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**George F. Fries, Dennis J. Paustenbach, and  
William J. Luksemburg**

U.S. Department of Agriculture, 10300 Baltimore Avenue, Beltsville,  
Maryland 20705, Exponent, 149 Commonwealth Drive, Menlo Park,  
California 94025, and Alta Analytical Laboratory, 5070 Robert J.  
Matthews, El Dorado Hills, California 95762

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## Complete Mass Balance of Dietary Polychlorinated Dibenzo-*p*-dioxins and Dibenzofurans in Dairy Cattle and Characterization of the Apparent Synthesis of Hepta- and Octachlorodioxins

GEORGE F. FRIES,<sup>\*,†</sup> DENNIS J. PAUSTENBACH,<sup>‡</sup> AND WILLIAM J. LUKSEMBURG<sup>§</sup>

U.S. Department of Agriculture, 10300 Baltimore Avenue, Beltsville, Maryland 20705, Exponent, 149 Commonwealth Drive, Menlo Park, California 94025, and Alta Analytical Laboratory, 5070 Robert J. Matthews, El Dorado Hills, California 95762

Mass balances of 2,3,7,8-substituted dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) were measured in cows following administration of pentachlorophenol (PCP)-treated wood. Fecal excretion accounted for the major fraction of all congeners. Recovery in feces increased with increasing chlorination, while storage in body fat and excretion in milk decreased with increasing chlorination. The PCDFs with no chlorines in the 4- and 6-positions were apparently metabolized because residues were not detected in milk and body fat. Storage and excretion of 1,2,3,4,6,7,8-HpCDD and 1,2,3,4,6,7,8,9-OCDD exceeded intake by factors of 1.7 and 3.4, but recovery of other PCDD/Fs did not exceed intake significantly. Excess excretion of OCDD, but not HpCDD, was confirmed in a follow-up study. Synthesis of HpCDD and OCDD did not occur when PCP-treated wood was fermented with rumen microorganisms, and enhanced concentrations of HpCDD and OCDD were not found in gastrointestinal tract contents of dosed animals. Formation of OCDD during incubation of feces spiked with PCP-treated wood led to the conclusion that synthesis was postexcretion during sample preparation.

**KEYWORDS:** Dioxins; furans; pentachlorophenol; wood; PCDD/Fs; synthesis

### INTRODUCTION

Animal products were identified as important contributors to human background exposure to dioxins and related compounds in the U.S. Environmental Protection Agency (EPA) reassessment (1). Contamination of pastures and forage crops by combustion emissions, both particulate and vapor, was considered to be the primary potential source of background residues. This contamination could arise from direct deposition on the crops or by volatilization of residues deposited on soil. However, analyses of tissue samples from a survey of beef cattle research facilities identified several facilities with high-level polychlorinated dibenzo-*p*-dioxin and dibenzofuran (PCDD/F) residues that did not match the congener profiles associated with combustion emissions (2). The concentrations of PCDFs in combustor emissions generally exceed the concentrations of PCDDs (3). In contrast, the residues in beef consisted primarily of PCDDs with the concentration of 1,2,3,6,7,8-HxCDD severalfold greater than the concentrations of the other HxCDDs. This congener profile most closely resembles the profile of pentachlorophenol (PCP) contaminants (3). Because cattle chew

wood, follow-up studies were conducted, and it was demonstrated that high-level residues in beef were associated with facilities in which animals had access to PCP-treated wood (4, 5).

We initiated studies to characterize the transfer of the PCDD and PCDF contaminants in PCP-treated wood to milk and tissues of dairy cows. The transfer of PCDD/Fs to milk during and following the dosing period has been discussed in a previous paper (6). This paper presents the mass balance measurements conducted during the last 5 days of a 58-day dosing period. Results of follow-up studies to confirm and characterize the apparent biosynthesis of HpCDD and OCDD are also included.

### MATERIALS AND METHODS

**Mass Balance Measurements.** The initial study was carried out at the Beltsville Agricultural Research Center (BARC) in 1996 under an experimental protocol and animal management system described previously (6). The animal care protocol was approved by the Beltsville Area Animal Use and Care Committee. The four Holstein cows in midlactation were confined in stalls fitted with rubber mats and bedding was not used. The complete mixed feed, primarily composed of corn and grass silages, hay, and a concentrate mixture, was the same as that fed to all lactating cows in the BARC. Cows were milked at 12-h intervals, and the amount of milk was recorded. The treatment was 3.0 g of ground PCP-treated wood administered once per day by gelatin capsule for 58 days.

\* Address correspondence to this author at 2205 Bucknell Terr., Silver Spring, MD 20902 (e-mail gffries@starpower.net).

† U.S. Department of Agriculture.

‡ Exponent.

§ Alta Analytical Laboratory.

Composite samples of feed, milk, and feces for the mass balance measurements were collected during the last 5 days of the dosing period (days 54–58). The cows were at steady state with respect to concentrations in milk (6). Weighed amounts of the mixed feed were offered to the cows twice daily, and unconsumed feed was collected, weighed and discarded daily. Samples of the feed were obtained daily and stored at 5 °C. The five feed samples were combined, mixed, dried at 60 °C, and ground prior to PCDD/F analysis. Dry matter content of the feed samples was recorded. Water was not sampled because it is not considered an important contributor to the background intake of PCDD/Fs (7).

Bladder catheters were inserted in the cows during the collection period in order to separate urine from feces. Urine was not sampled and analyzed because other work and the low solubility of PCDD/Fs suggested there is little likelihood of the presence of PCDD/Fs in urine (7). Feces were collected in stainless steel pans placed in the gutter, and the output was weighed, mixed, and sampled daily. A 2% aliquot sample of the feces was dried at 60 °C, and dry matter content was recorded. The five one-day samples were combined and ground prior to PCDD/F analysis.

The quantity of PCDD/Fs excreted in milk was derived from analysis of 3-L samples collected from each cow at a single milking on day 56. The fraction of the daily intake of PCDD/Fs that was stored in the body was estimated from the analyses of liver and perirenal fat samples obtained when the animals were slaughtered 32 days postdosing (8). It was assumed that concentrations in perirenal fat were representative of all body fat. The liver was evaluated separately because it is known to be an important storage site for several congeners. Liver weights and body fat contents were estimated from published direct body composition measurements of animals from the BARC herd (9). The quantities of PCDD/Fs excreted in milk from the end of the mass balance measurements until slaughter (day 90) were added to the measurements at time of slaughter to provide an estimate of the burden at the end of dosing. This value was divided by 58 to provide the value for daily net accumulation.

The extraction, cleanup, and quantification of the PCDD/F homologues by high-resolution gas chromatography–mass spectrometry was performed by Alta Analytical Laboratory (El Dorado Hills, CA). Two liters of milk was weighed and evenly divided among three 2-L separatory funnels. Each aliquot was spiked with a battery of  $^{13}\text{C}$  internal standards, and 1 L of ethanol and 42 mL of saturated sodium oxalate were added to each separatory funnel. Each subsample was extracted twice by vigorous shaking with a mixture of 1:1.5 ethanol and hexane and once with hexane only. The extracts were combined and dried by filtering through anhydrous sodium sulfate. The ground solid samples, such as feed (25 g), wood (1 g), feces (10 g), and tissues (25 g), were mixed with 60 g of precleaned sodium sulfate. The samples were spiked with the  $^{13}\text{C}$  internal standard mixture and then Soxhlet extracted for 16 h with a 1:1 mixture of methylene chloride and hexane. Matrix blanks were run with each set of samples. The cleanup of concentrated extracts followed standard methods (10). After cleanup, the extracts were analyzed using high-resolution gas chromatography/high-resolution mass spectrometry following the guidelines of EPA Method 1613A. Typically, recoveries of  $^{13}\text{C}$  internal standards were in the range of 80–100%, and average recoveries did not differ among congeners or matrices. Concentrations were reported on both a wet weight and lipid basis for milk and tissues and on a dry matter basis for feeds, feces, wood, and intestinal tract contents.

**Follow-up Studies.** The excretion of HpCDD and OCDD in feces during the initial mass balance study was 2–4-fold greater than intake. Several follow-up studies were conducted in 1997 and 1998 to confirm the result and to partially characterize the conditions of the apparent biosynthesis of PCDD/Fs. These studies in sequence included an *in vitro* fermentation study of PCP-treated wood by rumen microorganisms, a limited replication of the mass balance measurements with determination of congener concentrations in gastrointestinal tract contents at two locations, and incubation of fresh feces spiked with PCP-treated wood.

**In Vitro Fermentations.** Studies utilizing rumen microorganisms were carried out with an adaptation of a standard method for conducting *in vitro* rumen fermentations (11). The test substrate was ground

alfalfa–orchardgrass hay spiked with 1% of the same PCP-treated wood as used for dosing cows in the mass balance study. Hay without added wood was the untreated control. Rumen fluid, obtained from a Holstein cow fitted with a cannula, was mixed on an equivalent basis with artificial saliva under anaerobic conditions. The initial pH of the mixture was 6.8. Replicate samples of substrate were placed in 60 mL serum bottles with 30 mL of the rumen fluid mixture, flushed with  $\text{CO}_2$ , and incubated for 48 h at 39 °C. The fermentation was terminated by the addition of 1 mL of 50%  $\text{H}_2\text{SO}_4$ , and total contents of the bottles were analyzed for PCDD/Fs. Unfermented controls consisted of samples that were acidified immediately after preparation of the mixture.

**Repeat Mass Balance.** Four cows, two dosed and two untreated controls, were used in a second mass balance study to confirm the results of the original study. The two cows were dosed with the same wood at the same daily dose rate as in the original study. The dosing period was only 28 days because this length of time is sufficient to attain stable concentrations in the gastrointestinal tract and milk fat (6). All other aspects of animal feeding and management were identical to those of the original study. The collection period was 5 days, and milk samples were obtained a single time on the third day. The two cows dosed with wood were slaughtered within 24 h after the end of dosing. Samples of contents from several areas of the gastrointestinal tract were obtained and immediately acidified to pH 2 with phosphoric acid to inhibit microbial activity and inactivate enzymes. The samples were neutralized with sodium carbonate before drying and grinding in the usual manner. Contents of ash and several metals were determined in the samples of feed, intestinal tract contents, and feces samples. Sparingly absorbed metals (Zn, Cu, Mn, and Sr) were used as markers to adjust concentrations for dry matter loss in the gastrointestinal tract and to correct for the mass added to samples of tract contents by the acidification and neutralization procedures.

**Fecal Incubation.** Fresh cow feces was spiked with the wood–hay substrate as used in the fermentation study to determine if the synthesis of PCDD/Fs occurred in the period between excretion and the completion of sample preparation. The feces were spiked at the rate of 1 g of substrate/100 g of feces. Control samples were immediately acidified to stop microbial activity, whereas experimental samples were allowed to stand at ambient temperature (20 °C) for 24 h to simulate feces handling under barn conditions. Sample preparation for analysis followed the neutralization, drying, and grinding procedures outlined for the intestinal tract contents. As with gastrointestinal contents, metal content was used to correct concentrations in the control samples for the weight added by the acidification and neutralization procedures.

## RESULTS AND DISCUSSION

**Mass Balance Measurements.** The results of the original mass balance study with daily intake of PCDD/Fs partitioned among fecal excretion, milk excretion, and storage are presented in **Table 1**. Although stable concentrations of PCDD/Fs were reached in 28 days (6), it should not be inferred that the cows were at steady state because factors such as amount of milk produced and body fat pool sizes are never stable (9). The intake values include the PCDD/Fs present in both the feed and the PCP-treated wood, but feed made important contributions to intake only in the cases of TCDD, TCDF, and the PeCDFs. The concentrations of the three PCDFs without chlorines in the 4- and 6-positions (TCDF, 1,2,3,7,8-PeCDF, and 1,2,3,7,8,9-HxCDF) were below the detection limits. The absence of detectable residues is probably a result of metabolism because these congeners are metabolized in laboratory species (12, 13). The congener concentrations below the detection limits in one or more matrices are not shown because it is not possible to calculate a proper mass balance. The percentage of PCDD/Fs excreted in milk, commonly termed the carry-over ratio (COR), is within the range of values reported in the literature (6). Feces was the primary route of excretion of all congeners, and this route increased in importance with increased chlorination. Data for all congeners listed in **Table 1**, except HpCDD, OCDD,

**Table 1.** Fractional Disposition of the Daily Intake of PCDDs and PCDFs in Four Cows Administered Pentachlorophenol-Treated Wood for 58 Days

congener <sup>a</sup>	intake, pg/day	recovery, %			
		storage ± SD <sup>b</sup>	milk ± SD	feces ± SD	total ± SD
1,2,3,7,8-PeCDD	4,500	18.4 ± 5.2	22.6 ± 10.4	64.5 ± 11.6	105.5 ± 17.5
1,2,3,4,7,8-HxCDD	12,600	10.0 ± 3.5	12.8 ± 5.7	121.5 ± 22.1	144.3 ± 17.2
1,2,3,6,7,8-HxCDD	117,000	12.2 ± 3.2	15.3 ± 6.7	68.7 ± 12.9	96.1 ± 14.6
1,2,3,7,8,9-HxCDD	26,300	7.1 ± 2.0	11.0 ± 4.9	80.1 ± 25.1	108.2 ± 24.7
1,2,3,4,6,7,8-HpCDD	3,380,000	3.1 ± 0.9	3.1 ± 1.4	168.2 ± 21.4	174.4 ± 23.2
1,2,3,4,6,7,8,9-OCDD	17,400,000	0.4 ± 0.2	0.3 ± 0.1	338.2 ± 38.9	338.9 ± 39.2
1,2,3,4,7,8-HxCDF	13,400	9.3 ± 2.7	10.9 ± 4.6	66.8 ± 11.8	87.0 ± 15.0
1,2,3,6,7,8-HxCDF	13,600	8.6 ± 2.2	11.2 ± 5.2	72.1 ± 15.5	92.0 ± 17.1
2,3,4,6,7,8-HxCDF	13,800	5.8 ± 1.8	7.2 ± 3.5	99.0 ± 29.2	112.0 ± 28.3
1,2,3,4,6,7,8-HpCDF	683,000	1.8 ± 0.5	2.4 ± 1.1	87.3 ± 15.2	91.5 ± 16.4
1,2,3,4,7,8,9-HpCDF	32,100	2.6 ± 0.5	3.3 ± 1.6	83.9 ± 16.1	89.8 ± 16.7
1,2,3,4,6,7,8,9-OCDF	3,980,000	0.2 ± 0.1	0.2 ± 0.1	115.2 ± 27.2	115.6 ± 27.3

<sup>a</sup> Congeners not listed are those with concentrations below the limit of quantification in feed and the PCDFs that are metabolized (6). <sup>b</sup> Storage in body fat was estimated by measuring concentrations in perirenal fat and multiplying by the estimated fat content of the animal (9). Storage also includes estimates for liver, which was analyzed separately.

**Table 2.** Fractional Disposition of the Daily Dose of PCDDs and PCDFs in Two Cows Administered Pentachlorophenol-Treated Wood for 28 Days<sup>a</sup>

congener	intake, pg/day	recovery, %			
		storage <sup>b</sup>	milk	feces	total
1,2,3,7,8-PeCDD	4,500		66.5	62.6	
1,2,3,4,7,8-HxCDD	11,700	15.2	31.7	81.3	128.2
1,2,3,6,7,8-HxCDD	114,000	16.5	34.5	80.1	131.1
1,2,3,7,8,9-HxCDD	24,600	10.2	25.9	107.2	143.2
1,2,3,4,6,8,9-HpCDD	3,300,000	3.3	4.9	91.1	99.2
1,2,3,4,6,7,8,9-OCDD	16,500,000	2.4	0.9	183.8	187.1
1,2,3,4,7,8-HxCDF	9,900	12.8	32.1	83.1	128.0
1,2,3,6,7,8-HxCDF	12,000	6.8	31.4	123.4	161.5
2,3,4,6,7,8-HxCDF	114,000	10.2	21.4	70.8	102.3
1,2,3,4,6,7,8-HpCDF	660,000	1.9	4.6	99.1	105.6
1,2,3,4,7,8,9-HpCDF	30,000	4.3	7.3	74.7	86.4
1,2,3,4,6,7,8,9-OCDF	3,900,000	0.5	0.4	96.7	97.6

<sup>a</sup> Congeners not listed are those with concentrations below the limit of quantification in feed and the PCDFs that are metabolized (6). <sup>b</sup> Storage in body fat was estimated by measuring concentrations in perirenal fat and multiplying by the estimated fat content of the animal (9). Storage also includes estimates for liver, which was analyzed separately. No values are listed for storage of 1,2,3,7,8-PeCDD because concentrations in fat were below the detection limits.

and possibly 1,2,3,4,7,8-HxCDD, indicate that the cows were near steady state because 100% was included within the range of recovery values ± one standard deviation.

The excretion of HpCDD and OCDD in excess of intake was originally attributed to synthesis in the animal. The low concentrations of these congeners in milk at the start of dosing would indicate that body stores were too low to account for the level of excretion in feces even if there was a sudden release of residues (6). Fecal excretion in excess of intake of these congeners also has been reported for humans (14). Formation of these congeners during composting and digestion of sewage sludge, which are microbial processes, has been reported (15, 16). Because the gastrointestinal tracts of animals include active microbial systems, it was reasonable to consider these organs as the site, and fermentation by microorganisms as the mechanism, of formation of HpCDD and OCDD from precursors in the PCP-treated wood.

**Follow-up Mass Balance.** These measurements only partially confirmed the results of the first study (Table 2). The fractions of intake estimated to be stored in body fat were similar in both studies, but the fraction transferred to milk was greater in the second study. This was probably a function of the level of milk

**Table 3.** Concentrations of PCDDs and PCDFs Due to the Fermentation of Pentachlorophenol-Treated Wood by Rumen Microorganisms *In Vitro*<sup>a</sup>

congener	pg/sample			
	background (N = 1)	substrate (N = 1)	0 h (N = 2)	48 h (N = 3)
2,3,7,8-TCDD	(1.1)	0.75	(1.5)	(1.5)
1,2,3,7,8-PeCDD	(1.0)	6.5	9.0	8.2
1,2,3,4,7,8-HxCDD	(0.7)	19	24	23
1,2,3,6,7,8-HxCDD	(0.7)	185	185	200
1,2,3,7,8,9-HxCDD	(0.7)	50	57	41
1,2,3,4,6,7,8-HpCDD	43	7,000	7,200	7,300
1,2,3,4,6,7,8,9-OCDD	410	65,000	65,500	66,000
2,3,7,8-TCDF	(1.1)	0.39	(1.4)	(0.9)
1,2,3,7,8-PeCDF	(1.3)	1.6	4.0	2.4
2,3,4,7,8-PeCDF	(2.0)	1.8	4.9	3.4
1,2,3,4,7,8-HxCDF	(0.6)	16.5	25	21
1,2,3,6,7,8-HxCDF	(0.7)	15.5	24	25
2,3,4,6,7,8-HxCDF	3.4	18.5	26	20
1,2,3,7,8,9-HpCDF	(0.4)	2.4	7.8	3.4
1,2,3,4,6,7,8-HpCDF	14	1650	1,750	1,700
1,2,3,4,7,8,9-HpCDF	(0.6)	48	52	52
1,2,3,4,6,7,8,9-OCDF	48	6,000	7,350	7,300

<sup>a</sup> Values in parentheses are half the detection limit for concentrations below the detection limit. Each fermentation sample contained 0.5 g of substrate, which was 1% PCP-treated wood in ground alfalfa-orchardgrass hay. Fermentations were stopped by acidification to pH 2.

fat production, which was an average of 1.66 kg/day in the second study compared to an average of 0.85 kg/day in the first. The fractional excretion in feces provided the largest discrepancies between the first and second balance measurements. As in the first experiment, excretion of OCDD in feces significantly exceeded intake, but excess excretion of HpCDD was not confirmed. The large relative standard deviations for the fecal excretion values (Table 1) make it difficult to draw conclusions concerning the excess recovery of the other congeners in the second study. The variability of fecal values was not due to the basic experimental technique because dry matter disappearance, a basic calculation in balance measurements, was highly reproducible at 71 ± 3 and 70 ± 1% for the two studies.

**Microbial Fermentations.** The quantities of PCDD/Fs in the control and experimental fermentation bottles are presented in Table 3. The quantities of PCDDs in the 0- and 48-h samples do not differ from that provided by the substrate, which indicates there is no biosynthesis of HpCDD and OCDD under these conditions. A number of PCDFs, most notably OCDF, were

higher in amounts in 0- and 48-h samples than in the substrate sample, which did not have the added rumen fluid and mineral solution. The small number of replicates, however, does not permit one to draw conclusions concerning chemical synthesis because the differences are within the normal analytical variation. The *in vivo* results (Tables 1 and 2) also do not support the conclusion the PCDFs are synthesized during fermentation with rumen organisms.

The measured concentration of PCP in the substrate was 53  $\mu\text{g/g}$ , which is equivalent to  $\sim 0.8 \mu\text{g/mL}$  in the 30-mL fermentation mixture. Several measurements were used to determine if the level of PCP in the substrate had an adverse effect on microbial activity. Fatty acid production was increased slightly, whereas gas production was slightly decreased in the samples containing PCP-treated wood. These observations and others detailed elsewhere indicate that microbial activity was not affected significantly by the treatment (17). The effect of PCP on rumen microorganisms in comparable fermentation systems has been examined (18). No adverse effects on fiber digestion were noted when the PCP concentrations in the fermentation mixture were 10  $\mu\text{g/mL}$ . We conclude that the concentrations of PCP in our fermentations were too low to affect fermentation adversely and that reliable inferences concerning PCDD synthesis can be drawn from these samples.

The failure to demonstrate synthesis of HpCDD and OCDD in these fermentations with rumen organisms is not necessarily in conflict with the work on composting and sewage sludge digestion (15, 16). Our system is completely anaerobic, whereas composting is aerobic and the sewage sludge digestion system was described as semi-anaerobic. Peroxidase enzymes also have been shown to convert PCP to OCDD (19). Our negative results in an anaerobic system together with the literature reports suggest that the formation of HpCDD and OCDD in microbial systems is strictly an oxidative process.

**Gastrointestinal Tract Analyses.** The PCDD/F analysis of the contents of two sites in the gastrointestinal tract of animals slaughtered at the end of the 28-day dosing period was another means to determine if dioxins were formed *in vivo* (Table 4). The two sites selected were the rumen and the ileum. The rumen in the upper tract is the site of active anaerobic fermentation and would not be a site for recycling large molecules such as PCDD/Fs from the bloodstream. The ileum in lower tract would contain PCDD/Fs recycled to the gastrointestinal tract via the bile or diffusion across the intestinal wall if synthesis occurred in liver or other tissues. Concentrations in the diet were a calculated value based on the total PCDD/F intake from wood and feed divided by total feed intake. Approximately 70% of the dry matter in the diet is digested and absorbed during passage through the gastrointestinal tract. The concentrations in the tract contents and feces were adjusted for dry matter disappearance by use of unabsorbed metals as markers. These markers are listed in the Table 4. The adjusted concentrations of PCDD/Fs in the rumen did not differ significantly from the concentrations in diet, confirming that there was no synthesis or absorption at this site. The adjusted concentrations of PCDD/Fs in the ileum are somewhat lower than in the diet. In theory, this could be interpreted as absorption from this section of the gastrointestinal tract, but a better interpretation would be that the metal ions are not transported through the gastrointestinal tract at the same rate as the PCDD/Fs. Metal ions would be in aqueous solution, whereas the PCDD/Fs probably associated with the fiber fraction. As noted in mass balance results (Table 2), OCDD is the only congener with enhanced concentrations in feces.

**Table 4.** Relative Concentrations of PCDDs and PCDFs in Feed, Intestinal Tract Contents, and Feces of Two Cows Administered Pentachlorophenol-Treated Wood<sup>a</sup>

congener	pg/g			
	diet	rumen	ileum	feces
2,3,7,8-TCDD	0.02	(0.09)	(0.05)	(0.04)
1,2,3,7,8-PeCDD	0.25	0.24	0.26	0.14
1,2,3,4,7,8-HxCDD	0.62	0.76	0.78	.50
1,2,3,6,7,8-HxCDD	5.8	5.0	3.6	4.6
1,2,3,7,8,9-HxCDD	1.3	1.5	1.1	1.4
1,2,3,4,6,7,8-HpCDD	167	136	92	152
1,2,3,4,6,7,8,9-OCDD	860	940	620	1540
2,3,7,8-TCDF	0.07	(0.07)	(0.04)	(0.04)
1,2,3,7,8-PeCDF	0.10	0.08	0.13	0.04
2,3,4,7,8-PeCDF	0.10	0.10	0.14	0.06
1,2,3,4,7,8-HxCDF	0.66	0.60	0.61	0.48
1,2,3,6,7,8-HxCDF	0.67	0.62	0.71	0.80
2,3,4,6,7,8-HxCDF	0.68	0.69	0.82	0.48
1,2,3,7,8,9-HxCDF	0.04	0.10	0.29	(0.04)
1,2,3,4,6,7,8-HpCDF	34	37	23	34
1,2,3,4,7,8,9-HpCDF	1.6	1.4	1.1	1.2
1,2,3,4,6,7,8,9-OCDF	197	202	131	190

<sup>a</sup> Values in parentheses are half the detection limit for concentrations below the detection limit. All values except diet are an average of two cows. Concentrations in diet were calculated from the measured feed intake and the concentration in wood (6). Concentrations in gastrointestinal tract contents and feces were corrected for dry matter disappearance, and the mass added by acidification-neutralization to the original weight of dry matter represented in the diet using Zn, Cu, Mn, and Sr as markers.

A major impetus for the analysis of gastrointestinal tract contents was to examine the possibility that the synthesis could occur in the liver from suitable precursors. Synthesis of OCDD from the predioxin, nonachloro-2-phenoxyphenol, was demonstrated in laboratory animals (20). The rate of formation was increased when OCDD was administered concurrently. If synthesis had occurred, and if the material was recycled to the gastrointestinal tract, enhanced concentrations of OCDD should have been found in the ileum. The cows had enhanced concentrations of OCDD in the liver; it is not known whether the high concentrations were due to synthesis or the sequestering of OCDD that occurs when dose rates are high (21). Recycling via bile would not be expected because the PCDD/Fs have generally not been found in bile (21). These findings with the gastrointestinal contents support the conclusion that synthesis of OCDD occurred after the feces was excreted.

**Fecal Incubation.** The fecal incubation studies were carried out to examine the possibility that HpCDD and OCDD synthesis occurred postexcretion. The results (Table 5), although not great enough to be considered conclusive, did demonstrate increased concentrations of HpCDD and OCDD after incubation of feces for 24 h at ambient temperatures. This suggests the possibility that our original observations (Table 1) involved synthesis of the compounds after excretion while the feces was in the barn prior to collection and processing. This time period could be as long as 24 h with several additional hours for sample processing and the time required to reach a temperature in the drying oven that would destroy microorganisms and enzymes.

**Conclusions.** Our work demonstrated that OCDD is synthesized in cattle, or in the feces postexcretion, when the animals have been exposed to PCP-treated wood. Evidence for synthesis of HpCDD was inconsistent, but research with other systems such as sewage treatment and composting indicates that HpCDD is formed in these systems. Thus, it is likely that HpCDD is formed in our system. Although the evidence from our studies suggested that the synthesis is postexcretion, more comprehen-

**Table 5.** Concentrations of PCDDs and PCDFs in Feces Spiked with Pentachlorophenol-Treated Wood and Incubated at Ambient Temperatures for 24 h<sup>a</sup>

congener	control, pg/g	incubated, pg/g
2,3,7,8-TCDD	(0.21)	(0.3)
1,2,3,7,8-PeCDD	1.1	1.2
1,2,3,4,7,8-HxCDD	2.6	2.6
1,2,3,6,7,8-HxCDD	23.7	24.5
1,2,3,7,8,9-HxCDD	7.1	6.9
1,2,3,4,6,7,8-HpCDD	610	750
1,2,3,4,6,7,8,9-OCDD	7,100	8,340
2,3,7,8-TCDF	0.43	0.44
1,2,3,7,8-PeCDF	1.1	1.0
2,3,4,7,8-PeCDF	1.5	1.7
1,2,3,4,7,8-HxCDF	5.2	5.6
1,2,3,6,7,8-HxCDF	5.0	5.2
2,3,4,6,7,8-HxCDF	4.1	4.4
1,2,3,7,8,9-HxCDF	0.65	0.69
1,2,3,4,6,7,8-HpCDF	160	160
1,2,3,4,7,8,9-HpCDF	7.1	7.8
1,2,3,4,6,7,8,9-OCDF	900	890

<sup>a</sup> Values in parentheses are half the detection limit for concentrations below the detection limit. Concentrations in the control were adjusted for the weight added by acidification and neutralization based on the dilution of Zn, Cu, Ni, Mn, Fe, and Sr.

sive studies would be required to provide definitive answers to this question. There is no information on the precursors of HpCDD and OCDD, but there are a number of dimers such as the nonachlorophenoxyphenols that are contaminants in PCP (20, 22). These dimers can form dioxins under the appropriate conditions. The predioxin dimers are also logical as precursors because concentration would not be as important a factor in the reaction as it would be in attempting to react two monomers. It also has been suggested the dioxins could be formed during sample preparation and analysis if the predioxins are subjected to heat and basic conditions (20).

The significance of the observations from this and related studies is difficult to evaluate. Both HpCDD and OCDD have low toxicity equivalence so that these potential additions of HpCDD and OCDD to the environment are not of great health significance. Some of the contaminant dimers present in PCP would form PCDFs under the conditions cited for the formation of PCDDs, but no evidence for this formation was found in our studies. A possibly more important question is whether there are dimers with fewer chlorines that could form some of the more toxic PCDD congeners. The greatest difficulty in conducting definitive studies on this question involves the large relative variations associated with collecting and analyzing samples from some environmental matrices. The high costs of PCDD/F analyses probably preclude the resolution of these issues in studies with environmental samples, but properly designed laboratory studies with potential precursors might provide useful insights.

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