBs and then to uncontaminated corn silage.

MATERIALS AND METHODS

Chemicals. All of the organic solvents (Promochem) were of Picograde quality. Silica (Fluka), Florisil (Promochem), carbon (Supelco), sodium sulfate (Merck), sulfuric acid, ammonium sulfate, and potassium oxalate (SDS) were of superior analytical quality. Native and ^{13}C-labeled standards were purchased from Wellington Laboratories and Cambridge Isotope Laboratories.

Contaminated Matrices and Experimental Diets. Contaminated corn silage was collected in a rural area exposed to a long-term wood fire (St Cyprien, France). The soil of this area had been contaminated by PCBs and was used in part to extinguish the fire. Contaminated corn silage was stored in containers hermetically sealed before being used for this experiment. Uncontaminated corn silage was provided from the experimental farm of the Ecole Nationale Supérieure d'Agronomie et des Industries Alimentaires (ENSAIA) (Domaine de la Bouzule, Laneuvelotte, France). Levels of PCDD/Fs + DL-PCBs and NDL-PCBs in this corn silage (at 12% moisture) were 0.112 ng WHO_{2005}-TEQ/kg and 0.109 μg/kg, respectively; these values were under the regulatory limit values[2] (PCDD/Fs + DL-PCBs, 1.25 ng WHO_{2005}-TEQ/kg; NDL-PCBs, 10 μg/kg). These levels are close to the P50 of the sum of PCDD/Fs + DL-PCBs (0.10 ng WHO_{2005}-TEQ/kg) and to the P50 of the sum of NDL-PCBs (0.36 μg/kg) for feed materials of plant origin (oils excluded) established by EFSA.[18]

The corn silage fodder was completed by a commercial feed concentrate in a pellet form and based on corn, alfalfa, wheat, soybean, mineral mix, and sunflower oil. This concentrate feed as well as soybean meal was mixed daily with the corn silage to meet the requirements of the animals and to improve the palatability of this ration. Finally, meadow hay was daily distributed to the animals (INRA Mirecourt, France). During the experimental period, 50 g of each feed matrix (hay, soybean meal, commercial feed, contaminated, and uncontaminated silage) was collected every day. They were then pooled, mixed, and crushed per matrix. Thus, a representative sample of each matrix was obtained and analyzed to check the NDL-PCB, DL-PCB, and PCDD/F contamination and to assess the contribution of contaminated corn silage in the exposure of goats.

Goats and Management. Four Alpine multiparous goats (*Capra hircus*, 66 ± 11 kg BW) from the herd of the experimental station of ENSAIA were placed in individual boxes at a room temperature of 22 °C, under natural light conditions. The animal protocol was in accordance with the general directive on animal care.[19] Prior to the experiment, goats were allowed a 10 day adaptation period, during which uncontaminated corn silage was distributed gradually (from 1.9 to 3.3 kg of fresh matter) in the daily ration, which was established to meet the requirements for maintenance and milk production of goats yielding daily 3500 ± 500 mL of milk.[20] The goats were fed meadow hay (approximately 1.2 kg), commercial feed (0.9 kg), corn silage (on average over the entire experiment 3.1 kg), soybean meal (0.2 kg), water, and salt ad libitum. During the entire experiment, the animals were milked mechanically twice a day. Milk production and diet intake were individually recorded daily.

Experimental Procedure. After the adaptation period, the goats were given 3.1 kg of contaminated corn silage on average during the 39 day exposure period and, subsequently, the uncontaminated corn silage during the 20 day depuration period. At days 0, 14, 29, 34, 39, 43, 47, 51, 55, and 59, individual milk samples (100 mL) were taken from the two milkings of the day and mixed in proportion to the respective production. Immediately after sampling, milk samples were stored at −20 °C until analysis. Blood samples (100 mL) were taken by venupuncture at days 39, 43, 47, 51, and 59 and stored during 24 h at 4 °C before centrifugation at 1500*g* for 10 min to get serum samples, which were stored at −20 °C before analysis.

Analytical Method. Before extraction, 17 ^{13}C-labeled PCDD/Fs and 18 ^{13}C-labeled PCBs were added to the samples. After spiking, the feed samples were desiccated, powdered, and transferred into cells to be extracted by accelerated solvent extraction (ASE) using a Dionex ASE 300. Pressure and temperature were set to 100 bar and 120 °C, respectively. The extraction solvent was a mixture of toluene/acetone 70:30 (v/v), and three successive extraction cycles (5 min each) were performed.

Milk (30−40 mL) was extracted with a mixture of ether, ethanol, and pentane after the addition of potassium oxalate. The upper layer was washed with a solution of sodium sulfate and finally dried over sodium sulfate.

The serum samples (30−40 mL) were mixed for a few minutes with internal standards and diluted with the same volume of Milli-Q ultrapure water. The extraction procedure was performed as follows: addition of 20 mL of aqueous saturated ammonium sulfate, shaking for 1 min, addition of 80 mL of ethanol, shaking for 1 min, two extractions with 200 mL of hexane. The hexane layer was evaporated at 40 °C to dryness and reconstituted in hexane for sample cleanup. The total lipid content of the serum samples was determined using two colorimetric assays from a RANDOX kit (Mauguio, France) and DiaSys (Diagnostic Systems Gmbh, Holzheim, Allemagne) on a 50 μL aliquot.

Cleanup and fractionation of PCDD/F, non-ortho PCB, and mono-ortho PCB were carried out using classical liquid chromatography columns with sulfuric acid silica, Florisil, and carbon (Carbopack C) in that order.

Detection and identification of PCDD/Fs and PCBs were carried out using a Hewlett-Packard 6890 gas chromatograph, equipped with a DB-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) for dioxins and an HT8-PCB column (60 m × 0.25 mm i.d.) for PCBs, coupled respectively to a JEOL JMS-800D and JMS-700D (JEOL, Tokyo, Japan), operating at a resolution of 10000 in the selected ion monitoring acquisition mode after electroionization (40 eV). Two isotope masses were measured for each congener. The quantification was carried out using a five-point minimum calibration curve. All of the insurance requirements were in accordance with the analytical procedure described in the Commission Regulation (EC) No. 152/2009 and 1883/2006 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 feeding stuffs. The research project was conducted upon a certified system (ISO 9001 v. 2000), and the analyses were performed upon an accredited (ISO 17025) system. The procedure integrated a blank control and a sample from a reference material in each series of samples; PCDD/Fs and PCBs values were automatically corrected by taking into account the recovery rate of the ^{13}C labeled molecules, and the recoveries for individual congeners were within 30−140%; the chromatographic separation was checked (<25% peak to peak between 1,2,3,4,7,8-HxCDF and 1,2,2,6,7,8-HxCDF); the determination of the concentrations was performed according EPA Method 1613 revision B.

The limits of quantification (LOQ) for milk and serum were below 0.1 pg WHO_{2005}-TEQ/g fat for PCDD/Fs + DL-PCBs, corresponding to 0.01 pg/g of fat for most of the congeners of PCDD/Fs and DL-PCBs. The limits of quantification were 10 times higher for serum samples on fat basis.

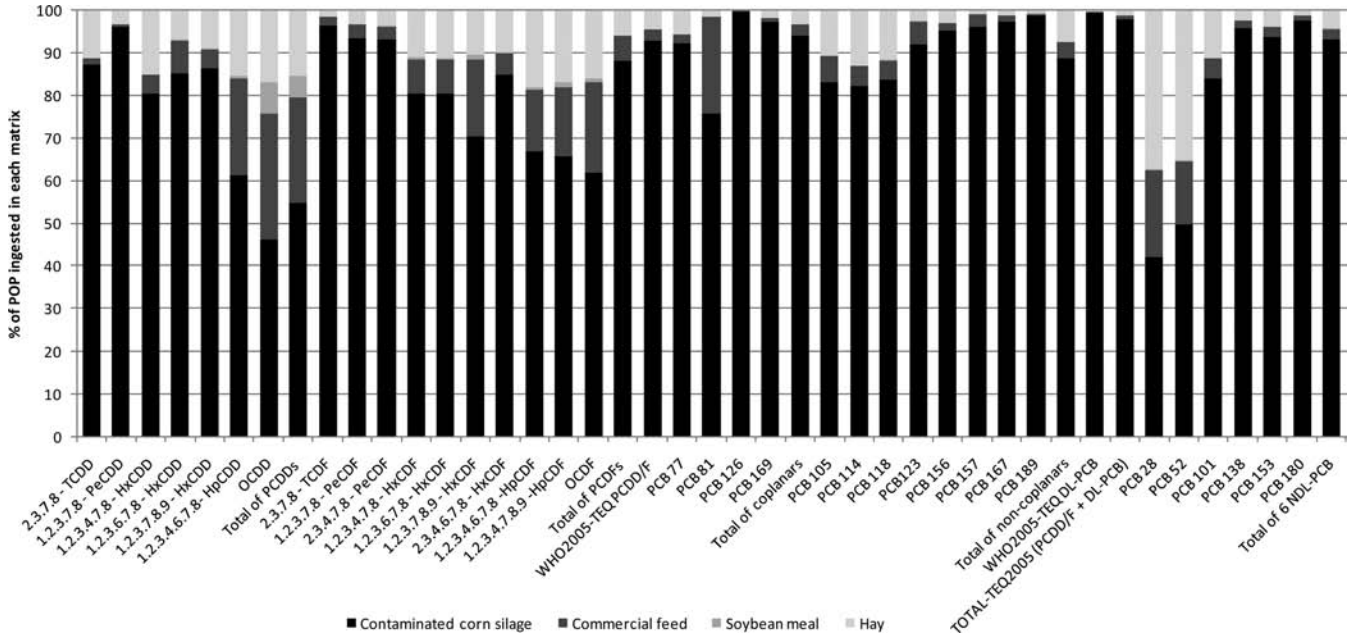

Figure 1. Percentage of POPs provided by each matrix in a standard ration.

Calculations and Statistical Analysis. Statistical analyses were performed by means of the Statistical Analysis Systems software package (SAS, version 9.1, SAS Institute, Cary, NC, USA). Concentrations of PCDD/Fs, DL-PCBs, NDL-PCBs, and each individual NDL-PCB in milk and serum were analyzed statistically as repeated measures using the MIXED procedure and considering the goat as the experimental unit. Milk production was introduced as a covariate in the model. The date of sampling was considered as a fixed effect. Student's t test was used for comparison of the means. Differences were considered to be significant for P values <0.05.

The objective was to assess a mathematical model of the milk contamination kinetic fitted on means by the NLIN procedure of SAS. The model was

$$Y = (I/k)(1 - \exp(-kt))$$

where t is time, Y is the concentration of POPs in milk at time t (days), I multiplied by mass of adipose tissue corresponds to the absorbed POPs dose per day, and I/k characterizes the level of the asymptote for $t = \infty$.

The data obtained during depuration were then submitted to a nonlinear regression using the NLIN procedure of SAS, to estimate the kinetic parameters of POPs depuration in milk. The concentrations of POPs in milk are usually[11] described by a two-component first-order system with the equation $C_t = C_1\ e^{-k_1 t} + C_2\ e^{-k_2 t}$. C_t is the concentration of POPs (per gram lipid weight) in milk at time t (days). C_1 is the initial concentration of the first component, C_2 is the initial concentration on the second component, and k_1 and k_2 are rate constants. The half-lives were calculated as $\ln(2)/k$.

The relationship between the concentrations in milk and in serum were tested with a regression procedure (Minitab Inc., State College, PA, USA).

RESULTS

Contribution of Ingested Matrices on POPs Exposure. The NDL-PCB level in the contaminated corn silage (12% moisture) was quantified at 9.58 μg/kg, that is, almost at the regulatory limit value[2] of 10 μg/kg and above the P99 (4.8 μg/kg dw) of the sum of POP level established by EFSA.[18] Figure 1 indicates the percentage of each NDL-PCB congener provided by each feed matrix in the standard ration distributed (3090 g of corn silage, 900 g of pellet, 200 g of soybean meal, 1200 g of hay). The part of NDL-PCBs provided by the contaminated corn silage of St Cyprien represented by far the most important contribution to the exposure, >95%. Only PCB 28 and PCB 52 were not mainly provided by the contaminated silage. The volatile characteristic of both these congeners certainly explains their dominance in the background atmospheric contamination of hay. The low proportion of these compounds (PCBs 28 and 52) provided by the silage is probably due to their volatilization during the fire, whereas compounds with low volatility were less affected (PCBs 138, 153, and 180).[21]

The level of the sum PCDD/Fs + DL-PCBs in the contaminated corn silage (12% moisture) was determined at 4.49 ng WHO_{2005}-TEQ/kg. This value was 3 times higher than the maximum limit[2] fixed at 1.25 ng WHO_{2005}-TEQ/kg and above the level of P99 (2.96 ng WHO_{2005}-TEQ/kg) of the sum of POP level established by EFSA.[18] Figure 1 indicates the percentage of each PCDD/F and DL-PCB congener provided by each matrix in the standard ration. The part of PCDD/Fs and DL-PCBs (expressed in WHO_{2005}-TEQ) provided by the contaminated corn silage of St Cyprien represented by far the highest contribution to the animal exposure, >95%. From the POPs profile in the contaminated corn silage, it appeared that DL-PCBs represented 83% of the total WHO_{2005}-TEQ and was largely dominated by PCB 126 (96% of the DL-PCBs (WHO_{2005}-TEQ)). Three major congeners were found for PCDD/Fs (WHO_{2005}-TEQ): 2,3,4,7,8-PeCDF (24%), 2,3,7,8-TCDF (29%), and 1,2,3,7,8-PeCDD (28%). This high proportion of PCDFs (59% of the sum of PCDD/Fs) in the profile is certainly linked to the origin of the contamination (wood fire).[22]

Kinetics of Contamination. The milking performance was unaffected by the exposure to contaminated corn silage ($P > 0.1$) and reached 3415 ± 440 g day^{-1} goat^{-1}.

POP concentrations in milk are presented in Table 1. Concentrations in milk varied according to the days ($P < 0.01$ for PCB 28 and $P < 0.001$ for the others). POPs were quantified in goat milk at time $t = 0$ at a level of 2.73 ng NDL-PCBs/g fat and 1.1 pg WHO_{2005}-TEQ/g fat for the sum of PCDD/Fs and DL-PCBs. During the exposure period, the POP

Table 1. Concentrations of POPs in Milk[a]

day	PCB 28		PCB 52		PCB 101		PCB 138		PCB 153		PCB 180		∑ 6 NDL-PCBs		∑ PCDD/Fs		∑ DL-PCBs		∑ PCDD/Fs + ∑ DL-PCBs	
0	0.244	ab	0.098	b	0.143	ab	0.434	a	1.01	a	0.399	a	2.73	a	0.191	a	0.702	a	0.90	a
14	0.292	b	0.103	bc	0.299	bc	5.72	b	10.4	b	8.68	bc	25.8	bc	1.14	bc	9.87	b	11.0	b
29	0.277	b	0.099	b	0.243	bc	7.53	b	12.6	b	10.5	bc	31.2	bc	1.49	c	13.2	bc	14.7	bc
34	0.339	b	0.157	c	0.333	c	9.05	b	13.5	b	11.8	c	35.0	c	1.75	c	16.3	c	18.1	c
39	0.281	b	0.068	ab	0.219	b	7.23	b	14.1	b	12.5	c	34.3	c	1.74	c	15.4	bc	17.1	bc
43	0.206	ab	0.040	a	0.098	a	5.80	b	11.1	b	7.46	b	24.6	bc	1.45	c	10.6	b	12.1	b
47	0.145	a	0.040	ab	0.062	a	3.82	ab	6.77	ab	4.49	a	15.5	ab	0.99	b	6.74	ab	7.72	ab
51	0.217	ab	0.038	a	0.066	a	3.51	ab	6.02	ab	3.80	a	13.6	ab	0.780	ab	6.00	ab	6.78	ab
55	0.223	ab	0.047	ab	0.080	a	2.63	ab	4.59	ab	3.51	a	11.0	ab	0.726	ab	5.05	ab	5.77	ab
59	0.181	ab	0.047	ab	0.073	a	1.97	ab	4.21	ab	3.09	a	9.72	ab	0.680	ab	4.13	ab	4.81	ab
P values																				
time	<0.01		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
av milk production	0.332		0.296		0.363		0.081		0.024		0.102		0.114		0.030		0.736		0.647	
RMSE	0.050		0.023		0.049		2.57		2.95		1.78		7.13		0.267		3.07		3.31	

[a]NDL-PCBs in ng/g fat; DL-PCBs and PCDD/Fs in pg WHO_{2005}-TEQ/g fat. Values are adjusted means ($n = 4$). Values within a column not followed by the same letter (a–c) significantly differ ($P < 0.05$).

concentration increased gradually to reach a maximum value at the end of this period (34.3 ng NDL-PCBs/g fat and 17.9 pg WHO_{2005}-TEQ/g fat for the sum of PCDD/Fs + DL-PCBs). The increase in the concentration was less marked for the following compounds: PCBs 28, 52, and 101. Concentrations of each POP family were not significantly different from days 29 to 39 ($P > 0.05$), suggesting that concentrations were stabilized, approaching the steady state.

The equation parameters modeling the kinetics of contamination could be identified (Table 2) and appear valid under the conditions of this study (level of exposure, physiological characteristics of the animal). Derived with respect to t, this equation modeling the contamination allows to the reduction of the contamination rate to be quantified. After 15 days of exposure, the contamination rate of milk decreased by about 50% for DL-PCBs and PCDD/Fs and by about 70% for NDL-PCBs. The ratio I/k is coherent with the value observed at the end of the exposure period, considered as the value at steady state.

Table 2. Parameters of the Contamination Mathematical Model[a]

	model				
	I	k	I/k	R^2	RMSE
∑ 6 NDL-PCBs	3.21	0.091	35.4	0.997	2.00
∑ PCDD/Fs	0.118	0.062	1.90	0.994	0.133
∑ DL-PCBs	1.00	0.056	17.8	0.996	1.04
∑ PCDD/Fs + ∑ DL-PCBs	1.12	0.057	19.6	0.996	1.15

[a]The equation of the model is $Y = (I/k)(1 - \exp(-kt))$, where t is time (days), Y is the concentration of POPs in milk at time t, I corresponds to the input of POPs per day and I/k (ng/g fat for NDL-PCBs and pgWHO_{2005}-TEQ/g fat for DL-PCBs and PCDD/Fs) characterizes the level of the asymptote for $t = \infty$, R^2 is the coefficient of determination calculated as the square of the correlation coefficient between predicted and observed values, and RMSE is the root mean square error calculated as the root squares of differences between predicted and observed values divided by number of observations.

Depuration and Half-lives. *Concentration of POPs in Milk and in Serum.* As soon as the exposure ceased, the level of PCDD/Fs and of PCBs in milk fat declined (Figure 2 and Table 1) in 12 days from 34.3 to 13.6 NDL-PCBs/g fat and from 17.9 to 7.14 pg WHO_{2005}-TEQ/g fat for the sum of PCDD/Fs and DL-PCBs. After 10 or 15 days the rate of depuration was quite slow ($P < 0.05$). This shape of curve is characteristic of a biphasic decontamination. After 20 days of depuration, the POP concentrations in milk were still higher than the initial values: 9.72 ng NDL-PCBs/g fat and 5.12 pg WHO_{2005}-TEQ/g fat for the sum of PCDD/Fs and DL-PCBs.

POP concentrations in serum at the end of the exposure period were 21.0 ng NDL-PCBs/g fat and 8.7 pg WHO_{2005}-TEQ/g fat for the sum of PCDD/Fs and DL-PCBs. These concentrations rapidly decreased during the first 5 days of the depuration period. Then the POP concentrations diminished at a slower rate to reach values of 5.0 ng NDL-PCBs/g fat and 3.2 pg WHO_{2005}-TEQ/g fat for the sum of PCDD/Fs +DL-PCBs, after 20 days of depuration (Table 3).

The relationship between the PCB concentrations in milk and in serum was clearly linear ($P < 0.001$), with a coefficient of determination over 0.95 (Figure 3). Moreover, the absence of a significant intercept ($P > 0.1$ for PCB) demonstrated a direct

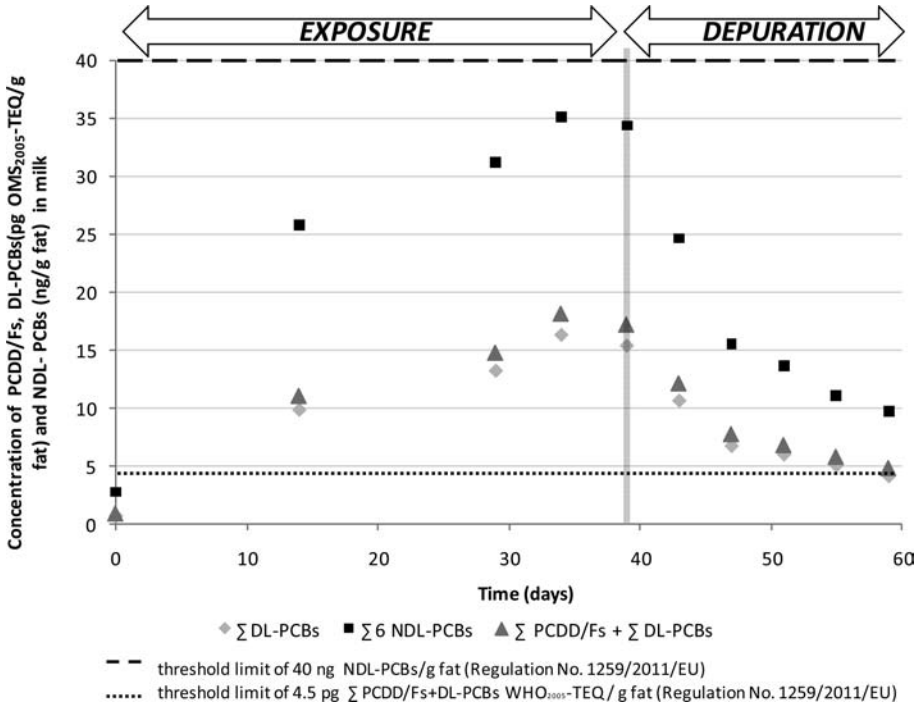

Figure 2. Kinetics of NDL-PCBs and sum of PCDD/Fs and DL-PCBs in goat milk during exposure (39 days) and depuration (20 days).

proportionality between the PCB concentrations of both biological compartments. However, the coefficient of proportionality is different from 1.

Half-lives. The estimated equation using lsmeans (n = 4) of the goats pooled together was $C_t = C_1\, e^{-k_1 t} + C_2\, e^{-k_2 t}$. Equations were fitted with coefficients of determination above 0.95. However, it was not possible to precisely estimate all of the parameters of the equation, especially concerning the second half-life, because of the limited number of observations. The estimates of the parameters of the second-order kinetics of POPs are presented in Table 4. Two estimated half-lives were calculated: depending on the congener, the half-life for the different sums of congeners was found between 4 and 7 days for the first and between 33 and 59 days for the second. The results of this study highlight the difference in half-life existing between the different congeners. For NDL-PCBs, two contrasting molecules can be compared: PCB 101 is rapidly metabolized compared to PCB 180. Indeed, the concentration of PCB 101 in the milk drops rapidly and is of the order of the background level (corresponding to more than half of the steady-state concentration) in the first sample following the cessation of exposure, that is, after 4 days of depuration. Therefore, we can suggest that the half-life of PCB 101 is <4 days. However, the concentrations of PCB 180 have decreased significantly during the first 8 days of decontamination (first half-life, 3 days). Beyond this time, concentrations were not significantly different from each other. However, they stayed 10 times higher than the background level, indicating that the second phase of decontamination is much longer (second half-life estimated at 32 days). Similarly, PCB 77 has a behavior that is similar to that of PCB 101, whereas PCB 126 has the same kinetics as the sum of DL-PCBs, which is relevant because it is the major contributor to the TEQ. Congeners of PCDD/Fs also present differences between them. For example, the first half-life is longer for TCDD than for TCDF (~10 vs ~3 days), and after 20 days of decontamination, the concentration of TCDD is still 4 times higher than the initial level against only 2 times for TCDF. These differences are also observed more generally between families of molecules: especially the PCDD/Fs half-life is longer than PCBs half-life.

DISCUSSION

Contamination of Milk. This study highlights that exposure of lactating goats to PCBs and PCDD/Fs via contaminated corn silage results in a fast contamination of milk. Such a result is in agreement with previous studies in goats[13,14] or in cows.[8]

Assuming that steady state is reached at the end of exposure, a carry-over rate (COR, %) could be evaluated. The COR is calculated as the ratio of the amount of POPs excreted in milk daily and the amount of POPs ingested daily and expressed in percentage. The estimated COR for this exposure was 37% for the sum of PCDD/Fs + DL-PCBs (from 1 to 42% for PCDD/Fs and from 5 to 73% for DL-PCB) and 43% for the sum of NDL-PCBs (from 3 to 49% for NDL-PCBs). These values are of the same order of magnitude as those estimated in goats' milk after an exposure via contaminated hay and contaminated soil.[13,14] This confrontation of results indicates that PCDD/Fs and PCBs are equally bioavailable after an exposure via corn silage, hay, or soil: levels of transfer to milk are the same.

This experiment with an original exposure via accidentally contaminated corn silage allows results to be compared with regulations for feed[1] and food[2] concerning the limit levels of DL-PCBs + PCDD/Fs and NDL-PCBs.

The food intake for goats during the experiment was evaluated at 1.9 ng WHO_{2005}-TEQ/kg for the sum PCDD/Fs + DL-PCBs at 12% moisture, that is, barely above below maximum limit. Despite this concentration of the bolus, the level of DL-PCBs + PCDD/Fs rises over 3.5 times higher than the regulatory limit in milk at the end of the exposure period. Similarly, the level of NDL-PCBs was 2 times lower than the maximum limit in the bolus and barely reached 90% of the 40

Table 3. Concentrations of POPs in Serum[a]

day	PCB 28	PCB 52	PCB 101		PCB 138		PCB 153		PCB 180		∑ 6 NDL-PCBs		∑ PCDD/Fs	∑ DL-PCBs		∑ PCDD/Fs + ∑ DL-PCBs	
39	0.129	0.030	0.196	b	4.139	b	8.59	b	7.91	b	21.0	c	0.93	7.45	b	8.38	b
43	0.122	0.026	0.027	a	2.454	a	4.19	a	4.50	a	11.3	b	0.83	4.88	a	5.71	a
47	0.134	0.037	0.048	a	1.467	a	2.91	a	2.68	a	7.22	a	0.747	3.12	a	3.86	a
51	0.126	0.021	0.058	a	1.314	a	2.37	a	2.43	a	6.32	a	0.710	3.10	a	3.81	a
59	0.125	0.028	0.024	a	0.916	a	1.97	a	1.93	a	4.99	a	0.617	2.41	a	3.02	a
P values																	
time	0.975	0.434	0.0021		<0.001		<0.001		<0.001		<0.001		0.296	<0.001		<0.001	
RMSE	0.027	0.009	0.050		1.344		1.511		2.267		4.214		0.450	1.089		1.154	

[a]NDL-PCBs in ng/g fat, DL-PCBs and PCDD/Fs in pg WHO_{2005}-TEQ/g fat. Values are adjusted means ($n = 4$). Values within a column not followed by the same letter (a−c) significantly differ ($P < 0.05$).

ng/g set by the regulation. These results highlight that for these families of molecules in the case of exceeding the regulatory maximal level in a part of the ration (in this study, corn silage presented levels 3.4 times higher or barely equal to the regulatory limit for DL-PCBs + PCDD/Fs and NDL-PCBs, respectively), the level of POPs in milk exceeds regulatory limit values in a few days or weeks according to the exposure levels.

Possible Depuration of the Animals. The depuration kinetics highlights that decontamination of the animals appears feasible, that is, the concentration of POPs in animal products can be reduced under the regulatory limits after a period during which animals are no longer exposed. This decontamination kinetics in milk is proportional to that observed in the serum, which supports the hypothesis that the animal is gradually decontaminated (Figure 3). In practice, although regulation concerns milk, which is the product consumed, the relative evolution of the POP concentration may be known by serum analysis. Serum is more easily collected than adipose tissue, especially when the amount of subcutaneous adipose tissue is too low to perform biopsies and/or if the samples are too close in time.

The time required to obtain uncontaminated milk that is in accordance with the regulation values depends on the speed of decontamination, that is, the half-life of the compounds in the organism. Indeed, the half-life is a function depending on the molecules' characteristics and the physiological status of the animals, such as level of lactation, turnover of fat due to this lactation and fatness.[23] Previous studies have shown that the PCB depuration kinetics in plasma follows a biphasic kinetic,[6,24] resulting in two successive half-lives. The present study supports these findings even if an experiment with a longer depuration period would be needed to specify the second half-life.

The half-life appeared to be congener dependent. Thus, results expressed in sums of congeners are insufficient to draw conclusions about the amount of time needed to decontaminate animals. In fact, this duration is directly dependent on the contamination profile of milk at the end of the exposure period, which depends of course on the contamination pattern.

The physiological characteristics of the animals also have an effect on the half-lives. The comparison of the second half-lives of PCDD/Fs + DL-PCBs in Limousine cows in lactation[25] (130 days) and Alpine lactating goat (between 35 and 55 days, about 3 times faster than in the cows) seems to illustrate the influence of fatness and level of milk production on the depuration speed. Indeed, in the Limousine cow, the fat amount is about 7−9 times higher than in the goat,[26,27] whereas the amount of excreted fat via milk is only 2−2.5 times higher than in dairy goats. PCDD/Fs and PCBs are lipophilic compounds, mainly stored in fat and excreted via milk. In addition, the goats in this study were highly productive (>3 kg milk per day), in a high lactation phase, which corresponds to a lipid mobilization.[28,29] In these conditions, the ratio of excreted/stored lipids is maximum, which logically promotes a faster decontamination in lactating goats (ratio[27] $\geq$ 2.1%) than in suckler cows (ratio[26,30,31] $\leq$ 0.9%). Therefore, depletion is a matter of the amount of fat excreted (milk yields × milk fat) and of the amount of fat stored in adipose tissues.

In addition to the factors influencing the half-life of the molecule in the organism, the time required to decontaminate the animals depends on the initial level of contamination, that is, the concentration at the first day of depuration. For example, if the initial value is 10 pg WHO_{2005}-TEQ PCDD/Fs + DL-

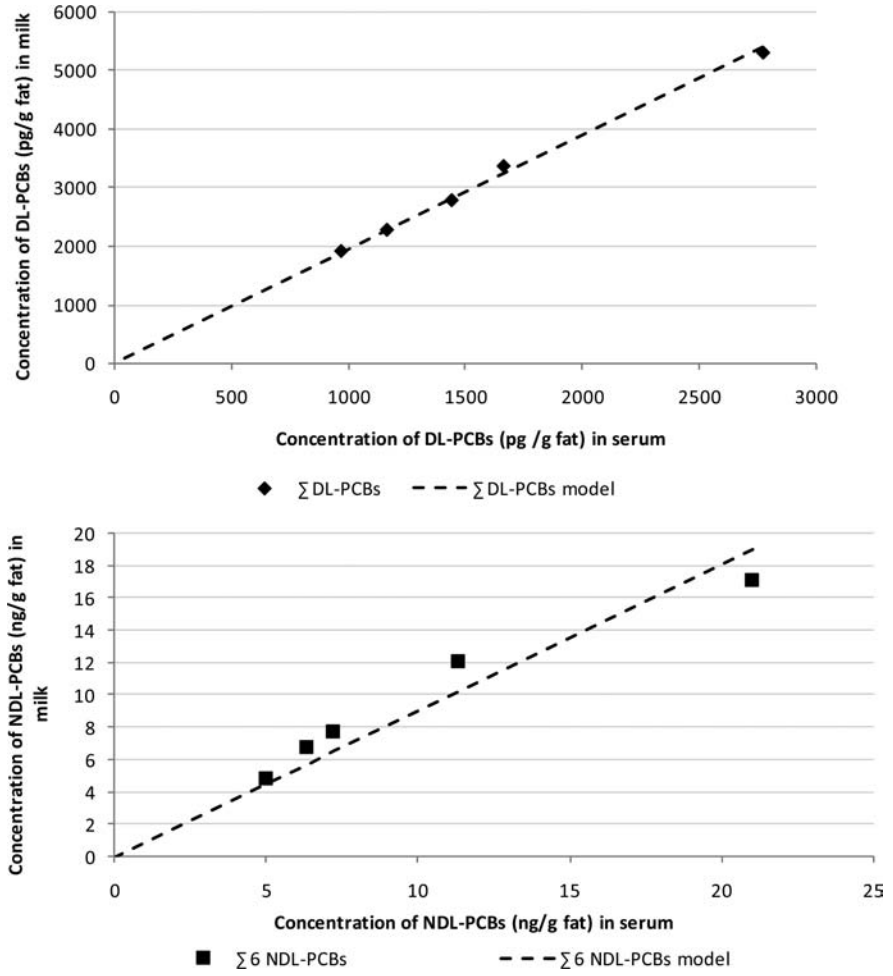

Figure 3. Linear relationship between concentrations of PCBs in milk and in serum.

Table 4. Parameters of the Second-Order Kinetics of POPs in Milk[a]

	model: $[POP]_{milk}\ t = a\ \exp(-k_1 t) + b\ \exp(-k_2 t)$							
POP	a	k_1	b	k_2	R^2	RMSE	$t_{1\ 1/2}$ (days)	$t_{2\ 1/2}$ (days)
∑ 6 NDL-PCBs	23.6	0.146	10.8	0.014	0.998	1.02	4.8	51
∑ PCDD/Fs	1.11	0.107	0.626	0.012	0.997	0.081	6.5	59
∑ DL-PCBs	9.69	0.183	6.08	0.021	0.998	0.450	3.8	33

[a]Half-life ($t_{1/2}$, days) was calculated as $\ln(2)/k$, R^2 is the coefficient of determination calculated as the square of the correlation coefficient between predicted and observed values, and RMSE is the root mean square error calculated as the root squares of differences between predicted and observed values divided by number of observations.

PCBs/g, the concentration of PCDD/Fs + DL-PCBs will approach the regulatory limit values in about 10 days. However, if the initial concentration is 20 pg WHO_{2005}-TEQ PCDD/Fs + DL-PCBs/g, it will reach about 10 pgWHO_{2005}-TEQ PCDD/Fs + DL-PCBs/g after 10 days and will continue to decrease at a slower speed to fall below the regulatory value after about 15 days.

The results of this experiment demonstrate that decontamination of ruminants, especially dairy goats, is feasible within a time frame that is entirely compatible with the lifetime of these animals. The potential of decontamination of animals fed uncontaminated fodder is therefore a good alternative to the current management of crises involving usually euthanasia of animals and avoiding the psychological impact on farmers. However, in the case of lactating animals, this approach would require a specific treatment of contaminated milk and monitoring of the level of POPs in milk to check its conformity to be marketed.

This study shows that POP concentration increases rapidly (within 15 days) in milk when dairy goats are exposed to a POP contaminated fodder. For a certain profile of exposure and a given animal, the daily amount of ingested POPs determines the maximum level of POPs in the milk, which can exceed the regulatory limit values, especially if the concentration of POPs in one of fodder exceeds the regulatory limit. However, the present study shows that contaminated animals when given an uncontaminated diet may undergo a rapid depuration process. After 20 days, the milk gets back to values that are compliant with the regulations. Half-life is dependent on the physiological status of the animals and on the physicochemical characteristics of the molecules involved (family of compounds). The duration of the decontamination period depends on (i) the level of

contamination of the organism at the end of the exposure period, (ii) the contamination profile, and (iii) the physiological status of the animals. It would be necessary to specify the decontamination process according to the physiological status of the animals, particularly studying the impact of different ratios of lipids stored/lipids excreted on POP concentrations kinetics, to better manage the decontamination situations during crises.

■ ASSOCIATED CONTENT

Supporting Information

Table of concentrations of POPs in each matrix of the bolus. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ABBREVIATIONS USED

ASE, accelerated solvent extraction; COR, carry-over rate; DL-PCB, dioxin-like polychlorinated biphenyl; LOQ, limit of quantification; NDL-PCB, non-dioxin-like polychlorinated biphenyl; PCB, polychlorinated biphenyl; PCDD, polychlorinated dibenzo-*p*-dioxin; PCDF, polychlorinated dibenzofuran; POP, persistant organic pollutant; RMSE, root mean square error; WHO-TEQ, World Health Organization toxic equivalent

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