

Transport of Chlorinated Dioxin and Furan Contaminants in Pentachlorophenol-treated Wood to Milk and Adipose Tissue of Dairy Cattle

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1. Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) have been found throughout the world in practically all media. The background body burden of PCDD/Fs in humans is believed to range between 4 and 13 picograms/gram (pg/g)¹⁾. It generally is accepted that animal food products, particularly meat and dairy products, are important sources of human exposure^{1,2,3)}. Feed and grazing have been suggested as major routes of PCDD/F uptake in cattle^{4,5,6)}. Though several studies have been conducted to examine the transfer of PCDD/Fs from feed to milk and the adipose tissue of dairy and beef cattle^{7,8,9,10,11)}, there remains some ambiguity in the interpretation of the results.

This study examines the significance of pentachlorophenol-treated wood, which is typically used in farm buildings and fences, as a potential source of PCDDs and PCDFs to livestock. The study describes the distribution of PCDD/Fs in milk and adipose tissue of dairy cows fed a diet of pulverized pentachlorophenol-treated wood, which served as a surrogate for various sources of PCDD/Fs. Elimination half-lives for selected PCDD/F congeners, as well as preliminary dioxin mass balance were calculated from the results of the feeding study. Four Holstein cows were fed 3 grams (g) of pentachlorophenol treated wood per day for 56 days. This dose resulted in a milk concentration of the hexa, hepta and octa PCDD/Fs of 10 to 20 times the background of the bulk milk used as a control in the study. The treatment period was followed by a 28-day depuration phase when the animals were not dosed. Milk samples were collected on days 0, 28, 56, and 84. Perirenal fat was collected from each animal at 84 days when they were euthanized. The PCDD/F homologues were quantitated by high resolution mass spectrometry. A 5-day composite feces sample for each

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cow was prepared from 1-day samples collected during the last 5 days of the dosing period (Days 53-58). Feed intake was also measured from a composite feed sample obtained during this period to calculate dioxin mass balances.

2. Methods

The study was conducted in the loose housing unit of the dairy facility at the Beltsville Agricultural Research Center (BARC). This facility was constructed within the last 5 years and contains no pentachlorophenol treated wood.

Cows. Four multiparous cows in mid-lactation were used. Multiparous cows were used in order to deplete potential residues accumulated during rearing. No animal was selected for the study if it was used in an experiment earlier in lactation, if it had a clinical condition that required treatment, or if it had a previous case of mastitis that resulted in the loss of a part of the mammary gland. Identification and characteristics of the animals are shown in Table 1.

Animal Care. Animals were fed the normal diet (Mix #1) that was fed to control animals at this stage of lactation. The composition of Mix #1 is shown in Table 2. Animals were housed in tie stalls fitted with rubber mats. Bedding was not used to prevent dioxin exposure from the sawdust bedding normally used. Cows were milked twice daily at twelve hour intervals. The amount of milk produced was recorded and the fat content of the milk was determined periodically. Milk was discarded to prevent its entry into the human food supply. Animals were exercised daily except during a 5-day fecal collection period that was conducted between Days 55 and 60 of the study.

Test Materials/Dose Administration. Pentachlorophenol-treated wood was identified in an existing animal housing structure at BARC. The wood was ground and a composite sample was analyzed. The congener profile of PCDD/Fs in the wood sample in this study was found to be broadly similar to a sample of sodium pentachlorophenate (Dowicide G)¹². It was projected that a daily dose of 3 g of this wood would produce a residue in milk at least 10 to 20 times the background concentration of the hexa, hepta and octa dioxins and furans in bulk milk at the BARC facility. The concentrations of tetra and penta dioxins and furans in the wood were not judged to be sufficient to provide milk residue concentrations high enough for unambiguous interpretation. All animals received the same 3.0 g dose of ground wood that was administered once per day by gelatin capsule.

Data of Initiation and Duration. Initial milk samples were collected on 3/28/96. Dosing began on 3/29/96 and continued for 56 days. The treatment period was followed by a 28-day depletion phase when the animals were not dosed. The animals were euthanized at the end of the study to prevent the movement of residues in human foods.

Sample Collection.

1) Milk - A 3 L milk sample was collected from each animal at a single milking on Days 0, 14, 28, 42, 56, 70, and 84. The corresponding sampling dates were 3/28, 4/11, 4/25, 5/9, 5/23, 6/6 and 6/20. Refrigerated samples were shipped to Alta Analytical Laboratory (El Dorado, CA) within 24 hours of collection. This study reports the results obtained from the analyses of samples from Days 0, 28, 56, and 84. The remaining samples were frozen for subsequent analysis.

2) Feces - Five-day total fecal collection was carried out during the last 5 days of the dosing period (Days 55-60). Animals were maintained in tie stalls with collection pans in the gutter. Bladder catheters were established to provide separation of urine and feces. Total fecal output was weighed, mixed and sampled daily. A 5-day composite feces sample for each cow was prepared from the 1-day samples. Feed intake was measured from a composite feed sample obtained during this period to calculate dioxin mass balances.

3) Tissue- Perirenal fat was collected from each animal after euthanasia.

4) Wood - Composite sample of the dosing material was collected while preparing the capsules. Random capsules were retained at various times during the study to address possible questions about the stability of the dosing material.

5) Feed - Feed (Mix 1) was sample weekly during the study. A single composite sample was obtained during the balance study. Only the sample collected during the balance study was analyzed initially. The periodic samples were not analyzed since the residue levels of the control milk samples suggest that there was no significant change in routine background exposure during the study.

6) Untreated Controls - Bulk milk samples from the BARC herd were obtained at all of the milk sampling periods. These animals received the same feed ingredients and were housed in the same facility as the experimental animals. The analyses of these samples was used to measure the stability of exposures from feeds and environmental sources.

PCDD/F Analyses. The cleanup, extraction, and quantitation of the PCDD/F homologues by high resolution gas chromatography, mass spectrometry was performed by Alta Analytical Laboratory.

3. Results

At the time of preparation of this abstract, the samples were undergoing analysis. The results of the experiment will be available for presentation at Dioxin 96. The results are expected to describe the distribution of PCDD/Fs in milk and adipose tissue of cows, and elimination half-lives for selected PCDD/F congeners. Results of a preliminary dioxin mass balance estimate will also be available.

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4. Discussion

Previous studies have evaluated the effect of pentachlorophenol in feed on dairy cattle, including subchronic administration of pentachlorophenol on the general health of lactating dairy cattle, pathologic changes and chronic toxicity of pentachlorophenol^{13,14}, and chemical analyses of tissue plasma pentachlorophenol concentrations in calves exposed to treated wood in the environment¹⁵. The effect of pentachlorophenol-treated wood in feed on PCDD/F concentrations in milk and adipose tissue of dairy cattle had not been established. In addition to understanding the role of pentachlorophenol treated wood, the results of this study are expected to provide a "finger print" of pentachlorophenol contaminants that will aid in the identification of potential sources of dioxin and furan exposure when contaminated market animals are detected. Ultimately, the results can be used by regulatory and action agencies to evaluate and possibly reduce human background exposure to dioxins and furans.

Several studies have been conducted on the distribution and elimination rates of PCDD/Fs in adipose tissue and milk of dairy cattle^{7,8,9,10}. However, interpretation of the results was compromised by the biological variability, differences in the starting concentrations of PCDD/Fs in the animals, changes in body weight over the experimental period, changes in dose over time, or not approaching steady state in the study^{7,8,16}. It has also been suggested that experimental and/or biological variability may be a more important factor than time-dependant changes in the depletion times of PCDD/Fs in animals⁷. This study is expected to reduce the inter-animal variability, permitting a meaningful analyses of time-dependant changes in the depletion times of selected PCDD/F congeners.

5. References

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**TABLE 1
CHARACTERISTICS OF COW**

Cow ID	Birth Date	Calving Date	Weight (kg) on 3/38/96	Production (On 2/28/96)		
				Milk (kg)	Fat (%)	Fat (kg)
9405	2/25/90	8/27/95, lactation #4	90	38	4.1	1.56
9406	3/22/90	11/4/95, lactation #4	604	41.7	3.4	1.42
1047	12/29/91	7/29/95, lactation #2	732	13.4	5	0.67
9838	9/8/92	8/27/95, lactation #2	506	26.2	3.7	0.97

**TABLE 2
COMPOSITION OF MIX #1**

Ingredient	Amount Offered (kg/day, wet wt.)	Amount Offered (kg/day, dry wt.)	Composition (%, dry wt.)
Corn Silage	17.01	6.4	27.5
Alfalfa-Grass Silage	14.87	5.23	22.5
High-Moisture Shelled Corn	7.06	5.22	22.4
Whole Cotton Seed	1.59	1.48	6.4
Roasted Soybeans	0.91	0.86	3.7
Protein Mix ¹	3.03	2.72	11.7
Mineral Mix	1.58	1.36	5.8
Total	46.05	23.27	100.0

¹Composition of Protein Mix:

Soybean Meal	63.5
Protein Blend	13
Corn Gluten Meal	9.6
Megalac	3.2
Minerals & Buffers	10.7
Total	100.0

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