THE TRANSFER RATE OF PCBs FROM FEED TO ADIPOSE TISSUE DEPENDS ON BODY FATNESS IN GROWING CATTLE

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Introduction

Although polychlorinated biphenyls (PCBs) have been banned worldwide in 2004 by the Stockholm Convention on Persistent Organic Pollutants (POPs), it is estimated that around 80% of the total PCB amount produced still remains in our environment[1,2]. Characterized by their easily disperse, highly lipophilic and poorly degradable properties, PCBs are highly persistent and bioaccumulate into the animal food chain. Thus, consumption of food of animal origin embodies the main PCB exposure route for humans, accounting for more than 90% of the overall human exposure[3]. Therefore, it is important to understand the transfer of such compounds in the food chain, in order to ensure the chemical safety of animal products and further reduce the human exposure.

In livestock, the fate of POPs is primarily investigated via feeding experiments, from which the biotransfer factor (BTF), the bioconcentration factor (BCF) or the assimilation efficiency (AE) can be derived for meat producing animals. Such transfer parameters are widely used in order to assess and manage the chemical risk in animal farming systems. Nonetheless, data for PCBs in meat producing growing cattle are scarce, since the few studies with beef animals focus mainly on polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), where no transfer rates were calculated[4,5]. Additionally, some studies in monogastrics highlight the dependency of BTF and BCF on animal physiology, especially on body lipid dynamics influenced by growing rate[6,7]. The aim of the present study was to better understand the transfer of PCBs in growing cattle depending on animal physiology. It was hypothesized that body fatness and growing rate will affect the transfer factors (BTF, BCF and AE).

Materials and methods

Feeding experiment. The feeding experiment was conducted at the experimental farm of Agroscope (Posieux, Switzerland). Eleven bulls aged 3.3-4.7 months at the initiation of the experiment were kept indoor in a free-stall barn with access to an outdoor walking area. In order to record the individual feed intake, the animals had controlled access to feed bunks. After an initial adaptation period, bulls were divided into three groups based on body weight and intake, and subsequently received three different total mixed feed rations for the duration of the fattening period. Rations were composed of maize and grass silage, as well as concentrate (76:24 forage/concentrate ratio), with three distinct energy levels [7.0, 7.4 and 7,5 MJ of net energy for meat production/kg dry matter (DM)]. The bulls were slaughtered when reaching a body weight of either 530 (n=4) or 600 kg (n=7) at an age of 12.2-15.6 months.

Sampling and chemical analyses. Total mixed feed ration pool samples were composited along the fattening period. Perirenal adipose tissue and the 11th rib of a half carcass were collected for each bull at slaughter. The 11th rib was dissected and the relative proportions of muscle, adipose tissue and bone were determined.

Feed samples were Soxhlet extracted with n-hexane for 16 h. The extracts were spiked with $^{13}C_{12}$-labeled internal PCB standards 28, 52, 101, 138, 153, 180 and the dioxin-like PCBs (dlPCBs) 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189 (Cambridge Isotope Laboratories Inc., USA). The concentrated extract was purified on a multilayered silica column (acid, neutral and basic silica), followed by fractionation on an alumina and activated carbon column (DexTech Plus, LCTech GmbH, Germany). Both fractions were reduced to 30 µl, after which the recovery standard 70 PCB (Cambridge Isotope Laboratories Inc., USA) was added. Lipids of the perirenal adipose tissue were extracted for 16 h with toluene via Hot-Soxhlet extraction (Büchi B811, Büchi Labortechnik AG, Switzerland). The extracted lipids were quantified gravimetrically and spiked with the same $^{13}C_{12}$-labeled PCB standards, followed by the same purification and fractionation steps than for feed samples.

The PCBs were quantified with a Finnigan MAT95 sector field mass spectrometer (Thermo Finnigan MAT, Germany) coupled to a Finnigan Trace GC Ultra equipped with a TriPlus auto sampler (Thermo Electron

Corporation, USA). A SGE HT8 column (30 m x 0.22 mm with film thickness 0.25 µm) was used with helium at 100 kPa for the gas chromatographic separation. Injection of the sample happened splitless at a source temperature of 220°C. The column had an initial temperature of 100°C for 1 min, followed by an increase of 20°C/min until 180°C. Thereafter, it further increased 3°C/min until 250°C and 20°C/min until 300°C for 5 min.

Calculations and statistical analyses. The 11th rib adipose tissue and the carcass weight were used to estimate the carcass lipid content and mass applying the predictive equation of Robelin *et al.*[8]. Carcass PCB burdens were further derived by multiplying the PCB lipid-normalized concentrations in perirenal adipose tissue by the carcass lipid mass, assuming equal PCB lipid-normalized concentrations in both carcass and perirenal adipose tissue[9]. The transfer descriptors were calculated based on the following equations[10,11]:

BTF = PCB concentration in adipose tissue (ng/kg lipid) / daily PCB intake (ng/day)

BCF = PCB concentration in adipose tissue (ng/kg lipid) / PCB concentration in feed (ng/kg DM)

AE (%) = [PCB body burden (ng)] / [PCB concentration in total mixed feed ration (ng/kg DM) x feed intake along the fattening period (kg DM)] x 100

To test our hypotheses, the animals were classified based on low, medium or high carcass lipid content [LL > 0.5 standard deviation (SD) below, ML ± 0.5 SD from and HL > 0.5 SD above the carcass lipid content mean, resp.], as well as slow, medium or fast growing rate (SG > 0.5 SD below, MG ± 0.5 SD from and FG > 0.5 SD above the growing rate mean, resp.). A mixed model in total randomization was used to determine the effects of carcass lipid content and growing rate class on BTF, BCF and AE for each PCB congener. The model included feeding treatment, carcass lipid content class (LL, ML, HL), growing rate class (SG, MG, FG) and their interaction as fixed effect, and animal as a random effect. Feeding treatment and interactions were never significant ($P > 0.10$) and were removed from the final model. Experimental data was logarithmically transformed to comply with the assumptions of normality and homoscedasticity of residuals, when needed. To assess the individual effect of carcass lipid content and growing rate on BTF, BCF and AE, simple and multiple linear regression analyses were performed using the GLM procedure. All statistical analyses were done using SAS 9.3. (SAS Institute Inc. USA). Significance was declared at $P < 0.05$. Values reported are least square means and standard error.

Results and discussion

PCB concentrations in feed and adipose tissue. Between the three total mixed feed rations, there were slight differences in the sum of iPCBs (2'450, 2'236 and 1'652 ng/kg DM) and dlPCBs (0.078, 0.059 and 0.045 ng TEQ_{05}/kg DM, for low, medium and high energy level rations, resp.), presumably due to increasing proportions of grass silage from high to low energy level rations. The pattern of iPCBs in feed was dominated by the congeners 52 and 101 (34% and 31% resp.), whereas the dlPCBs were dominated by 126, 118 and 105 based on ng TEQ_{05}/kg DM (73%, 16% and 5% resp.). For the adipose tissue, the iPCB concentrations varied between 2'774 and 11'966 ng/kg lipids and the dlPCB concentrations were between 0.28 and 0.66 ng TEQ_{05}/kg lipids, with 138, 153 (average 35%, 34% resp.) for iPCBs and 126, 118 and 169 (average 85%, 8% and 3% resp.) for dlPCBs contributing the most. All those concentrations are representative for background levels, as they are largely under the regulatory limits.

Transfer factors depending on PCB chlorination degree. Despite the fact that PCBs are seen as POPs, large differences in persistency and bioaccumulation among congeners were observed. By calculating the BTF (the same for BCF and AE), three groups could be identified (Figure 1), which correspond mainly to the chlorination degree of the congeners. The tri- to penta-chlorinated PCBs 28, 52, 101, 105, 114, 118 and 123 with low BTFs (mean per congener < 0.60) could clearly be classified as labile compounds, probably because they are highly metabolized by cattle[10]. The congeners 77 and 81 had also low BTFs (< 0.66), but they behaved differently compared to the other seven labile congeners measured, as more variability among animals was recorded. Some animals (with BTFs > 1.5) were probably less potent in metabolizing these two latter compounds. The persistent PCBs were the hexa- to hepta-chlorinated congeners 169, 180 and 189 with BTFs > 1.70, representing a clear bioaccumulation from feed to adipose tissue. The penta- to hexa-chlorinated PCBs 126, 138, 153, 156, 157 and 167 had BTFs in between (1.10 < BTF < 1.29) and were classified here as semipersistent. These findings correspond well to the statement of Travis *et al.*[12], who suggested that the BTF is directly proportional to the partition coefficient (K_{ow}) and therefore to the chlorination degree. This statement is however, questioned by several authors as observations showed that the BTF decreases again when $\log K_{ow} > 7$ [10]. Indeed, by comparing

these results with the classification of other authors stupendous differences could be observed, e.g. 118 and 123 were allocated to the persistent PCBs by McLachlan *et al.*[13], as well as by Huwe *et al.*[14] for 118, compared to the labile classification of both congeners in the present study. This difference demonstrates that the transfer rates seem to vary not only from one PCB congener to another, but also depend on the physiological status of the animal (i.e. lactating cows in McLachlan *et al.*[13] and Huwe *et al.*[14] vs. growing cattle in the present study).

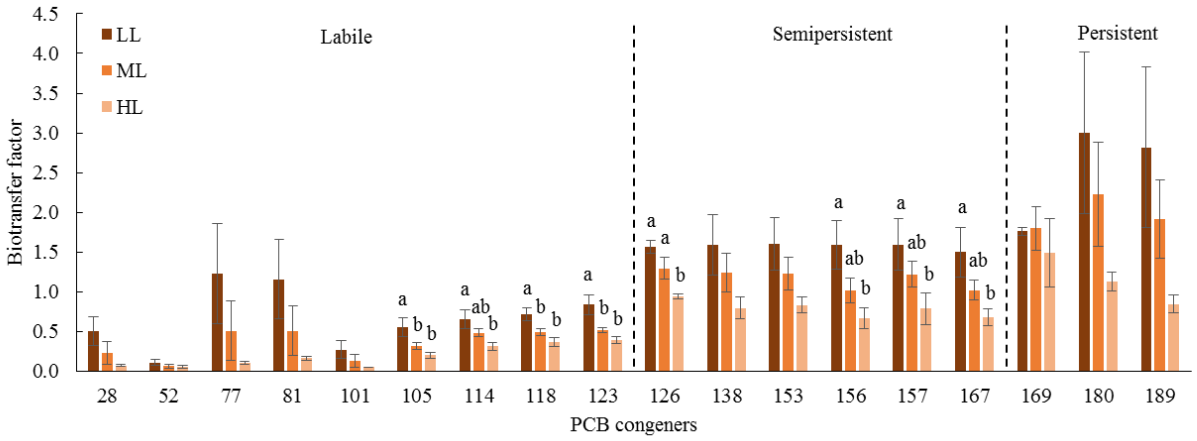

Figure 1: Biotransfer factor affected by carcass lipid content. Biotransfer factor of PCB congeners classified based on low (LL), medium (ML) or high (HL) carcass lipid content are represented. Means with distinct superscripts (a-b) differ significantly between classes ($P < 0.05$). The arithmetic standard error is displayed.

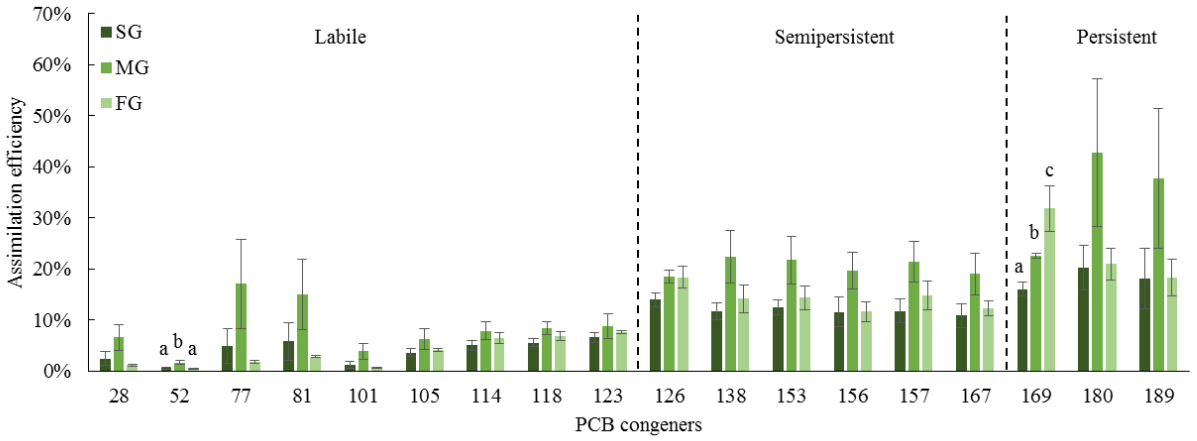

Figure 2: Assimilation efficiency affected by growing rate. Assimilation efficiency of individual PCB congeners classified based on slow (SG), medium (MG) or fast (FG) growing rate are represented. Means with different superscripts (a-c) differ significantly between classes ($P < 0.05$). The arithmetic standard error is displayed.

Transfer factors depending on growing rate. Growing rate ranged from 1.35 to 1.65 kg/d and had no effect ($P > 0.10$) on the BTF or BCF of any tested congener (data not shown). The AE was also mostly unaffected by the growing rate class, except for one significant increase with increasing growing rate (Figure 2) in the case of the persistent PCB 169. An increased AE of POPs in fast growing compared to slow growing was previously demonstrated in broilers for α-hexabromocyclododecane (α-HBCDD)[11]. Along the fattening period, an increased growing rate is concomitant with a faster adipose tissue mass increase, leading to a lower PCB 169 concentration in body lipids and subsequently in blood. This putative lower blood PCB 169 concentration, in the case of FG compared to SG animals, could generate a concentration gradient from the digestive tract to the blood, so that the absorption rate and the AE of PCB 169 is presumably increased.

Transfer factors depending on carcass lipid content. Clear decreasing trends in both BCFs (data not shown) and BTFs (Figure 1) were seen from LL to HL cattle. Also on an individual basis by performing linear regression

this influencing effect of carcass fatness was confirmed (Table 1). This consistent decrease of BCFs and BTFs from low to high carcass lipid content, compared to an unaffected AE (data not shown), suggests that a dilution process occurred in the case of fatter cattle. Indeed, similar AEs suggest that whatever the body fatness, equal amounts of PCBs were transferred to the carcass for given amounts of PCB intake. Therefore, concentrations, and further BCFs and BTFs were diminished when equal PCB carcass burdens were diluted in a higher carcass lipid mass. The insensitivity of AE to the physiological status (i.e. amount ratio, rather than concentration ratio as for BCF and BTF) is comparable to the independence of the carry over rate (COR, POP amount excreted by milk/POP amount taken up by feed) to milk fat yield, body lipid dynamic and feed intake in lactating cows[15].

Table 1: PCB biotransfer (BTF) and bioconcentration (BCF) factor prediction equations depending on carcass lipid content of growing cattle. Only regression models with $P < 0.10$ for the effect of carcass lipid content on the slope are reported.

	BTF (Y)*					BCF (Y)*				
PCB	Slope (a)	Intercept (b)	rSD	*P*	R^2	Slope (a)	Intercept (b)	rSD	*P*	R^2
105	-0.073	1.24	0.18	0.057	0.35	-0.45	7.92	1.28	0.098	0.27
114	-0.067	1.29	0.19	0.091	0.28					
118	-0.072	1.39	0.15	0.033	0.41	-0.39	8.49	1.05	0.080	0.30
123	-0.092	1.68	0.20	0.042	0.38	-0.53	10.46	1.48	0.095	0.28
126	-0.146	3.03	0.21	0.006	0.59	-0.76	18.38	1.40	0.017	0.49
156	-0.174	3.19	0.49	0.097	0.28					

*Linear regression equation where *Y = a x carcass lipid content + b ± residual standard deviation (rSD).*

In conclusion, this study demonstrated that carcass lipid content had a decreasing effect on BTF and BCF, but not on AE, whereas growing rate had only an increasing effect on AE for PCB 169. This underpins the complex interaction between POP physiological properties and animal physiology, which leads to difficulties in interpreting average transfer factors to support chemical risk assessment and management in livestock farming systems. Due to the insensitivity of AE to the animal physiological status, it could be retained as a reliable transfer descriptor in growing cattle. However, this descriptor is difficult to calculate, since the body burden is needed. Therefore, BCF is probably easier to use for assessors, since only the concentrations in the target tissue, as well as in feed are needed. In that instance, the use of the equations provided in Table 1 could help to decrease the uncertainties in BCF calculations, when one dispose of a reliable estimate of the carcass lipid content.

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