Transgenerational Absorption, Distribution, Metabolism, and Excretion of PCBs in Beef Cows and Calves

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1 Introduction

Polychlorinated biphenyls (PCBs) are anthropogenic persistent organic pollutants (POPs) that are ubiquitous within the environment. Despite their worldwide ban in 2004 by the Stockholm Convention on POPs (*chm.pops.int*), up to 53% of the European citizens exceed the tolerable weekly PCB intake, with the ingestion of food of animal origin as the main exposure pathway[1]. Only bovine meat and dairy products already contribute more than 70% to the total dietary PCB exposure[2]. To reduce this exposure, authorities have set maximum regulatory levels in food[3], to which suckling cow herds have been shown to be occasionally non-compliant[4,5]. Their exposure risk is enhanced by involuntary ingestion of soil (up to 10% of the total diet) while grazing[6], whereas suckling by the calves results in bioaccumulation[7,8]. To ensure the chemical food safety and sustainability of grass-based suckling beef husbandries, it is mandatory to characterize the transgenerational fate of PCBs from diffusive grass and soil sources. Thus far, studies examining the PCB fate, including a detailed assessment of absorption, distribution, metabolism, and excretion (ADME) processes, in suckling cow-calf pairs are scarce[7,8], as it has primarily been described in dairy cows and non-lactating ewes[9,10]. Additionally, studies have shown that physiological traits are important to consider, as these can influence the POP fate[11,12] and obviously differ among dairy cows, suckling cows, and growing calves. The aims were (i) to characterize PCB congener-specific ADME kinetics in cows and calves when exposed via a diffusive soil source, and (ii) to quantify the PCB milk accumulation and decontamination toxicokinetics during full lactation.

2 Materials and Methods

Animal feeding experiment. The experiment mimicking a suckling cow husbandry was reported by Driesen *et al.*[8]. In brief, 12 pregnant Simmental cows (six primi-/six multiparous) were fed a grass silage-based diet from 109±11.5 d *prepartum* until 288±4.5 d in milk (DIM). Four cows were assigned to the control and eight to the exposed treatment, balanced for parity and physiological traits. After 164±4.4 DIM, four of the exposed cow-calf pairs underwent a decontamination. Control and decontaminated cows received only grass silage, whereas for the exposed treatment 2.5% [dry matter (DM) basis] environmentally PCB-loaded soil was mixed to the silage.
The calves were separated from the mother cows immediately after birth, and fed twice daily with the milk of their respective mothers, next to hay and concentrate (after reaching 3 months of age for the latter). After DIM30, two calves deceased due to rumen milk drinking (data reported as 3-month-old exposed calves). At DIM288, remaining cows and calves were slaughtered as detailed in Xavier *et al.*[13].

Measurements and sampling. Individual solid feed DM intake was determined based on the fresh matter intake and bi-weekly offered feed DM content determinations. For PCB quantification, pools of grass silage, grass silage-soil mixture, hay, mineral, salt, and concentrate were composited per lot from weekly or bi-weekly dry subsamples. Individual milk yield and milk intake were recorded at each milking. Milk pools (DIM1-164, DIM165-288), representative of milk excretion or intake, were composited from bi-weekly milk subsamples collected over two successive milking. Additionally, milk samples were sampled eleven times at specific DIM throughout the experiment. Individual feces pools (*prepartum*, DIM1-164, DIM165-288) were composited from bi-weekly dry subsamples collected straight from the rectum in proportion to DM intake and estimated digestibility. Fecal flux mass was further determined using acid-insoluble ashes as an indigestible marker in diet and feces.
After slaughter, the entire left half-carcass and rest of empty body (full body minus digesta, urine, exsanguinated blood, and carcass) were frozen at -20°C, followed by serial grinding and homogenizing steps, before sampling.

Chemical analyses. Chemical analyses performed were detailed by Xavier *et al.*[13] and Driesen *et al.*[8]. Briefly, feedstuffs and feces were oven-dried (50 °C, 72 h) and ground before DM, lipids, and acid-insoluble ashes were determined. The analytical PCB procedure was previously reported[14] and was based on Soxhlet (feedstuffs, feces) and liquid-liquid extraction (milk), with a gravimetrical lipid quantification for the latter. After spiking $^{13}C_{12}$-labeled indicator PCB (iPCB) and dioxin-like PCB (dlPCB) standards, silica, alumina and carbon column chromatography was used for purification, whereas the PCB quantification was done using a Q-Exactive Orbitrap GC-HRMS.

Calculations and statistical analyses. The detailed calculations were previously described by Driesen et al.[14]. Especially the initial (DIM-109 and at calving for cows and calves, respectively) and final (at slaughter) body burdens, as well as the total intake and excretion through feces or milk for the specific *prepartum*, DIM1-164, and DIM165-288 periods, were computed. Additional equations applied were[15]:

Metabolism (ng) = (initial burden + oral intake) – (final burden + calf burden$_{parturition}$ + fecal excretion + milk excretion),

Apparent absorption rate (AR; %) = (oral intake – fecal excretion)/oral intake × 100,

where the calf burden and milk excretion are only applicable for cows and oral calf intake includes solid and milk intake. The PCB concentration kinetics in milk were analyzed by a MIXED model (SAS, 9.4) with dietary treatment, parity nested within dietary treatment, time, and their interactions as fixed effects and animal as random effect. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

3 Results

Input-output balances in cows. Control grass silage and exposed grass silage-soil mixture showed concentrations of 1.6±0.5 and 6.6±0.8 µg of iPCBs kg$_{DM}^{-1}$, respectively, to which PCB101 contributed 33% and 19%, and PCB153 16% and 28%[8]. Additional data regarding dlPCBs and PCDD/Fs are available in Driesen *et al.*[8,14].

The input-output balances for cows are represented in figure 1A-D for a labile (PCB101) and persistent (PCB153) PCB congener. For both, the highest input contribution resulted from the feed (>87%), rather than from the initial burden (≤13%). During the entire experiment, PCB101 and PCB153 intake by control cows was 2.0- and 6.6-fold lower than in exposed, and 1.6- and 4.3-fold lower than in decontaminated cows. During the decontamination period, 1.3- and 1.5-fold higher input levels were found in exposed compared to decontaminated cows.

Larger differences among labile and persistent congeners were observed for the total output (sum of final body burden, calving, milk and fecal excretions, and metabolism). The PCB101 output was characterized by a high fecal excretion (18-46% relative to total input) and a negligible accumulation, resulting in a high estimated metabolism (54-82%), whereas the PCB153 output was characterized by high fecal excretion (23-79%), with in addition milk excretion (10-19%) and accumulation (final burden of 7-13%). Calving excretion played a minor role in both cases (≤0.4%).

The estimated metabolism for PCB101 was 1.4-fold higher in control cows compared to both exposed and decontaminated and for PCB153 3.1- and 23-fold higher, respectively. Next to that, the input of primiparous cows was 1.3-fold and milk excretion 1.4-fold lower than in multiparous, whereas the final burden was generally higher in primiparous cows.

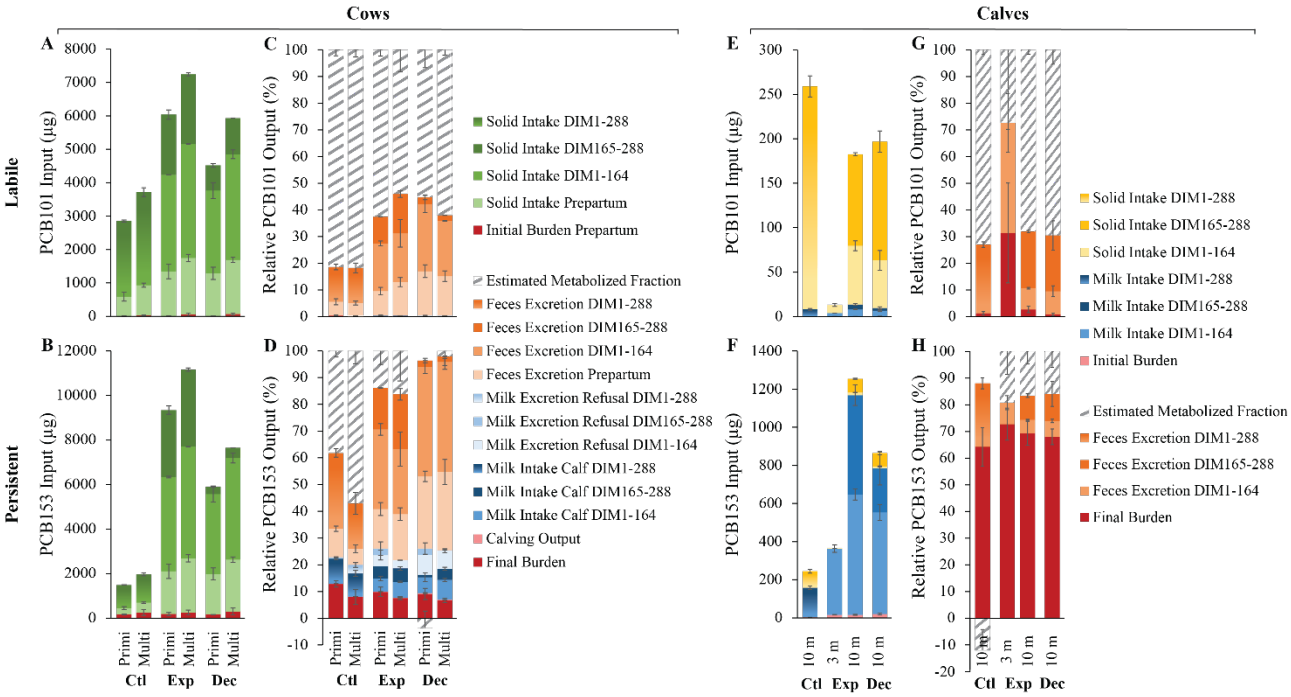

Figure 1: Input (A, B, E, F) and output (C, D, G, H, normalized to input) mass balances of PCB101 and 153 in cows (left panels) and calves (right panels). Cows were divided into primi- and multiparous within each control (Ctl), exposed (Exp), and decontaminated (Dec) treatment (n = 2 each). Calves were divided into 10-month-old Ctl (n = 4), Exp (n = 3), and Dec (n = 3) and 3-month-old Exp (n = 2). 'Milk Excretion Refusal' refers to the milk excreted but not ingested by calves. Data of the Dec animals' output should be interpreted with caution due to the diet switch at DIM165 and the use of relative values. Arithmetic means and standard errors are presented.

Input-output balances in calves. The hay contained 1.6±1.2 µg of iPCBs kg$_{DM}^{-1}$, to which PCB101 contributed 23% and PCB153 8.4%[8]. For the milk, average control and exposed concentrations of 3.3±0.3 and 21.3±1.9 µg of iPCBs kg$_{lipids}^{-1}$ were found, with PCB101 contributing 2.3% and 0.5%, respectively, and PCB153 contributing 45% for both[14].

The input-output balances for calves are represented in figure 1E-H. In 10-month-old calves, the main input for PCB101 was coming from the solid feed intake (hay) with 95%. Conversely, PCB153 was mainly taken up via milk (63% for control and 90% for treated calves). The initial burden at parturition was negligible for both congener types (≤2.3%).

The PCB101 intake of the control calves was 1.4-fold higher than in 10-month-old exposed and decontaminated calves, resulting from a slightly higher hay intake, whereas it was 4.3-fold lower for PCB153. The 3-month-old calves mainly drank milk, resulting in a low PCB101 intake.
The output showed also differences among PCB101 and 153, with a high estimated metabolism for the former (70% relative to total input), high fecal excretions (28%), and a low final burden contribution (1.6%) in the 10-month-old calves. The 3-month-old calves formed an exception for PCB101, with a lower estimated metabolism, resulting in a higher accumulation (31%). Conversely, the output of PCB153 was characterized by a high accumulation (74%) in 3- and 10-month-old calves and consequently resulted in lower estimated metabolism and fecal excretion.

Apparent dietary absorption rate. The apparent dietary AR of PCBs are represented in figure 2. The ARs of cows decreased with increasing lipophilicity (i.e., log K_{OW}), which is linked to a higher chlorination degree. This decline was stronger for the exposed than for the control cows. The ARs of calves did not decrease with log K_{OW} and were higher the younger the animals were, resulting in higher ARs than those reported in cows.

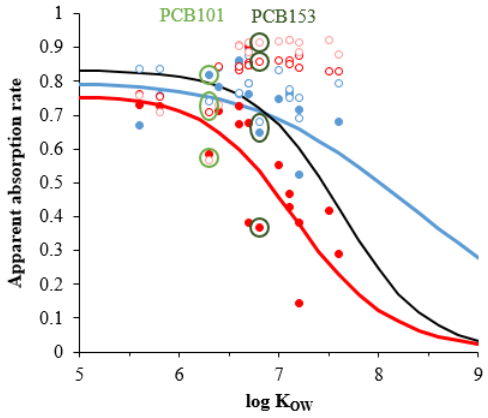

Figure 2: Apparent dietary absorption rates (ARs) of indicator and dioxin-like PCBs in cows and calves (● and ○, respectively) against their respective chemical log K_{OW}[16]. A Hill function was fitted for control (Ctl, $n = 4$, blue): $AR = (0.77 \times \log K_{OW}^{-9.38})/(8.79^{-9.38} + \log K_{OW}^{-9.38})$ and exposed cows (Exp, $n = 4$, red): $AR = (0.75 \times \log K_{OW}^{-19.03})/(7.23^{-19.03} + \log K_{OW}^{-19.03})$, and a non-linear regression for dairy cows (black)[17]: $AR = 1/(2.9 \times 10^{-8} \times K_{OW} + 1.2)$. For calves no relationship could be fitted. PCB101 and PCB153 were indicated with a circle.

Transgenerational transfer through milk. The milk concentration kinetics during one lactation cycle are shown in figure 3. Whereas milk PCB101 concentrations showed almost no difference among dietary treatments or over time (average 0.10±0.04 ng g_{lipids}^{-1}), the PCB153 concentration was 6.4-fold higher in exposed compared to control milk. Additionally, primiparous cows showed generally higher PCB153 milk concentrations than multiparous cows, which was significant ($P < 0.05$) at the beginning of lactation. The dynamics of PCB153 were characterized by a high level at parturition, which declined 1.7-fold until DIM7, followed by a slight upward kinetic until DIM288. Further, a bi-exponential decontamination was observed for PCB153 when switching the decontaminated cows from the exposed to the control diet at DIM164. The decontamination was divided in a fast elimination phase (α), followed by a slower phase (β), both of which took longer for primiparous cows ($t_{1/2}\alpha$ = 7.6 and $t_{1/2}\beta$ = 311 d for primi- vs. $t_{1/2}\alpha$ = 4.8 and $t_{1/2}\beta$ = 170 d for multiparous cows)[8].

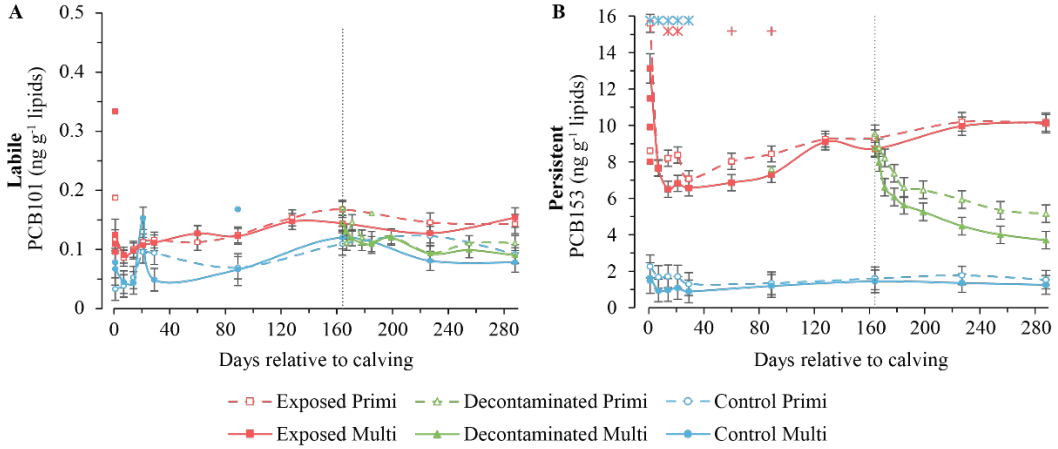

Figure 3: Cow milk concentrations of PCB101 and 153. Cows were divided into primi- (average milk yield = 5.9 kg_{milk} d^{-1}) and multiparous (milk yield = 9.7 kg_{milk} d^{-1}) animals within each control, exposed, and decontaminated treatment ($n = 2$ each). The vertical dotted line at day 164 indicates the initiation of decontamination. Least squares means and standard errors are displayed. Outliers are represented as separate points. Significant differences between parities at the corresponding time point are indicated as $^{*}P < 0.05$ and trends as $^{+}P < 0.10$.

4 Discussion

ADME in lactating beef cows. The highest PCB input investigated in this study, resulted from the intake of grass silage or grass silage-soil mixture independent of dietary treatment or congener properties. Those two factors, however, affected the apparent AR. The exposed diet reduced the AR progressively from penta- to hepta-chlorinated PCBs. This presumably resulted from a reduced bioaccessibility of highly chlorinated PCBs, when carried to the cow via soil. Such effect was previously not observed by Richter and Mclachlan (2001)[18], when comparing the POP ARs in cows fed with forages contaminated by atmospheric deposition or soiling. Additionally, a higher chlorination degree is linked to a higher lipophilicity (log $K_{OW} > 6.5$), which might reduce the absorption along the unstirred water layer surrounding the intestinal wall[9,18]. Still, the present results are in broad agreement with the ones in dairy cows exposed to pasture PCB and PCDD/F background levels (Figure 2 black curve)[9,17].

The PCB101 fraction not excreted via feces was estimated to be metabolized, as the accumulated (final burden) as well as milk excreted fractions were negligible. In contrast, PCB153 showed a lower metabolization, confirming Mclachlan[9] that PCB persistency is partly linked to the 4,4'-substitution pattern. This higher persistence resulted in a higher accumulation (final burden) and milk excretion of PCB153.

The milk concentration kinetics confirmed the differences among PCBs, with higher concentrations for the more persistent congener. The changes in concentration occurring along the lactation highly depended on dynamics in body weight, milk fat yield as well as DM intake[16], which might also explain, at least in part, differences among parities. Primiparous cows showed generally higher PCB153 concentrations, which might result from their lower body weight as well as milk fat yield compared to multiparous cows, being accordingly less efficient in diluting and excreting the compound. This is also in agreement with the higher decontamination half-lives for primiparous cows, as a lower milk fat yield might result in a slower excretion rate[19].

ADME in suckling calves. The exposure of calves to PCBs started prenatally, as the placental barrier was shown to be ineffective[20]. Still, the contribution of the initial body burden at calving was slight. The transgenerational transfer occurred mainly *postpartum* through suckling. Since the milk PCB101 concentration was low, the input was mainly originating from the solid feed. On the contrary, PCB153 was mainly taken up via the milk, since in contrast to cows no decrease in AR with an increase in chlorination degree was seen in calves[7]. The absorption might be promoted by the richer content in highly bioavailable lipids (triglycerides) in milk compared to grass silage or soil. Such steady AR in pre-weaning calves matched with previous observations in monogastrics[17]. Additionally, as no milk excretion route occurred in calves, their final PCB153 body burden relative to total input was much higher than in cows.

The 3-month-old calves showed the lowest metabolism, resulting in a higher accumulation of also PCB101. This was coupled with a lower relative PCB concentration in the liver of 3-month-old calves (data not shown), which might result in less cytochrome binding and consequently a reduced metabolization by hepatic enzymes. Similar observations were made in humans, where infants younger than 6 months showed lower xenobiotic metabolization capacities than adults[21].

5 Conclusions

This study quantified the transgenerational ADME fate of labile and persistent PCB congeners in beef cows and calves exposed via a grass silage-soil mixture. It was shown that the ADME was affected by the physicochemical PCB properties, exposure matrix, and animal physiology. To ensure chemical meat safety in suckling beef husbandries, it is mandatory to consider the complex interplay among those parameters. Physiologically-based toxicokinetic (PBTK) models are useful tools to handle and integrate such interplays[22, 23]. However, mechanistic PBTK models need fine calibrations and further evaluations, which will be facilitated by the detailed transgenerational toxicokinetic dataset reported here.

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