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A Mass Balance of Tri-Hexabrominated Diphenyl Ethers in Lactating Cows

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Beef and dairy products can be important vectors of human exposure to polybrominated diphenylethers (BDEs), and hence an understanding of BDE transfer from feed to cows' milk and tissue is important for BDE exposure assessment. The fate of tri- to hexaBDEs in lactating cows exposed to a naturally contaminated diet was studied by analyzing feed, feces, and milk samples from a mass balance study. Tissue distribution was studied in one cow slaughtered after the experiment. The carryover rates from feed to milk ranged from 0.15 to 0.35 for the major congeners. Lower values were observed for several of the tetrabrominated congeners, and this was attributed to metabolism. The dietary absorption efficiency decreased with increasing octanol−water partition coefficient of the BDE congener. The absorption behavior was consistent with a model based on chemical lipophilicity, but agreed less well with a model based on effective molecular diameter, and it violated Lipinski's "rule of 5". The lipid normalized concentrations were similar in all tissues analyzed including liver and milk, suggesting that tissue distribution is governed by partitioning into lipids. Overall, the behavior of the tri- to hexaBDEs was consistent with that observed for other classes of halogenated aromatic contaminants such as PCBs and PCDD/Fs, but it differed markedly from the behavior of the hepta- decaBDEs.

Introduction

Polybrominated diphenyl ethers (PBDE) are additive flame retardants widely used in plastics, textiles, and electronics (*1*). The pentabrominated diphenyl ether (pentaBDE) product contains primarily BDE-47 and -99, with BDE-28, -66, -85, -100, -153, and -154 as minor components (*2*).

Previous studies have shown that food is a major route of human exposure for organochlorines such as PCBs and PCDD/Fs, with fish, meat, and dairy products as the major sources (*3, 4*). For lower brominated BDEs, a few studies also indicate that fish, meat, and dairy products may be important human exposure routes depending on dietary habits (*5*−*13*).

PCB and PCDD/F contamination in meat and dairy products occurs primarily via atmospheric deposition on plants, uptake in grazing animals, and subsequent transfer to tissues and milk (*3, 4*). Mass balance studies have been performed for PCBs and PCDD/Fs in cows to understand the role of physicochemical properties for their uptake from feed, transfer to meat and milk, and accumulation in terrestrial organisms (*14*−*16*). However, no such studies have been carried out for the lower brominated BDEs. For BDEs, soil ingestion by cattle may also be an important vector of human exposure, since agricultural land that has been treated with sewage sludge can be highly contaminated with BDEs (*17, 18*). Consequently, an understanding of the transfer of BDEs from feed to meat and milk is important for evaluating the importance of atmospheric deposition and sewage sludge as sources of human exposure to these chemicals.

The aim of the present investigation was to perform a mass balance study of tri-decaBDEs, and to understand the digestive tract absorption, tissue distribution, and excretion via milk in cows, based on exposure via "naturally" contaminated feed. Stored samples from a previous mass balance study of PCBs in lactating cows, performed in Devon, UK (*14*), were analyzed for PBDEs. Due to variations in concentrations of the higher brominated BDEs in feed, only tri-hexaBDEs could be used for mass balance calculations and these results are reported here. The results for hepta−decaBDEs are reported elsewhere (*19*).

Materials and Methods

Samples. The study was performed over a period of three months in an experimental husbandry farm in Devon, a rural area in southwest England. The experiment was originally designed for a long-term mass balance of PCBs (*14*). Details of the study design and sample collection and handling are presented in ref *14* and will only briefly be described below.

The cows were kept indoors where the feed consumption and milk production were measured on a daily basis. Both milk and feces were subsampled once a week for 13 weeks from bulked morning and evening samples. The feed consisted of silage (unlimited access), concentrate, and a mineral supplement. The silage was produced on the experimental farm and stored in tightly covered clamps. Prior to the experiment the cows had received feed from the same sources. The average consumption of feed and production of milk and feces are summarized in Table S1 in the Supporting Information (SI). For the present study, samples from two cows (cows 1 and 2) were analyzed, one of which (cow 2) was slaughtered after the experiment. The 13 weekly samples of feces and milk fat were pooled into five samples, three representing 3-week periods, and two representing 2-week periods. Fat tissues and liver, kidney, heart, and leg muscle were collected from the slaughtered cow. All samples were kept frozen in hexane rinsed glass containers until analysis.

Chemicals. Chemicals, including the standards used are listed in the SI.

Sample Preparation and Cleanup. Subsamples of 1−2 g of grass silage, concentrate, and feces were taken for dry weight determinations (105 °C, overnight). The fat content of milk and tissues was determined gravimetrically.

Organs and Fat Tissues. Approximately 1 g of fat tissue and 10 g of the organs were extracted according to the original Jensen method (*20*). In brief, the samples were spiked with surrogate standards, $^{13}C_{12}$−BDE28, $^{13}C_{12}$−BDE47,

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$^{13}C_{12}$−BDE99, $^{13}C_{12}$−BDE153, and $^{13}C_{12}$−BDE154 (SI Table S2), homogenized, and solvent extracted with acetone/*n*-hexane/diethyl ether. The extracts were further cleaned up with concentrated sulfuric acid and an acidified silica column (1 g, 2:1 silica: sulfuric acid w/w).

Milk Fat. The samples were pooled to a combined weight of 5.5 g. Surrogate standards were added and the fat was solvent extracted in test tubes according to the original Jensen method (*20*). After solvent evaporation the bulk fat dissolved in *n*-hexane was removed by treatment with potassium hydroxide (1 M in 96% ethanol) for 1 h at 60 °C. To obtain two phases, aqueous 0.9% sodium chloride was added. The organic phase was collected and the aqueous phase was re-extracted twice with *n*-hexane. The total extract was cleaned up on a combined silica (1.5 g, 2% deactivated in sealed glass ampoules) and acidified silica column (2 g). The PBDEs were eluted with *n*-hexane (1−6 mL discarded, 6−29 mL collected).

Feces. The pooled sample was homogenized and 18 g was subsampled for extraction. After the addition of surrogate standards (overnight) the sample was extracted in test tubes with isopropanol/diethyl ether/*n*-hexane in a slightly modified version of the method (Jensen modification II) described in ref *20*. The combined extract was washed with aqueous potassium hydroxide solution (0.5 M in 0.9% NaCl). The organic phase was subsequently evaporated and treated with concentrated sulfuric acid. Additional clean up was achieved with acidified silica (1 g) and activated silica (1.5 g) columns.

Silage. A subsample of grass silage (25 g), precut into 0.5 cm lengths, was frozen with liquid nitrogen and pulverized together with 50 g anhydrous sodium sulfate in a Waring blender with a dry grinding attachment. The ground silage mixture was transferred to a pre-extracted thimble, spiked with the surrogate standards overnight, and Soxhlet extracted with 450 mL of DCM (8 h). After evaporation the solvent was changed to *n*-hexane. The extract was treated with concentrated sulfuric acid and further cleaned up as the milk fat.

Concentrate, Mineral. The feed supplements were ground to a fine powder. A subsample of 10 g was spiked with surrogate standards overnight and extracted in 50 mL test tubes according to the original Jensen method (*20*). After sulfuric acid treatment the following clean up was performed as described for milk fat.

Volumetric standards were added to all sample extracts before GC/MS analysis. The final volume of the extracts was adjusted to 25−100 μL depending on the matrix and detection method.

Analysis. The analysis of tri- to hexaBDEs was performed using HRMS on a Micromass AutoSpec Ultima MS (EI). The instrumental conditions and a list of the ions monitored are presented in the SI.

Quantification. The following BDE congeners were detected and quantified: 28, 47, 49, 66, 85, 99, 100, 153, and 154. The identification was based on the relative retention time versus the surrogate standards differing by less than 0.005 from that of the calibration standards.

The limit of quantification (LOQ) was established based on 5 times the total amount in the blanks or 5 times the noise. The LOQ differed between the matrices and with sample size and was in the range of 0.2−150 pg/g lipid weight or dry matter. The absolute recoveries of the labeled surrogate standards varied between 60 and 98%. More information on LOQ and recoveries are given in the SI (Tables S4 and S5).

Quality Assurance. All glassware was heated to 450 °C overnight and further rinsed with solvents before use. The extracts were at all times shielded from UV-light and from deposition of dust/particles in the laboratory air. GC-MS analysis was performed mixing samples and calibration standards randomly. The samples were quantified with standard solutions using 10 different concentration levels, which were analyzed 1−3 times. Procedural blanks covering the whole procedure were run in parallel with the samples on all extraction occasions.

Silage (two out of three samples), concentrate, and mineral samples were analyzed in duplicate. One feces sample, a control feces sample, and one milk sample were also analyzed in duplicate. The difference between duplicates was, on average, 7−24%, calculated as the difference compared to the mean.

Results and Discussion

Concentrations of PBDEs. Concentrations of tri- to hexaBDE in the different matrices are presented in SI Table S6. All of the congeners could be quantified in all of the samples, with the exception of the muscle sample and BDE99 in the kidney sample. The data for the congeners in the organ samples with no matching internal standard were excluded due to strong matrix interferences in these samples. For 19 of 293 data points (largely BDEs 85 and 153 in feces), the measured values lay between the limit of detection and the limit of quantification. After careful scrutiny of the consistency of these data points with the remaining data, it was decided to retain them for the mass balance calculations. The measured concentrations were considered to be a better estimate of the true value than the LOQ.

All of the BDE congeners studied were detected in the three types of feed at similar concentrations on a dry weight (dw) basis (290−510 pg/g dw for ΣPBDE) (SI Table S6). The congener pattern in the mineral supplement and the concentrate were similar, whereas the grass silage had a higher fraction of the congeners 85, 99, and to some extent 153 and less of 47 and 49 (SI Table S6). The BDE concentrations in the three different silage clamps were similar, with the CV ranging from 14 to 27% for BDE-47, -85, -99, -153, -154 up to 41% for BDE-100. All three matrices had congener patterns that were dominated by BDE-47 and -99.

The congener pattern in silage (collected in 1995) was similar to that seen in herbage from Rothamsted, UK (42 km north of London) collected in the same year (*21*) as well as in air samples collected in Chilton, UK (southwest UK) in 2001 (*22*) (SI Figure S1). This pattern was also very similar to that of the pentaBDE technical products (*2, 23*) (SI Figure S1). This indicates that deposition from air is probably a major route of contamination for the silage and that emissions of the pentaBDE product are the primary source. The ΣPBDE concentrations were also fairly similar in the herbage sample (740 pg/g dw) and the silage (mean of 420 pg/g dw).

Organs and Fat Tissues. The lipid normalized concentrations of the individual BDE congeners in the different fat tissues from cow 2 were very similar, ranging from 1300 to 2700 pg/g lw (CV 17−31%) for ΣPBDE (Figure 1, SI Table S6). The congener profiles were dominated by BDE-99, -47, and -153. Elevated concentrations of most congeners were obtained in the dorsal thorax fat sample, but this may be due to the anomalously low lipid content obtained for this sample (42%).

There was no evidence of elevated lipid normalized BDE concentrations in the liver compared to fat (Table 1). This is in agreement with observations made for BDEs in rats (*24, 25*), but stands in contrast to the PCB data from the same cow, which showed an enrichment in the liver of up to a factor of 4.7 compared to fat (*14*). PCDD/Fs have been reported to have up to 188 times higher lipid normalized concentrations in the liver of lactating cows than in other tissues (*26*). For PCDD/Fs, elevated concentrations in the liver of laboratory animals have been attributed to binding to specific proteins (*27*). This is apparently not an important factor in the behavior of BDEs in cows and rats. As a consequence, only ∼1% of the BDE body burden of the cow was present in the liver. The great majority of the BDEs were sequestered in lipid-rich tissues.

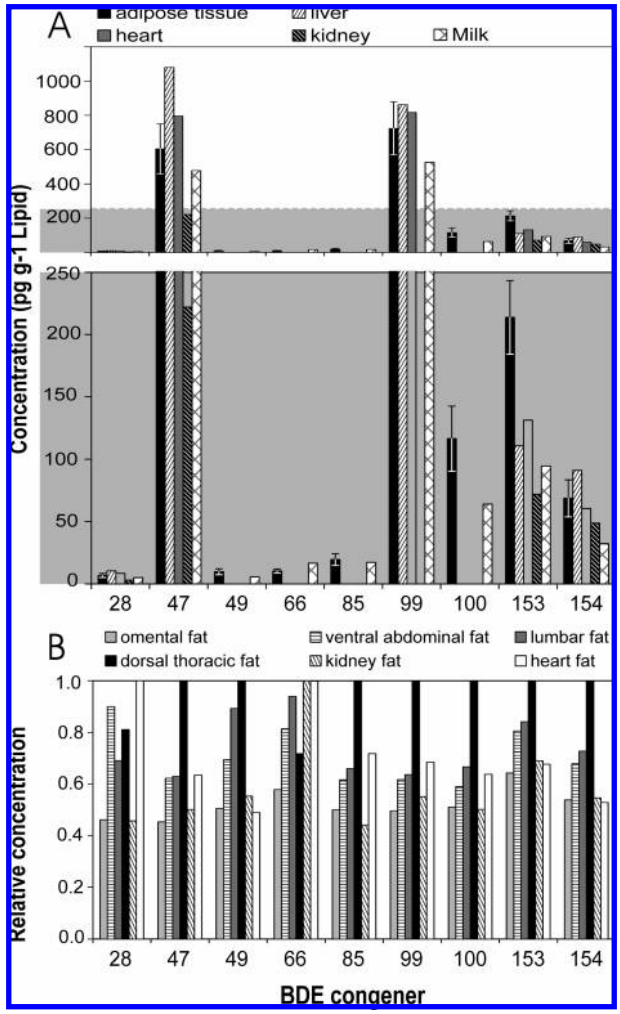

FIGURE 1. (A) Comparison of the lipid normalized BDE concentrations in milk and different tissues of a slaughtered cow. The adipose tissue concentrations are presented as the mean ($n = 6$) with 95% confidence interval. Note that the lower graph is an expansion of the lower part of the upper graph. (B) Comparison of the lipid normalized BDE concentrations in the different adipose tissues. For each congener, the concentrations have been normalized to the concentration in the tissue with the highest levels.

The lipid-normalized BDE concentrations in milk were also very similar to the concentrations in the fat tissues for most of the congeners, ranging from 1100 to 2600 pg/g lw for ΣPBDE (Figure 1, SI Table S6). This indicates that there was a partitioning equilibrium between the cows' tissues and the milk for these substances. However, the concentrations of the two most hydrophobic congeners, BDEs 153 and 154, were about 50% lower in the milk compared to the fat tissues. This may be an indication that the transfer of the more hydrophobic BDEs to milk is subject to kinetic constraints that prevent the milk from reaching equilibrium with the BDE reservoirs in the cow. A much greater difference was observed for the hepta- to decaBDE congeners in the same cows (*19*).

BDE Mass Balance. Assuming that feed was the dominant source of BDEs to the cows and that feces and milk were the dominant elimination pathways of the native compounds, the mass balance is defined by the following equation:

$$\Delta S = I + F - E - L - M \quad (1)$$

where ΔS is the change in BDE storage in the cow, I is the ingestion flux, F is formation, E is egestion (via feces), L is lactation, and M is metabolism, all in ng d^{-1}. The experimental design allowed ΔS, I, E, and L to be quantified, whereas F and M were inferred from the difference.

The contaminant mass balance of a cow varies during the lactation cycle as a result of changes in the quantity of feed ingested, the quantity of milk produced, and the body lipid mass of the cow (*14*). To obtain the best possible estimate of the average mass balance of a cow throughout the lactation cycle, the different terms in eq 1 were calculated for the full length of the experiment. I, E, and L were calculated by multiplying the BDE concentrations in the relevant matrix (feed, feces, milk) by the ingestion/excretion rate for a given day/period and adding this up for the duration of the experiment (SI Table S7). ΔS was estimated based on the change in body mass of the cows and the change in the milk concentrations (see the footnote to SI Table S7 for details).

The results of the mass balance for both cows are shown in Figure 2. The change in BDE storage was a small term in the mass balance, amounting to <5% of the input (with the exception of BDE100 in cow 2 and BDE 154 in both cows). It was also less than 21% of the lactation flux (with the same exceptions), which indicates that once the BDEs had been absorbed through the digestive tract, deposition of BDEs to body fat was small compared to excretion via milk (averaged over the whole experiment).

The constancy of the BDE concentrations in the feed (SI Table S6) and the absence of a large change in the BDE storage within the animals indicated that the cows were close to steady state. This facilitates the interpretation of further aspects of the mass balance such as dietary absorption, BDE carryover into the milk, and metabolism.

Gastrointestinal Absorption. The net absorption efficiency (Abs.) (Table 1) was calculated as the fraction of the ingested chemical that was not excreted via the feces (note that this presumes that any metabolism of the BDEs occurred after absorption, not in the digestive tract itself, see below):

$$\text{Abs.} = 1 - \text{feces flux/input flux} \quad (2)$$

The results are plotted in Figure 3 against the log octanol–water partition coefficients (K_{OW}) taken from ref *28*. The absorption efficiency decreased with increasing K_{OW} of the BDE congener. About 80% of each tri- and tetrabrominated congener was absorbed, while for the penta- and hexabrominated congeners absorption was only ~50%. The absorption efficiencies in cows are considerably lower than in rats, which ranged from ~95% for BDE-47 to ~90% for BDE-154 when fed BDEs in peanut/corn oil (*25, 29*), and were ~80% for all congeners reported here when fed BDEs in dust (*29*). Cows are known to have generally lower absorption efficiencies for organic contaminants than humans (*30*), which may be related to their vegetarian diet and ruminant digestive system.

Figure 3 also shows the net absorption efficiencies obtained for a range of PCB congeners in the same cows (*14*). The PCBs show a similar trend of decreasing net absorption efficiency with increasing K_{OW}. At a given K_{OW}, the PCBs and the BDEs show similar net absorption efficiencies, with the exception of the pentabrominated congeners, which were absorbed to a lesser extent than PCBs of similar K_{OW}.

A conceptual model of the absorption process has been used to explain the K_{OW} dependency of the net absorption efficiency that was first observed in fish (*30, 31*). In this model chemical absorption is described as a diffusive process driven by the thermodynamic (chemical potential) gradient between the lumen and the organism, and limited by two resistances acting in series. One resistance is considered to have lipid-like properties while the second is an aqueous barrier. This diffusive uptake competes with advection of the chemical out of the digestive tract with the feces. At low K_{OW} values

TABLE 1. Liver enrichment ratios (LER, pg/g liver fat/pg/g body fat), mean net dietary absorption efficiency (Abs, %), bioaccumulation factors[a] (BAF, pg/g lipid weight in tissue/pg/g dry matter in feed), estimated first order metabolic rate constants (MRC, d^{-1}) and carryover rates (COR) of different BDE congeners

	cow	BDE-28	BDE-47	BDE-49	BDE-66	BDE-85	BDE-99	BDE-100	BDE-153	BDE-154
LER	2	1.6	1.8				1.2		0.5	1.3
Abs.	1, 2	84	72	88	79	42	54	50	49	35
BAF[a]	2	0.92	4.8	0.43	0.82	2.3	3.8	5.8	10	6.5
MRC	1	0.3	0.04	0.5	0.3	0.05	0.03	0.03	0.007	0.01
	2	0.2	0.02	0.5	0.2	0.02	0.01	0.009	0.001	−0.002
COR	1	0.035	0.20	0.015	0.072	0.091	0.17	0.15	0.26	0.16
	2	0.043	0.28	0.018	0.089	0.16	0.24	0.20	0.34	0.23

[a] Mean concentration for six different fat types.

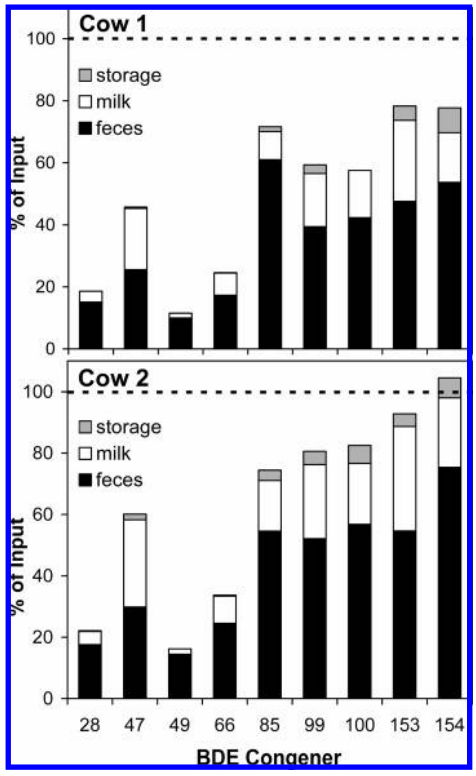

FIGURE 2. Results of the input/output mass balance of the BDE congeners in the two cows. The cumulative output in milk and feces over the whole experiment as well as the quantity of chemical estimated to have been stored in the cows over this period are plotted as a percentage of the cumulative input via feed.

the lipid barrier is the dominant resistance for absorption, and the absorption efficiency is independent of K_{OW}. However, as K_{OW} increases, diffusive transport through the aqueous barrier becomes increasingly limited and the absorption efficiency decreases.

This conceptual model has been applied to cows. The following equation was developed from a mass balance study of the dietary absorption of a range of chlorinated contaminants including PCBs and PCDD/Fs (*32*) and later modified to account for better estimates of K_{OW} values (*33*).

$$\% \text{ Absorption} = 1.295 + 3.226 \times 10^{-8} K_{OW} \qquad (3)$$

This equation was plotted together with the experimental data from this study (Figure 3), and reasonable agreement was observed, particularly considering the interanimal variation in the net absorption efficiency. This provides independent confirmation of the utility of the equation and shows that it can also be applied to BDEs, albeit with reduced accuracy for the pentabrominated congeners.

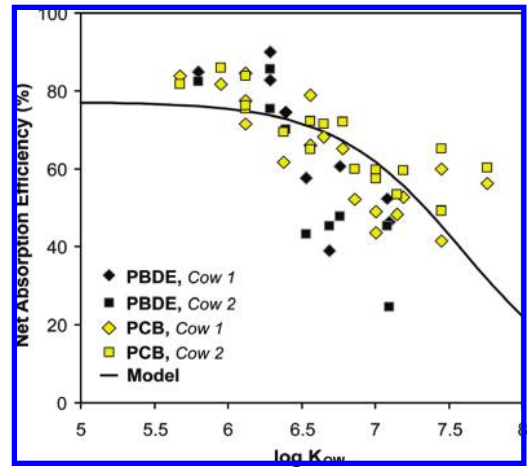

FIGURE 3. Plot of the net dietary absorption efficiency versus the log K_{OW} for the BDE congeners reported here and for the PCB congeners in the same study reported earlier (*14*). The K_{OW} values were taken from Wania and Dugani (*28*) (BDEs) and Schenker et al. (*41*) (PCBs). The equation developed by McLachlan (*32*) to predict the net dietary absorption efficiency from K_{OW} and modified by Czub and McLachlan (*33*) (to take into consideration better K_{OW} estimates) is also shown.

Another chemical property that has been hypothesized to influence the transport of contaminants across membranes is molecular diameter. It has been suggested that if a molecule exceeds an effective diameter of 9.5 Å it will not be able to pass through the gaps between adjacent fatty acids in the lipid bilayer structure of the membrane (*34*). In the two cows studied, the PCBs and PBDEs of a given degree of halogenation had similar net dietary absorption rates, although the PBDEs are markedly larger. In SI Figure S2) the net absorption rate is plotted against the effective molecular diameter D_{eff} as defined and calculated by Schüürmann (*35*). While there was a general trend of decreasing absorption with increasing D_{eff}, there was no evidence of a cutoff in absorption at 9.5 Å. Even PBDEs with D_{eff} in excess of 10 Å had a net absorption in excess of 40%.

A further approach to predicting dietary absorption is Lipinski's "rule of 5", which was developed to predict drug delivery and states that absorption will be poor when the chemical has more than 5 H-bond donors, 10 H-bond acceptors, a molecular weight greater than 500, or a log K_{OW} greater than 5 (*36*). The pentaBDE and hexaBDE congeners in this study exceeded the two latter thresholds, but their dietary absorption was good. This indicates that there are limitations in applying the "rule of 5".

Bioaccumulation, Biotransformation and Carry over Rates of BDEs. Bioaccumulation of individual BDE congeners from feed to lipid stores can be estimated by calculating fat to feed ratios. Highest bioaccumulation is seen for BDE-153 (BAF of 10) (Table 1) followed by BDE-154, -100, and -47 (BAFs of 5−6).

The transformation of BDEs was not measured directly in the experiment, but it can be evaluated from the difference between intake and the total output of the native chemical. Transformation could have occurred in the digestive tract (e.g., in the rumen) or within the cow following absorption. Since the BDE congener pattern in the feces resembled that in the feed (with the exception of the somewhat greater predominance of the more lipophilic congeners as a consequence of poorer absorption, see above), whereas the pattern in milk and tissues was clearly different than in the feed for some congeners (particularly BDE 28 and BDE 49, see SI Figure S3), it was concluded that transformation occurred primarily after absorption as a result of metabolism in the cow.

The metabolism flux was calculated as the difference between the total input and the total output of the native BDEs. A first order rate constant for metabolic transformation was then estimated from the quotient of the metabolism flux and the body burden of the chemical (Table 1). Relatively high metabolic rate constants, ranging from 0.2 to 0.5 d^{-1}, were obtained for BDE-28, -49, and -66. This indicates that these congeners are effectively metabolized in the cow. As a result, the elimination of the native compound via the milk was very low (Figure 2). Evidence for metabolism of BDE 28 has also been reported in rats (*29*).

The metabolic rate constants were considerably lower for the remaining BDEs, ranging from −0.002 to 0.05 d^{-1}, and were lowest for BDE-154 and -153. For these congeners the metabolism flux could be of similar magnitude to the flux in the milk (Figure 2). However, since the metabolism flux was estimated indirectly from the difference between the measured input and output fluxes, it is sensitive to errors in the measured fluxes. This is reflected in the large relative difference in the metabolic rate constants estimated for the two cows. Due to these uncertainties, it cannot be concluded that any of the congeners apart from BDE-28, -49, and -66 are metabolized in cows. In rats, BDE-154 was reported to be metabolized (*29*), which stands in contrast to the behavior in cows.

The carryover rate is a parameter commonly used in the risk assessment of organic contaminants in dairy cows. It is defined as the fraction of the chemical intake of the cow that is transferred to the milk at steady state. The carryover rates were calculated as the quotient of the milk flux and the input flux (Table 1). For BDE-47, -99, -100, -153, and -154, values of 15−34% were obtained. This is considerably lower than the carryover rates measured for more persistent PCB congeners in the same cows. However, it is comparable to the carryover rates for tetra- to hexachlorinated dibenzo-p-dioxins and dibenzofurans (*37*−*39*), for which dairy products are a major source of human exposure (*4, 40*). The carryover rates of BDE-28, -49, and -66 were considerably lower. This can be attributed to their efficient metabolism in the cow (see above). Finally, the carryover rates are based on measurements in just two cows, and hence their absolute values may not be representative for other cows. However, the relative carryover of the different contaminants is expected to be more consistent between cows.

Implications for Risk Assessment. Since several PBDEs have carryover rates comparable to those of some PCDD/Fs, this suggests that milk and beef may also be important vectors for human exposure to BDEs. This is supported by a few studies that show that meat and dairy products represent 10−37% of the dietary intake of lower brominated BDEs (*5*−*7, 9, 12, 13*).

A major source of PBDE contamination in the silage used in this study was probably from atmospheric deposition, as suggested from the similarity in congener profile and concentrations in the silage, herbage, and in comparison to the profile in air samples (SI Figure S1). Although not relevant for this study, the use of sewage sludge as fertilizer on agricultural land may also represent a serious vector of exposure as sewage sludge has been shown to be more important than atmospheric deposition for soil contamination with PBDEs (*17, 18*). This exposure pathway warrants further study.

The behavior of the BDEs in cows reported here is consistent with the behavior of other previously studied lipophilic organic contaminants such as PCBs and PCDD/Fs. In particular, the dietary absorption can be described with the same model (Figure 3), and the distribution among different tissues and milk reflects equilibrium partitioning controlled by the lipid content of the matrix. Hence predictive models of contaminant behavior in cows that are based on these principles (*32, 33*) should be applicable to the lower brominated diphenyl ethers studied in this paper. The hepta- to decaBDE congeners, on the other hand, showed a different behavior characterized by a strong tendency to accumulate in tissue and low carryover into the milk (*19*).

Acknowledgments

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Supporting Information Available

Seven tables with information on the analytical conditions and performance, BDE concentrations and fluxes, and three figures (comparison of BDE concentrations in silage, herbage, air and PentaBDE, net dietary absorption versus effective molecular diameter, and congener composition in silage, feces, milk and fat). This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) de Wit, C. A. An overview of brominated flame retardants in the environment. *Chemosphere* **2002**, *46*, 583–624.
(2) Sjödin, A.; Jakobsson, E.; Kierkegaard, A.; Marsh, G.; Sellström, U. Gas chromatographic identification and quantification of polybrominated diphenyl ethers in a commercial product, Bromkal 70−5DE. *J. Chromatogr., A* **1998**, *822*, 83–89.
(3) Duarte-Davidson, R.; Jones, K. C. Polychlorinated-biphenyls (PCBs) in the UK population-Estimated intake, exposure and body burden. *Sci. Total Environ.* **1994**, *151*, 131–152.
(4) Fürst, P.; Fürst, C.; Groebel, W. Levels of PCDDs and PCDFs in foodstuffs from the Federal Republic of Germany. *Chemosphere* **1990**, *20*, 787–792.
(5) Ohta, S.; Ishizuka, D.; Nishimura, H.; Nakao, T.; Aozasa, O.; Shimidzu, Y.; Ochiai, F.; Kida, T.; Nishi, M.; Miyata, H. Comparison of polybrominated diphenyl ethers in fish, vegetables, and meats and levels in human milk of nursing women in Japan. *Chemosphere* **2002**, *46*, 689–696.
(6) Bocio, A.; Llobet, J. M.; Domingo, J. L.; Corbella, J.; Teixido, A.; Casas, C. Polybrominated diphenyl ethers (PBDEs) in foodstuffs: Human exposure through the diet. *J. Agric. Food Chem.* **2003**, *51*, 3191–3195.
(7) Kiviranta, H.; Ovaskainen, M. A. L.; Vartiainen, T. Market basket study on dietary intake of PCDD/Fs, PCBs, and PBDEs in Finland. *Environ. Int.* **2004**, *30*, 923–932.
(8) Huwe, J. K.; Larsen, G. L. Polychlorinated dioxins, furans, and biphenyls, and polybrominated diphenyl ethers in a US meat market basket and estimates of dietary intake. *Environ. Sci. Technol.* **2005**, *39*, 5606–5611.
(9) Darnerud, P. O.; Atuma, S.; Aune, M.; Bjerselius, R.; Glynn, A.; Grawe, K. P.; Becker, W. Dietary intake estimations of orga-

nohalogen contaminants (dioxins, PCB, PBDE and chlorinated pesticides, e.g. DDT) based on Swedish market basket data. *Food Chem. Toxicol.* **2006**, *44*, 1597–1606.
(10) Gomara, B.; Herrero, L.; Gonzalez, M. J. Survey of polybrominated diphenyl ether levels in Spanish commercial foodstuffs. *Environ. Sci. Technol.* **2006**, *40*, 7541–7547.
(11) Schecter, A.; Papke, O.; Harris, T. R.; Tung, K. C.; Musumba, A.; Olson, J.; Birnbaum, L. Polybrominated diphenyl ether (PBDE) levels in an expanded market basket survey of US food and estimated PBDE dietary intake by age and sex. *Environ. Health Perspect.* **2006**, *114*, 1515–1520.
(12) Voorspoels, S.; Covaci, A.; Neels, H.; Schepens, P. Dietary PBDE intake: A market-basket study in Belgium. *Environ. Int.* **2007**, *33*, 93–97.
(13) Domingo, J. L.; Marti-Cid, R.; Castell, V.; Llobet, J. M. Human exposure to PBDEs through the diet in Catalonia, Spain: Temporal trend-A review of recent literature on dietary PBDE intake. *Toxicology* **2008**, *248*, 25–32.
(14) Thomas, G. O.; Sweetman, A. J.; Jones, K. C. Input-output balance of polychlorinated biphenyls in a long-term study of lactating daily cows. *Environ. Sci. Technol.* **1999**, *33*, 104–112.
(15) McLachlan, M. S. Mass balance of polychlorinated-biphenyls and other organochlorine compounds in a lactating cow. *J. Agric. Food Chem.* **1993**, *41*, 474–480.
(16) McLachlan, M. S.; Thoma, H.; Reissinger, M.; Hutzinger, O. PCDD/F in an agricultural food-chain 0.1. PCDD/F mass balance of a lactating cow. *Chemosphere* **1990**, *20*, 1013–1020.
(17) Matscheko, N.; Tysklind, M.; de Wit, C.; Bergek, S.; Andersson, R.; Sellström, U. Application of sewage sludge to arable land-soil concentrations of polybrominated diphenyl ethers and polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls, and their accumulation in earthworms. *Environ. Toxicol. Chem.* **2002**, *21*, 2515–2525.
(18) Sellström, U.; de Wit, C. A.; Lundgren, N.; Tysklind, M. Effect of sewage-sludge application on concentrations of higher-brominated diphenyl ethers in soils and earthworms. *Environ. Sci. Technol.* **2005**, *39*, 9064–9070.
(19) Kierkegaard, A.; Asplund, L.; de Wit, C. A.; McLachlan, M. S.; Thomas, G. O.; Sweetman, A. J.; Jones, K. C. The fate of higher brominated PBDEs in lactating cows. *Environ. Sci. Technol.* **2007**, *41*, 417–423.
(20) Jensen, S.; Haggberg, L.; Jorundsdottir, H.; Odham, G. A quantitative lipid extraction method for residue analysis of fish involving nonhalogenated solvents. *J. Agric. Food Chem.* **2003**, *51*, 5607–5611.
(21) Hassanin, A.; Johnston, A. E.; Thomas, G. O.; Jones, K. C. Time trends of atmospheric PBDEs inferred from archived UK herbage. *Environ. Sci. Technol.* **2005**, *39*, 2436–2441.
(22) Lee, R. G. M.; Thomas, G. O.; Jones, K. C. PBDEs in the atmosphere of three locations in western Europe. *Environ. Sci. Technol.* **2004**, *38*, 699–706.
(23) La Guardia, M. J.; Hale, R. C.; Harvey, E. Detailed polybrominated diphenyl ether (PBDE) congener composition of the widely used penta-, octa-, and deca-PBDE technical flame-retardant mixtures. *Environ. Sci. Technol.* **2006**, *40*, 6247–6254.
(24) Huwe, J. K.; Hakk, H.; Birnbaum, L. S. Tissue distribution of polybrominated diphenyl ethers in male rats and implications for biomonitoring. *Environ. Sci. Technol.* **2008**, *42*, 7018–7024.
(25) Huwe, J.; Hakk, H.; Lorentzsen, M. Bioavailability and mass balance studies of a commercial pentabromodiphenyl ether mixture in male Sprague-Dawley rats. *Chemosphere* **2007**, *66*, 259–266.
(26) Richter, W.; McLachlan, M. S. Uptake and transfer of PCDD/Fs by cattle fed naturally contaminated feedstuffs and feed contaminated as a result of sewage sludge application. 2. Nonlactating cows. *J. Agric. Food Chem.* **2001**, *49*, 5857–5865.
(27) Leung, H. W.; Paustenbach, D. J.; Murray, F. J.; Andersen, M. E. A physiological pharmacokinetic description of the tissue distribution and enzyme-inducing properties of 2,3,7,8-tetrachlorodibenzo-*para*-dioxin in the rat. *Toxicol. Appl. Pharmacol.* **1990**, *103*, 399–410.
(28) Wania, F.; Dugani, C. B. Assessing the long-range transport potential of polybrominated diphenyl ethers: A comparison of four multimedia models. *Environ. Toxicol. Chem.* **2003**, *22*, 1252–1261.
(29) Huwe, J. K.; Hakk, H.; Smith, D. J.; Diliberto, J. J.; Richardson, V.; Stapleton, H. M.; Birnbaum, L. S. Comparative absorption and bioaccumulation of polybrominated diphenyl ethers following ingestion via dust and oil in male rats. *Environ. Sci. Technol.* **2008**, *42*, 2694–2700.
(30) Kelly, B. C.; Gobas, F. A. P. C.; McLachlan, M. S. Intestinal absorption and biomagnification of organic contaminants in fish, wildlife, and humans. *Environ. Toxicol. Chem.* **2004**, *23*, 2324–2336.
(31) Gobas, F. A. P. C.; Muir, D. C. G.; Mackay, D. Dynamics of dietary bioaccumulation and faecal elimination of hydrophobic organic chemicals in fish. *Chemosphere* **1988**, *17*, 943–962.
(32) McLachlan, M. S. Model of the fate of hydrophobic contaminants in cows. *Environ. Sci. Technol.* **1994**, *28*, 2407–2414.
(33) Czub, G.; McLachlan, M. S. A food chain model to predict the levels of lipophilic organic contaminants in humans. *Environ. Toxicol. Chem.* **2004**, *23*, 2356–2366.
(34) Opperhuizen, A.; van der Velde, E. W.; Gobas, F. A. P. C.; Liem, D. A. K.; van der Steen, J. M. D.; Hutzinger, O. Relationship between bioconcentration in fish and steric factors of hydrophobic chemicals. *Chemosphere* **1985**, *14*, 1871–1896.
(35) Schüürmann, G. QSAR Analysis of the acute fish toxicity of organic phosphorothionates using theoretically derived molecular descriptors. *Environ. Toxicol. Chem.* **1990**, *9*, 417–428.
(36) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* **1997**, *23*, 3–25.
(37) Jensen, D. J.; Hummel, R. A. Secretion of TCDD in milk and cream following the feeding of TCDD to lactating dairy-cows. *Bull. Environ. Contam. Toxicol.* **1982**, *29*, 440–446.
(38) Olling, M.; Derks, H. J. G. M.; Berende, P. L. M.; Liem, A. K. D.; Dejong, A. P. J. M. Toxicokinetics of 8 C-13-labeled polychlorinated dibenzo-p-dioxins and dibenzo-p-furans in lactating cows. *Chemosphere* **1991**, *23*, 1377–1385.
(39) McLachlan, M.; Richter, W. Uptake and transfer of PCDD/Fs by cattle fed naturally contaminated feedstuffs and feed contaminated as a result of sewage sludge application. 1. Lactating cows. *J. Agric. Food Chem.* **1998**, *46*, 1166–1172.
(40) Theelen, R. M. C.; Liem, A. K. D.; Slob, W.; Vanwijnen, J. H. Intake of 2,3,7,8 chlorine substituted dioxins, furans, and planar PCBs from food in the Netherlands-Median and distribution. *Chemosphere* **1993**, *27*, 1625–1635.
(41) Schenker, U.; MacLeod, M.; Scheringer, M.; Hungerbuhler, K. Improving data quality for environmental fate models: A least-squares adjustment procedure for harmonizing physicochemical properties of organic compounds. *Environ. Sci. Technol.* **2005**, *39*, 8434–8441.

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