

A Congener Specific Evaluation of Transfer of Chlorinated Dibenzop-dioxins and Dibenzofurans to Milk of Cows following Ingestion of Pentachlorophenol-Treated Wood

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Pentachlorophenol (PCP) treated wood has been hypothesized as an important source of dioxins in milk and beef. This phase of studies to evaluate the hypothesis involved the administration of PCP-treated wood to cows and measurement of the transfer of polychlorinated dibenzo-*p*-dioxins (PCDD) and dibenzofurans (PCDF) to milk. The 3 g/day dose of ground wood was administered to four cows for 56 days. This dose provided a PCP intake of 4.8 mg/day and PCDD/F intakes in the range of 0.3 μ g/day for 2,3,7,8-TCDD to 17 000 μ g/day for 1,2,3,4,6,7,8,9-OCDD. Steady state with respect to excretion in milk was reached because concentrations of PCDD/Fs in milk fat were reasonably constant from day 28 through day 56. The PCDFs without chlorine in the 4 and 6 positions were metabolized and not transported to milk. The other PCDFs and all PCDDs had intake adjusted concentrations in milk that were inverse to the number of chlorines. Variations among cows in concentrations of specific congeners were small and were not related to body weight, dry matter intake, or production of milk and milk fat. The transfer coefficients calculated for the PCDD/Fs in this study provide tools for reducing uncertainty in risk assessments.

Introduction

The background concentrations of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in foods are considered to be the primary source responsible for producing mean body burdens of 28 pg/g TCDD toxic equivalents (TEQ) in adipose tissue of the general population of the United States (1). Meat and dairy products are thought to provide as much as 90% of human exposure

from foods (2, 3). Forages and pastures contaminated by aerial deposition of PCDD/Fs from combustion sources are considered the major pathways of uptake in cattle. Papers and guidance documents for calculating estimated exposures by these pathways are available (2, 4, 5).

The most prevalent PCDD/Fs detected in surveys of the United States beef supply were 1,2,3,6,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD, 1,2,3,4,6,7,8-HpCDD, and OCDD (6, 7). High concentrations of PCDDs relative to PCDFs, and of 1,2,3,6,7,8-HxCDD relative to other HxCDDs, are typical of the contaminants of chlorophenol, whereas PCDFs are typically the dominant chemicals in combustion emissions (8). Animal confinement and housing facilities built before uses of pentachlorophenol (PCP) were restricted during the 1980s often contain PCP-treated wood (9). The importance of these facilities as reservoir sources of PCDD/Fs was suggested by finding PCP-treated wood in several facilities that produced beef with higher than average PCDD/F concentrations (10).

Reliable measurements of the transfer coefficients of PCDD/Fs from feed to milk and adipose tissue of cattle are required to produce dependable exposure assessments and to infer sources based on congener profiles found in meat and milk. Transfer of PCDD/Fs to milk and tissues of lactating cattle has been measured in studies that have one or more deficiencies (11-16). These deficiencies include inadequate replication, use of animals in nonrepresentative stages of lactation, failure to collect or report data on feed intake and production, and the failure to obtain data on the full range of toxic congeners.

This paper describes the first phase of studies designed to characterize the transport of PCDD/Fs to the milk and tissues of dairy cows that had been dosed with PCP-treated wood. An additional objective was to extend and improve the reliability of the database on the uptake and elimination of PCDD/Fs in milk and tissues. The transport of PCDD/Fs to milk is evaluated in this paper. Mass balance, depuration, tissue residues, and evaluation of the significance of PCP-treated wood as a PCDD/F source will be included in subsequent papers.

Materials and Methods

Four Holstein cows in mid to late lactation were used in the study (Table 1). The work, conducted at the Beltsville Agricultural Research Center (BARC), followed a protocol approved by the Beltsville Area Animal Use and Care Committee. The design and methods are described in more detail in the Supporting Information on the ES&T Web site.

The housing facilities for the cows contained no PCP-treated wood, and the animals had no known past exposure to treated wood. The cows were offered a complete mixed diet that was typical of diets for lactating cows at BARC (Table S.1, Supporting Information section). Feed intake was measured from day 54 through day 58 of dosing. The PCDD/F analysis of the feed is shown in Table 2. Milk production was measured daily, and fat content was determined monthly as part of the routine record keeping at BARC.

A 3.0 g/day dose of ground PCP-treated wood was administered to each cow for 58 days. The treated wood was obtained from an unused structure at BARC. The date of treatment of the wood and the manufacturer of the PCP are not known. The analysis of a composite wood sample is shown in Table 2.

Milk samples for this phase of the study were collected from each cow at a single milking at 14-day intervals. Samples of the bulk milk from the BARC herd were obtained at the

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The average values of the transfer coefficients are presented in Table 3. The concentrations of 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD in feed were below the detection limits. Concentrations of half the detection limits were used for feed when calculating the coefficients listed for these PCDDs.

where C_{MF} is the concentration in milk fat (pg/g) and C_{diet} is the concentration in dry matter of the diet (pg/g) and C_{milk} is the concentration in whole milk (pg/kg) and I is the intake (mg/day), and

$$BTF = C_{milk}/I \quad (2)$$

where C_{milk} is the concentration in whole milk (pg/kg) and I is the intake (mg/day), and

$$CR = 100 \cdot A_{milk}/I \quad (3)$$

where CR is expressed as percent (%), A_{milk} is amount excreted in milk (pg/day), and I is intake (pg/day). The three transport coefficients were calculated using data from all sampling times during the dosing period. Feed intake

was measured only during the 5-day period beginning on day 54, but daily dry matter intake for cows such as those in this study would vary less than 5% over the period from day 28 to day 56 (24). Therefore, it is reasonable to use the intake measured from day 54 to day 58 as the value for dry matter intake at days 28 and 42 when calculating total congener intake and the apparent concentrations in the total diet.

FIGURE 2. Concentrations of 2,3,7,8-substituted furans in the milk fat of cows administered 3 g/day pentachlorophenol-treated wood for 58 days. Each point is the mean and standard deviation of four cows. The 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, and 1,2,3,7,8-HxCDF congeners are not shown because occurrence of residues at concentrations greater than the 0.01 to 0.02 pg/g detection limits were inconsistent.

FIGURE 1. Concentrations of 2,3,7,8-substituted dioxins in the milk fat of cows administered 3 g/day pentachlorophenol-treated wood for 58 days. Each point is the mean and standard deviation of four cows.

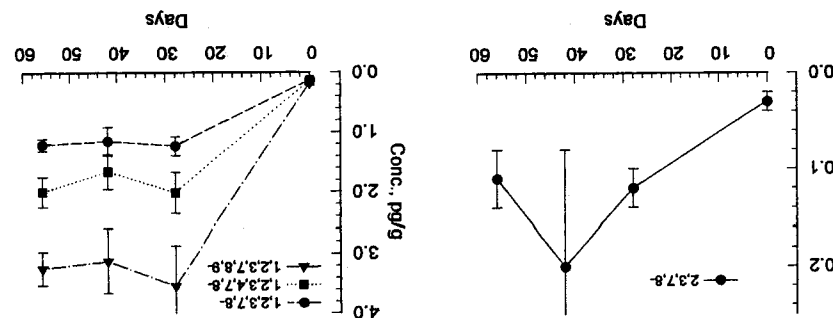
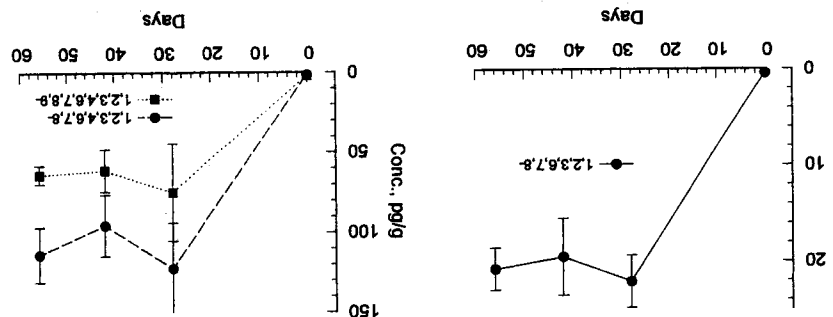
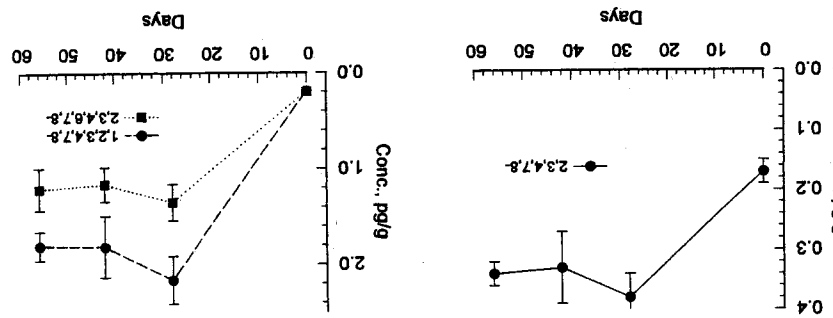
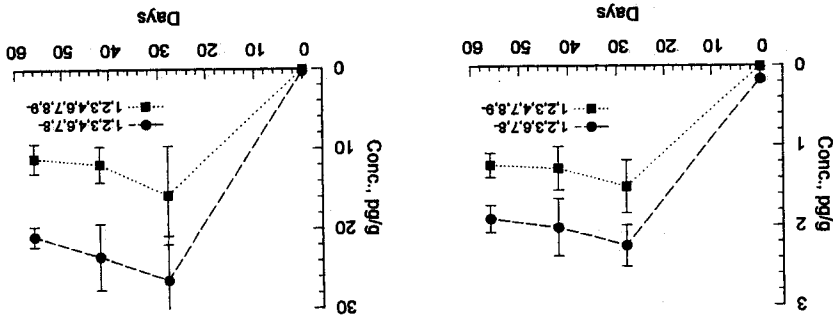


TABLE 3. Mean Bioconcentration Factors (BCFs), Biotransfer Factors (BTFs), and Carry-Over Rates (CR) of PCDD and PCDF Congeners of Four Cows Dosed with PCP-Treated Wood*

Congener	BCF		BTF, (pg/kg)/(ng/d)		CR, %	
	mean ± SD	CV	mean ± SD	CV	mean ± SD	CV
2,3,7,8-PCDDs	7.1 ± 4.2	58	14 ± 7.5	52	35 ± 25	73
1,2,3,7,8-PCDD	5.0 ± 1.2	24	10 ± 1.0	10	24 ± 10	42
1,2,3,4,7,8-PCDD	3.1 ± 0.8	26	6.2 ± 0.8	13	15 ± 6	42
1,2,3,6,7,8-PCDD	3.7 ± 0.9	24	7.4 ± 0.8	10	18 ± 7	41
1,2,3,7,8,9-PCDD	2.6 ± 0.7	26	5.2 ± 0.6	11	13 ± 6	43
1,2,3,4,6,7,8-PCDD	0.68 ± 0.22	32	1.4 ± 0.3	18	3.3 ± 1.6	45
1,2,3,4,6,7,8,9-PCDD	0.08 ± 0.03	40	0.16 ± 0.04	27	0.39 ± 0.22	57
2,3,7,8-PCDFs	<0.01		<0.1		<0.1	
1,2,3,7,8-PCDF	3.5 ± 0.7	19	7.2 ± 1.1	15	17 ± 6	37
1,2,3,4,7,8-PCDF	3.0 ± 0.7	23	6.0 ± 0.7	12	14 ± 6	41
1,2,3,6,7,8-PCDF	3.1 ± 0.8	24	6.3 ± 0.7	10	15 ± 6	42
2,3,4,6,7,8-PCDF	1.9 ± 0.5	26	3.7 ± 0.5	13	8.9 ± 3.9	43
1,2,3,7,8,9-PCDF	<0.01		<0.10		<0.1	
1,2,3,4,6,7,8-PCDF	0.72 ± 0.21	30	1.4 ± 0.2	16	3.5 ± 1.7	47
1,2,3,4,6,7,8-PCDF	0.87 ± 0.27	31	1.7 ± 0.3	16	4.3 ± 2.1	48
1,2,3,4,6,7,8,9-PCDF	0.07 ± 0.03	35	0.14 ± 0.04	31	0.33 ± 0.21	63

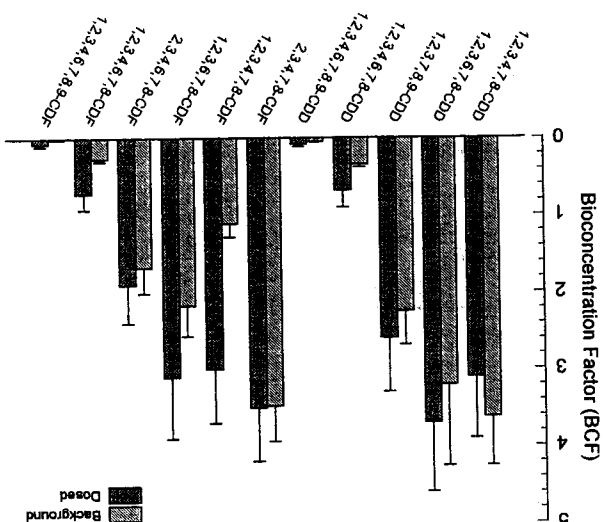
* Values are the mean ± standard deviation (SD) of 12 observations involving four cows at three times. CV is the coefficient of variation (%). Concentrations of 2,3,7,8- and 1,2,3,7,8-PCDD in feed were below the limit of quantitation, and half of the detection limit was used in calculations for these congeners. Detection of 2,3,7,8- and 1,2,3,7,8-PCDF was inconsistent, and only the nominal upper limits based on the detection limits are shown.

This assumption is not serious in the case of 1,2,3,7,8-PCDD because approximately 95% of the estimated intake was from PCP-treated wood. In the case of 2,3,7,8-TCDD, however, less than 50% of the estimated intake was from wood and the coefficients could be in serious error if the actual concentration in feed was markedly above or below half the detection limit.

The differences in transfer coefficients among the three sampling periods were not significant. The greatest source of variation among the transfer coefficients for individual congeners was attributed to differences among cows. The coefficients of variation (CVs) were calculated to determine which of the methods of expressing transfer provided the least variation among animals. The BTFs, which essentially are PCDD/F concentrations in whole milk normalized to a unit of intake (eq 2), had lower CVs than other measures of biotransfer. The biological basis for this finding is not clear. The PCDD/Fs are fat soluble and are transported through biological compartments by processes dependent on concentration gradients in the lipid fractions. Thus, the comparability of the concentrations in whole milk, which is largely water, is somewhat unexpected. On the other hand, the low variation in BTFs may be an artifact related to the small variation in the fat concentrations in the milk (Table 1).

The carry-over rates were the most variable of the coefficients (Table 3). The large variations in CRs mainly reflect the 3-fold variation in milk production among the cows (Table 1). Because of the influence of the amount of milk and fat produced on CRs, the use of CRs in exposure assessments requires adjustment for any differences in milk production between the experimental cows used to determine the CRs and cow population in the field situation. The greater variability of BCFs than BTFs was related to the differences in dry matter intake (Table 1). Intake of PCDD/Fs was essentially the same for all cows with the result that calculated concentrations in the diet were inverse to dry matter intake. **Transfer Rates at Background Intake.** Concentrations of PCDD/Fs in milk fat reached a stable level within 28 days of the initiation of dosing, but it cannot be inferred that body burdens were at steady state. Longer times would be required to perfuse PCDD/Fs to deep fat stores than the time required

FIGURE 3. Comparison of bioconcentration factors calculated using data from cows exposed to background PCDD/F sources only (day 0) and with the same cows dosed with PCP-treated wood (days 28, 42, and 56). Only the congeners consistently occurring at concentrations above background are shown. Error bars represent the standard deviations.



to reach stable concentrations in milk fat (20). The fortuitous finding that background concentrations in milk fat were relatively stable (Figure S.1 of the Supporting Information) suggested a stable background concentration in feed with that the cows at day 0 were probably near steady state with their environment. Because changes in daily dry matter intake over a 56-day period would not be great (24), it is reasonable to calculate the transfer coefficients for day 0 using the intake and analyses values from day 56 (Tables 1 and 2). The BCFs calculated for day 0 and those calculated for the dosing periods are compared in Figure 3. The BCFs were comparable for many congeners, but the background values were lower in the case of several PCDFs. The differences were relatively large for 1,2,3,4,7,8-HxCDF and 1,2,3,4,6,7,8-HxCDF and somewhat smaller for 1,2,3,6,7,8-HxCDF. The

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