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Laboratory and On-Farm Studies on the Bioaccumulation and Elimination of Dioxins from a Contaminated Mineral Supplement Fed To Dairy Cows

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A dioxin-contaminated mineral supplement was used to study the bioaccumulation and elimination of dioxins in two dairy cows. The supplement was mixed into the total maintenance ration and fed to the cows for 40 days after which unfortified diets were fed for 40 additional days. Dioxins and coplanar polychlorinated biphenyls (PCBs) were measured twice a week in the milk and in selected tissues of the cows, one at death (day 10 of withdrawal) and one at slaughter (day 40 of withdrawal). The dioxins and PCBs were readily transferred into the milk, and at steady state, total toxic equivalents were concentrated 6-fold into the milk fat from the diet. Bioaccumulation was inversely related to chlorination number. The elimination of dioxins and PCBs in milk was biphasic. With the exception of 1,2,3,4,6,7,8-heptachlorodioxin and both octachlorinated congeners, dioxin and furan half-lives in milk were approximately 3−5 days for the α-phase and 35−50 days for the β-phase. PCB-169 had a longer half-life: 11 (α) and 200 days (β). When milk and feed samples from Minnesota farms that had used similar contaminated mineral supplements were analyzed, no elevated dioxin levels were found in milk. It appeared that although the dioxins from the mineral supplements have the potential to bioaccumulate, dilution into the total diet was sufficient to prevent a significant rise in the dioxin concentrations in the milk at these farms.

KEYWORDS: Dioxins; polychlorinated biphenyls; cows; milk; bioaccumulation; half-life; mineral supplement

INTRODUCTION

Dioxins are a class of ubiquitous environmental pollutants, which include polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and coplanar polychlorinated biphenyls (PCBs). In recent years, several incidents of elevated dioxin levels in food products have occurred due to the use of contaminated animal feed components. In the United States, dioxin-contaminated ball clay, used as an anticaking agent in feeds, resulted in chickens (*1*) and catfish (*2*) with dioxin levels 10−50 times higher than the average of unexposed animals. In Europe, contaminated citrus pulp fed to cows increased the dioxin concentration in dairy products 2−8 times over the background (*3*). In Belgium, PCB-contaminated oil was mixed with rendered fat and added to animal feeds leading to widespread contamination of animals and products (*4*). Outcomes of these incidents included recalls of feed, voluntary or mandatory recalls of products, and the establishment of temporary or permanent maximum residue limits in feeds and food products.

In March of 2002, a dioxin-contaminated mineral supplement fortified with an organic binder was discovered during routine screening in Europe. Subsequent testing by the U.S. Food and Drug Administration showed copper, iron, magnesium, zinc, and trace mineral supplements with elevated dioxin contents (*5*). The supplements were approved for use in all food animals and had been used by poultry, pork, and dairy producers in the United States. The contaminated supplements were removed from the market, but food products were not recalled because the small amounts of mineral supplement added to livestock feeds were deemed a negligible contributor of dioxins and thought to pose no significant food safety risk. To investigate the potential dioxin exposure associated with the contaminated minerals, we conducted a controlled feeding study in dairy cows and also collected and analyzed samples from local dairy farms that had purchased the contaminated supplements.

The bioavailability of dioxins is known to vary with exposure matrix. Fly ash-bound dioxins were relatively unavailable to cattle (0.5% carried into milk), while dioxin-contaminated grass provided better uptake (7% into milk) (*6*). Other studies have shown that dioxins in mixed diets may afford higher bioavailability (20−30% of major congeners into milk) (*3*, *7*). The current feeding study was designed to determine the bioavailability of dioxins from a contaminated mineral supplement under typical dietary usage, the bioconcentration into milk, and also to estimate withdrawal times needed for cows after a contami-

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Table 1. Concentration (pg/g Dry Weight) of PCDD/F and PCB TEQs in Environmental and Feed Components[a]

	N	D/F TEQ	PCB TEQ
wood shavings	1	0.09 (0.04)	0.003 (0)
MgO[b]	3	294 ± 39.4	2.91 ± 0.5
		(304 ± 47.5)	(2.91 ± 0.5)
forage	3	0.22 ± 0.04	0.01 ± 0.01
		(0.33 ± 0.06)	(0.01 ± 0.01)
concentrate	3	0.05 ± 0.03	0.04 ± 0.01
		(0.20 ± 0.06)	(0.04 ± 0.01)
concentrate + MgO	6	12.5 ± 1.27	0.11 ± 0.01
		(12.7 ± 1.28)	(0.11 ± 0.01)
control TMR	3	0.19 ± 0.09	0.02 ± 0.01
		(0.23 ± 0.07)	(0.02 ± 0.01)

[a] Values are upper bound (n = LOD) with lower bound (nd = 0) in parentheses. N = number of replicates. [b] Wet weight concentration.

nation. These data would be valuable in the event of future discoveries of contaminated minerals or feeds. In addition, data on the distribution of dioxins into edible tissues and organs were gathered. The results of the feeding study were compared to the findings from field-collected samples. Because of limitations in financial and physical resources, only two dairy cows were included in the feeding study.

MATERIALS AND METHODS

Dosing Experiment. Two healthy Holstein cows (nos. 2232 and 2189) ages 2 and 3 years, respectively, were purchased from the North Dakota State University (NDSU) dairy barn in July, 2002. Cow 2232 had calved once and was 184 days into the milking cycle. Cow 2189 had calved twice and was 262 days into the milking cycle. The two cows were related on the maternal side (mothers were sisters), and both had been raised since birth at the NDSU dairy.

For the duration of the experiment, the cows were tethered to stanchions in an open barn area with concrete flooring and did not have contact with one another. Rubber mats and wood shavings served as bedding and prevented access to the concrete. The shavings were changed daily as needed to provide a clean environment. Each cow had free access to feed from individual plastic feeders and water from individual metal watering cups. No materials that were suspected of containing PCDD/Fs or PCBs (such as pentachlorophenol-treated wood) were in the vicinity of the cows. The cows were acclimated to their new surroundings for 1 week before dosing began.

The cows' rations remained the same as while at the NDSU dairy. A supply of total maintenance ration (TMR) was received daily from NDSU to feed the cows during the acclimation and withdrawal periods. The TMR consisted of 71% forage and 29% grain concentrate by weight (55:45 by dry weight). During the dosing portion of the experiment, the forage component of the TMR was obtained daily from NDSU and mixed on-site with a grain concentrate containing the dioxin dose. The dioxin-containing grain concentrate was prepared in the following manner: 4.13 kg of a dioxin-contaminated magnesium oxide supplement (10% Mg polysaccharide complex, obtained from the MN Department of Agriculture) was added in three portions to 150 kg of the grain concentrate and mixed in a Davis mixer for 5 min between additions and 10 min after the final addition. The final dosing TMR contained 0.13% Mg and 5.8 ppt dioxin toxic equivalency (TEQ) on a dry weight basis. Samples of the control grain concentrate and each 150 kg batch of dose-containing concentrate were collected for dioxin analysis. Grab samples were also collected weekly from the NDSU forage, TMR, and wood shavings to provide composite samples of each for dioxin analysis. The analytical results for each of these components are given in **Table 1**.

The cows were milked twice daily with a portable milking machine, and daily milk production was recorded. Samples of milk were collected for analysis from each cow twice a week and consisted of pooled aliquots (125 mL) from the morning and evening milkings. Four days into the withdrawal phase of the experiment, cow 2189 went off feed and was treated under veterinary care for indigestion (vitamin B complex injections and Poly Ox II boluses). After 2 days of treatment, she seemed to improve but died suddenly on day 10 of withdrawal. A complete necropsy revealed gastrointestinal inflammation but provided no definitive cause of death (report from Neil Dyer, DVM, NDSU Veterinary Diagnostic Laboratory. Histopathology: Cerebrum, cerebellum, hippocampus, heart, lung, diaphragm, liver, kidney, spleen, ileum, colon, abomasum, and rumen were examined; diffuse distention in the ileum and colon were found. Acid fast stains were negative for *Mycobacteria*. Bacteriology: mixed contaminants in ileum; no *Salmonella* recovered. Virology: ileum negative for BVDV.). Samples of liver, kidney, adipose tissue from around the kidney, and muscle from the back were collected at necropsy and frozen. The remaining cow was slaughtered (stun gun and exsanguination) after the 40 day withdrawal period, and tissue samples were collected and frozen as before.

MN Milk and Feed Sampling. The MN Department of Agriculture identified dairy farms within the state that had purchased products containing the dioxin-contaminated mineral supplements. Ten farms were randomly selected for sample collection. State milk inspectors collected milk samples from the bulk tanks along with feed samples from each of these dairies. A questionnaire was also completed at each farm, which provided information on the type and amount of mineral supplements mixed into the feed and the duration of feeding. In addition, milk samples were collected by the inspectors from two nearby farms, which had not used the contaminated mineral supplements. The frozen samples and the questionnaires were shipped to the ARS laboratory in Fargo, ND, for analysis.

Sample Analysis. All samples were processed and analyzed for PCDDs, PCDFs, and coplanar PCBs by a modification of EPA Method 1613 (Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, 1994) to include the coplanar PCBs (IUPAC nos. 77, 126, and 169). The chemical standards used for the analysis were purchased from Wellington Laboratories (Guelph, ON). Prior to chromatography, the samples were extracted by previously reported methods as briefly described here. Milk samples (100 mL) were treated with potassium oxalate and ethanol and extracted into ether:hexane (*8*). Tissue samples were homogenized, and subsamples (10 g of liver and 50 g of kidney or muscle) were extracted in an Accelerated Solvent Extractor (Dionex, Sunnyvale, CA) using 2-propanol:hexane:methylene chloride (35:30:35) (*9*) and subsequently washed with 20% potassium hydroxide and concentrated sulfuric acid (*10*). Feed and wood samples were homogenized, freeze-dried or oven-dried, and subsamples (10 or 100 g) were sonicated in toluene or toluene:acetone (70:30) for 1 h. Adipose tissue samples were homogenized, and subsamples (5 g) were dissolved in methylene chloride. For milk and tissue samples, lipid percentages were measured gravimetrically from the extracts after drying with sodium sulfate.

All samples were applied in hexane to a PowerPrep automated dioxin cleanup instrument (Fluid Management Systems, Waltham, MA) for chromatography on triphasic silica, basic alumina, and carbon cartridges. A 2 μL aliquot of the final sample in 10 or 20 μL of dodecane was analyzed by HRGC−HRMS using an Autospec Ultima mass spectrometer (Micromass, Beverly, MA) coupled to an Agilent 6890 gas chromatograph. The limits of detection (LODs) were determined by the MS operating system as S/N = 3. Average congener LODs were 0.03−0.12 pg/g lipid for milk, 0.02−0.15 pg/g dry matter for feeds, and 0.2−0.8 pg/g lipid for tissues. For feed samples containing the contaminated MgO supplement, LODs were elevated to 1−3 pg/g dry matter in the TCDF, PeCDF, and HxCDF regions due to interferences in the gas chromatography/mass spectrometry (including multiple non-2,3,7,8-congeners).

TEQs were calculated by summing the products of each congener concentration and its toxic equivalency factor (TEF) (*11*). Both upper-bound (nd = LOD) and lower-bound (nd = 0) TEQ values were calculated. For the dosing experiment, upper-bound and lower-bound values were generally the same; upper-bound values were used in calculations of bioaccumulation parameters.

Quality Assurance/Quality Control. Our laboratory has validated the analytical method for lipid-rich matrices using low level spikes of the 20 dioxin and PCB congeners. With this method, we have

Table 2. Average Milk Production ± Standard Deviations for Cows 2189 and 2232 during the Overall Study and during the Steady State Period of the Study (Days 17–38); Estimated Average Feed Intake over the Entire Study

	daily milk production (kg)		% milk fat		estimated daily feed intake (kg)	
cow no.	overall	days 17–38	overall	days 17–38	wet wt	dry wt
2189	19.5 ± 1.7	19.4 ± 1.2	3.01 ± 0.15	3.06 ± 0.18	39.8	23.7
2232	25.2 ± 1.4	25.7 ± 1.8	3.08 ± 0.25	3.15 ± 0.14	39.8	23.7

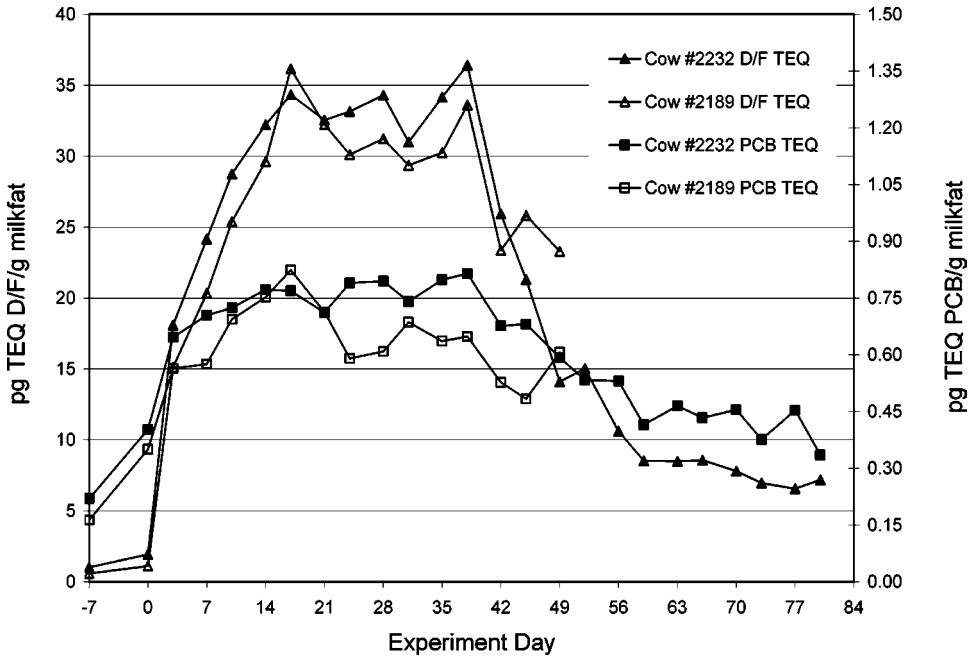

Figure 1. Accretion and depletion of PCDD/F and PCB TEQs in the milk of cows 2189 and 2232. Dosing began midday of day 0 and withdrawal began on day 40.

successfully participated in an interlaboratory exercise for the analysis of dioxins and PCBs in food matrices. For ongoing quality control, a method or matrix blank and a method or matrix spike were run with every eight samples. Blanks were evaluated to ensure no laboratory contamination, and spiked samples were required to be within 25% accuracy and precision.

Modeling. Depletion of PCDD/Fs and PCBs from the milk of cow 2232 was fitted to a two compartmental model [$C(t) = Ae^{-\alpha t} + Be^{-\beta t}$] using WinNonlin Software (vs 1.5; Scientific Consulting, Inc.; Cary, NC). Because milk accumulation and steady state were not modeled, the model is not appropriate for predicting milk concentrations of PCDD/Fs and PCBs. However, within the limitation that data were from one animal only, the model is appropriate for estimating the half-lives of PCDD/Fs and PCBs in the milk. The average concentrations during steady state were used for day zero values in the model to remove some of the sample-to-sample variability. Correlations (r^2) (observed vs predicted) of the modeled curves were between 0.9357 and 0.9985 for PCDD/F and PCB data.

RESULTS

The two dairy cows used in this study were from the local NDSU dairy where bulk milk samples had previously been analyzed and found to be low in PCDD/Fs (0.87 ± 0.24 pg TEQ/g lipid) and PCBs (0.26 ± 0.03 pg TEQ/g lipid) and similar to the national averages [0.88 pg PCDD/F TEQ/g lipid and 0.5 pg PCB TEQ/g lipid (*12*, *13*)]. The cows were kept on the same TMR supplied by the university with one exception: A dioxin-contaminated magnesium supplement was substituted for the magnesium oxide in the ration during the dosing phase. During the dosing phase, weighed amounts of TMR were offered to the cows daily. Because the majority of the TMR was consumed, the offered amounts gave an estimate of the average daily intake for each cow (**Table 2**). After 10 days in their new surroundings, the cows reached milk production rates that remained steady throughout the study (**Table 2**). Cow 2232 averaged 90% of her milk production at the university; cow 2189 averaged 73%. The lowered milk production by cow 2189 was attributed to her late stage of lactation (262 days at arrival).

Over the 40 days of dosing, each cow received a daily dose of approximately 135 ng TEQ in the total ration (5.8 ppt dry weight). **Figure 1** shows that the TEQs in the milk fat rose quickly and appeared to reach a steady state after 17 days. Although the initial TEQ increases on day zero may be due to day-to-day variations, it may also represent a rapid breakthrough of PCDD/Fs and PCBs into the milk because dosing began midday on day zero.

After withdrawal of the contaminated feed, the TEQ resulting from PCDD/Fs (D/F TEQ) dropped nearly 50% in the first week (days 40−49) and then began a more gradual decrease. The TEQ resulting from PCBs (PCB TEQ) showed a slower decline. Unfortunately, cow 2189 died at day 49, so only data from cow 2232 were available for the entire withdrawal period. The estimated half-lives for the biphasic milk depletion curves are 4.7 and 62.2 days for the D/F TEQ and 6.1 and 87.1 days for the PCB TEQ (cow 2232). Following 40 days of withdrawal, the milk had not yet reached the predose levels of either D/F or PCB TEQs.

The uptake and withdrawal curves for the dominant congeners in the milk paralleled the TEQ curves (data not shown). The half-lives for individual congeners in the milk for cow 2232 are shown in **Table 3**. Except for OCDF, OCDD, and the α-half-life of 1,2,3,4,6,7,8-HpCDD, the half-lives of the PCDD/Fs were

Table 3. Estimated α- and β-Half-Lives (Days) for Major PCDD/F and PCB Congeners and TEQs

congener	α-half-life	β-half-life
23478-PeCDF	4.6	42.5
123478-HxCDF	4.4	50.5
123678-HxCDF	3.3	34.5
234678-HxCDF	4.1	40.8
1234678-HpCDF	4.9	45.8
OCDF	0.1	14.1
12378-PeCDD	4.1	51.4
123478-HxCDD	5.4	46.2
123678-HxCDD	3.8	35.8
123789-HxCDD	4.0	42.3
1234678-HpCDD	14.5	54.3
OCDD	0.2	72.6
PCB-126	10.7	196.4
PCB-169	1.5	38.8
D/F TEQ	4.7	62.2
PCB TEQ	6.1	87.1

Table 4. TEFs and Average Levels of 19 PCDDs, PCDFs, PCBs, and TEQs in the TMR (Dry Weight Basis) and the Milk Fat at Steady State for Each Cow (Average of Days 17−38)[a]

		congener levels (ppt)							
			milk fat, steady state		carry over (%)		BCF	
congener	TEF	TMR[b]	cow 2189	cow 2232	cow 2189	cow 2232	cow 2189	cow 2232
2378-TCDF	0.1	1.5[c]	0.1	0.1	0.2	0.3	0.1	0.1
12378-PeCDF	0.05	1.2	0.9	0.9	1.8	2.8	0.7	0.8
23478-PeCDF	0.5	2.4	17.8	20.1	18.7	28.8	7.5	8.4
123478-HxCDF	0.1	8.0	58.0	57.5	18.1	24.5	7.2	7.2
123678-HxCDF	0.1	6.1	42.3	42.5	17.4	23.8	6.9	7.0
234678-HxCDF	0.1	8.7	49.6	52.3	14.4	20.6	5.7	6.0
123789-HxCDF	0.1	0.3[c]	1.4	1.3	12.4	15.6	4.9	4.6
1234678-HpCDF	0.01	59.8	98.5	82.7	4.1	4.7	1.7	1.4
1234789-HpCDF	0.01	7.7	15.6	13.7	5.1	6.1	2.0	1.8
OCDF	0.0001	50.8	8.5	7.5	0.4	0.5	0.2	0.2
2378-TCDD	1	0.3[c]	0.3	0.3	2.5	4.2	1.0	1.2
12378-PeCDD	1	0.4	3.1	3.4	21.4	32.3	8.5	9.4
123478-HxCDD	0.1	1.1	7.0	8.1	15.5	24.4	6.2	7.1
123678-HxCDD	0.1	1.7	14.2	16.1	21.5	33.1	8.6	9.7
123789-HxCDD	0.1	1.3	7.1	7.2	13.3	18.2	5.3	5.3
1234678-HpCDD	0.01	22.9	38.9	35.7	4.3	5.3	1.7	1.6
OCDD	0.0001	59.6	13.8	12.1	0.6	0.7	0.2	0.2
PCB-126	0.1	0.8	6.2	7.0	20.5	31.7	8.2	9.3
PCB-169	0.01	0.5	5.3	7.1	26.7	48.4	10.6	14.2
D/F TEQ		5.76	31.9	33.7	13.9	20.0	5.5	5.9
PCB TEQ		0.07	0.7	0.8	24.0	38.1	9.6	11.1
total TEQ		5.83	32.5	34.5	14.0	20.2	5.6	5.9

[a] Carry over percentages and bioconcentration factors (BCF) into the milk fat are calculated from the following formulas: carry over = pg excreted daily in milk/pg daily intake; daily milk fat production and dietary intake are taken from Table 1; BCF = ppt milk fat/ppt diet. Results of calculations may not appear exact due to rounding of concentrations. [b] Calculated from the analyses of MgO-containing concentrate and forage. [c] Values are nondetects set to the LOD.

similar (3−5 days for the α-phase and 35−55 days for the β-phase) and did not show a trend based on chlorine number. 1,2,3,4,6,7,8-HpCDD had an α-half-life almost three times longer than the other congeners (14.5 days) while OCDF and OCDD showed extremely rapid distribution half-lives in the milk (0.1−0.2 days). For the individual PCBs, PCB-126 had half-lives over five times longer than PCB-169 and at least twice that of the average PCDD/Fs.

Table 4 gives the individual congener concentrations and TEQs in the feed and in the milk fat from each cow during the steady state period. Bioconcentration factors (ppt milk fat/ppt feed, dry weight) and carry over rates (pg excreted daily in milk/pg daily intake) were calculated from the data. Because TCDF, 1,2,3,7,8,9-HxCDF, and TCDD were below the LODs in the feed, the results for these three congeners are problematic and are not included in further discussions. PCB-77 was not included due to high laboratory blank contaminations. The congeners detected in the feed provide more reliable data and show that BCFs generally decrease with increasing chlorination from 8 for penta-CDD/Fs to 0.2 for the octa-CDD/Fs. The coplanar PCBs had the highest BCFs at 8−14. The percent of the dose carried over each day into the milk showed a similar decreasing trend from 30% for PCBs from 20% for penta-CDD/Fs to less than 1% for octa-CDD/Fs. The apparent difference in carry over rates between the two cows was due to the estimation that both ate the same amount of feed, but cow 2232 produced more milk and, therefore, excreted more PCDD/Fs and PCBs on a daily basis.

To determine the distribution of PCDD/Fs and coplanar PCBs into various tissue compartments, milk, adipose tissue from around the kidney, back muscle, kidney, and liver were analyzed from each cow. With the exception of liver, the concentrations of most congeners were equivalent in each tissue on a lipid weight basis (**Figure 2**); concentrations of hepta- and octa-CDD/Fs were less comparable. It appeared that hepta- and octacongeners remained higher in the tissues than in the milk after 40 days of withdrawal (cow 2232), suggesting a slower redistribution of these congeners from the lipid stores. In both cows, OCDD concentrations were 2−15 times higher in the kidney than in the milk, adipose, or muscle on a lipid weight basis. The concentrations of all the PCDD/Fs and PCBs were 2−10 times lower in cow 2232 than in cow 2189, most likely due to the longer withdrawal time.

The livers of both cows selectively accumulated PCDD/Fs and PCB-126 on a lipid weight basis as compared to other tissues. PCB-169 did not appear to accumulate to a higher degree in the liver fat. For cow 2189 (early in withdrawal), the liver-to-adipose ratios ranged from 2 to 12 for penta- and hexa-CDD/Fs and PCB-126 (**Table 5**). For cow 2232 (late in withdrawal), the penta-CDD/F, hexa-CDD/F, and PCB-126 ratios were higher than for cow 2189 and ranged from 8 to 20. A similar trend was seen for the milk, kidney, and intramuscular lipids (kidney and muscle data not shown). Assuming no dramatic redistributions due to the health problems of cow 2189, these data suggest that the lower chlorinated PCDD/Fs and PCB-126 are redistributed out of lipid stores during withdrawal more quickly than from the liver. The concentrations of individual congeners in the livers were up to five times lower in cow 2332 but in some cases were not lower than cow 2189 (**Table 5**).

For the hepta- and octa-CDD/Fs, liver-to-adipose ratios ranged from 12 to 500 for cow 2189 and from 15 to 90 for cow 2232 (**Table 5**). Although these congeners concentrated in the liver to a higher extent than the lower chlorinated congeners, they appeared to deplete at the same rate or more quickly from the liver than from the adipose tissue stores resulting in equal or lower ratios in the cow with a longer withdrawal time. No strong trend emerged when liver:kidney and liver:muscle lipid ratios were compared between the two cows; however, the general trend showed equivalent or lower ratios for cow 2232 again suggesting similar depletion rates of hepta- and octacongeners between the tissue compartments. For the milk fat compartment, an opposite tendency was seen for hepta- and octacongeners. Liver:milk lipid ratios were three times higher in cow 2232 than

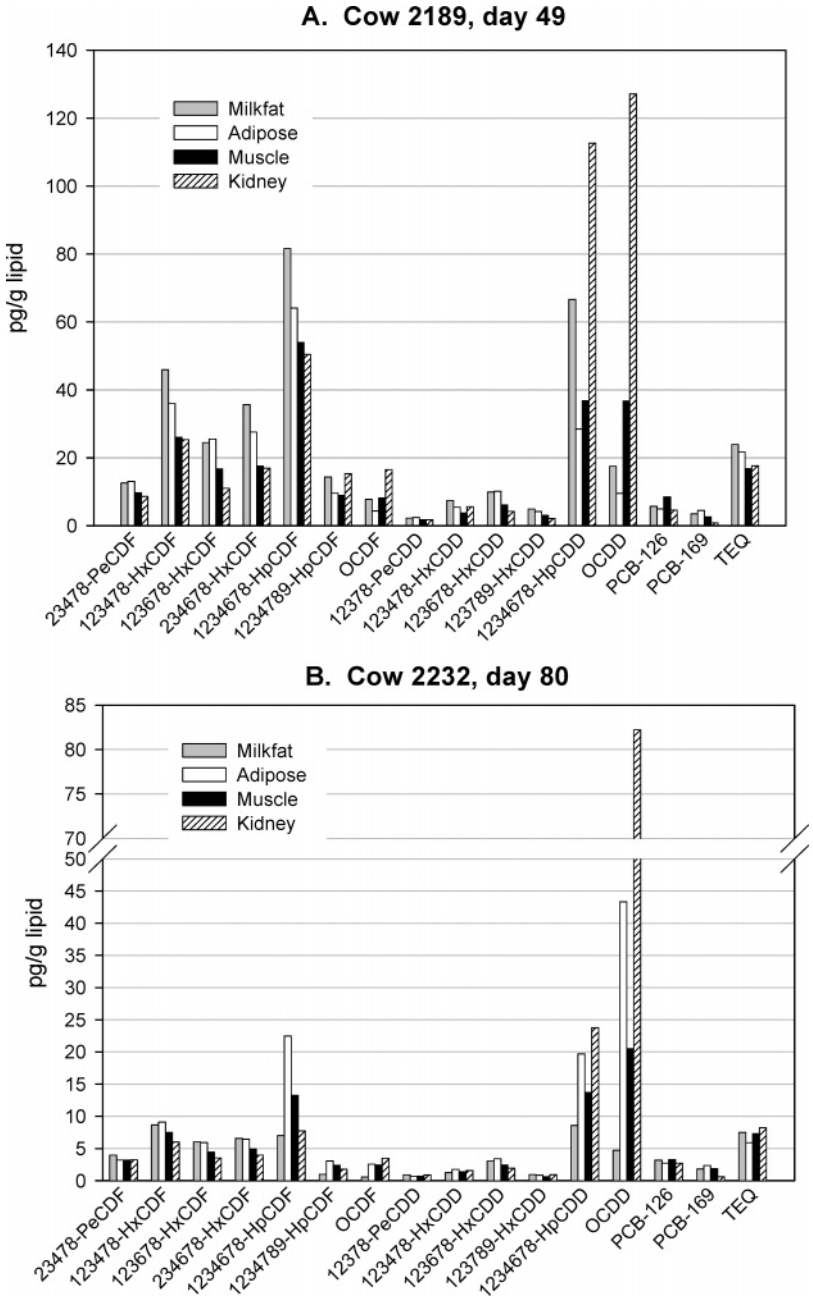

Figure 2. Concentrations of individual congeners and total TEQ in milk, adipose, muscle, and kidney on a lipid weight basis for (**A**) cow 2189 at day 49 and (**B**) cow 2232 at day 80.

in cow 2189 (**Table 5**) suggesting that the higher chlorinated congeners are depleted from milk fat more rapidly than from liver.

When milk and feed samples from 10 Minnesota dairy farms that had purchased one of the contaminated mineral supplements were analyzed, only one feed sample showed elevated dioxin levels (**Table 6**). This sample appeared to be a mineral concentrate and had a TEQ equal to 5.23 ppt. The corresponding milk sample did not have elevated dioxin levels. All milk samples from the "contaminated" farms had dioxin levels comparable to the control farms where no dioxin-containing supplements were used. The average TEQ for all the farms was 1.05 pg/g lipid.

DISCUSSION

The magnesium oxide supplement used for this dosing study had high levels of dioxins and furans (TEQ ≅ 300 ppt) and accounted for 95% of the TEQ found in the dosing TMR. The supplement contained lower levels of coplanar PCBs but contributed approximately 60% of the daily PCB dose. The three major hexa-CDFs contributed 40% of the total TEQ to the dose followed by 2,3,4,7,8-PeCDF, which contributed 21%. The daily dose of PCDD/Fs and PCBs received by the cows was not anticipated to cause any acute toxicity or adverse health effects. In a previous study with beef cattle, animals received a TEQ dose twice this amount (272 ng/day) for 120 days and showed no signs of health problems (*10*). The premature death of cow 2189 was, therefore, not attributed to the dose but to a sudden physical malady.

The two cows were remarkably similar in their uptake and elimination of the PCDD/Fs and PCBs in the milk. The D/F TEQ withdrawal curve modeled for cow 2232 is qualitatively similar to the ones observed by Tuinstra et al. (*14*) who measured depletion rates in the milk of dosed cows after calving

Table 5. Concentrations of Major Dosed Dioxins, Furans, Coplanar PCBs, and TEQs in Liver (pg/g Lipid) and Ratio of Liver Fat to Adipose and Milk Fat Concentrations for Cow 2189 at Day 49 and Cow 2232 at Day 80

	liver concn (pg/g lipid)		liver fat/ adipose ratio		liver fat/ milk fat ratio	
	no. 2189	no. 2232	no. 2189	no. 2232	no. 2189	no. 2232
23478-PeCDF	42	33	3.3	10.4	3.4	8.4
123478-HxCDF	194	106	5.4	11.6	4.2	12.2
123678-HxCDF	71	53	2.8	9.0	2.9	8.9
234678-HxCDF	131	81	4.7	12.5	3.7	12.3
1234678-HpCDF	803	277	12.5	12.3	9.8	39.6
1234789-HpCDF	211	44	22.0	14.5	14.7	44.8
OCDF	1052	211	241.3	83.4	135.0	370.8
12378-PeCDD	5	5	2.1	7.5	2.2	5.7
123478-HxCDD	69	38	12.6	21.9	9.3	30.9
123678-HxCDD	31	33	3.1	9.8	3.2	10.9
123789-HxCDD	24	16	5.8	19.6	5.0	17.3
1234678-HpCDD	2099	867	73.8	44.0	31.5	101.2
OCDD	4995	3926	523.3	90.6	285.4	835.6
PCB-126	13	20	2.7	7.2	2.3	6.2
PCB-169	2	3	0.5	1.5	0.7	1.9
D/F TEQ	111	69	5.2	12.3	4.76	9.62
PCB TEQ	1.4	2.0	2.6	6.8	2.27	5.93
total TEQ	112	71	5.2	12.0	4.70	9.46

Table 6. TEQ Values in Milk (pg/g Lipid) and Feed (pg/g Dry Weight) Collected from 10 Dairy Farms in Minnesota Known to Have Used Dioxin-Contaminated Supplements and Two Dairy Farms that Did Not Use the Contaminated Supplements[a]

farm	milk TEQ	feed TEQ
1	0.53 (0.48)	0.036 (0.007)
2	1.08 (0.98)	5.23[b] (4.50)
3	0.92 (0.91)	0.019 (0.003)
4	0.96 (0.96)	0.020 (0.005)
5	0.95 (0.94)	0.138 (0.127)
6	1.49 (1.47)	0.024 (0.002)
7	0.90 (0.84)	0.065 (0.059)
8	0.79 (0.74)	0.197 (0.171)
9	1.55 (1.42)	0.048 (0.008)
10	0.99 (0.94)	0.197 (0.006)
control 1	0.81 (0.57)	NA
control 2	1.69 (1.46)	NA
average	1.05 ± 0.33 (0.98 ± 0.31)	

[a] Values are upper bound (nd = LOD) with lower bounds (nd = 0) in parentheses. NA = not available. [b] Average of three analyses (RSD = 25%).

and Malisch (*3*) who measured dioxin depletion in the bulk milk after a contaminated feed was removed from the farm. Tuinstra defined an initial TEQ distribution phase of approximately 1 week in which the average TEQ half-life was 1.6 days. A slower β-depletion phase (elimination phase) had an average TEQ half-life of 84 days, but for individual cows, β-half-lives varied from 41 to 87 days. In the present study, the estimated α- and β-D/F TEQ half-lives for cow 2232 were 4.7 and 62.2 days, respectively. At best, the estimation for the β-depletion phase in this study is based on a relatively short withdrawal and, as a result, could contain significant errors.

Because a dioxin TEQ is composed of the weighted sum of numerous congeners, it is valuable to calculate data for individual congeners. Neither the distribution (α) nor the elimination (β) half-lives showed a trend based on chlorination number but remained relatively constant for all the penta- to hepta-CDD/Fs. Tuinstra et al. (*14*) also saw no trend based on chlorination number but observed a wide range of half-lives in four individual cows with HxCDD/Fs having the longest half-lives (40−220 days for the β-phase). Although a short distribution half-life was estimated for the octacongeners in the present study (0.2 days), it should be noted that this is the half-life in milk and does not necessarily represent the whole body half-life. Other tissue compartments showed longer retentions of OCDD and OCDF (**Figure 2** and **Table 5**), and the true whole body half-life is most likely much longer than the half-life in the milk.

The PCB TEQ in the milk samples declined more slowly than the D/F TEQ, with estimated α- and β-half-lives of 6.1 and 87.1 days, respectively. Again, because of the relatively few data points used to calculate the β-elimination portion of the depletion curve and the natural background level of PCBs in the feed, these values at best represent rough estimates. Of the individual PCBs, PCB-126 was more persistent than PCB-169. The PCB-169 half-lives were similar or shorter than half-lives reported for PCBs found in Aroclor 1254, which averaged 2.2 days (α) and 69 days (β) (*15*); however, PBC-126 half-lives were up to five times longer. In the Aroclor study, Fries also noted a 3-fold range in the depletion rates from individual cows during the PCB elimination phase and speculated that multiple factors, including body fat content, might contribute to large variations between animals. Although no obvious factors have been correlated to PCB or PCDD/F half-lives in cows, each of the studies has shown an initial withdrawal phase of approximately 1 week in which PCDD/F and PCB concentrations decreased by approximately 50%. Depending on the extent of dioxin contamination, this 1 week period may be enough to lower an elevated TEQ in milk to an acceptable level.

The PCB carry over rates in this study were similar to those reported by Slob et al. (*6*) and Kerst at al. (*16*) for cows grazing in pastures (31−49 and 31−65% for PCBs 126 and 169, respectively) but lower than rates reported for other persistent PCBs such as PCB 138 and 153 (74 and 83%, respectively) (*17*). In general, the persistent PCBs appear to bioconcentrate to a higher level than the PCDD/Fs.

The PCDD/F carry over rates were in the range of those reported in previous studies where cows were fed contaminated feeds for 3 weeks or more (*3*, *7*, *18*); however, carry over rates among these studies varied greatly. For example, the carry over rates for two typical congeners (2,3,4,7,8-PeCDF and 1,2,3,4,7,8-HxCDF) ranged from 17 to 58% and from 14 to 33%, respectively. These variations may partly be explained by differences in uptake of dioxins from the different matrices (citrus pulp, sludge, and wood). In the present study, the two cows also displayed differences in carry over rates: 18.7 and 28.8% for 2,3,4,7,8-PeCDF and 18.1 and 24.5% for 1,2,3,4,7,8-HxCDF; therefore, other factors effecting carry over rates include feed intake and milk production. Cow 2189 appeared to eat the same amount of feed as cow 2232 but produced 25% less milk leading to roughly 30% lower daily excretion in milk.

Another measure of bioaccumulation that was less variable in the present study was a bioconcentration factor. Because the dose was mixed in the entire ration, the daily amount of feed consumed was not a variable in this calculation. **Table 7** compares the BCFs calculated in this study to BCFs reported or calculated from several other studies with dairy cows (*3*, *6*, *7*, *16*−*21*). The BCFs from this study were somewhat higher than in other studies but comparable to those values reported from a U.S. dairy herd by Lorber et al. (*19*). The effect of matrix on bioavailability can clearly be seen for PCDD/Fs bound to flyash (*6*) where BCFs were 1−2 orders of magnitude lower than other studies. Grass contaminated by a municipal solid

Table 7. Comparison of Average Bioconcentration Factors (BCFs) (pg/g Milk Fat/pg/g Feed, Dry Weight) Reported or Calculated from Data in Various Studies with Dairy Cattle[a]

ref	this	*3*	*7*[b]	*15*	*6*	*6*	*16*	*17*	*7*[b]	*19*	*15*	*20*	*21*[c]
matrix	mineral suppl.	citrus pulp	sludge-feed	PCP-wood	MSW-grass	flyash	grass	feed	feed	feed	feed	feed	feed
congener													
2378-TCDF	0.10	0.19			0.13					2.93		0.40	1.30
12378-PeCDF	0.77	0.25			0.08					4.00		0.80	1.11
23478-PeCDF	7.94	3.84	10.60	3.50	1.92	0.13			6.92	6.72	3.50	10.00	4.64
123478-HxCDF	7.18	2.21	3.75	3.00	0.69	0.05			4.15	6.19	1.10	1.40	3.53
123678-HxCDF	6.96	2.02	4.40	3.10	0.58	0.04			3.24	5.74	2.20		2.97
234678-HxCDF	5.88	1.29	3.18	1.90	0.67	0.06			3.27	4.86	1.70		2.60
123789-HxCDF	4.75					0.01				4.03			0.00
1234678-HpCDF	1.51	0.21	0.31	0.72	0.06	0.01			0.59	0.99	0.30	0.20	0.56
1234789-HpCDF	1.89	0.28	0.59	0.87	0.08	0.01				2.04			1.49
OCDF	0.16	0.02	0.05	0.07						0.46		0.20	0.19
2378-TCDD	1.12	3.89	8.31	7.10	2.40	0.11			6.57	4.08		6.00	6.50
12378-PeCDD	8.99	3.24	4.40	5.00	1.60	0.11			6.75	4.92		4.00	6.13
123478-HxCDD	6.64	3.40	3.42	3.10	0.90	0.05			5.71	5.25	3.60	1.60	3.16
123678-HxCDD	9.12	5.10	2.15	3.70	1.02	0.05			5.71	6.69	3.20		2.60
123789-HxCDD	5.31	2.30	1.61	2.60	0.50	0.02			2.77	4.06	2.30		3.34
1234678-HpCDD	1.63	1.20	0.33	0.68	0.10	0.01			0.59	1.16	0.30	0.40	0.56
OCDD	0.22	0.24	0.05	0.08	0.02				0.12	0.13		0.16	0.74
PCB-77	0.11				0.20		0.42						
PCB-126	8.72				5.65		6.83						
PCB169	12.39				4.95		8.83						
PCB-118							5.81	12					
PCB-138								11					
PCB-153								13					

[a] Six of the studies include amended or contaminated feeds or forage (matrix indicated), and six studies were done with background levels (grass or feed). [b] Milk fat estimated at 4% to calculate BCFs. [c] Moisture content of feed estimated at 50% to calculate BCFs.

waste incinerator also appeared to have lower BCFs than other matrices, perhaps due to particulate binding similar to that of the flyash matrix (*6*). In general, the average BCFs from the studies (excluding the flyash) showed a relative variability of 10−130% (average 66%). Each study showed an inverse relationship between bioconcentration and chlorination number.

The BCFs for PCB-126 and 169 in this study were also somewhat higher than those found in grazing cattle in Europe (*6*, *16*) but are in the same range as other persistent PCBs, that is, PBC-118, -138, and -153 fed to cows in naturally contaminated feed concentrate and silage (*17*). The difference in bioavailabilities may again be due to the matrix on which the PCBs are adsorbed. Grazing cattle may receive some or most of their exposure from contaminants bound to soils or particulates, as opposed to contaminants bound to feeds, which may be a more lipid-rich matrix due to corn or grain components.

Cow 2189 died shortly into the withdrawal phase and provided data that estimated tissue distributions near the steady state or immediately following the distribution phase. Data from cow 2232 reflected tissue distribution further into the elimination phase of withdrawal. In both cows, milk fat provided a good estimate of the PCDD/F and PCB concentrations in the adipose tissue, kidney fat, and intramuscular fat; however, hepta- and octacongeners were less predictable. Similar observations have been made in dairy cows for PCDD/Fs by Fries et al. (*22*) and for total PCBs by Thomas et al. (*23*). Because the lower chlorinated D/Fs have higher toxicities, they generally dominate the total TEQs; therefore, concentrations in milk fat are a good estimate of whole body TEQ levels in dairy cattle.

The livers of both cows showed selectively higher accumulation of PCDD/Fs and PCB-126. Hepatic sequestration of PCDD/Fs and certain PCBs, relative to other lipid stores, has been shown to occur in dairy cows (*22*, *23*), nonlactating cattle (*10*, *24*), and several other species (*25*). In mice, the inducible protein cytochrome P450 1A2 has been implicated in the sequestration process (*26*). No studies have investigated the mechanism of sequestration in cattle; however, sequestration does occur and appears to result in longer retention of specific congeners in the liver leading to increased whole body half-lives.

In addition, OCDD appeared to be elevated in the kidneys as compared to milk or other tissues on a lipid weight basis. This observation has been noted by Richter in nonlactating cows for not only kidney but also lung and spleen (*24*). Whether this is due to selective binding or merely differences in perfusion rates of various organs is not clear.

This feeding study showed that when used at levels typical of a dietary mineral supplement (1.3% of diet) the contaminated magnesium oxide could elevate the D/F TEQ 30-fold over typical levels in the milk fat. Overall, the dioxins in the mineral supplement were bioavailable to the cows with up to 20% of the D/F TEQ and 38% of the PCB TEQ excreted into the milk. However, when milk from dairy farms that had been identified as having purchased a contaminated supplement was analyzed, no elevated milk levels were found. The one elevated feed sample (5.23 ppt) was a premixed mineral supplement. In completed questionnaires, the farmers indicated that this supplement was typically added at a rate of 0.3−3% in the total ration. At this rate, the cows' ration would contain only 0.016−0.16 ppt TEQ. Assuming 50% moisture content in the ration and a bioconcentration factor of 6, the milk would then contain 0.19−1.9 pg TEQ/g lipid. In fact, the TEQ in milk from this farm was 1.08 ppt.

Although the TEQ range in the milk at the MN farms (0.53−1.55 pg TEQ/g lipid, upper-bound) could indicate contamination from low levels of supplements, the congener pattern in the farms' milk did not indicate that one of these contaminated mineral supplements was the major source of dioxins. In the contaminated supplements and the milk from cows fed the supplement, furans predominated over the dioxins (*5*, this study). The ratio of total hexaCDFs to total hexaCDDs in the milk from cows 2189 and 2232 was 5; however, this ratio in the milk from MN farms averaged 0.7 and ranged from 0.4 to 1.5 [national

average ratio = 0.46 (*12*)]. Likewise, furans contributed 77% of the TEQ in milk from cows 2189 and 2232 but only 30% in the MN milk. Therefore, the contaminated mineral supplements at best contributed only a portion (40%) of the total TEQ found in the MN milk.

Other reasons for the lack of elevated dioxin levels from the on-farm samples are that (i) several farms reported using the mineral supplements only in calves, heifers, and dry cows, and (ii) the dioxin contamination varied greatly from batch to batch of mineral product (personal communication with Kyle Brokken). Although mineral supplements were identified as "contaminated", there was no way to know the level of contamination without complete analysis of each batch. Three different supplements obtained by our laboratory were a copper supplement with TEQ = 1070 ppt, a magnesium supplement with TEQ = 300 ppt, and a zinc supplement with TEQ = 9 ppt.

In conclusion, while dioxins from the magnesium mineral supplement were found to be bioavailable to cows in a feeding study, no widespread contamination was observed in the milk supply from local farms that had purchased the contaminated products. We found that bioconcentration factors were the least variable measure of bioaccumulation between animals and that milk fat gave a good estimate of tissue and whole body TEQ concentrations. Although bioaccumulation varied inversely with chlorination number, withdrawal rates in the milk did not appear to follow this general trend.

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Supporting Information Available: Experimental data for milk, tissue, feeds, and MNmilk. This material is available free of charge via the Internet at http://pubs.acs.org.

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