A Mass Balance of PCBs and Other Organochlorine Compounds in a Lactating Cow

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1. Introduction

Food has been shown to be the most important route of exposure for the general population to highly lipophilic ubiquitous organic contaminants. Milk products in particular are known to contribute significantly to the total exposure, accounting for 50% of the total polychlorinated dibenzo-p-dioxin and -furan (PCDD/F) uptake in a Canadian study (Environment Ontario, 1988), and 30% of the polychlorinated biphenyl (PCB) exposure in a German study (Sümmermann et al., 1978). In order to better understand the phenomena determining human exposure to these kinds of pollutants, we selected an air/soil-plant-cow-milk food chain for more extensive investigation. A farm in northeastern Bavaria well removed from known significant contaminant sources and with no history of sewage sludge use was selected. The first study was a contaminant mass balance of a lactating cow. Although many experiments using artificially contaminated feed are present in the literature, there is little information available on the pollutant transfer behaviour in cows under natural conditions. At DIOXIN 89 we presented the results of the PCDD/F mass balance (McLachlan et al., 1990). In this paper we report on the mass balance for polychlorinated biphenyls (PCBs) and other chlorinated compounds.

2. The Study Conditions, Sampling and Analysis

Due to the limited space we'd like to refer you to the PCDD/F paper for a description of the study conditions, sampling and extraction. The same samples were analysed. Prior to extraction all samples were dosed with a mixture of ^{13}C labelled standards (pentachlorobenzene (QCB), hexachlorobenzene (HCB), lindane (γ-HCH), pentachloroanisole (PCA), p,p'-DDE, p,p'-DDT, PCB-101 and PCB 153). The cleanup consisted of gel permeation and florisil and alox column chromatography. HRGC/LRMS analysis was performed in the SIM mode on a HP-MSD 5970 equipped with a Gerstel cold injector and an HP Ultra 2 fused silica capillary columnn. The isomer specific PCB analysis was conducted on a Finnigan MAT 8230 at a resolution of 2000. Isomers identification was accomplished using 15 pure standards and the relative retention times published by Mullin et al. (1984) and Schulz et al. (1989). The p-dimethoxytetrachlorobenzene (p-DMTCB) was quantified using the recovery of the PCA standard, α-HCH using that of β-HCH, tri-, tetra- and penta-chlorobiphenyl using that of PCB 101, and all other PCBs using that of PCB 153. The response factors of PCBs 28, 52, 101, 153, 180, 194 and 209 were used to quantify all isomers in their respective homologue groups.

3. Results and Discussion

The results for the input side of the mass balance are presented in Table 1. Feed was responsible for virtually all of the cow's exposure to the compounds studied. This was true despite the use of worst case assumptions, namely that the retention of airborne substance in the lungs was 100%, and that the concentrations in the water were equal to the detection limits (None of the compounds were detected).

Table 1 Mass Flows into the Cow

Substance	Flows (ng/d)		
	Air	Water	Feed
QCB	20	<7	2400
HCB	80	<45	12300
γ-HCH	96	<15	106000
α-HCH	35	<8	30000
p-DMTCB	59	<1	5800
PCA	12	<4	4100
p,p'-DDE	2.3	<5	4500
p,p'-DDT	3	<70	7600
PCB 28	2.1	<6	1800
PCB 52	4	<5	920
PCB 101	4.2	<8	2400
PCB 153	3.0	<16	3300
PCB 138	2.2	<21	3200
PCB 180	0.6	<6	1600
PCB 194	<0.1	<7	112
PCB 209	<0.1	<4	30

The overall mass balance is summarized in Table 2. The total input, excretion through the feces and through the milk, and the amount necessary to balance the mass flux are presented. Although some compounds were identified in the urine, they were not present in large enough quantities to affect the mass balance. The value in the last column can be attributed to either storage/release in the animal, degradation, or experimental error, and is refered to as the SDE term. Only those PCB isomers are presented that showed a persistance in the milk significantly larger than that of the least persistent isomers in their homologue group. The remaining isomers are summarized under the designation "other".

The great variability in the SDE term indicates a wide range in the persistence of the compounds studied. Particularly startling are the large negative values for some substances, which indicate that up to 50% more compound left the cow than entered it. It is possible that these compounds (HCB, p,p'-DDE, various PCBs) were actually formed as products of the degradation of other compounds in the cow. However, we consider it more likely that these results arise from a combination of three other factors:

1. The cow ingested more than estimated by taking feed from cows adjacent to her. She is known as a particularly hearty eater.
2. The cow was not quite at steady state. Although the milk samples did not show any decreasing trend in the milk concentrations, it is possible that she was excreting contaminants accumulated during the dry weeks prior to the birth of her last calf.
3. Experimental error. This is always possible when looking at the difference between several different measurements.

Of the compounds studied, PCA and p-DMTCB were apparently completely degraded. The transfer of the HCHs to the milk was also low. Interestingly, the fraction of the α-isomer ingested appearing in the milk (22%) was five times higher than the fraction of the γ-isomer (4.4%), suggesting that the latter is more labile in the cow. Since only 18% of the QCB intake is excreted in the milk, compared to all of the resorbed HCB, the presence of 1 unsubstituted position on the benzene ring appears to ease degradation. Also, while p,p'-DDE is quantitatively excreted, only a very small fraction of the ingested p,p'-DDT appears in the milk.

From the 77 PCB peaks quantified, 50 displayed a low but for each homologue group uniform transfer rate to the milk, ranging from 1.8% for the trichlorinated isomers to 11% for the octachlorinated isomers. Our explanation is that a small fraction of the labile isomers resorbed are carried in the blood to the milk secreting glands before having been degraded. This effect could be larger for the higher chlorinated isomers because of their greater lipophilicity.

The chlorine substitution of the persistent PCB isomers are included in Table 2. With two exceptions (PCBs 146 and 130), all isomers have a 2,4,4' configuration. Also, no isomer with this configuration was among the non-persistent peaks quantified. Where one peak with and one without this pattern coeluted, an estimate of the relative concentrations in feed was made using the sum of the concentrations reported in 4 clophen mixtures by Schulz et al. (1989). The excretion of the non-2,4,4'-substituted isomer was calculated using the fractions for the non-persistent isomers of that chlorine number, and the difference in the excretion fluxes was use to estimate the fluxes of the 2,4,4'-substituted compound.

Table 2 Cow Mass Balance

Cl Nr	Substance	Substitution 23456-23456	Fr[1]	Flows (ng/d) Inflow	Feces	% In[2]	Milk	% In[2]	SDE	% In
3	PCB 28	x x - x		950	160	17	30	3	760	80
	Other			2800	540	19	51	2	2200	79
4	PCB 47/48			310	64		79		167	
	47	x x -x x	52	160	42	26	75	47	43	27
	PCB 74	x xx - x		440	88	20	370	84	-20	-4
	PCB 66	x x - xx		840	130	16	110	13	600	71
	PCB 56/60			620	81		44		500	
	60	xxx - x	39	240	24	10	33	14	183	76
	Other			3500	510	15	100	3	2900	82
5	PCB 99	x xx -x x		460	88	19	600	130	-228	-49
	PCB 87/115			590	110		57		420	
	115	xxx x- x	9	54	24	44	41	76	-11	-20
	PCB 85	xxx -x x		190	49	26	170	89	-29	-15
	PCB 118/ 123	x xx - xx x x - xxx		1550	460	30	1800	115	-700	-45
	Other			6600	1070	16	190	3	5300	81
6	PCB 146	x xx -xx x		500	100	20	300	60	100	20
	PCB 153	x xx -x xx		3300	820	25	4200	127	-1700	-42
	PCB 130	xxx -xx x		49	15	31	61	124	27	-55
	PCB 138	xxx -x xx		3200	890	28	3200	100	-890	-28
	PCB 158	xxx x- xx		340	80	24	470	140	-210	-64
	PCB 128	xxx -xxx		410	130	33	390	95	-110	-28
	PCB 156	xxxx - xx		270	80	29	300	111	-110	-40
	PCB 157	xxx - xxx		51	16	31	56	110	-21	-41
	Other			5200	840	16	250	5	4100	79
7	PCB 183	xxx x-x xx		530	175	33	450	85	-100	-18
	PCB 171	xxx x-xxx		260	78	30	146	55	36	15
	PCB 180	xxxx -x xx		1600	520	33	1300	81	-220	-14
	PCB 170/ 190	xxxx -xxx xxxxx- xx		790	280	35	710	90	-200	-25
	Other			3000	760	25	300	10	1940	65
8	PCB 197	xxx x-xxx x		12.7	4.7	37	4.1	32	3.9	31
	PCB 196/ 203	xxx x-xxxx xxxxx-x xx		150	70	47	105	71	-25	-18
	PCB 195	xxxxx-xxx		48	22	45	29	60	-3	-5
	PCB 194	xxxx -xxxx		112	50	44	68	61	-6	-5
	PCB 205	xxxxx- xxx		7	3	43	5.5	79	-1.5	-22
	Other			190	74	39	22	11	94	50
10	PCB 209	xxxxx-xxxxx		30	22	73	17	57	-9	-30
	QCB			2450	420	17	450	18	1600	65
	HCB			12300	2700	22	12600	102	-3000	-24
	γ-HCH			106000	2500	2	4400	4	99000	93
	α-HCH			30000	2170	7	6760	22	21100	70
	p-DMTCB			5800	44	1	0	0	5800	99
	PCA			4100	870	22	0	0	3200	78
	p,p'-DDE			4500	820	18	4300	95	-600	-13
	p,p'-DDT			7600	1900	25	330	4	5400	71

[1]- Fraction of the specified isomer in the coeluting peak in a mixture of equal parts Clophen A30, A40, A50 and A60 according to Schulz et al., 1989.

[2]- Outflow as a percentage of the total inflow into the cow.

The 2,4,4' configuration would appear to be the key to PCB persistence in this cow. However, the lability of the 2,4,4'-Cl_3B illustrates that another chlorine atom is necessary to protect the molecule. The varying persistence of the 2,4,4'-substituted Cl_4B isomers suggests that the 2' and 5 positions are more beneficial in this regard than the 3 or 3' positions. The 2',3,4,4',5 configuration is stable though, indicating that additional substitution of a 2,3',4,4' configuration further hinders degradation.

Of the 6 PCB isomers specified in most current analytical protocols (28, 52,101, 138, 153 and 180), the first three are of little relevance for this milk. However, the last 3 account for over half of the total PCBs excreted (assuming that the variation of the MS response factors is not enormous). Other isomers that are much more predominant than 28, 52 and 101 are 74, 99 and 118/123.

A measure of the intestinal resorption is obtained by comparing the feed and feces fluxes of the non-persistent substances in Table 2. The resorption of the more polar compounds is almost complete, while that of the less polar PCBs decreases with increasing chlorination. The same behaviour was observed for PCDD/F.

A closer examination of the results in Table 2 indicates that the feces excretion of persistent compounds is almost always greater than the feces excretion of non-persistent compounds of similar log K_{ow}. This was also observed for PCDD/F. We attribute this to back diffusion of substance in the intestinal blood and tissue into the lumenal contents.

4. Conclusions

1. Under normal conditions the PCBs, HCHs, DDT, DDE and higher chlorinated benzenes found in cows milk originate in the feed.
2. More polar compounds are more readily resorbed in the cow's digestive tract than very non-polar compounds.
3. Persistent lipophilic compounds in the cow can be excreted in both the milk and the feces.
4. α-HCH is more persistent than γ-HCH, p,p'-DDE more than p,p'-DDT, and HCB more than QCB. PCA and p-DMTCB are completely degraded in the cow.
5. All major 2,4,4'-substitiuted PCBs with 3 exceptions (28, 60 and 66) are persistent in the cow and are excreted mainly through the milk.
6. All major non-2,4,4'-substituted PCBs with two exceptions (130 and 146) are not persistent in the cow and are transfered to the milk in only small amounts.

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