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Rates of Elimination of Polychlorinated Dibenzo-*p*-dioxins and Dibenzofurans from Lactating Dairy Cattle

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Abstract

Excretion of 2,3,7,8- substituted dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) in milk of cows was measured for 32 days following administration of pentachlorophenol (PCP) treated wood for 58 days. Declines in concentrations of the more highly chlorinated congeners were greater than for the less chlorinated congeners. These rapid rates of decline were associated with higher relative concentrations of the highly chlorinated congeners in the liver.

Introduction

The U. S. Environmental Protection Agency in the 1994 reassessment of dioxins and related compounds cited animal products as important contributors to human background exposure.¹⁾ Animal exposures to combustion emissions deposited on pasture and forage crops were considered the primary source, but later studies provided evidence that contact with PCP-treated wood may be an important pathway of animal exposure to PCDDs and PCDFs.^{2,3)}

We administered PCP-treated wood to dairy cows to quantitatively understand the transfer of PCDD/F contaminants to milk and tissues.⁴⁾ This paper presents data on excretion of PCDDs/Fs in milk for the period after administration of PCP-treated wood ended, and relates the rates of concentration decline to body stores in liver and adipose tissue.

Materials and Methods

A detailed description of the design of the cattle study is available.⁴⁾ The study followed an animal care protocol approved by the Beltsville Area Animal Use and Care Committee. The four Holstein cows in mid-lactation were confined in stalls fitted with rubber mats and bedding was not used. Cows were milked at twelve hour intervals, the amount of milk was recorded. The cows were administered 3.0 g of ground PCP-treated wood once per day by gelatin capsule for 58 days. Wood and feed samples were obtained during the dosing period and the daily intake of each congener during the dosing period is presented in Table 1. A 3 L milk sample was collected from each cow at a single milking on Day 56, and additional sample were collected at 12 and 32 days post-dosing. Samples of adipose tissue and liver were obtained when the animals were sacrificed 32 days post dosing. Extraction, cleanup, and quantitation of the PCDD/F homologs by high resolution gas chromatography-mass spectrometry was performed by Alta Analytical Laboratory (El Dorado Hills, CA) following USEPA methods 1613A and 8290.

Table 1. Average Daily Intake of PCDDs and PCDFs During the Dosing Period.

Congener	Wood, µg/day	Feed, µg/day	Total, µg/day	Wood, %
2,3,7,8-CDD	0.14	(0.21)	0.35	39
1,2,3,7,8-CDD	4.5	(0.42)	4.9	92
1,2,3,4,7,8-CDD	11.7	0.83	12.5	93
1,2,3,6,7,8-CDD	114	2.9	117	98
1,2,3,7,8,9-CDD	24.6	1.7	26.3	94
1,2,3,4,6,7,8-CDD	3,300	85	3,390	98
1,2,3,4,6,7,8,9-CDD	16,500	955	17,450	95
2,3,7,8-CDF	0.08	1.45	1.53	5
1,2,3,7,8-CDF	0.78	1.25	2.03	39
2,3,4,7,8-CDF	1.02	1.04	2.06	50
1,2,3,4,7,8-CDF	9.9	3.53	13.4	74
1,2,3,6,7,8-CDF	12.0	1.66	13.7	88
2,3,4,6,7,8-CDF	11.4	2.49	13.9	82
1,2,3,7,8,9-CDF	0.66	(0.21)	0.87	76
1,2,3,4,6,7,8-CDF	660	22.8	683	97
1,2,3,4,7,8,9-CDF	30	2.08	32.1	94
1,2,3,4,6,7,8,9-CDF	3,900	79	3,980	98

Note: Values in () were not detected. Intake was estimated using half the detection limit.

Results and Discussion

The concentrations PCDDs and PCDFs in milk fat at the end of the dosing period, and at 12 and 32 days post dosing are shown in Figure 1. Congeners with concentrations below the detection limit (2,3,7,8-CDF, 1,2,3,7,8-CDF, and 1,2,3,7,8,9-CDF) and those from which more than 50% of the intake was from feed (2,3,7,8-CDD and 2,3,4,7,8-CDF) are not shown. The decline in concentration of persistent halogenated organics in milk after the end of dosing can be described by a two-compartment first order system.⁵⁾ This system is defined by the following equation

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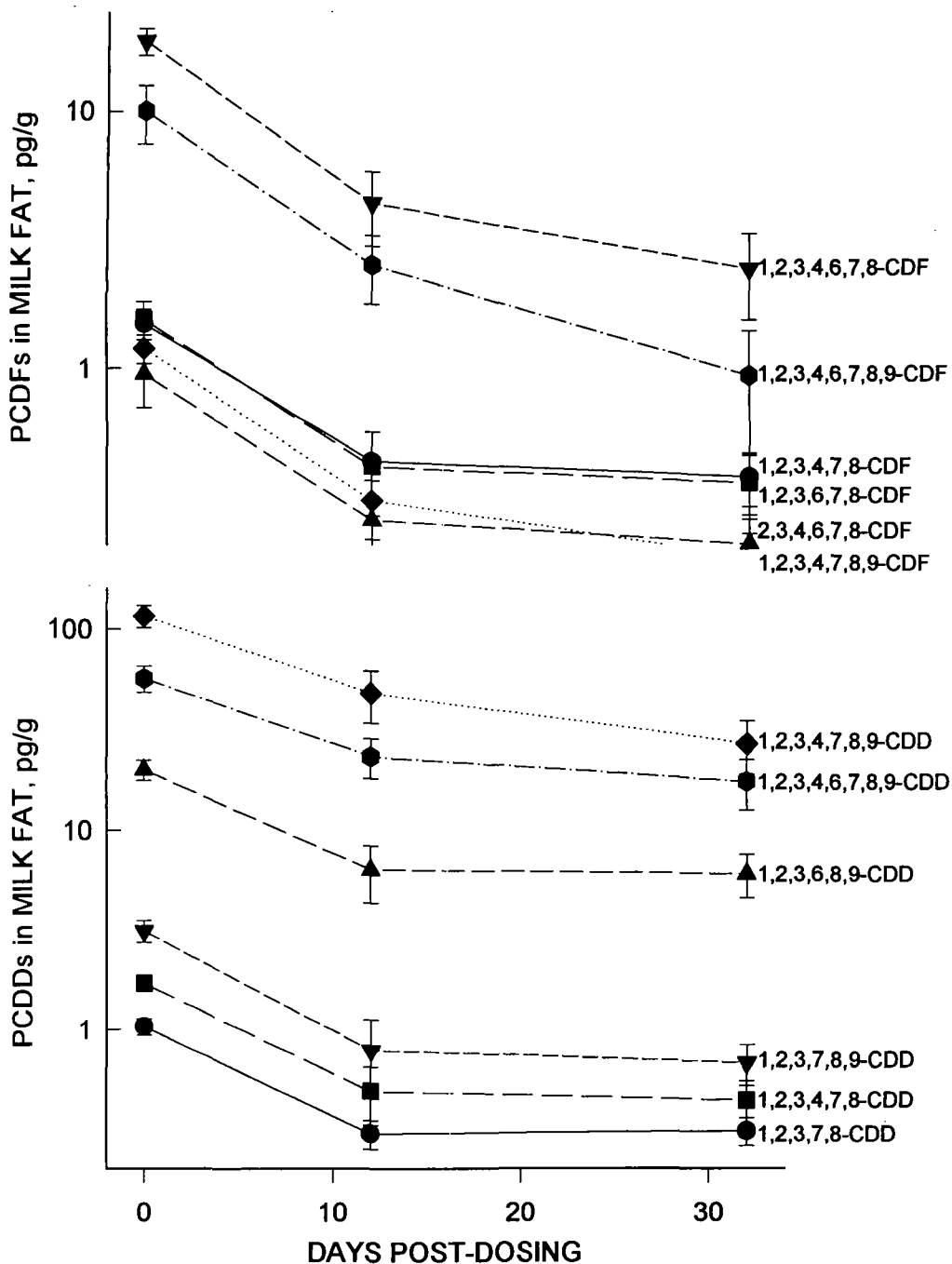


Figure 1. The concentrations PCDDs and PCDFs in milk fat after the end of a 58-day period of administration of pentachlorophenol-treated wood. Congeners with concentrations below the detection limit and those from which more than 50% of the residue was from feed are not shown.

$$C = C_1e^{-k_1t} + C_2e^{-k_2t} \quad (1)$$

where C is concentration at any time, C_1 is the initial concentration of the first compartment, C_2 is the initial concentration of the second compartment, k_1 and k_2 are constants, and t is time. The first term is related to clearance of the gut pool, and the magnitude of k_1 is too large to accurately estimate with 12-hr periods between milkings. The contribution of this term to the milk residue is negligible after 10 days. The second term reflects elimination from storage in lipids. The parameters of this term cannot be estimated accurately in this study because only two points were measured and the time interval between points was much shorter than the typical half-lives of persistent halogenated organics.⁵⁾

The number of data points are inadequate to estimate the parameters of Eq. (1), but the shapes of the concentration curves (Figure 1) are consistent with the model. The data may be used, however, to compare the relative behaviors of the congeners because any animal or analytical factor that affects one congener would affect all. The fractional decline between 0 and 12 days post-dosing would provide a measure of the relative contribution of the two pools (C_1 and C_2) to the initial concentration, and the decline between 12 and 32 days would provide a measure of the relative half-lives of the tissue depletion phase.

The relative declines in concentrations of the congeners for which there is adequate data are shown in Table 2. The results this study indicate that PCDD/Fs behave differently in cattle than one would predict from behavior of other halogenated organics administered under comparable conditions.⁵⁾ Typically, the rate of transport of halogenated organics to or from adipose tissue is inversely related to molecular weight or degree of chlorination. The fraction decline from 0 to 12 days post-dosing should increase with increased chlorination to reflect the slower rate of tissue penetration. Except marginally in the case of 1,2,3,4,7,8,9-CDD and 1,2,3,4,6,7,8,9-CDD, the expected differences did not occur. The results are more anomalous for the 12- to 32-day data when fractional decline is inversely related to half-life. The data suggest that the half-lives of hepta- and octa-CDD/Fs are much shorter than those of the less chlorinated congeners. This is opposite of expectations from work with other compounds.⁶⁾ The shorter half-lives for the highly chlorinated PCDD/Fs have been reported for studies a single dose and or multiple doses during the dry period.^{6,7)}

Data for concentrations in liver and adipose tissue (Table 2) provides a basis for explaining for the unexpectedly rapid rates of elimination of the highly chlorinated PCDD/Fs. All congeners were present in the liver at higher concentrations on a lipid basis than were present in perirenal adipose tissue. Retention in liver was enhanced with increasing chlorination and the enhancement was especially pronounced with hepta- and octa-CDD. The elimination of PCDD/Fs in milk during post-dosing period in this study appears to be a reflection of mobilization from liver rather than adipose tissue.

Conclusions

The pharmacokinetic parameters of PCDD/Fs in cattle appear to differ from other halogenated organics in that the half-lives are shorter than would be predicted for compounds with comparable molecular weights or degrees of halogenation. The high concentrations of PCDD/Fs in the liver may be a result of induction of protein to which the PCDD/Fs may be bound. The significance of the higher concentrations in liver, and the possible differential storage in other tissues should be investigated in future studies. The results suggest that typical pharmacokinetic models halogenated organics may require modifications for PCDD/Fs.

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Table 2. Fractional Concentration Reductions in Milk Fat and Concentrations in Milk and Tissue Lipids at Termination of the Experiment.

Congener	Fractional Reduction		Concentration in Lipid, pg/g		
	Phase 1	Phase 2	Liver	Adipose	Milk
2,3,7,8-CDD	nc	nc	2.1±0.7	0.06±0.01	0.02±0.01
1,2,3,7,8-CDD	0.71±0.6	-0.05±0.19	1.5±0.2	0.39±0.09	0.31±0.05
1,2,3,4,7,8-CDD	0.71±0.27	0.08±0.15	5.9±1.1	0.57±0.20	0.44±0.11
1,2,3,6,7,8-CDD	0.68±0.06	0.05±0.06	20.6±3.9	6.2±1.8	6.0±1.5
1,2,3,7,8,9-CDD	0.74±0.09	0.10±0.20	5.1±1.2	0.81±0.23	0.68±0.16
1,2,3,4,6,7,8-CDD	0.59±0.06	0.44±0.12	1,200±260	50.2±16.7	26.9±7.9
1,2,3,4,6,7,8,9-CDD	0.59±0.04	0.25±0.16	13,500±2,050	29.1±9.8	17.4±4.9
2,3,7,8-CDF	nc	nc	nd(0.7)	nd(0.04)	nd(0.02)
1,2,3,7,8-CDF	nc	nc	nd(0.5)	nd(0.06)	nd(0.02)
2,3,4,7,8-CDF	nc	nc	0.86±0.18	0.16±0.01	0.03±0.03
1,2,3,4,7,8-CDF	0.72±0.04	0.15±0.18	3.4±0.6	0.58±0.15	0.37±0.09
1,2,3,6,7,8-CDF	0.74±0.05	0.14±0.09	2.2±0.5	0.54±0.13	0.35±0.10
2,3,4,6,7,8-CDF	0.74±0.10	0.09±0.35	4.3±0.4	0.38±0.08	0.20±0.06
1,2,3,7,8,9-CDF	nc	nc	nd(0.5)	nd(0.02)	nd(0.03)
1,2,3,4,6,7,8-CDF	0.76±0.04	0.45±0.13	40.0±9.5	5.8±1.8	2.46±0.91
1,2,3,4,7,8,9-CDF	0.75±0.04	0.44±0.12	3.5±0.5	0.41±0.12	0.17±0.05
1,2,3,4,6,7,8,9-CDF	0.74±0.02	0.65±0.12	199±71	3.3±1.3	0.93±0.47

Notes: Means of four cows ± standard deviation. NC - Not calculated because feed accounted for more than 50% of intake while dosing. ND - Nondetected with the detection limit ().

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PCDD/PCDFs, PCBs AND OCPs IN HUMAN BREAST ADIPOSE TISSUE

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Abstract

An isotope dilution procedure is described for the analysis of PCDD/PCDF, PCBs and Organochlorine Pesticides in human breast adipose tissue. The small sample size (~1 g), requires special considerations to optimize the analysis. Preliminary results on 10 US patients show background levels for most targeted analytes, with PCBs #180, 153/132, 138, 74, 170/190 contributing over 50% of the total PCBs. The most prevalent OCPs are p,p'-DDE, trans-nonachlor, oxychlorodane, HCB, dieldrin, beta-HCH and p,p'-DDT.

Introduction

Certain PCBs and organochlorine pesticides (OCPs) have recently been implicated as possible contributors to breast cancer¹⁻⁵. As more evidence becomes available on the estrogenic/antiestrogenic activity of certain PCB and PCDD/PCDF congeners, reliable analytical methods are necessary to conduct epidemiologic studies on the possible links of these chemicals to breast cancer. A challenge to analysts involved in such studies is the limited amount of material available for analysis. In this paper, an isotope dilution procedure is described for the analysis of PCDD/PCDF, PCBs and OCPs in 1 g of human breast adipose tissue.

Experimental methods

Sample preparation. Samples were stored at -20°C until analysis. Samples were thawed, weighed, mixed with anhydrous Na₂SO₄ and homogenized with 1:1 dichloromethane:hexane. Following centrifugation, 1/10 of the volume was analysed for OCPs and PCBs and the remaining 9/10 was analysed for PCDD/PCDFs and coplanar PCBs, as shown in Fig. 1. Labelled internal standards were used for PCDD/PCDFs (all 17 congeners), for PCBs (#77, 126, 169, 28, 52, 47, 101, 105, 118, 153, 180, 194, 209) and for OCPs (HCB, β-HCH, DDT, DDE, Mirex, Dieldrin).

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OCP and PCB analysis. Samples were processed through an automated GPC column (FMS) linked to Florisil columns. The extract was concentrated gently to about 10 μ L and recovery standards were added. The same extract was injected into a HRGC/HRMS (EI) to measure PCBs and into a HRGC/LRMS (NCI) to measure OCPs. The latter procedure is described in detail in a separate abstract⁶⁾.

PCDD/PCDF and coplanar PCB analysis. Samples were passed through columns containing anhydrous Na_2SO_4 and AX21 carbon. The carbon column was eluted with toluene in the reverse direction and the eluate,

concentrated and exchanged to hexane, was cleaned up with a silica and a alumina column. A recovery standard was added and the sample concentrated to 5 μ L. PCDD/Fs and PCBs were analyzed by HRGC/HRMS (Finnigan MAT 90) with a 60m, 0.25 mm id, 0.25 μ m film thickness, DB - 5ms column. The samples were introduced into the GC through a SPI injector. The temperature was held at 220 $^\circ$ C for 2 minutes, increased to 260 $^\circ$ C for at 5 $^\circ$ C/min, and then to 300 $^\circ$ C at 1 $^\circ$ C/min. PFK was used for the lock masses and the MS was operated in a EI mode with multiple ion monitoring.

Lipid determination. Fat content was determined gravimetrically during the PCDD/PCDF cleanup. Standard QA/QC procedures were followed (Method blanks, recovery standards, duplicate analysis)⁷⁾.

Results and discussion

Women undergoing breast surgery (biopsy, lumpectomy, or mastectomy) at Stanford University, California, were recruited in the study. The total study population consists of 50 cases and 50 controls and is expected to be completed by the time of this presentation. Preliminary data from 10 breast adipose samples are shown for PCDD/PCDFs and for the most prevalent PCBs on Tables 1, and 2. The most prevalent PCBs are 180, 153/132, 138, 74, 170/190, comprising over 50% of the Total PCBs measured. Of the 84 PCB congeners

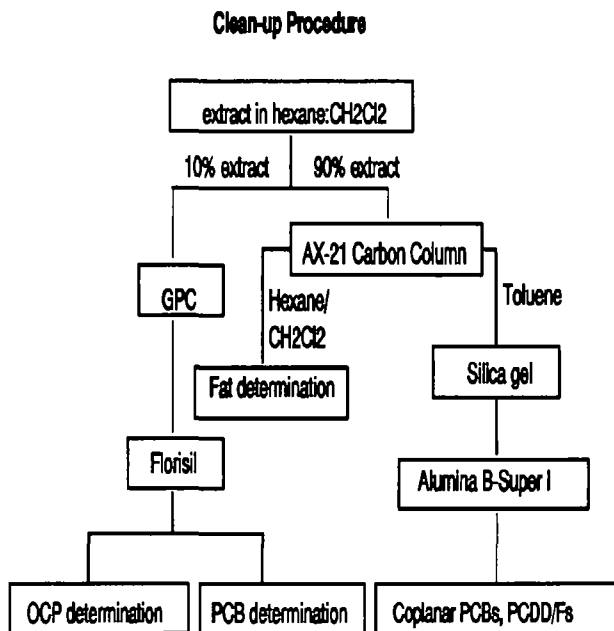


Figure 1: Clean up procedure for analysis of PCDD/F, PCB and OCP in human breast tissue

measured, the 27 shown on Table 2 comprise approximately 90% of the Total PCBs. The most prevalent OCPs in this set of samples are p,p'-DDE, trans-nonachlor, oxychlorane, HCB, dieldrin, β -beta-HCH and p,p'-DDT⁽⁶⁾.

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Table 1: PCDD/PCDF and coplanar PCB concentrations (pg/g) in 10 breast adipose samples

	Min	Max	Median	Mean	SD	CV%
2,3,7,8-Cl4DD	<1	-	-	-	-	-
1,2,3,7,8-Cl5DD	<1	-	-	-	-	-
1,2,3,4,7,8-Cl6DD	2.8	15.1	7.1	8.2	4.2	52
1,2,3,6,7,8-Cl6DD	38.7	93.8	57.3	61.9	18	28
1,2,3,7,8,9-Cl6DD	3.0	16.4	5.8	7.5	4.1	54
1,2,3,4,6,7,8-Cl7DD	32.4	198	67.1	79.3	47	59
OCDD	265	768	420	465	173	37
2,3,7,8-Cl4DF	<1	-	-	-	-	-
1,2,3,7,8-Cl5DF	<1	-	-	-	-	-
2,3,4,7,8-Cl5DF	4.1	15.4	7.6	8.4	3.5	42
1,2,3,4,7,8-Cl6DF	3.5	11.9	4.4	5.8	2.9	51
1,2,3,6,7,8-Cl6DF	3.0	16.8	3.9	6.5	4.6	71
1,2,3,7,8,9-Cl6DF	<1	-	-	-	-	-
2,3,4,6,7,8-Cl6DF	<1	23.3	5.3	7.5	7.5	101
1,2,3,4,6,7,8-Cl7DF	6.8	22.3	11.0	12.0	4.7	39
1,2,3,4,7,8,9-Cl7DF	<2	-	-	-	-	-
OCDF	<2	-	-	-	-	-
PCB-77	<1	75.0	17.7	24.1	26	108
PCB-126	<1	97.3	37.3	41.5	40	97
PCB-169	<2	74.0	17.7	26.5	30	111
I-TEQ	7.8	22.5	14.0	14.7	5.4	37
PCB-TEQ(coplanars)	0.14	10.5	4.0	4.4	4.2	96

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Table 2: Concentrations (ng/g fat) of PCB congeners in 10 breast adipose samples (in decreasing order)

	% of Total	Cumulative %	Min	Max	Median	Mean	SD	CV%
180	17.7	17.7	82	242	136	155	51	33
153/132	13.8	31.5	44	226	96	121	56	46
138	9.8	41.3	16	194	68	86	51	59
74	7.3	48.5	27	177	47	64	45	70
170/190	5.9	54.4	28	79	47	52	17	32
182/187	4.4	58.8	25	57	37	39	10	25
118	4.3	63.1	12	79	37	38	19	50
196/203	4.0	67.1	20	48	34	35	10	29
194	3.9	71.0	18	53	33	34	11	33
156	3.4	74.3	4	97	18	29	28	96
199	3.2	77.6	15	39	30	28	9	32
113/99	2.0	79.5	8	31	15	17	7	41
206	1.7	81.3	7	25	12	15	7	48
183	1.7	82.9	11	20	14	15	4	27
177	1.6	84.5	4	23	13	14	6	43
146/161	1.0	85.5	3	14	9	9	4	44
105/127	0.9	86.4	2	15	7	8	4	50
178	0.9	87.4	4	12	8	8	2	25
171	0.9	88.2	5	10	7	8	2	25
209	0.9	89.1	3	19	6	7	5	71
Total PCBs	89.1	100	451	1501	805	876	303	35

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