Mass Balance of Polychlorinated Biphenyls and Other Organochlorine Compounds in a Lactating Cow†

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A contaminant mass balance was conducted of a lactating cow in its natural state. PCBs, HCHs, DDE, DDT, HCB, and several other chlorinated substances were investigated. It was found that virtually all of the cow's exposure was through feed. The contaminant absorption in the cow and hence the carry-over rate of persistent compounds was found to be a function of K_{ow}, with approximately constant values up to a log K_{ow} of 6.5 and thereafter rapidly decreasing absorption with increasing lipophilicity of the contaminant. The key to PCB persistence in the cow was the 4,4′ substitution pattern. The 2,3,5 substitution was a less effective hindrance for PCB metabolism.

1. INTRODUCTION

The main route of human exposure to many chlorinated organic compounds is through food, whereby milk is the single most important source. Sümmermann et al. (1978) found that 28% of the polychlorinated biphenyl (PCB) uptake in Germany is from this food group. Fürst et al. (1990) attributed 32% of the polychlorinated dibenzo-*p*-dioxin and dibenzofuran (PCDD/F) exposure to milk and milk products. Both authors found that the contribution from meat products was approximately equal to that from dairy products. An understanding of the behavior of PCBs, PCDD/Fs, and many other organochlorine compounds in agricultural food chains is therefore important if human exposure to these substances is to be reduced. This study examined the transfer of organochlorine compounds from feed to milk in lactating cows.

The most common approach to investigating feed/animal transfer of contaminants is the feeding study [e.g., Ewers et al. (1987), Fries et al. (1973), Jensen and Hummel (1982)]. The animals receive a daily dose of the compound, either through gavage or by mixing the contaminant with the feed. The dosage is significantly above the normal uptake of the animal, making it possible to monitor the effect of the dosage on the contaminant concentrations in the milk and/or tissue of the animal. After a certain period, generally several weeks, the treatment is stopped. In the following clearance phase the decrease in contaminant concentration over time is monitored. Feeding studies directly measure the kinetics of the transfer process and, if the accumulation phase is long enough, the steady-state behavior as well. When a steady state is not reached, the steady state behavior is extrapolated with the help of pharmacokinetic models. Simple first-order kinetic models are most often applied.

In a review of the environmental applicability of the published literature, several shortcomings common to many studies were identified:

1. There is some uncertainty associated with the extrapolation of the steady-state transfer rate. This is particularly true when physiological parameters such as body fat weight or milk production change during the study, complicating the kinetic behavior. Most commercial milk cows are close to a steady state, and hence the estimate of transfer at steady state is the most important parameter in the overall description of the transfer phenomenon.

2. The contaminant is usually applied at levels at least an order of magnitude above those normally present in the feed, although this problem has been circumvented in some studies by using labeled compounds [e.g., Jones et al. (1987)]. Fries (1977) suggested that contaminant behavior is a function of the concentration. This would result in errors when the bulk of the feeding study results is linearly extrapolated to typical agricultural conditions. Recently, Willett et al. (1990) studied the dose-related feed–milk transfer of PCBs and proposed a nonlinear equation to describe the behavior. However, the lowest exposure level in their dataset was about 50 times higher than the PCB ingestion under background conditions in Bayreuth. It is not yet clear whether their equation can be applied at these levels.

3. The method of contaminant application, for instance, mixing the compound with the feed or dissolving it in vegetable oil, does not necessarily reflect the form in which the contaminants are present in the feed under natural conditions. Matrix effects can strongly influence the behavior of organochlorines in animals. For instance, Van den Berg et al. (1983) found that the bioavailability of PCDD/F to rats was a factor of 3–20 lower in fly ash than in fly ash extract. It is possible that such an effect is also present in cows. This particular example is especially relevant to the dioxin risk analysis discussion, where some authors feel that fly ash deposited on plants is the main source of PCDD/F in feed (Travis and Hattemer-Frey, 1987; Stevens and Gerbec, 1988).

In light of these findings it was decided to attempt a contaminant mass balance of a cow under natural conditions as it was thought that such an experiment would avoid the possible shortcomings outlined above. Perhaps more importantly, this study design allows the simultaneous examination of the behavior of many different compounds in one animal. It is currently difficult to draw conclusions about the role of physical chemical properties on contaminant transfer in cows as the feeding studies are generally conducted with just one or several compounds and comparison between studies is confounded by the between-animal and interstudy variation.

In this work PCDD/Fs, PCBs, and several other chlorinated organic compounds were investigated. The results for PCDD/Fs were published previously (McLachlan et al., 1990). A more complete dataset with corrected feces fluxes is found in McLachlan (1992). This paper presents the results for the PCBs and the other organochlorine

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Table I. Feed Ration before and during the Mass Balance

feed	amount, kg of FW/day	feed	amount, kg of FW/day
fodder beets	12	hay, 1st cut	1.7
corn silage	12.5	hay, 2nd cut	4.3
grass silage	14	concentrate	8
		minerals	0.15

Table II. Sampling Schedule

	no. of samples					
matrix	Feb 16	Feb 23	March 2	March 9	March 16	March 23
feed					2	2
feces					1	2
urine						1
milk	1			1	1	3

compounds. The experimental methods are repeated here for the sake of completeness.

2. EXPERIMENTAL PROCEDURES

2.1. Experimental Design. The study was conducted on a teaching farm (Landwirtschaftliche Lehranstalten des Bezirks Oberfranken) on the southern outskirts of the small city of Bayreuth (population 70 000). Bayreuth lies in a rural region of northeastern Bavaria, and there are no known significant point sources of the study compounds in the area. The prevailing winds are southwesterly, so the influence of the city on the agricultural environment is thought to be negligible. The farm has no history of sewage sludge use, and chlorinated pesticides have not been employed in recent years. This site is considered to represent the typical background situation for the area.

The mass balance was conducted on a 4-year-old, 650-kg, Simmenthal cow, one of a herd of 40. Her environment was not modified in any way due to the lack of appropriate facilities and in order to avoid any disturbances in milk production.

The sampling period was selected to maximize the likelihood of the cow being in a steady state with respect to the chlorinated compounds. The first samples were collected on February 16, 1989, approximately 2 months after calving (December 22, 1988). With a milk fat production of ca. 1.4 kg/day and an estimated body fat mass of 50 kg, the cow excreted about 1.5 times her body fat mass in milk fat between calving and the first sampling. Thus, any effects of calving and the preceding dry period were expected to have largely disappeared.

The cows at the teaching farm were kept inside in the winter, tied in open stalls in a barn. The cement floors were covered in straw. No possible contaminant sources in the form of treated wood were present. The study cow was on the same feed ration (see Table I) from the beginning of December 1988 until the end of the study on March 24, 1989. With the exception of soya, which made up 20% of the grain ration, all of the feed had been produced on the farm. This increased the likelihood of a homogeneous feed composition and reduced the risk of "imported" contamination. It was possible for the cow to take feed from her neighbors, but this was not a significant factor in her total uptake.

Feed, water, and air were considered as possible contaminant routes to the cow. Dermal absorption was assumed to be minimal. Milk, feces, and urine were the output pathways analyzed. The difference between the sum of the inputs and the sum of the outputs was attributed to either storage in the animal (S), metabolism (M), or experimental error (E) (abbreviated SME).

2.2. Sampling and Flux Parameters. The sampling schedule is given in Table II. The milk samples were taken over a 5-week period to test the steady-state assumption. Although the sampling program was limited by the costs of the PCDD/F analyses, more samples were planned. Unfortunately, the experiment had to be terminated early due to an unforeseen change in the cow's environment.

Samples of the individual feed components were collected just before they were distributed to the animals. Two composites, each equivalent to 1% of the cow's daily ration, were assembled in the laboratory on the day of sampling. One was extracted immediately, and the other was stored at −20 °C and extracted several weeks later.

A 3-L water sample was taken directly from the farm water supply outside the barn and extracted immediately. The cow's average daily water consumption was estimated to be 30 L.

Twelve consecutive air samples of 1-week duration collected sequentially on the farm from July 12 to October 4, 1989, were used to estimate respiratory exposure to the contaminants. The sampling method and the results of this monitoring program will be published in detail elsewhere. A respiration rate of 150 m^3/day (Stevens and Gerbec, 1988) was adopted for this work, and the conservative assumption of 100% retention of inhaled contaminant was made.

Milking was conducted twice daily using a milking machine. A 2-L sample was collected on sampling days and transported to the laboratory where it was centrifuged at 3000 rpm and 4 °C for 20 min. The fat layer was then skimmed off and frozen for future workup. With the exception of two samples from March 23, all milk samples were from the morning milking. Over the sampling period the cow produced an average of 27 L/day of milk containing 5% fat.

The feces and urine samples were collected in glass containers at the moment of excretion. They were transported to the laboratory and extracted immediately. The feces flux was calculated using standard digestibility values for the different feed types (DLG-Futterwerttabellen). The resulting dry mass flux was 33% of the dry mass intake, or 7 kg/day. A urine flux of 20 L/day was assumed.

2.3. Analysis. *2.3.1. Extraction.* The urine and water samples were transferred to a shaking flask. They were dosed with the internal standard mixture dissolved in toluene. An amount of toluene equal to 20% of the sample volume was added, and the mixture was shaken for 5 min. The fractions were separated, and the procedure was repeated twice. The toluene extract was then dried over Na_2SO_4.

The feed samples were blended in an acetone/water mixture (1:1) using an Ultra-Turrax blender. They were then dosed with the internal standard and placed in an ultrasonic bath for 10 min. The extract was filtered and the residue washed with acetone and Soxhlet extracted in toluene for 48. The acetone/water extract was concentrated on a rotary evaporator, and the water was back extracted in a shaking flask using 200 mL of *n*-hexane (three repetitions). The two extracts were combined.

The feces samples were extracted using the method above but without the blending step.

The milk fat samples were mixed with 500 g of Na_2SO_4 and filled in a column. The standard was applied to the top of the column, and the sample was eluted with 1000 mL of acetone/*n*-hexane (1:2).

2.3.2. Cleanup. Ninety percent of the extract was removed for analysis of the PCDD/F (McLachlan et al., 1990). An elaborate cleanup was developed for the remaining extract that could be successfully employed for a wide range of matrices and would give low detection limits on a mass selective detector (MSD). Three liquid chromatography columns were used.

The first cleanup step was gel chromatography. The sample was transferred to a column filled with bio-beads SX-3 (i.d. = 25 mm, L = 30 cm) equilibrated in *n*-hexane/dichloromethane (1:1). The column was then eluted with this solvent mixture. The first 95 mL was discarded, and the following 75 mL were collected.

This was followed by a Florisil column (i.d. = 10 mm, L = 20 cm, 4% water). The sample was transferred to the column and eluted with 150 mL of *n*-hexane.

The final step was an alumina column (i.d. = 6 mm, L = 17 cm, neutral alumina with 6% water). The sample was transferred to the column and eluted with a small quantity of *n*-hexane; 3 mL was collected and discarded. The sample was then eluted from the column with 10 mL of dichloromethane.

The method proved to be very robust, yielding extracts from which 10% of the cleaned up sample (1% of the original sample: e.g., 20 mL of milk or 2 g DW of feed) could be injected on a capillary column with satisfactory chromatography results.

2.3.3. Measurement. Eight chlorinated pesticides or related compounds along with PCBs with three or more chlorine atoms were analyzed. The measurements were conducted on an HP-5890 gas chromatograph (GC) coupled to an HP-5970 mass selective detector. A Gerstel cold injector was employed. The

Table III. Compounds Analyzed, Internal Standards Used, and Their Recoveries

compound	abbrev	internal standard	standard recovery, %
trichlorobiphenyl	Cl_3Bi	$^{13}C_{12}$ PCB 101	80–100
tetrachlorobiphenyl	Cl_4Bi	$^{13}C_{12}$ PCB 101	
pentachlorobiphenyl	Cl_5Bi	$^{13}C_{12}$ PCB 101	
hexachlorobiphenyl	Cl_6Bi	$^{13}C_{12}$ PCB 153	80–100
heptachlorobiphenyl	Cl_7Bi	$^{13}C_{12}$ PCB 153	
octachlorobiphenyl	Cl_8Bi	$^{13}C_{12}$ PCB 153	
decachlorobiphenyl	$Cl_{10}Bi$	$^{13}C_{12}$ PCB 153	
pentachlorobenzene	QCB	$^{13}C_6$ QCB	15–30
hexachlorobenzene	HCB	$^{13}C_6$ HCB	40–70
γ-hexachlorocyclohexane	γ-HCH	D_6 γ-HCH	80–100
α-hexachlorocyclohexane	α-HCH	D_6 γ-HCH	
p-dimethoxytetrachlorobenzene	*p*-DMTCB	$^{13}C_6$ PCA	40–70
pentachloroanisole	PCA	$^{13}C_6$ PCA	
p,p'-DDE	DDE	$^{13}C_{12}$ *p,p'*-DDE	80–100
p,p'-DDT	DDT	$^{13}C_{12}$ *p,p'*-DDT	80–100

desorption of the sample was conducted at 2 °C/s to a final temperature of 240 °C. It was found that faster heating rates and higher end temperatures led to degradation of the hexachlorocyclohexanes (HCHs) and a conversion of *p,p'*-DDT to *p,p'*-DDD in the injector. The GC column was an HP-Ultra 2 (0.2 mm × 0.33 μm × 25 m) capillary with helium as the carrier gas.

The quantification was conducted using isotope dilution. The labeled internal standards that were used are listed in Table III. At least two masses per standard and analyte were monitored. Four criteria had to be satisfied for analyte identification:

1. The substance had to successfully pass through the cleanup procedure.

2. The relative retention time of the analyte had to correspond to the relative retention time of the corresponding standard.

3. A signal at the mass of the molecule had to be detected.

4. The ratio of the signal between two isotopes of the analyte had to lie within 15% of the theoretical ratio arising from the natural distribution of chlorine in the environment.

The isomer-specific quantification of the PCBs was conducted on a Varian 3400 gas chromatograph coupled to a Finnigan 8230 double-focusing mass spectrometer. The injector and the column were the same as for the other measurements. This system had lower detection limits and a higher resolution (2000), useful properties when PCB congeners present in very small quantities are quantified. The relative retention times of 18 PCB congeners were measured for this column. The remaining congeners were identified using the relative retention times of the 209 PCBs published by Mullin et al. (1984) and Schulz et al. (1989) for this column phase. The ability of the mass spectrometer to differentiate between the homologue groups was very useful in identifying the congeners. The relative intensities of the isomers in a particular homologue group in the feed samples corresponded well with the relative intensities determined in the commercial mixtures by Schulz et al. (1989). It was thus decided to estimate the relative concentrations of coeluting isomers on the basis of a 1:1:1:1 mixture of Clophens A30, A40, A50, and A60 as characterized by Schulz et al. (1989). The response factors for all isomers in a homologue group were assumed to be identical. The reference congeners for quantification were PCBs 28, 52, 101, 153, 180, 194, and 209 [numbering according to Ballschmitter and Zell (1980)]. As the mass balance was concerned with relative concentrations, this assumption was not significant.

2.3.4. Blanks and Recoveries. Laboratory blanks were regularly analyzed. The amounts in the blanks in the few cases where an analyte was detected were at least 10 times less than the amounts present in the samples.

The quality of the method was continuously monitored through the recoveries of the internal standards. These are summarized in Table III. The recoveries were satisfactory for most of the compounds analyzed. However, the complexity of the cleanup with the repeated evaporation of the extract resulted in considerable losses of the more volatile compounds: pentachlorobenzene (QCB), hexachlorobenzene (HCB), and pentachloroanisole (PCA). It was possible to correct for these losses through the internal standard.

Table IV. Routes of Contaminant Uptake in the Cow

	air		water		feed	
substance	ng/day	% total	ng/day	% total	ng/day	% total
PCB 28	2.1	<1	<10	<1	1800	>99
PCB 52	4	<1	<8	<1	920	>98
PCB 101	4.2	<1	<13	<1	2400	>99
PCB 153	3.0	<1	<27	<1	3300	>99
PCB 138	2.2	<1	<32	<1	3200	>99
PCB 180	0.6	<1	<10	<1	1600	>99
PCB 194	<0.1	<1	<12	<10	112	>90
PCB 209	<0.1	<1	<7	<20	30	>80
QCB	20	<1	<12	<1	2400	>99
HCB	80	<1	<75	<1	12300	>99
γ-HCH	96	<1	<25	<1	106000	>99
α-HCH	35	<1	<13	<1	30000	>99
p-DMTCB	59	1	<2	<1	5800	99
PCA	12	<1	<7	<1	4100	>99
p,p'-DDE	2.3	<1	<8	<1	4500	>99
p,p'-DDT	3	<1	<120	<2	7600	>98

3. RESULTS AND DISCUSSION

3.1. Sources of the Cow's Exposure. The results of the input side of the mass balance for several representative compounds are summarized in Table IV. Despite the use of conservative assumptions in calculating the inputs from air and water, virtually all of the cow's exposure to all of the study compounds occurred through the feed.

The air contributed very little to the overall uptake, but it is interesting to note that the fractional contribution to the total exposure increased with increasing volatility of the compounds, from 0.04% for PCB 180 to 0.8% for QCB. It is possible that the air is a significant contributor to the cow's uptake of more volatile organic contaminants.

None of the substances were detected in the water. The insignificance of this pathway corroborates the findings of Ruoff et al. (1988) for PCBs.

3.2. Validity of the Steady-State Assumption. The existence of an approximate steady state was a prerequisite for conducting the mass balance as it was not possible to independently determine the metabolism and storage of substance in the cow. Since the contaminant uptake and the contaminant storage capacity of the cow (the body fat weight) were relatively constant, the existence of an approximate steady state could be verified by measuring the contaminant output. The concentrations in milk samples collected over a period of 5 weeks previous to and during the mass balance measurements are listed for four representative compounds in Table V. The concentrations are relatively constant in all cases. It was concluded that the steady-state assumption was justified.

3.3. Results of the Mass Balance. The results of the mass balance are summarized in Table VI. None of the compounds were detected in the urine. Seventy-five PCB

Table V. Milk Concentrations (Nanograms per Gram of Fat) vs Time for Four Representative Compounds

substance	date			
	Feb 16	March 9	March 16	March 23[a]
HCB	10.3	10.4	11.3	7.9
lindane	3.0	3.3	2.9	3.1
p,p'-DDE	3.1	3.1	3.4	2.6
PCB 99	0.45	0.45	0.49	0.36

[a] Average of three samples.

peaks were measured containing 88 congeners (those congeners comprising <5% of a peak were not included). There were distinct patterns in the results which allowed the congeners to be classified into three groups: persistent, semipersistent, and labile PCBs. The behavior of each group of substances was virtually identical within a homologue, and hence the data have been presented in this summarized form. The negative values of the SME parameter for HCB and the persistent PCBs indicate that up to 40% more of these compounds was excreted than was taken up.

The possibility that the negative mass balance was due to the cow losing body fat and thus releasing stored contaminant cannot be completely ruled out as no measure of storage was possible. However, assuming the tissue fat and milk fat concentrations were similar, the cow would have had to lose ca. 14 kg of fat (28% of the estimated total tissue fat mass) over the 5-week measurement period to account for the mass balance deficit of 40%. Such a fat loss would be unusual for a healthy Simmenthal cow on this diet. Furthermore, according to the experienced farm personnel, there was no observable change in the cow's weight. The fact that the mass balance functioned very well for the persistent PCDD/F (McLachlan, 1992) and for DDE indicates that the cause of the deficit was compound-specific. The most likely explanation was a secondary contamination of the feed through binder twine that had been treated with recycled oil. This is known to be a source of PCBs in milk in Germany (Carl, 1989). This contamination is heterogeneous, and hence the feed samples may not have represented the average levels of these compounds in the feed.

The results in Table VI indicate that a large fraction of the persistent compounds is excreted in the milk. However, small amounts of all labile PCBs were also found. The proportion of the PCBs excreted in the feces increases with increasing degree of chlorination, and the feces flux is generally higher for the persistent congeners than for the labile congeners in a homologue group. These observations are discussed in more detail below.

3.4. Metabolism. *3.4.1. PCBs.* Of the 88 PCB congeners measured, 52 were classified as labile due to their high SME values and low rates of excretion in the milk. Some 26 congeners were characterized by low (or negative) SME values and high rates of excretion in the milk. These congeners were classified as persistent. A third group of 10 congeners displayed an intermediate behavior and were classified as semipersistent. The chlorine substitution patterns of the persistent and semipersistent congeners are shown in Figure 1. Twenty-five of the 26 persistent congeners are substituted at both para positions (4,4'), as are the three Cl_3 and Cl_4 congeners in the semipersistent group. One persistent compound and the remaining semipersistent compounds are para-substituted on one ring and have a 2,3,5-substitution pattern on the second ring. On the other hand, all members of the labile group of compounds had at least one ring that was neither 4- nor 2,3,5-substituted, with one exception (PCB 201). Thus, it was concluded that the 4,4' substitution pattern is the key to PCB persistence in the cow and, furthermore, that the 2,3,5 pattern is a partially effective alternative. The 4,4' substitution is not in itself sufficient protection as illustrated by the significant metabolism of PCBs 28, 60, and 66. The non-ortho-substituted PCBs were not measured, so their behavior can only be inferred from the results.

It has been shown that the metabolism of PCBs in mammals occurs by hydroxylation at the 2- and 4-positions through the hepatic cytochrome P-448 and P-450 enzyme system, respectively (Matthews and Dedrick, 1984). There are large differences between species, however (Lutz and Dedrick, 1987; Sipes and Schnellmann, 1987), so that the results for other species cannot be extrapolated to cows. Gardner et al. (1976) and Safe et al. (1975) demonstrated that 4-hydroxy-PCBs are the primary metabolites of several congeners in cows. This is in agreement with the observations made in this study, as the presence of a chlorine atom at the 4-position or the presence of chlorine atoms at both of the adjacent 3- and 5-positions would inhibit the formation of the intermediate arene oxide at the 4-position.

3.4.2. HCHs. Neither of the HCH congeners was persistent in the cow. The low rates of excretion in the feces suggest that the compound is metabolized in the digestive tract. This agrees with the results of Blüthgen et al. (1983), who measured half-lives of 12 and 35 h for γ-HCH and α-HCH, respectively, in rumen juices.

Other studies indicate that HCH is quite persistent once it enters the tissue of the cow. Van den Hoek et al. (1975) found half-lives of about 2 weeks for α-HCH and 1 week for γ-HCH in the cow's milk. These are in the same range as the half-life of 2.5 weeks reported for the persistent substance HCB.

The presence of HCHs in the milk indicates that a small portion of the ingested HCH is absorbed before it can be metabolized in the rumen. Blüthgen et al. (1983) found HCHs in the blood of a cow minutes after injecting it into the rumen. This demonstrates that the absorption of organic compounds can occur very quickly. The transfer of HCHs from the feed to the milk would appear to be determined by the two competing processes in the rumen—metabolism vs absorption.

3.4.3. Methoxybenzenes. PCA and p-DMTCB were only found in very small quantities in the feces (2 and 1%, respectively), which suggests that these compounds are also metabolized by bacteria in the digestive tract. This hypothesis is supported by the work of Hippelein and Schramm (1990), who measured a relatively rapid degradation of these compounds in soil (half-lives of 7 and 3 days, respectively).

These compounds were not found in the milk at all. This indicates that, in contrast to the HCHs, PCA and p-DMTCB are significantly metabolized in cow tissue. Although no information regarding the persistence of these compounds in mammals was found, Opperhuizen and Voors (1987) found that PCA is metabolized to pentachlorophenol in fish.

3.4.4. p,p'-DDE and p,p'-DDT. DDT and DDE have been studied extensively in the cow, so the results are of a confirmatory nature. DDE was persistent in the cow, a conclusion that is supported by the long half-lives measured in feeding experiments [e.g., Fries (1977)]. DDT was, on the other hand, not persistent. Only 4% of the ingested substance was excreted in the milk. Fries (1977) reported that DDT is metabolized in the rumen to DDD, which is then further metabolized in the cow. That portion

Table VI. Results of the Mass Balance with Uptake Flux and Output Fluxes (Nanograms per Day) and as a Percent of the Uptake Flux

no. of Cl	substance	uptake, ng/day	flux: feces ng/day	feces %	flux: milk ng/day	milk %	flux: SME[a] ng/day	SME[a] %
	PCBs (Isomers Are Grouped Together According to Their Persistence)							
3	semipers	950	190	20	28	3	730	77
	labile	2800	640	23	48	2	2100	75
4	persistent	600	150	25	410	68	40	7
	semipers	1080	180	16	130	12	770	72
	labile	4000	690	17	100	3	3200	80
5	persistent	2300	740	32	2500	109	-940	-41
	semipers	106	29	28	39	37	38	35
	labile	7100	1400	20	200	3	5500	77
6	persistent	7600	2400	31	8100	107	-2900	-38
	semipers	500	118	24	280	56	100	20
	labile	5200	1020	20	230	4	4000	76
7	persistent	3200	1200	38	2400	75	-400	-13
	semipers	1800	540	30	270	15	990	55
	labile	1400	450	32	37	3	910	65
8	persistent	330	180	53	190	59	-40	-12
	semipers	33	15	45	4	13	14	42
	labile	160	73	45	16	10	71	45
10	persistent	30	26	87	16	53	-12	-40
	Other Compounds							
	QCB	2450	490	20	420	17	1540	63
	HCB	12300	3200	26	11800	96	-2700	-22
	γ-HCH	106000	2900	3	4100	4	99000	93
	α-HCH	30000	2600	9	6300	21	21100	70
	p-DMTCB	5800	52	1	0	0	5800	99
	PCA	4100	100	2	0	0	4000	98
	p,p′-DDE	4500	970	22	4000	89	-470	-11
	p,p′-DDT	7600	1700	22	310	4	5600	74

[a] SME, storage or metabolism in the cow or experimental error.

Persistent Congeners

Cl Nr	PCB Nr.	Substitution Pattern 2	3	4	5	6	-	2	3	4	5	6
4	47	X		X			-	X		X		
	74	X		X	X		-			X		
5	99	X		X	X		-	X		X		
	115	X	X	X		X	-			X		
	85	X	X	X			-	X		X		
	118	X		X	X		-		X	X		
	123	X		X			-		X	X	X	
6	153	X		X	X		-	X		X	X	
	130	X	X	X			-	X	X		X	
	138	X	X	X			-	X		X	X	
	158	X	X	X		X	-		X	X		
	128	X	X	X			-	X	X	X		
	156	X	X	X	X		-		X	X		
	157	X	X	X			-		X	X	X	
7	183	X	X	X		X	-	X		X	X	
	171	X	X	X		X	-	X	X	X		
	180	X	X	X	X		-	X		X	X	
	170	X	X	X	X		-	X	X	X		
	190	X	X	X	X	X	-		X	X		
8	197	X	X	X		X	-	X	X	X		X
	196	X	X	X		X	-	X	X	X	X	
	203	X	X	X	X	X	-	X		X	X	
	195	X	X	X	X	X	-	X	X	X		
	194	X	X	X	X		-	X	X	X	X	
	205	X	X	X	X	X	-		X	X	X	
10	209	X	X	X	X	X	-	X	X	X	X	X

Semi-Persistent Congeners

3	28	X		X			-			X		
4	66	X		X			-		X	X		
	60	X	X	X			-			X		
5	107		X	X			-	X	X		X	
6	146	X		X	X		-	X	X		X	
7	187	X		X	X		-	X	X		X	X
	177	X	X	X			-	X	X		X	X
	172	X	X	X	X		-	X	X		X	
	178	X	X		X	X	-	X	X		X	
8	202	X	X		X	X	-	X	X		X	X

Figure 1. Substitution pattern of the persistent and semipersistent PCBs.

of DDT which is directly absorbed would appear to be persistent, as indicated by the long half-life measured by Fries. The behavior of DDT is similar to that of the HCHs.

Table VII. Comparison of the Measured Carry-Over Rates with Values in the Literature

substance	source: a	b	c	d	e	f
PCB 153	0.78	0.23	0.71			
PCB 138	0.63	0.18	0.75			
PCB 180	0.63	0.21	0.68			
γ-HCH	0.04			0.03	0.02	
α-HCH	0.21			0.09	0.12	
HCH	0.79				0.23	0.53
p,p′-DDE	0.80					0.50
p,p′-DDT	0.04					0.03

[a] This work. [b] Tuinstra et al. (1981) (calculated by the author). [c] Heeschen and Blüthgen (1986). [d] Heeschen and Blüthgen (1985). [e] van den Hoek et al. (1975). [f] Fries (1977).

3.5. Carry-Over Rates. Heeschen and Blüthgen (1986) define the carry-over rate as follows:

$$r = \frac{\text{substance flux in the milk (ng/day)}}{\text{substance flux to the cow (ng/day)}} \quad (1)$$

The carry-over rate is the fraction of the ingested compound that is excreted in the milk.

The calculation of the carry-over rates was complicated by the discrepancies in the mass balance. It was assumed, for the reasons discussed above, that the feed concentrations were not representative. Therefore, the carry-over rate for the persistent compounds was calculated according to

$$r = \frac{\text{substance flux in the milk (ng/day)}}{\text{flux in the milk + flux in the feces (ng/day)}} \quad (2)$$

In Table VII the carry-over rates from this work are compared with the results of several feeding experiments

in the literature. There are significant differences among the studies.

The PCB values from this study and from Heeschen and Blüthgen (1986) agree well, whereas those from Tuinstra et al. (1981) lie considerably lower. One explanation for this could be the more primitive chromatography available at the time of the Tuinstra study. It is possible that coeluting congeners in the Arochlor mixtures in the feed samples led to an overestimation of the feed uptake.

The carry-over rates for γ-HCH agree reasonably well with each other, while the measured value for α-HCH is considerably higher. As the carry-over is determined by the competing metabolism and absorption in the rumen, it is possible that the carry-over rate is dependent on the species, feed, or other factors that could affect the state of the rumen. This would mean a high natural variation in carry-over of these compounds.

The values for HCB and DDE are much higher in this study than in the two studies found in the literature. The low value of 0.23 measured by Van den Hoek et al. (1975) can be partly attributed to the fact that this value was not calculated on the basis of an extrapolated steady state. It was the ratio of the two substance fluxes at the end of the feeding period, although the authors recognized that steady state had not been reached. The dosage dependency of the carry-over rate reported by Willet et al. (1990) may be another explanation for the discrepancies. The much lower dosage rates in this study could have led to higher carry-over rates than those from the other studies, where the dosage was higher. Another hypothesis can be deduced from the results of Heeschen and Blüthgen (1986). Two cows with different milk production (18.1 vs 23.3 L/day) showed different carry-over rates. The cow with the lower production had carry-over rates that were about 25% lower for all substances. The higher carry-over rates measured in this study could then be related to the higher milk production. These hypotheses are speculative, however, and require further investigation.

It should be noted that many of the environmental parameters in this study were not well controlled and that this could have affected the reliability of the carry-over data. The good agreement with published carry-over rates for most of the compounds measured [i.e., the PCBs and the previously published PCDD/F results (McLachlan, 1991)] is thus particularly encouraging. The experience gained here shows that a mass balance under natural conditions is a viable experimental approach that can supplement the information gained from feeding studies. It is recommended that future investigations be conducted in better facilities where more exact measurements of the feed, feces, and milk fluxes as well as the storage are possible.

3.6. Absorption. The net absorption of contaminant from the digestive tract into the cow's tissue is the crucial process determining the carry-over of persistent compounds. If a compound is not metabolized, then the amount absorbed is equal to the amount excreted in the milk at steady state. The net absorption, defined as

$$r_e = \frac{\text{flux in the feed} - \text{flux in the feces (ng/day)}}{\text{flux in the feed (ng/day)}} \quad (3)$$

was calculated for the study compounds using the corrected feed fluxes. The same correction applied to the persistent isomers in a PCB homologue group was also applied to the semipersistent and labile isomers.

The results are plotted against the logarithm of the 1-octanol/water partition coefficient (log K_{ow}) in Figure 2. The absorption is relatively constant at about 80% up to a log K_{ow} of about 6.5. After this, the absorption declines with increasing hydrophobicity. Fries (1977) observed decreasing feed/milk transfer rates with increasing chlorination for several PCB and polybrominated biphenyl congeners. He hypothesized that the absorption was decreasing due to increasing molecular mass. The results presented here agree with the previously published observations made for PCDD/F (McLachlan, 1991). A similar behavior was also reported by Gobas et al. (1988) for organic contaminants in fish, although in this case the absorption plateau was at ca. 50%, not 80%. It is also interesting to note that the absorption of the persistent compounds is on average somewhat lower than the absorption of the semipersistent or labile compounds. Further research is needed to determine if other families of organic contaminants behave in the same way.

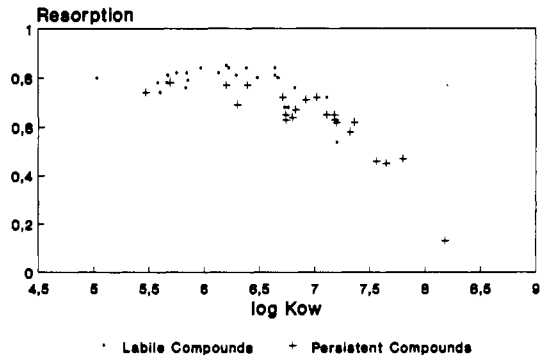

Figure 2. Substance absorption in the mass balance experiment vs log K_{ow}.

4. CONCLUSIONS

1. Feed is the primary source of the lipophilic chlorinated aromatic compounds present in the milk.
2. The 4,4'-substitution pattern is the key to PCB persistence in the cow. The 2,3,5 substitution is a less effective hindrance for metabolism.
3. The "natural" mass balance is a useful supplement to standard feeding studies for investigations of contaminant behavior in animals.
4. The absorption in the cow of the compounds studied can be described quite well using the lipophilicity of the compound. Whereas the absorption is relatively constant up to a log K_{ow} value of ca. 6.5, it decreases sharply as the lipophilicity of the compound increases beyond this point.

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