Chlorinated Dibenzo-*p*-dioxin and Dibenzofuran Concentrations in Beef Animals from a Feeding Study

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Four calves were fed polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans for 120 days at levels somewhat higher than what may be found in forage near some waste incinerators and manufacturing plants. Four calves were fed identical diets but without the chemicals. Using bioelectrical impedance measurements of total body fat, 30−50% of the dosed 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, and 2,3,4,7,8-PeCDF was estimated to be retained by the animals. Although these same congeners were bioconcentrated in adipose tissue (BCF ∼ 10), consumer products such as ribeye showed concentrations less than what were found in the animal feed (BCF ∼ 0.1). Distribution of the dioxins and furans into various lipid compartments appeared to be rather uniform in back fat, perirenal fat, and ribeye for tetra to hexa congeners. Ribeye, serum, and liver lipids had higher concentrations of the higher chlorinated congeners, due in part to not reaching a steady state. An unexpected source of dioxin and furan contamination was discovered during the experiment, resulting in the control animals having concentrations of some congeners that were equal to or in some cases greater than those of the dosed animals. Pentachlorophenol-treated wood components in the pole barn where the feeding experiment was conducted were found to have contributed to the animals' exposure.

Keywords: *Dioxins; furans; cattle; tissue; pentachlorophenol*

INTRODUCTION

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and the coplanar polychlorinated biphenyls, often designated "dioxins" because of similarities in structure and biological properties, are considered to be potential chronic toxins in humans. Human exposure has been proposed to be predominately from dairy products, meat, and fish, with beef being possibly the largest contributor in the U.S. diet (U.S. EPA, 1994).

A common hypothesis explaining the presence of dioxins in beef is that animals consume feed that has been contaminated by emissions from combustion sources via atmospheric deposition. The results of a statistical survey conducted by the U.S. Department of Agriculture Food Safety Inspection Service and the U.S. Environmental Protection Agency on dioxin levels in beef back fat have been reported (Winters et al., 1996), but no information on production sites, feeding regimen, or distribution of dioxin/furan congeners in the edible tissues was obtained in the survey. A study of dioxin/furan levels in beef from some specific production facilities has been reported (Feil et al., 1995), and trace-back investigations have been done on some sites that produced animals with above average dioxin/furan levels (Feil et al., 1997). Dioxin levels in food samples collected at supermarkets and at fast food restaurants have also been published (Schecter et al., 1994, 1997). However, little work has been done to investigate the transfer of dioxins from the feed source to the whole animal and finally to the retailed cuts of meat for beef animals.

Several studies on uptake, depletion, and transport of dioxins/furans in lactating animals have been conducted. Bioavailabilities and carry-over rates have been calculated by measuring amounts excreted in the milk. For 2,3,7,8-TCDD bioavailabilies were 35% for a cow fed naturally incurred dioxins and furans in the feed (McLachlan et al., 1990), 15% for cows grazing near a municipal solid waste incinerator (Slob et al., 1995), and 30% for cows given a single intraruminal dose of ^{13}C-labeled dioxins/furans in oil (Olling et al., 1991). Fries et al. (1999) found the carry-over rate for 2,3,7,8-TCDD into milk was 35% for cows fed pentachlorophenol-treated wood in their diet. Both carry-over rates and bioavailabilities decreased with increasing amounts of chlorination. Chang et al. (1989) analyzed fat and liver from one cow raised in an area with dioxin-contami-

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Table 1. Dose Components and Amounts Fed

dioxin	toxic equivalency factor[a]	daily dose per animal (ng)	total dose per animal (μg)	estimated concentration in daily feed[b] (ng/kg)
1,2,7,8-TCDD[c]	0	750	90	97.4
1,3,7,8-TCDD[d]	0	750	90	97.4
1,4,7,8-TCDD[d]	0	750	90	97.4
2,3,7,8-TCDD[d]	1	83.3	10	10.8
1,2,3,7,8-PeCDD[d]	1	83.3	10	10.8
1,2,3,6,7,8-HxCDD[d]	0.1	150	18	19.5
1,2,3,4,6,7,8-HpCDD[d]	0.01	750	90	97.4
OCDD[d]	0.0001	750	90	97.4
2,3,7,8-TCDF[d]	0.1	150	18	19.5
2,3,4,7,8-PeCDF[e]	0.5	83.3	10	10.8
1,2,3,4,6,7,8-HpCDF[e]	0.01	150	18	19.5
OCDF[d]	0.0001	750	90	97.4
3,3′,4,4′,5′-PCB (IUPAC No. 126)[d]	0.1	150	18	19.5
2,3′,4,4′,5-PCB (IUPAC No. 118)[d]	0.0001	750	90	97.4
2,3,3′,4,4′,5,5′-PCB (IUPAC No. 189)[d]	0.0001	750	90	97.4

[a] van den Berg et al. (1998). [b] Average daily feed intake = 7.7 kg of hay and corn. [c] Synthesized from 4,5-dichlorocatechol and 2,3,4-trichloronitrobenzene. [d] Purchased from Cambridge Isotope Laboratories, Inc., Andover, MA. [e] Purchased from Chemsyn Science Laboratories, Lenexa, KS.

nated soil and found that penta through octa congeners accumulated at much higher levels in the liver than in fat on a lipid adjusted basis. However, chickens from the same area showed only small differences in concentrations in the liver and fat. 2,3,7,8-TCDD was not analyzed in this study.

Few studies have been done on beef cattle to determine bioavailability and distribution into tissues. After a 4 week dosing period, Jensen et al. (1981) looked at the distribution of 2,3,7,8-TCDD among several tissues and found that fat contained 9 times the amounts in the liver or kidney on a wet weight basis. On a lipid-adjusted basis muscle had a concentration similar to that of fat; however, the value was at the limit of detection for the study, that is, 2 ppt. Thorpe et al. (1999) analyzed several matrices in beef cattle 1 week after discontinuing a 4 week dose and found that liver and muscle had 10 and 5 times, respectively, higher concentrations of five dioxins and furans than fat stores on a lipid-adjusted basis. No estimates of total dioxin body burden or bioavailability were made in either study, and animals were most likely not at steady states.

To address these issues, we conducted a 17 week feeding experiment with eight dioxins, four furans, and three coplanar PCBs to determine the relative uptake efficiencies and the distribution of the congeners among various tissues in a typical beef production setting. We report herein the results for the dioxins and furans in several major compartments (liver, serum, back fat, perirenal fat, and ribeye). The results for the PCBs and other compartments will be presented in future publications. We also report here the serendipitous discovery that animal exposure to relatively small surface areas of PCP-treated wood can be a major source of dioxins and furans in beef.

The congeners used in this study (Table 1) were fed at levels that would minimize contamination of the immediate experimental site but would generate tissue concentrations significantly above our limits of detection. These levels were within an order of magnitude of concentrations found in feed from moderately industrialized sites such as Rothamsted, England (Kjeller et al., 1991), and Elk River, MN (Reed et al., 1990). Whereas corn from the Elk River site was found to contain 3.7 and 170 ppt of 2,3,7,8-TCDD and OCDD, respectively, we fed them at levels (based on estimates of forage consumption) comparable to 11 and 100 ppt, respectively. The pattern of the congeners fed was chosen to represent most homologue groups and to mimic patterns seen from typical combustion sources (Cleverly et al., 1997) and in soils and vegetation from a semirural area (Kjeller et al., 1991). Nontoxic congeners were included in the dose for possible comparison of uptake/excretion evaluations and because they are present in the environment (Swerev and Ballschmiter, 1989; Kjeller et al., 1991; Ferrario et al., 1999).

MATERIALS AND METHODS

Location. The Carrington Research Extension Center, Carrington, ND, was selected because it was in a rural area, remote from any municipal or industrial incinerators. A medical waste incinerator with a short stack and attached to a small hospital located ~3 mi south of the Center was not expected to contribute to the dioxin levels in animals as southerly winds are rare.

Cattle. Eight steer calves weighing 220−262 kg from cows that had completed at least two previous lactations were used. The calves and their mothers were raised in confinement at the Center. The calves were weaned and accustomed to eating hay and grain before being assigned to the experiment. The eight calves were randomly divided into two groups of four: one group was the control, and one group was fed a dioxin supplement.

Bioelectrical Impedance. Bioelectrical impedance measurements (Marchello and Slanger, 1992, 1994; Berg and Marchello, 1994) were taken to estimate body fat at the beginning, midpoint, and end of the experiment. Impedance measurements were taken by inserting 23 gauge × 12.7 mm needles fully into the live animals along the dorsal midline 10 and 20 cm caudal from the top of the shoulder (first thoracic vertebrae) and at the tail head (first coxygeal vertebrae) and 10 cm cranial to it. The distance between electrodes varied from 55 to 92 cm. Fatfree mass has been empirically correlated to bioelectrical impedance measurements on live swine, beef, and lamb animals and on chilled carcasses (Marchello and Slanger, 1992; Swantek et al., 1992). Regression analyses from the live animal data yielded the following equation to estimate the amount of fat in an animal: fat (kg) = $2[-24.59 + 0.148W - 0.029(L^2/R)]$, where W is the weight of the animal in kg, L is the distance between electrodes in cm, and R is the resistance in ohms.

Feed. Corn (*Zea mays identata*, processed by dry rolling) and second-cutting alfalfa hay (*Medicago sativa*, chopped) grown at the location served as the common feed source for

all calves during the experiment. Dried molasses was fed to all calves for 14 days from the third day of the experiment to encourage more consumption of grain (225 g of molasses on day 3 and 113 g on each of the next 13 days). A block of mineral supplement was allowed.

Husbandry. Beginning with the initial sampling, the calves were housed in a pole barn with a concrete floor and open to the south. Individual feeding pens, about 1.2 × 2.5 m, with "J" style concrete bunks were provided for each calf. The calves were fed once per day these feeds in order: first, 250 g of ground corn supplement with or without test chemicals; next, ground corn in varying and increasing amounts as the experiment progressed; and last, chopped alfalfa hay, also in varying amounts. The goal was to feed 3.6 kg of corn daily the first 2 months of the experiment and 7.2 kg of corn daily after 2 months. The latter amount was not achieved, but averaged ~5.5 kg. Hay was weighed but was fed to appetite. The average daily feed intake was 7.7 kg (corn and hay) for each steer. About 3 h was allowed for eating, whereupon the calves were locked out of the feeding pens and allowed access to water outside of the barn. The control calves were separated from the dioxin-fed calves by a solid plywood wall within the barn and by a larger fenced area between the pens holding the calves outside the barn. Thus, feeding, watering, and exercising areas for the two groups were completely separate. Contact between the groups was not allowed.

Duration. The feeding period was 120 days long, extending from November 21, 1994, to March 22, 1995.

Supplement Preparation. The dioxins, furans, and PCBs (Table 1) were dissolved in acetone or acetone and small amounts of dichloromethane and then diluted with acetone so that 1 mL of solvent transferred the desired amount of all the compounds to the 250 g daily allotment of corn supplement described below.

Ground corn (250 g) containing 150 mg of lasalocid (Bovatec, Hoffmann-La Roche, Inc., Nutley, NJ) was weighed into each of 500 polyethylene containers (1 pint frozen food containers). A 50 g sample was set aside for dioxin analysis for every 100 samples weighed; thus, a single 250 g sample was collected for analysis. One milliliter of acetone (no dioxins) was then pipetted into the center of each container of feed, thus making the supplement for the control calves. The containers of feed were kept uncovered at room temperature overnight to allow the acetone to evaporate and were then capped until used.

After the control supplements were capped, the dioxin-laced supplements were made in the same manner except that the acetone contained the dioxins. All polyethylene containers were rinsed with a 1:1 solution of hexane/dichloromethane before use.

Sampling/Data Collection. On November 15, 1994, the calves were weighed and initial samples were collected. Blood (a 250-mL sample and an 8-mL sample) for serum was collected via jugular puncture, and feces were collected per rectum (~300 g). The 8-mL blood sample was analyzed for serum γ-glutamyl transferase and alanine amino transferase by Heartland Medical Center, Fargo, ND.

Dioxin feeding began on November 21, 1994. Subsequent weighings and blood and fecal collections were done days 30, 60, 86, 120 (control animals), and 121 (dosed animals). The control calves were slaughtered on March 21 at Shepard Arena, North Dakota State University, Fargo, ND, and the dioxin-fed calves were slaughtered at the same place on March 22. Adrenals, brain, bronchial tubes, heart, and kidneys were totally collected from all calves at slaughter. Samples (~1 kg) of backfat (subcutaneous), kidney fat (perirenal), liver, ribeye (longissimus), and tenderloin (psoas major and minor) were collected. Portions of these tissues were ground for dioxin analysis, and portions were kept for cooking experiments. The storage containers were polyethylene, solvent rinsed as described previously. All samples were stored at −60 °C until analyzed.

Dioxin Analysis. The analytical procedures used to determine PCDDs and PCDFs in the various beef matrixes in this study were similar to those described in U.S. EPA Method 8290A, "Polychlorinated Dibenzodioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) by High-Resolution Gas Chromatography/High-Resolution Mass Spectrometry (HRGC/HRMS)" (U.S. EPA, 1998).

Aliquots of the beef tissue samples, which had been ground and homogenized in a laboratory food mill, were blended with increasing amounts of anhydrous sodium sulfate until a free-flowing mixture was obtained with each sample. Subsamples of these mixtures (25−75 g) were spiked with nine isotopically labeled dioxins and furans (Cambridge Isotope Laboratories, Inc., Andover, MA) before Soxhlet extraction with methylene chloride/hexane (1:1, 300 mL). The concentrated crude organic extracts were initially purified by washing them sequentially with aqueous potassium hydroxide (20% w/v, 30 mL), double-distilled water (30 mL), concentrated sulfuric acid (30 mL), and double-distilled water (30 mL). Further purification of the organic extracts included a sequence of liquid chromatographic procedures described in Method 8290A but substituting basic alumina (Activity Grade 1, ICN Pharmaceuticals, Cleveland, OH) for neutral alumina.

Serum samples (40−50 g) were spiked with the nine isotopically labeled dioxins and furans (Cambridge Isotope Laboratories) and then diluted with water saturated with ammonium sulfate (40 mL), ethanol (25 mL), and hexane (40 mL). The mixture was shaken on a wrist action shaker for 15 min. The organic phase was collected,and the remaining aqueous phase was re-extracted twice with hexane. The pooled hexane fractions were concentrated and further purified according to the liquid chromatographic procedures described in Method 8290A and modified as mentioned previously. Just prior to GC-MS analysis, all tissues and serum residues were reconstituted by adding 10 μL of a standard solution containing $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,3,7,8,9-HxCDD to monitor recoveries achieved during the GC-MS analysis.

The levels of the PCDD/Fs in the purified beef extracts were determined using HRGC-HRMS on a Carlo-Erba Mega series gas chromatograph, coupled to a Kratos MS-890 mass spectrometer. The identification criteria specified in U.S. EPA Method 8290 with respect to the GC column performance (separations capability) and mass spectrometer performance (resolution and sensitivity) were fully satisfied by the analytical data obtained in this study. Laboratory blanks were analyzed along with the samples and showed acceptably low (or nondetectable) background levels of PCDD/Fs. Matrix spikes and matrix spike duplicates analyzed as quality control measures exhibited satisfactory recoveries (within 40−135%) and precision for these analyses. Lipid values were calculated gravimetrically using 5% of the initial organic extracts.

Pentachlorophenol (PCP) Analysis. One gram of perirenal fat was weighed into a 10 mL test tube, and then 6 mL of ethyl acetate, 10 μL of $^{13}C_6$-PCP (1.0 ng/μL) (Cambridge Isotope Laboratories), and a magnetic stir bar (~2 cm in length) were added. The test tube was placed into a well of a heating block/magnetic stirrer at 80° C. The solution was stirred for 5 min, and the clear liquid was transferred to a 50 mL Erlenmeyer flask. The extraction was repeated with an additional 6 mL portion of ethyl acetate and then two or three 3 mL portions of methylene chloride (if the solution was not clear, more methylene chloride or ethyl acetate was added). The combined ethyl acetate/methylene chloride solution was passed first through an HDPE filter (CC-23-M, Image Molding Inc., Denver, CO) and then through a column of 1 g of alumina (Woelm, basic) in an HDPE filter tube. The alumina column was eluted with three 6 mL portions of methylene chloride, then three 6 mL portions of ethyl acetate, and finally a 5 mL portion of methylene chloride. These eluates were discarded. The column was then eluted with two 3 mL portions of 1:1 acetone/methanol. Dodecane (15 μL) was added as a keeper solvent, and the acetone/methanol solution was concentrated to near dryness with a stream of nitrogen. An ether solution of diazomethane (0.5 mL, 0.4 mmol/mL) was added, the solution was allowed to react overnight at room temperature, the solvent was allowed to evaporate (concentration was best done without assistance of a stream of nitrogen as the pentachloroanisole was quite volatile), and the residue was dissolved in 0.1 mL of methylene chloride. The solution was

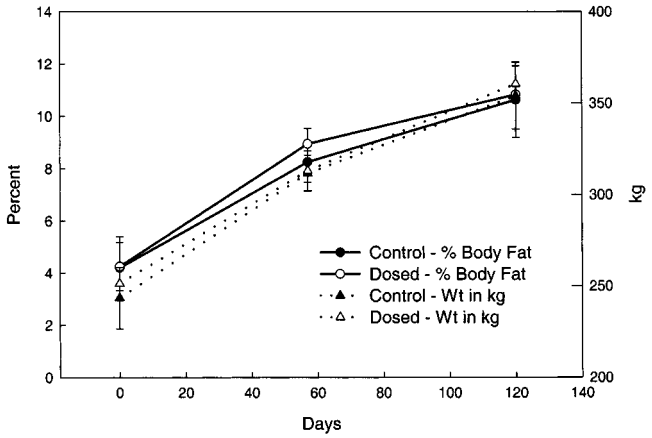

Figure 1. Average weight gain and percent body fat estimated from bioelectrical impedance measurements.

passed through a small chromatographic column made from a disposable pipet (5 mm i.d.) by adding approximately 1 cm of neutral silica gel and 1 cm of sulfuric acid/silica gel (3:7 w/w). The column was eluted with an additional 1 mL of methylene chloride. The solution was concentrated, 5 μL of $^{13}C_6$-hexachlorobenzene (2.0 ng/μL) (Cambridge Isotope Laboratories) was added as a recovery standard, and the solution was analyzed by GC-MS (Carlo-Erba 8000 GC, Micromass Autospec-Ultima) at a resolution of 15000 (10% valley) in the single-ion monitoring mode. The GC conditions were as follows: 80 °C, 2 min; 80−160 °C at 20 °C/min; 160−200 °C at 5 °C/min; 200−300 °C at 20 °C/min. The ions monitored were the M^{+2} and M^{+4} peaks for the native and $^{13}C_6$-labeled pentachloroanisole (279.8597, 281.8568, 285.8798, and 287.8769).

RESULTS AND DISCUSSION

The dosed animals showed no signs of toxicity throughout the experiment. Serum glutamyl transferase and alanine amino transferase analyses were normal, and no abnormalities were observed at slaughter. Weight gains and fat depositions as measured by the bioelectrical impedance technique were similar for control and dosed animals (Figure 1). The congeners fed represented a range of dioxins and furans from nontoxic to the most toxic based on toxic equivalency factors (TEF) (Van den Berg et al., 1998) and also different degrees of chlorination (tetra through octa). Tables 2−5 give the levels of dioxin and furan congeners measured in serum, perirenal (kidney) fat, ribeye (longissimus), and liver. The nontoxic congeners were not detected in any of the sera or tissues analyzed, indicating no accumulation, probably due to extensive metabolism. Studies have shown substantially more metabolism for the less toxic congeners than for 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD (Larsen et al., 1996; Huwe et al., 1998; Petroske et al., 1997; Hakk et al., 1997, 1998).

The 2,3,7,8-substituted congeners were low or below the detection limits for all animals at the beginning of the experiment as measured in initial sera (Tables 2 and 3; Figure 2). Congener levels were monitored in serum during the course of the experiment. Tetra and penta congeners appeared to reach steady state by the end of the experiment. During the course of the feeding experiment, the control animals accumulated levels of the higher chlorinated congeners similar to those of the dosed animals, indicating a secondary source of these compounds.

One of the objectives of this experiment was the determination of the uptake of various dioxin and furan

Table 2. Concentrations in Serum of Control Animals (Picograms per Gram of Wet Weight, ppt)

	initial serum				4 week serum				8 week serum				12 week serum				final serum			
	321[a]	345[a]	355[a]	489[a]	321	345	355	489	321	345	355	489	321	345	355	489	321	345	355	489
% lipid	0.26	0.27	0.27	0.30	0.32	0.16	0.26	0.34	0.27	0.30	0.32	0.36	0.27	0.27	0.26	0.33	0.32	0.30	0.26	0.31
2,3,7,8-TCDF	(0.02)[b]	(0.02)	(0.02)	(0.03)	(0.02)	(0.02)	(0.02)	(0.01)	(0.02)	(0.02)	(0.03)	(0.02)	(0.05)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.03)
2,3,7,8-TCDD	(0.03)	(0.02)	(0.03)	(0.03)	(0.03)	(0.03)	(0.03)	(0.02)	(0.02)	(0.02)	(0.03)	(0.03)	(0.08)	(0.04)	(0.04)	(0.05)	(0.03)	(0.03)	(0.04)	(0.05)
1,2,3,7,8-PeCDF	(0.03)	(0.03)	(0.03)	(0.02)	(0.03)	(0.03)	(0.04)	(0.02)	(0.03)	(0.06)	(0.04)	(0.03)	(0.08)	(0.03)	(0.03)	(0.04)	(0.03)	(0.03)	(0.04)	(0.04)
2,3,4,7,8-PeCDF	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.03)	(0.03)	(0.02)	(0.02)	(0.05)	(0.03)	(0.02)	(0.06)	(0.02)	(0.02)	(0.03)	(0.02)	(0.02)	(0.03)	(0.03)
1,2,3,7,8-PeCDD	(0.03)	(0.03)	(0.03)	(0.03)	(0.04)	(0.04)	(0.04)	(0.02)	(0.03)	(0.03)	(0.04)	(0.03)	(0.09)	(0.03)	(0.03)	(0.04)	(0.03)	(0.03)	(0.04)	(0.05)
1,2,3,4,7,8-HxCDF	(0.03)	(0.04)	(0.03)	(0.03)	0.08	(0.02)	0.06	0.04	(0.08)	(0.06)	(0.06)	(0.03)	0.08	0.05	0.06	0.05	0.07	0.04	0.07	0.06
1,2,3,6,7,8-HxCDF	(0.05)	(0.07)	0.16	(0.07)	0.16	0.13	0.18	0.15	0.22	0.13	0.19	0.09	0.24	0.23	0.15	0.15	0.18	0.13	0.15	0.16
2,3,4,6,7,8-HxCDF	(0.03)	(0.04)	(0.03)	(0.03)	0.05	(0.03)	(0.04)	0.04	0.05	(0.05)	0.08	(0.03)	(0.09)	0.05	0.04	0.04	0.04	0.03	0.05	(0.04)
1,2,3,7,8,9-HxCDF	(0.04)	(0.05)	(0.04)	(0.03)	(0.04)	(0.03)	(0.05)	(0.02)	(0.06)	(0.04)	(0.05)	(0.04)	(0.10)	(0.03)	(0.04)	(0.05)	(0.03)	(0.04)	(0.05)	(0.05)
1,2,3,4,7,8-HxCDD	(0.05)	(0.05)	0.05	(0.05)	(0.05)	(0.05)	(0.06)	(0.03)	(0.05)	(0.07)	(0.07)	(0.08)	(0.13)	(0.04)	(0.05)	(0.07)	(0.04)	(0.05)	(0.06)	(0.07)
1,2,3,6,7,8-HxCDD	0.37	0.57	0.99	0.35	2.01	1.40	1.44	1.33	2.16	1.31	2.06	0.91	2.05	1.52	1.71	1.06	2.09	1.28	1.57	1.30
1,2,3,7,8,9-HxCDD	(0.05)	0.07	0.20	(0.04)	0.32	(0.21)	0.22	0.20	(0.25)	0.14	0.31	0.15	0.27	0.26	0.26	0.30	0.27	0.16	0.18	0.23
1,2,3,4,6,7,8-HpCDF	0.20	0.57	1.03	0.23	1.62	0.97	1.08	0.96	1.48	0.78	1.18	0.62	1.44	1.51	1.28	0.94	1.26	0.90	1.30	1.16
1,2,3,4,7,8,9- HpCDF	(0.08)	(0.08)	(0.14)	(0.06)	(1.16)	(0.08)	(0.10)	(0.04)	(0.12)	(0.08)	(0.09)	(0.09)	(0.22)	(0.06)	(0.07)	(0.10)	(0.06)	(0.07)	(0.10)	(0.11)
1,2,3,4,6,7,8-HpCDD	0.41	1.11	1.24	48.0	3.72	1.49	2.69	2.55	2.88	1.63	2.92	1.52	2.87	1.89	3.44	1.57	2.94	1.75	2.89	1.93
OCDF	(0.08)	(0.07)	(0.12)	(0.06)	0.30	0.12	(0.21)	0.22	0.95	0.20	(0.37)	(0.22)	(0.26)	0.17	0.27	1.63	0.17	0.20	0.23	0.27
OCDD	0.89	3.48	3.77	1.03	4.34	1.52	3.55	3.02	5.40	2.81	6.10	3.02	4.99	2.71	5.90	2.62	4.87	2.88	5.30	3.06

[a] Animal number. [b] Numbers in parentheses represent limits of quantitation.

Table 3. Concentrations in Serum of Dosed Animals (Picograms per Gram of Wet Weight, ppt)

	initial serum				4 week serum				8 week serum				12 week serum				final serum			
	249[a]	277[a]	353[a]	419[a]	249	277	353	419	249	277	353	419	249	277	353	419	249	277	353	419
% lipid	0.21	0.06	0.17	0.01	0.20	0.15	0.10	0.10	0.18	0.14	0.09	0.16	0.11	0.02	0.07	0.05	0.14	0.21	0.15	0.13
2,3,7,8-TCDF	(0.02)[b]	(0.03)	(0.02)	(0.02)	0.04	(0.10)	(0.02)	(0.02)	(0.02)	0.01	(0.42)	(0.02)	0.03	(0.02)	(0.02)	(0.01)	0.02	(0.02)	0.02	(0.02)
2,3,7,8-TCDD	(0.03)	(0.03)	(0.03)	(0.03)	0.34	0.26	0.15	0.11	0.34	0.27	0.33	0.17	0.39	0.17	0.21	0.20	0.34	0.32	0.21	0.18
1,2,3,7,8-PeCDF	(0.02)	(0.07)	(0.04)	(0.03)	(0.02)	(0.03)	(0.02)	(0.02)	(0.03)	(0.02)	(0.03)	(0.03)	(0.02)	(0.02)	(0.02)	(0.03)	(0.02)	(0.02)	(0.02)	(0.04)
2,3,4,7,8-PeCDF	(0.02)	(0.05)	(0.03)	(0.02)	0.27	0.19	0.11	0.12	0.29	0.24	0.19	0.14	0.31	0.18	0.18	0.22	0.36	0.31	0.22	0.20
1,2,3,7,8-PeCDD	(0.02)	(0.03)	(0.04)	(0.04)	0.30	0.34	0.18	0.18	0.31	0.33	0.21	0.29	0.35	0.24	0.30	0.27	0.39	0.41	0.27	0.25
1,2,3,4,7,8-HxCDF	(0.02)	(0.04)	(0.02)	(0.04)	(0.04)	(0.03)	(0.02)	(0.02)	(0.02)	(0.02)	(0.03)	(0.03)	(0.02)	0.05	0.02	(0.02)	(0.02)	0.10	0.03	(0.02)
1,2,3,6,7,8-HxCDF	(0.04)	(0.07)	(0.04)	(0.07)	(0.10)	0.19	(0.04)	(0.23)	0.14	0.20	(0.05)	(0.18)	0.17	(0.19)	(0.04)	0.14	0.18	(0.29)	0.11	(0.13)
2,3,4,6,7,8-HxCDF	(0.02)	(0.04)	(0.03)	(0.04)	(0.05)	0.06	(0.03)	(0.02)	(0.03)	0.05	(0.03)	(0.03)	(0.03)	(0.04)	(0.02)	(0.03)	(0.02)	0.08	0.05	(0.03)
1,2,3,7,8,9-HxCDF	(0.03)	(0.05)	(0.03)	(0.05)	(0.04)	(0.04)	(0.03)	(0.03)	(0.03)	(0.03)	(0.04)	(0.04)	(0.03)	(0.03)	(0.03)	(0.03)	(0.03)	(0.04)	(0.02)	(0.03)
1,2,3,4,7,8-HxCDD	(0.05)	(0.29)	(0.05)	(0.05)	(0.05)	(0.05)	(0.06)	(0.04)	(0.05)	(0.04)	(0.05)	(0.04)	(0.04)	(0.05)	(0.04)	(0.05)	(0.04)	(0.04)	(0.04)	(0.04)
1,2,3,6,7,8-HxCDD	0.62	(0.29)	(0.19)	0.47	2.05	2.04	0.87	1.64	2.34	2.93	1.11	1.95	3.12	3.21	1.50	2.08	3.15	4.40	2.01	2.05
1,2,3,7,8,9-HxCDD	0.06	(0.18)	(0.04)	(0.04)	0.16	0.30	(0.07)	(0.03)	0.23	0.24	0.10	0.33	0.36	0.36	0.09	0.21	0.38	(0.35)	(0.22)	(0.29)
1,2,3,4,6,7,8-HpCDF	0.40	(0.28)	(0.15)	0.20	1.11	1.00	0.59	0.81	1.05	1.52	0.67	1.05	1.49	2.11	0.90	1.29	1.44	2.70	1.31	1.13
1,2,3,4,7,8,9-HpCDF	(0.06)	(0.12)	(0.06)	(0.09)	(0.05)	(0.06)	(0.07)	(0.05)	(0.07)	(0.05)	(0.07)	(0.08)	(0.06)	(0.14)	(0.05)	(0.07)	(0.07)	(0.06)	(0.05)	(0.06)
1,2,3,4,6,7,8-HpCDD	0.92	0.55	(0.19)	0.45	2.38	2.20	1.10	1.62	1.92	2.91	1.45	1.94	2.70	3.17	1.56	2.04	2.64	5.48	2.35	2.24
OCDF	(0.07)	(0.13)	(0.08)	(0.12)	0.25	0.22	0.14	(0.14)	0.29	0.41	0.25	0.21	0.23	0.31	0.22	0.27	0.23	0.56	0.33	0.21
OCDD	1.57	0.70	0.43	0.72	3.01	2.40	1.01	1.33	2.51	4.19	2.25	2.75	3.29	3.89	1.43	2.56	3.15	6.49	2.52	2.69

[a] Animal number. [b] Numbers in parentheses represent limits of quantitation.

Table 4. Concentrations in Perirenal Fat, Back Fat, Ribeye, Liver, and Final Feces of Control Animals (Picograms per Gram of Wet Weight, ppt)

	perirenal fat				back fat				ribeye				liver				final feces			
	321[a]	345[a]	355[a]	489[a]	321	345	355	489	321	345	355	489	321	345	355	489	321	345	355	489
% lipid	92.2	74.7	97.5	96.6	67.0	74.0	76.0	79.0	1.0	2.0	2.0	3.0	4.0	5.0	4.0	4.0	22	25	24.6	30
2,3,7,8-TCDF	0.20	(0.16)[b]	(0.10)	(0.09)	0.04	(0.07)	(0.03)	(0.03)	(0.01)	(0.03)	(0.01)	(0.01)	0.19	(0.02)	(0.02)	(0.04)	(0.21)	0.31	(0.51)	(0.41)
2,3,7,8-TCDD	0.82	0.71	0.68	0.83	0.31	(0.23)	(0.19)	(0.30)	(0.03)	(0.06)	(0.02)	(0.01)	13.8	(0.04)	(0.21)	(0.08)	(0.33)	(0.24)	(0.70)	(0.84)
1,2,3,7,8-PeCDF	(0.26)	(0.18)	(0.16)	(0.16)	(0.37)	(0.37)	(0.32)	(0.31)	(0.08)	(0.30)	(0.02)	(0.01)	0.70	(0.09)	(0.13)	(0.22)	(0.27)	(0.40)	(1.04)	(0.23)
2,3,4,7,8-PeCDF	4.91	3.54	3.62	2.26	2.14	2.03	1.71	1.20	(0.09)	(0.33)	(0.06)	(0.01)	1.81	0.88	1.23	0.54	(0.21)	(0.46)	(0.82)	0.67
1,2,3,7,8-PeCDD	8.12	5.79	7.27	8.33	4.41	4.80	4.42	4.21	(0.19)	(0.63)	(0.06)	(0.01)	55.2	2.47	2.62	2.28	0.99	1.32	(1.25)	3.62
1,2,3,4,7,8-HxCDF	24.4	15.1	16.2	10.2	11.8	7.66	8.75	5.35	0.19	(0.12)	0.32	0.21	13.1	10.2	11.8	6.58	6.64	5.41	3.38	10.2
1,2,3,6,7,8-HxCDF	26.7	20.9	20.3	15.3	(5.24)	5.36	5.29	3.90	(0.05)	(0.10)	0.14	0.15	6.44	4.04	4.60	3.25	6.24	6.05	5.67	12.5
2,3,4,6,7,8-HxCDF	11.4	8.33	9.72	6.67	5.15	4.21	5.70	3.46	(0.04)	(0.13)	0.15	(0.16)	7.06	6.18	7.29	4.37	2.69	2.60	(2.44)	4.80
1,2,3,7,8,9-HxCDF	(0.47)	(0.24)	(0.38)	(0.39)	(0.19)	(0.33)	(0.19	(0.24)	(0.05)	(0.18)	(0.03)	(0.02)	(0.06)	(0.08)	(0.12)	(0.12)	(0.54)	(0.42)	(1.12)	(0.40)
1,2,3,4,7,8-HxCDD	18.7	12.7	13.4	12.7	9.89	7.14	7.53	(0.34)	(0.12)	(0.21)	(0.10)	(0.18)	52.9	14.6	19.5	16.2	3.87	5.20	4.93	14.2
1,2,3,6,7,8-HxCDD	251	144	167	112	108	65.7	71.8	51.2	1.90	1.74	2.24	2.31	147	59.3	77.0	40.2	43.5	39.2	22.8	69.1
1,2,3,7,8,9-HxCDD	29.6	21.6	26.8	18.9	12.9	8.35	(0.19)	8.97	(0.13)	(0.23)	(0.23)	0.45	89.0	17.4	21.7	15.8	11.0	13.4	11.4	38.8
1,2,3,4,6,7,8-HpCDF	123	86.4	89.6	62.0	46.2	35.8	37.5	24.5	1.12	1.36	1.41	1.61	107	103	145	92.8	209	151	98.3	326
1,2,3,4,7,8,9-HpCDF	15.7	8.80	(10.1)	6.47	2.37	(0.68)	1.76	(0.54)	(0.09)	(0.44)	(0.09)	(0.04)	8.45	8.66	15.2	6.54	29.7	19.4	(11.6)	47.8
1,2,3,4,6,7,8-HpCDD	707	386	476	316	352	200	237	163	9.39	(0.46)	9.21	9.75	2420	1625	2300	1286	808	683	467	1357
OCDF	29.0	23.1	17.1	14.9	8.53	6.53	5.33	3.07	(0.45)	(0.29)	0.34	0.54	169	152	227	136	459	531	251	1182
OCDD	850	780	750	410	270	286	292	120	18.7	12.4	19.7	21.7	10116	5925	8751	5012	5210	4272	3733	11174

[a] Animal number. [b] Numbers in parentheses represent limits of quantitation.

Table 5. Concentrations in Perirenal Fat, Back Fat, Ribeye, Liver, and Final Feces of Dosed Animals (Picograms per Gram of Wet Weight, ppt)

	perirenal fat			back fat			ribeye			liver			final feces		
	249[a]	277[a]	353[a]	419[a]	249	277	353	419	249	277	353	419	249	277	353	419	249	277	353	419
% lipid	95.5	94.4	95.6	77.7	97	74.8	84.6	75.5	1.06	1.21	1.07	0.93	3.94	2.90	3.72	2.91	22	20	21	18
2,3,7,8-TCDF	7.17	3.37	4.99	4.27	5.94	2.91	4.65	4.36	(0.05)[b]	(0.09)	(0.05)	(0.06)	0.25	0.26	0.31	0.22	12.5	6.69	12.7	9.02
2,3,7,8-TCDD	152	124	126	115	113	109	110	125	3.22	3.41	3.32	2.33	10.1	9.80	9.22	8.45	18.1	13.4	19.6	13.8
1,2,3,7,8-PeCDF	(0.23)	(0.26)	(0.17)	(0.18)	(0.17)	(0.15)	(0.16)	(0.18)	(0.09)	(0.09)	(0.08)	(0.07)	(0.05)	(0.07)	(0.07)	(0.05)	0.60	(0.32)	(0.75)	(0.39)
2,3,4,7,8-PeCDF	123	108	95.7	90.9	94.2	90.2	79.4	88.6	1.45	1.52	1.37	0.92	41.2	38.8	32.8	31.7	12.5	11.2	14.9	9.65
1,2,3,7,8-PeCDD	96.5	103	78.7	77.5	78.4	76.6	63.1	79.6	1.13	1.46	1.19	0.90	23.9	27.8	21.8	22.1	13.9	9.48	14.9	12.0
1,2,3,4,7,8-HxCDF	9.9	19.2	7.11	10.9	6.84	12.2	4.83	10.2	(0.09)	(0.35)	(0.14)	(0.16)	8.09	16.8	7.0	7.43	4.44	16.2	32.8	14.5
1,2,3,6,7,8-HxCDF	12.5	26.6	8.7	19.1	8.81	17.0	6.4	17.9	(0.16)	(0.40)	(0.24)	(0.27)	8.55	15.0	7.35	9.76	6.14	17.7	37.0	15.0
2,3,4,6,7,8-HxCDF	7.55	11.6	5.27	10.4	5.13	8.62	3.72	9.98	(0.10)	(0.18)	(0.16)	(0.17)	6.23	10.2	5.46	7.24	6.22	(7.00)	12.6	8.61
1,2,3,7,8,9-HxCDF	(0.34)	(0.37)	(0.28)	(0.22)	(0.34)	(0.29)	(0.19)	(0.31)	(0.12)	(0.21)	(0.18)	(0.20)	0.17	(0.13)	(0.14)	(0.14)	(2.34)	(0.63)	(1.44)	(0.60)
1,2,3,4,7,8-HxCDD	(0.55)	22.6	(0.57)	15.1	(0.31)	15.2	5.95	15.6	(0.30)	(0.30)	(0.31)	(0.14)	21.8	33.6	25.8	25.7	(1.15)	9.44	22.7	11.0
1,2,3,6,7,8-HxCDD	224	345	169	208	160	225	114	188	2.38	4.97	2.81	2.3	122	187	97.9	104	71.2	116	202	116
1,2,3,7,8,9-HxCDD	22.9	41.4	8.67	19.9	11.3	22.8	9.02	17.1	(0.25)	(0.24)	(0.25)	(0.11)	16.1	29.3	16.2	23.1	14.1	23.5	33.5	31.3
1,2,3,4,6,7,8-HpCDF	85.6	155	71.3	87.7	53.5	80.5	45.9	71.3	1.21	2.59	1.63	1.18	119	185	132	121	235	540	668	494
1,2,3,4,7,8,9-HpCDF	9.31	15.6	6.74	6.06	4.28	7.53	3.44	4.66	(0.25)	(0.26)	(0.33)	(0.19)	12.2	32.3	17.8	16.2	38.3	106	279	97.2
1,2,3,4,6,7,8-HpCDD	457	647	316	416	285	319	204	337	7.3	13.7	7.2	6.6	929	1585	900	1077	1099	1533	2335	1773
OCDF	22.7	33.8	20.2	22.9	13.3	16.5	13.0	16.5	0.83	1.01	0.93	0.74	302	557	387	308	899	1979	3833	1891
OCDD	532	560	264	428	272	317	158	299	13.4	27	12	12.1	3768	792	4184	3845	6094	14504	24637	18155

[a] Animal number. [b] Numbers in parentheses represent limits of quantitation.

congeners by animals in a typical beef production setting. The experiment was conducted at a research facility that is typical of rural production sites. Beef production protocols will rarely provide a lactation period, and significant depletion will prevail only if the dioxin source is removed quite early in an animal's life as indicated by the long half-lives (100−200 days) estimated for certain PCDD/Fs (Startin et al., 1994; Thorpe et al., 1999). The uptake determinations were compromised by an unexpected contamination at the site and could only be calculated for the tetra and penta congeners. Bioavailabilities were determined using retention percentages, similar to carry-over rates, defined as the total amount of congener in fat (nanograms) divided by the total intake (nanograms), on a percentage basis. Estimates of the fat contents of the live animals were determined by the bioelectrical impedance measurements (Figure 1). Using the final measurements of total animal fat, body weight, and the individual congener concentrations in back fat, average retention percentages were calculated to be 52.4, 33.7, 1.0, and 40.2 for 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 2,3,7,8-TCDF, and 2,3,4,7,8-PeCDF, respectively. Retention percentages based on perirenal fat were 53.2, 36.7, 1.2, and 42.9, respectively, for these same congeners. These retention percentages should give a conservative estimate of the uptake of the lower chlorinated congeners by beef animals raised in typical production settings (see tissue distribution discussion below). Fries et al. (1999) compared carry-over rates from their study with carry-over rates reported or calculated from data presented in five publications involving lactating cows and found values of 15−35 for 2,3,7,8-TCDD, 10−55 for 1,2,3,7,8-PeCDD, and 12−36 for 2,3,4,7,8-PeCDF. The values obtained in our study are in reasonable agreement considering that feeding of the dioxins and furans took place until the time of slaughter and that lactation was not involved. Low retention of 2,3,7,8-TCDF is likely due to the high rate of metabolism of this congener relative to the others (Birnbaum et al., 1980; Poiger et al., 1989).

Bioconcentration factors (BCFs) can be estimated for the tetra and penta congeners if it is assumed that because the dose and the daily feed were given at essentially the same time the mixture was homogeneous. BCFs for the higher chlorinated congeners cannot be obtained due to the secondary site contamination. Average BCFs (picograms per gram of wet tissue/nanograms per kilogram of feed) for 2,3,7,8-TCDD, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDD, and 2,3,4,7,8-PeCDF were, respectively, 11.3, 0.2, 7.6, and 8.9 for perirenal and back fats; 0.9, 0.01, 2.2, and 3.3 for liver; 0.3, 0.0, 0.1, and 0.1 for ribeye. The bioconcentration values in fat were almost 3 times higher than what was observed by Jensen et al. (1981) in beef cattle or by Fries et al. (1999) in bovine milk fat (on a wet feed weight basis); however, the animals in those studies were most likely not at steady state after only 28 and 58 days of dosing. When compared to BCFs in chickens fed similar concentrations of PCDD/Fs for 164 days (Stephens et al., 1995), two notable exceptions were seen. 2,3,7,8-TCDF was bioconcentrated in fat, liver, and muscle to a much higher extent in chickens than in beef (50−130 times), probably indicating a lower capacity to metabolize TCDF in the chickens. In chicken thigh muscle 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, and 2,3,4,7,8-PeCDF were bioconcentrated 11, 25, and 33 times more, respectively, than in beef muscle (ribeye). This difference cannot be

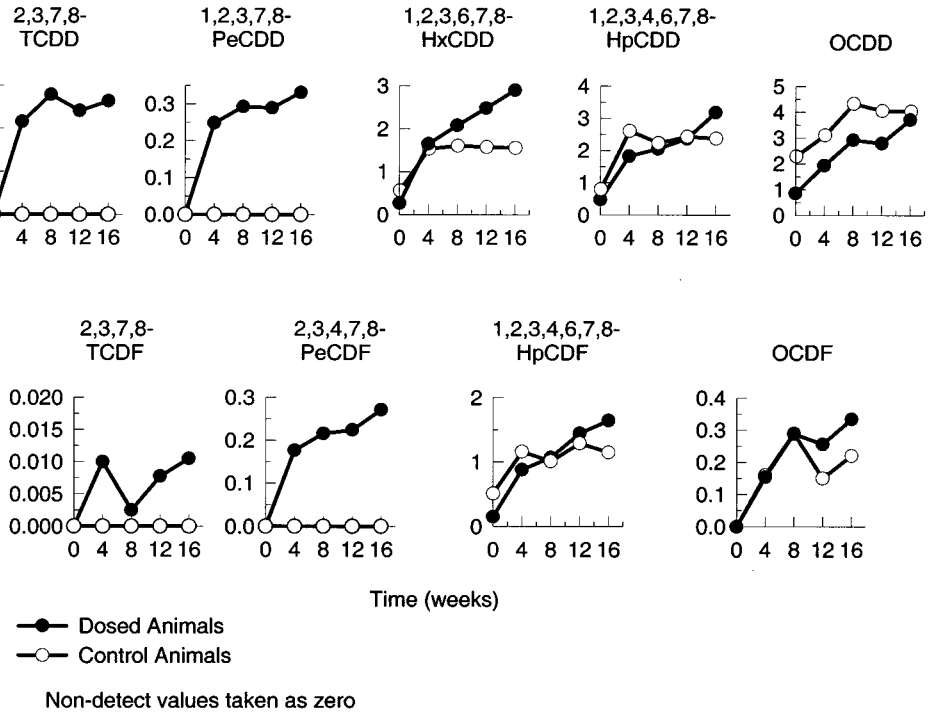

Figure 2. Average serum concentrations of dosed congeners on a wet weight basis (pg/g, ppt).

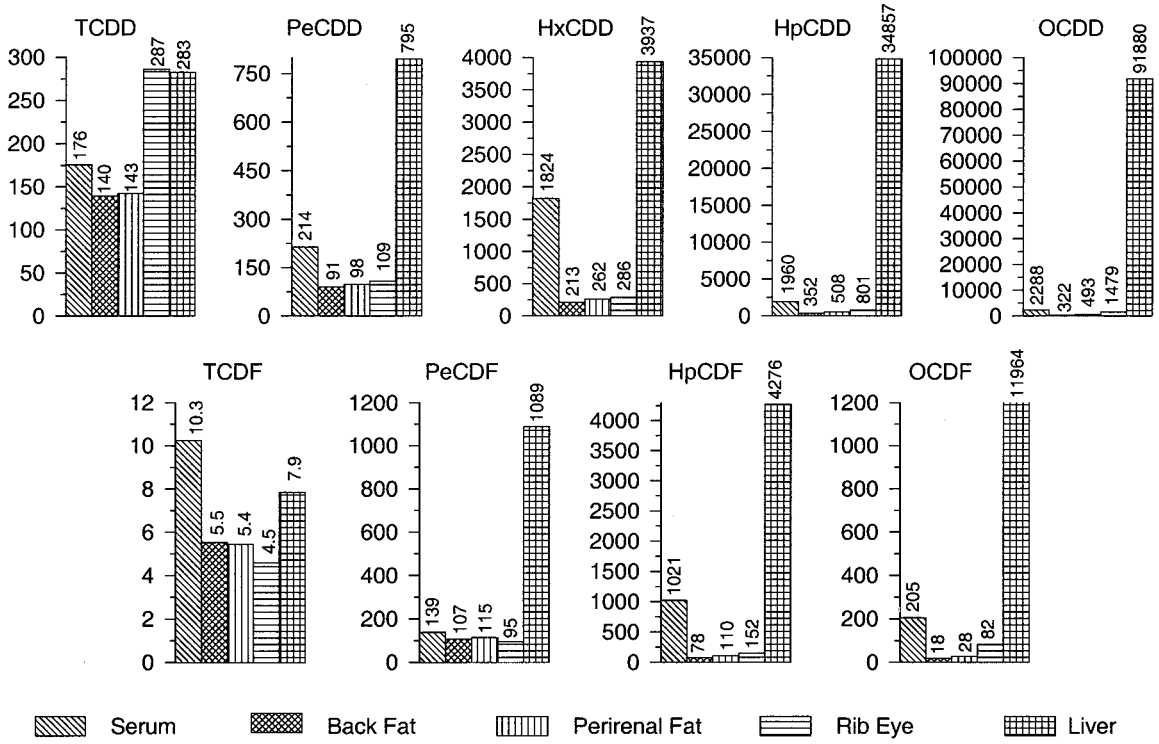

Figure 3. Congener distribution on a lipid-adjusted basis of dosed congeners in dosed animals (average of the four dosed animals, pg/g, ppt).

entirely explained by the different percentages of fat in the muscles but may reflect differences such as rates of metabolism, percentages of the total body fat present in muscle, or the composition of the fat.

The other objective of this study was to determine distribution profiles in various tissues. Figure 3 shows the distribution of the fed congeners on a lipid-adjusted basis. Back fat, perirenal (kidney) fat, and the marbling fat of muscle tissue (represented by ribeye in our experiment) have concentrations of the same magnitude, indicating that either back fat or perirenal fat would be a good sampling matrix for estimating levels of dioxins in retail cuts. The 2−3-fold higher concentrations of 2,3,7,8-TCDD, hepta, and octa congeners in ribeye present a problem not only because ribeye is a typical consumer meat product but also because there are, to our knowledge, no good estimates of what percentage of the total animal fat is stored in this compartment. Although not reaching a steady state may explain the difference for hepta and octa congeners, we

Table 6. Dioxin/Furan Concentrations in Components of the Feeding Facility (Picograms per Gram of Wet Weight, ppt)

	posts in animal stalls												
	control				dosed				feed bunk sandings	feed bunk drillings	alfalfa hay	ground corn	composite walls/posts
	321[a]	345[a]	355[a]	489[a]	249[a]	277[a]	353[a]	419[a]					
2,3,7,8-TCDF	(3.5)[b]	(3.0)	(2.5)	(3.0)	(8.0)	9.6	(6.5)	(3.0)	26.4	(1.45)	(0.13)	(0.09)	188
2,3,7,8-TCDD	187	379	325	(5.5)	8.5	(11.0)	(8.5)	32.3	69.5	(2.39)	(0.22)	(0.10)	1529
1,2,3,7,8-PeCDF	10.0	4.3	(66.5)	12.2	29.8	66.2	(7.0)	20.6	138	(3.64)	(0.21)	(0.23)	1606
2,3,4,7,8-PeCDF	20.1	3.9	(6.0)	21.5	41.7	121	28.0	32.5	145	(2.86)	(0.15)	(0.18)	3276
1,2,3,7,8-PeCDD	20.2	3.3	(9.0)	23.9	97.5	204	29.8	43.6	1350	(2.75)	(0.31)	(0.15)	11780
1,2,3,4,7,8-HxCDF	210	139	228	258	344	1372	424	255	4856	(5.54)	(0.38)	(0.15)	14498
1,2,3,6,7,8-HxCDF	123	35.3	93.7	156	294	1226	261	204	6408	(9.76)	(0.60)	(0.27)	22575
2,3,4,6,7,8-HxCDF	451	265	442	473	808	3250	1277	650	2532	(6.26)	(0.40)	(0.17)	(6370)
1,2,3,7,8,9-HxCDF	(22.5)	19.5	35.3	40.5	(35.5)	(137)	102	(47.5)	(10.3)	(7.33)	(0.47)	(0.20)	(4134)
1,2,3,4,7,8-HxCDD	197	28.7	136	199	354	1420	294	248	7304	(5.09)	(0.58)	(0.27)	23906
1,2,3,6,7,8-HxCDD	871	673	1024	1101	1069	4003	1475	841	21878	30.7	(0.50)	(0.25)	92009
1,2,3,7,8,9-HxCDD	395	65.8	278	410	722	3034	579	448	13156	(14.1)	(0.46)	(0.22)	53508
1,2,3,4,6,7,8-HpCDF	8946	5938	9755	9784	10245	47846	16970	9580	37858	51.9	(0.62)	0.62	355799
1,2,3,4,7,8,9-HpCDF	1546	1350	1956	1658	(14.0)	7296	(13.0)	2045	14626	13.5	(0.91)	(0.63)	(37262)
1,2,3,4,6,7,8-HpCDD	61787	62380	83665	79530	56561	204763	113454	54043	196824	978	0.84	(0.40)	1700000
OCDF	86179	113046	133475	109093	71887	198888	161489	74305	123176	130	0.96	2.50	1100000
OCDD	616963	805509	951698	798041	531856	1605048	1147381	565144	699602	7995	6.21	3.01	1500000

[a] Animal number. [b] Numbers in parentheses represent limits of quantitation.

have no experimental evidence to account for the difference seen for TCDD. Factors such as time of fat deposition in the growth of the animal, time of exposure to dioxins, lipid concentration in the tissues, and lipid makeup (i.e., the ratio of phospholipids, triglycerides, diglycerides, etc.) may be involved.

Liver and serum lipids had greater concentrations of most congeners; however, these compartments are relatively small fat depots (ca. 0.4 and 0.1% of the total body fat). Liver has been shown to accumulate PCDD/Fs, especially the more substituted congeners, at higher levels on a lipid basis than adipose tissue in rats (Birnbaum and Couture, 1988; Rose et al., 1976), chickens (Stephens et al., 1995), and cattle (Chang et al., 1989; Thorpe et al., 1999). On a lipid-adjusted basis, the beef in this study had approximate liver/adipose concentrations of 2 for TCDD and TDCF, 10 for the penta congeners, 20 for HxCDD, 50−100 for the hepta congeners, and >300 for OCDD and OCDF. These values are similar to ratios reported by Chang et al. (1989) in a foraging cow and also fall in the range of values reported by Thorpe et al. (1999). Unlike chickens, in which liver amounts of all congeners were low (Stephens et al., 1995), the high liver-to-adipose ratios in cattle resulted in the liver accounting for approximately half of the total body burden of OCDD and OCDF. Preferential sequestration in beef liver may imply higher levels of cytochrome P450 1A2, a known sequestering protein in mice (Diliberto et al. 1997), in cattle than in chickens.

Serum, although not a consumer concern, is a useful sampling matrix for estimating body burdens on live animals. For congeners that had approached steady state (tetra and penta), serum values gave a reasonable estimate of the concentrations in other fat compartments. However, large differences between serum and adipose stores were observed in the hexa through octa congener levels, most likely reflecting the continuing and varying exposure of these animals to a secondary contamination source. This uncontrolled exposure did not allow the animals to reach steady state for the hexa, hepta, or octa congeners during the experiment and, therefore, serum did not adequately predict the body burden of these congeners.

A secondary contamination source is obvious from Tables 2−5 and Figure 2, which show the concentrations of some PCDD/Fs in the control steers at similar or greater levels than those found in the dosed animals. It appeared that the site contained an additional source of dioxins and furans that contributed larger amounts of some congeners than the dose. The corn and hay had PCDD/F levels below the detection limits for most congeners (Table 6) and were identical for control and dosed animals. A survey of the feeding facility indicated that walls and posts were the likely source of dioxins and furans (Table 6). The primary source for each animal was one post in each stall. Although the posts had significantly different PCDD/F concentrations, there was no correlation between post concentrations and animal fat concentrations, likely due to variable tendencies of animals to lick and chew. Surface contamination was quite prevalent as indicated by concentrations in sanding samples of the concrete feed bunks versus concentrations in drilling samples. The surface contamination was almost certainly due to contamination by feces, which were shown to contain excreted PCDD/Fs (Tables 4 and 5). The most logical source of the PCDD/F contamination in our experiment is PCP-treated wood in the facility. The use of PCP-treated wood was extensive during the 1950−1980 time period (Shull et al., 1981) when the facility was built. The wood samples from our feeding facility contained large concentrations of PCP relative to PCDD/Fs (V. J. Feil, unpublished studies) and are similar to concentrations reported by Fries et al. (1998).

PCP concentrations in the perirenal fat of control animals were determined and correlate quite well with concentrations of the higher chlorinated congeners (Figure 4) probably because ad libitum exposure to the PCP-treated wood prevailed until the day of slaughter. PCDD/Fs are not readily excreted, having half-lives of 100−200 days (Startin et al., 1994; Thorpe et al. 1999), whereas PCP has a half-life of only a few days in cattle (Firestone et al., 1979; Osweiler et al., 1984). Therefore, the PCP levels found in our animals likely reflect the amount of licking and chewing a given animal did in the day or two before slaughter, whereas the PCDD/F levels represent accumulation from ingestion over the entire experimental time. Animals that have large exposures to PCP early in their lives and are then transferred to a facility free of dioxins, furans, and PCP for fattening would at the time of slaughter still have

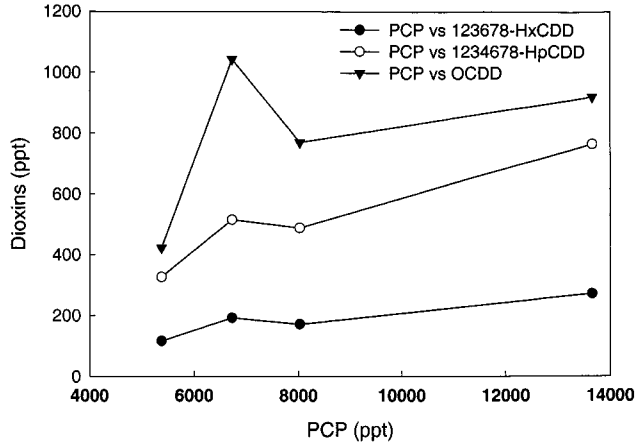

Figure 4. Relationship of PCP with 1,2,3,6,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD, and OCDD in perirenal fat (average of the four control animals).

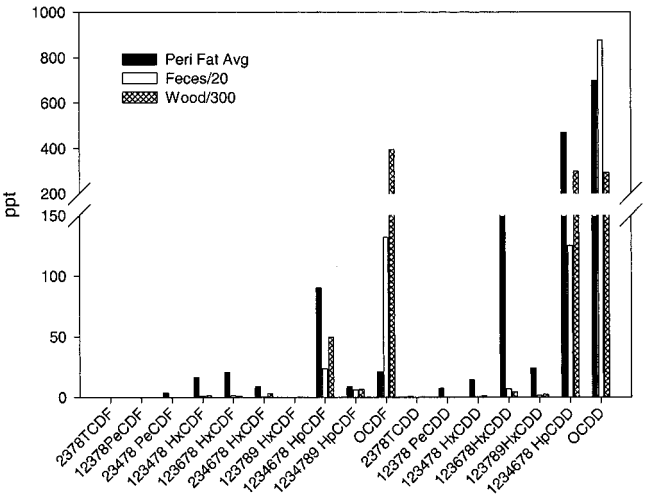

Figure 5. Congener patterns in perirenal fat, final feces, and wood (average of the four control animals for fat and feces and average of eight wood samples).

significant levels of dioxins and furans but no PCP residues. Conversely, animals that have been raised in a dioxin-free environment but had exposure to PCP-treated wood a day or two before slaughter would have significant levels of PCP but no PCDD/Fs.

Figure 5 shows the pattern of congeners in feces and perirenal fat of control animals as well as in wood. The changes that occurred in congener patterns between PCP-treated wood and beef fat are compatible with the BCFs reported by Fries et al. (1999) in lactating cattle fed ground PCP-treated wood and by Stephens et al. (1995) in adipose tissue of chickens "fed contaminated soil from a farm in the vicinity of a PCP facility". These BCF values were 3.7, 0.68, 0.08 0.72, and 0.07 in cows and 6.84, 1.61, 0.36, 1.43, and 0.31 in chickens for 1,2,3,6,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD, OCDD, 1,2,3,4,6,7,8-HpCDF, and OCDF, respectively. The most prevalent congeners in PCP formulations have relatively low toxicities (TEFs of 0.0001 for OCDD/F and 0.01 for HpCDD/Fs) in addition to the low BCFs. However, the congener that bioaccumulates to the greatest extent, that is, 1,2,3,6,7,8-HxCDD, has a TEF of 0.1. On a toxicity basis, 1,2,3,6,7,8-HxCDD accounted for 35−45% of the total toxic equivalency (TEQ) in the control animals. These control animals had some of the highest TEQs on a lipid weight basis (32.8−60.9 ppt) that we have seen in our work, suggesting that exposure to PCP-treated wood could present significant risks if such beef entered the market. Other studies have shown that technical grade PCP is more toxic to cattle than analytical grade (McConnell et al., 1980; Parker et al., 1980), mainly because of the PCDD/F contaminants found in technical PCP formulations.

The serendipitous discovery that licking and chewing of relatively small surface areas of wood by cattle can generate high levels of PCDD/Fs in fat has been valuable in some of our other studies. The relatively consistent congener pattern found in beef fat after PCP exposure, which is somewhat different from that found in wood (Figure 5), has also been of great value. Investigation of the sites in our national survey that produced animals with high levels of PCDD/Fs and characteristic PCP congener patterns revealed PCP-treated wood components in feeding facilities in every case (Feil et al., 1995, and unpublished studies). The characteristic congener pattern also led us to do a trace-back investigation on PCDD/F levels found in elk from two North Dakota areas (Feil et al., 1998). We found that local hunting groups had constructed feeding facilities from utility poles and PCP-treated lumber to improve animal survival during severe winter weather. Even the limited contact with these facilities caused an increase in PCDD/F levels in some animals.

In conclusion, although a secondary dioxin contamination compromised some of the experimental goals, uptake and distribution information was obtained for several important dioxin congeners. The unexpected site contamination (PCP-treated wood) raised concerns because the extent of PCP-treated wood currently in use in farm settings is not adequately known, nor is the extent to which it may contribute to levels in beef.

SAFETY

Dioxins are considered to be extremely toxic; 2,3,7,8-TCDD is a known human carcinogen. Appropriate measures should be used to minimize exposure when handling dioxins and dioxin-like compounds.

ABBREVIATIONS USED

PCDD, polychlorinated dibenzo-*p*-dioxin; PCDF, polychlorinated dibenzofuran; PCB, polychlorinated biphenyl; TCDD, tetrachlorodibenzo-*p*-dioxin; TCDF, tetrachlorodibenzofuran (other prefixes: Pe, penta; Hx, hexa; Hp, hepta; O, octa); PCP, pentachlorophenol; BCF, bioconcentration factor; TEF, toxic equivalency factor; TEQ, toxic equivalency.

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