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Survey of Dioxin-like Compounds in Dairy Feeds in the United States

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The United States Environmental Protection Agency (USEPA) has completed a survey of dioxin-like compounds (including 17 dioxin and furan (CDD/F) congeners and 12 coplanar polychlorinated biphenyl (PCBs) congeners) in dairy feeds from 10 dairy research facilities around the United States, sampling the overall mixtures and the major and minor feed components. Low levels of dioxin were found in all feed mixtures with an average concentration of 0.05 pg/g (ppt) toxic equivalent (TEQ) dry weight. This is lower than previously found in dairy feeds by about a factor of 4. While it is possible that generally lower levels of dioxins in the environment in recent years may explain this result, examinations of the data suggest that the oven drying used to prepare the wet feed samples could have resulted in a loss of dioxins from the feed materials. The percentage of the total TEQ due to CDD/Fs was about four times that of PCBs. Leafy vegetations in the feed (the silages and the hays) had concentrations about twice as high as nonleafy, protected vegetation of the feeds (the ground or meal corn, cottonseed, and others). Minor components did not significantly influence the final feed mixture concentration of dioxin TEQ. However, in one of the feed mixtures, a minor component with a high concentration of 38.5 ppt TEQ effectively doubled the concentration of the overall feed mixture.

KEYWORDS: Diary feeds; dioxin-like compounds

BACKGROUND

The primary route for exposure to dioxin-like compounds for the general population is through the consumption of animal fats with consumption of dioxins in milk and dairy products comprising about 37% of total dioxin exposure in the United States (*1*). The major route of dioxin exposure hypothesized for terrestrial food animals is airborne deposition onto the leaves of feed crops, followed by consumption of those feed crops. Over the past few years, additional pathways of exposure have been identified associated with contaminated feed additives such as ball clay, mineral supplements, waste oils, and animal byproducts (*2*−*4*). Studies by the United States Department of Agriculture (USDA) have shown that incidental contact with pentachlorophenol (PCP)-treated wood by cattle resulted in elevated tissue and milk levels (*5*). National surveys by the EPA on dioxins in milk have shown background levels at about 0.7−0.9 pg TEQ/g lipid weight basis (ppt TEQ lwt), typical of average levels found in other countries (*6*−*8*). This suggests that unusual dioxin exposures of dairy cows leading to high milk concentrations of dioxins are not typical for the U.S. milk supply. The purpose of this study was to measure dairy feeds around the country to gain insight into the pathways of dairy cattle exposure.

The EPA and our federal colleagues have undertaken three studies on the dioxin content of animal feeds and the role these feeds play on the dioxin levels in food. The first study was on the mass balance of dioxins in lactating cows, conducted with the USDA, which occurred between 1997 and 1999 (*9*−*11*). The primary objective of that study was to quantify the role feeds play in total dairy cow exposure to dioxin-like compounds by measuring the dioxins in their feeds and comparing that to the dioxins excreted in milk and feces. A second objective was to use the mass balance data to derive steady-state bioaccumulation factors such as bioconcentration factors, biotransfer factors, and carryover ratios. This study enabled an initial examination of the role of different feed components in

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delivering the dose to the cows. The sampling occurred when the lactating cows were expected to be at steady state: when the dioxin dose to the animal via animal feeds is expected to roughly equal the dioxins excreted in milk and feces. This assumes no unusual nonfeed-related exposures, such as contact with PCP-treated wood. It was found that dioxins excreted were roughly 70% of dioxins ingested, with the difference attributed to storage in the animal or metabolism. These results were consistent with similar mass balance studies reported in the literature (*12*). On the basis of the results of the mass balance study, the EPA was confident that, barring unusual circumstances, dairy cattle feed was responsible for the dioxin dose received by the cow, and therefore, studies on dairy feeds would provide knowledge on the lactating cow exposures to dioxins.

The second study, conducted between 2000 and 2002, focused on minor components of animal feeds (*3*). These components were not specific to dairy feeds; they were used in various types of animal feeds. Together with the U.S. Food and Drug Administration (FDA), the EPA collected a total of 47 samples. There was a focus on components derived from animal fats, including beef fat, pork fat, fat, meat, and bone meal from mixed animal species, poultry byproducts, fish meal, and egg samples. Some samples of plant origin, including deodorizer distillates and molasses, were also taken. Concentrations of dioxin-like compounds in the samples of animal origin ranged from about 0.2 to 4.0 ppt TEQ lwt, with the samples from menhaden fish the highest among the animal-derived samples, averaging 2.9 ppt TEQ lwt. The deodorizer distillates were the highest of all in the survey, averaging 4.4 pg of TEQ/g dry weight basis (ppt TEQ dwt), with a maximum of 7.1 ppt TEQ dwt. While some of these concentrations are higher than reported vegetation concentrations ($\leq$0.5 ppt TEQ dwt), they are not nearly as high as the minor feed component—ball clay, which was found in the mid-1990s to have a strong influence on poultry which fed on feed containing ball clay (*2*). Some ball clay samples measured well over 1000 ppt TEQ dwt, and the associated poultry samples were found to have elevated dioxin level measurements of about 30 ppt TEQ lwt. This is significantly higher than the $\leq$1 ppt TEQ lwt concentration that is typical for poultry products in the United States. Since minor components make up a small percentage of total animal feeds, on the order of 1−5%, only an unusually high concentration, perhaps well above 100 ppt TEQ like the ball clay, would likely influence overall feed mixtures significantly.

The EPA's third study, which was a survey of dioxins in dairy feeds, is presented here. Samples were taken between 2002 and 2003. Completion of the analysis of all samples occurred in 2005, and partial sets of results were reported in 2004 (*13*) and 2006 (*14*). The full results are presented here including additional sample analyses, interpretations, and discussions. This study entailed collection of the dairy feed total mixed ration (TMR), forage components, concentrate components, and minor components at 10 U.S. government and state university research facilities which raise dairy cattle in a manner similar to commercial dairy operations. The purposes of this study were to determine the concentrations of dioxin-like compounds in feed in different parts of the country, determine the relative contribution of various feed components to the total dioxin content of dairy feeds, and test the air-to-leaf hypothesis. Research has shown that dioxins do not translocate to within-plant parts, so grains and bulky vegetation would not be as impacted by atmospheric deposition as thin, leafy vegetation. Therefore, the theory is that the primary vector for terrestrial animal impacts is through their consumption of leafy vegetation, even though leafy vegetation may not dominate their intake of dry matter.

MATERIALS AND METHODS

Study Design. Dairy feeds are often classified as forages and concentrates. Forages are characterized by being more fibrous (higher than 30% neutral detergent fiber) and generally represent the vegetative portion of a plant. The major groups of forages include pasture and range plants, hays, and silages. The forages comprise the "leafy" portion of an animal's feed intake. Concentrates include grains, grain byproducts, oilseed meals, animal byproducts, and fruit and sugar processing byproducts. Corn is the primary grain included in dairy feed and is fed in similar proportions in all regions of the country, with soybeans and cottonseed also being primary components of dairy feed concentrate. For purposes of clarity in the data evaluations, forages will be termed "leafy" and concentrates will be termed "nonleafy". In addition to leafy and nonleafy major feed components, minor components such as minerals, vitamins, animal fat, and other additives are also included in dairy feeds.

Composite samples of dairy feed TMR, leafy and nonleafy major components, and minor components were collected at 10 U.S. government and state university research facilities that raise dairy cattle in a manner similar to commercial dairy operations. The selected research facilities are located in New York, Virginia, Florida, Michigan, Wisconsin, Oklahoma, Utah, Nebraska, Oregon, and Washington. The facilities were sampled between April 2002, and January 2003. EPA personnel traveled to each facility and over the course of a few days collected, boxed, and shipped the samples to the USDA Dairy Forage Research Center in Prairie du Sac, WI (WDFRC). At the WDFRC, samples were refrigerated until being dried and ground to a fine powder in preparation for their analysis at the EPA Environmental Chemistry Laboratory at the John C. Stennis Space Center, MS. The samples were dried at 55 °C for 48 h in an oven specifically used for feed sample drying and then ground using stainless steel equipment. Ground samples were packed tightly in dry ice in coolers and shipped to the EPA laboratory for refrigeration storage until chemical analysis.

Composite milk and feces samples were also taken from lactating cows. The purpose of these additional milk and feces samples was to evaluate whether any unusually high concentrations found in feed could be identified in feces and milk as well. The results did not suggest unusually high concentrations; however, milk samples were eventually analyzed in 6 of 10 stations in 2005.

Chemical and Data Analysis Methods. Chemical analysis generally followed a modified version of EPA Method 1613: Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, with modifications designed to achieve the lowest possible detection limits. Approximately 30 g of dried and homogenized dry matter sample were weighed into an extraction thimble and mixed with anhydrous sodium sulfate. All samples were fortified with a mixture containing each of the 17 ^{13}C-labeled 2,3,7,8,-Cl-substituted dioxins/furans (CDD/Fs) as well as the 12 dioxin-like polychlorinated biphenyls (PCBs). The 12 dioxin-like PCBs were measured in most samples; a small subset only measured seven of these PCBs. The samples were extracted with 75%/25% hexane/methylene chloride by soxhelation for 24 h. Dry weight basis limits of detection (LODs) for CDD/Fs ranged from 0.01 pg/g for the lower chlorinated congeners to 0.20 pg/g for OCDD, and dry weight basis LODs for the polychlorinated biphenyls (PCBs) ranged from 0.01 pg/g for PCB 169, 0.02 pg/g for the most toxic PCB congener, PCB 126, to 4.5 pg/g for PCB 118. Analysis of CDD/Fs in milk followed the procedures outlined in Schuam et al. (*7*). Further details on EPA Methods for CDD/F/PCBs are found in Ferrario et al. (*15*, *16*).

Average concentrations of the congeners were determined assuming nondetects (NDs) were equal to one-half limit of detection (LOD). Similarly, toxic equivalent, or TEQ, concentrations were determined at ND = 1/2LOD using the 1998 WHO recommendations (*17*) for assignment of TEF values. While the detection limits were low, the assumption of 1/2DL for NDs did have some influence on TEQ calculations: the average TEQ concentrations dropped by approximately 20% with ND = 0 as compared to ND = 1/2DL.

Besides sampling and analyzing a TMR sample, the concentrations of dioxin-like compounds in this TMR sample were predicted fromthe weighted average concentration of the major and minor feed components. If the prediction of TMR concentrations matched the measurements reasonably well, then this would provide confidence in the information provided on the composition of the TMR, as well as the analytical chemistry which developed the concentrations, and also confidence in future work where analysis of individual feed components could be used to quantify their influence on the overall quality of the feed mixture. Mathematically, a simple weighted average concentration of congener ‘i’, WC_i (pg/g dry), can be predicted from the concentrations in the feed components ‘j’ as

$$WC_{\mathrm{i}} = \frac{\sum_{j=1}^{\mathrm{n}} (C_{\mathrm{i,j}} \cdot FC_{\mathrm{j}})}{\sum_{j=1}^{n} FC_{\mathrm{j}}} \qquad (1)$$

where $C_{\mathrm{i,j}}$ is the concentration of congener i in the feed component j (pg/g dry; up to n total components) and FC_{j} is the fraction (<1) of the TMR which is feed component j. The sum of the fractions of feed components sampled in this study, ΣFC_{j}, was near 1.00 in all cases; in 8 of 10 facilities, essentially all of the components of the mixed feed were sampled, but in 2 of the facilities, only about 75% ($\Sigma FC_{\mathrm{j}} = 0.75$) of the components were sampled.

Congener profiles of the CDD/Fs (PCBs not included in the profiles) in a few samples were also examined for trends. A congener profile is generated by summing the concentrations of the 17 CDD/F congeners and then determining the percent each congener contributes to the sum total.

RESULTS AND DISCUSSION

1. Overall. Table 1 provides a list of the components sampled in each facility and the percentage of mass that each component contributes to TMR. The components were separated into three groups: leafy, nonleafy, and minor components. Leafy components typically made up the majority of the mass of the feed, averaging 62% for the 10 facilities. Nonleafy components averaged 29%, and minor components averaged 4% (the sum of the three does not add to 100% because less than 100% of the feed components, 77% and 79%, were sampled in two of the stations).

Table 2 contains the TEQ concentration for each major and minor component sample set as well as the TEQ concentration of the TMR samples. The TEQ concentrations include both the CDD/Fs and the dioxin-like PCBs. For the TMR TEQ concentrations, the portion which is due to CDD/F congeners is distinguished from the portion due to PCB congeners. The contribution to TMR TEQ from CDD/Fs was higher than from PCBs, by about a factor of about 4. This discrepancy was the highest for Oregon and Washington, where CDD/Fs contributed more than PCBs by factors of 5 and 6, respectively.

The CDD/F/PCB TEQ concentrations of the TMR were very low, all less than 0.10 ppt TEQ dwt, and 6 of the 9 TMR samples were less than or equal to 0.05 ppt TEQ dwt. The average over the 10 facilities was 0.05 ppt TEQ dwt. This includes the weighted average predicted concentration for the one facility for which there was no TMR taken—Wisconsin. The weighted average concentration was 0.06 ppt TEQ dwt at this facility, of which 0.04 ppt TEQ dwt was from CDD/Fs and 0.01 ppt TEQ dwt was from PCBs. The highest TMR concentration was 0.09 ppt TEQ dwt from Michigan.

Table 1. Percentage (by weight) of Each Feed Component in the Total Mixed Ration (TMR) for Each of the 10 Research Facilities[a]

ingredient	NY	VA	FL	MI	WI	OK	NE	UT	WA	OR
I. forages										
corn silage	51	73	42	34	31		18	15		30
alfalfa silage/hay	21	8	5	34	41	13	29	22	48	12
grass hay	3					5				25
sorghum silage			19			36				
II. nonforages										
soybean (meal, roasted)	8	4	7	6	7	10		2		3
cottonseed (hulls, whole)		3	3	5		3	10	6	6	6
beet (b)/citrus (c) pulp			6 (c)					5 (b)		
corn (ground, meal, gluten)	15	6	11	11	14	25	39	12		
barley		5								19
wheat						4			4	
concentrate mix							2	11		
pelleted grain mix									21	
III. minor components										
minerals/vitamins	0.7	0.5	2	4						3
blood meal					0.7					
limestone/lime	0.6	0.3	0.1		0.4					
yeast			0.1		0.1	2				
animal fat additives						0.7				
sodium bicarbonate	0.6	0.4	0.3		0.4					
molasses								1		
Flobond	0.1									
soyplus			4				1			
other minor	0.1			3	0.4		1	3		
total, %	100	100	100	97	95	99	100	77	79	98

[a] Key: NY = Cornell University, New York; VA = Virginia Tech University; FL = University of Florida; MI = Michigan State University; WI = USDA Dairy Forage Research Center, Wisconsin; OK = Oklahoma State University; NE = University of Nebraska; UT = Utah State University; WA = Washington State University; OR = Oregon State University.

In contrast, the dairy TMR measured in the EPA’s mass balance study conducted between 1997 and 1999 ranged from 0.13 to 0.22 ppt TEQ dwt, dioxins and furans only, with a mean of 0.17 ppt TEQ dwt (*9*). Samples from the national dairy feed study were collected only a few years later in 2002 and 2003. Also, the procedures to collect, process, and analyze the samples were very similar in both studies. Other measurements of animal feeds of vegetative origin in the literature also have higher concentrations. Samples collected in 2003 in different European countries as part of the European Union Directives regarding food and feed showed an average of 0.32 ppt TEQ dwt (0.19 CDD/Fs and 0.13 PCBs) over 50 samples (*18*). Over a smaller collection of 14 samples from Denmark in 2004, the average from animal feed of vegetative origin was 0.26 ppt TEQ (0.21 CDD/Fs and 0.05 PCBs) (*19*). A more detailed examination below suggests that oven drying may possibly have resulted in the loss of dioxins and PCBs from the feeds in this study.

2. Evaluation of Major Feed Components. As discussed above, dioxins sorb to outer portions of vegetation with very little within-plant translocation. For this reason, vegetation which is leafy with a higher surface area to volume ratio, such as grasses, would be expected to have higher concentrations as compared to nonleafy vegetative components, such as grains or seeds. To see if this holds true for these data, the major feed component samples were broken into two groups: 36 samples of leafy components, which included corn silage, alfalfa (hay, silage), sorghum silage, and grass (Bermuda, hay), and 42 samples of nonleafy components, which included products derived from corn (gluten, ground, meal), soybean (meal, roasted), cottonseed (whole, hulls), and dried pulps from citrus

Table 2. Toxic Equivalent, TEQ, Concentration of Total Mixed Ration, Major Feed Components, and Minor Feed Components for Each of 10 Sites

ingredient, number of samples	NY	VA	FL	MI	WI	OK	NE	UT	WA	OR	mean (SD)
I. forages, pg/g TEQ dry weight											
corn silage, $n = 9$	0.02	0.03	0.03	0.24	0.05		0.04	0.04		0.11	0.09 (0.10)
alfalfa silage/hay, $n = 20$	0.08	0.13	0.03	0.13	0.15		0.09	0.79	0.18	0.02	0.15 (0.16)
grass hay, $n = 5$	0.06					0.12				0.27	0.12 (0.08)
sorghum silage, $n = 2$			0.04			0.14					0.09 (0.05)
II. nonforages, pg/g TEQ dry weight											
soybean (meal, roasted), $n = 12$	0.02	0.03	0.02	0.04	0.05	0.02		0.02		0.18	0.05 (0.05)
cottonseed (hulls, wh), $n = 10$		0.02	0.03	0.06		0.03	0.02	0.03	0.02	0.03	0.03 (0.01)
beet/citrus pulp, $n = 2$			0.02					0.02			0.02 (0.00)
corn (grd, meal, gluten), $n = 11$	0.09	0.03	0.05	0.03	0.03	0.03	0.05	0.02			0.04 (0.03)
barley, $n = 2$		0.02								0.02	0.02 (0.00)
wheat, $n = 2$						0.06			0.06		0.06 (0.00)
concentrate mix, $n = 2$							0.03	0.13			0.08 (0.05)
pelleted grain mix, $n = 1$									0.03		0.03 (NA)
III. minor components, pg/g TEQ dry weight											
minerals/vitamins, $n = 5$	0.02	0.02	<0.01	0.02						0.06	0.02 (0.02)
blood meal, $n = 1$					0.03						0.03 (NA)
limestone/lime, $n = 4$	0.02	0.02	0.02		0.02						0.02 (0.00)
yeast, $n = 3$			0.02		0.05	0.02					0.03 (0.01)
animal fat additives, $n = 1$						0.60					0.60 (NA)
sodium bicarbonate, $n = 4$	0.02	0.02	0.02		0.02						0.02 (0.00)
molasses, $n = 1$								0.07			0.07 (NA)
Flobond, $n = 1$	38.5										38.5 (NA)
soyplus, $n = 2$			0.02					0.02			0.02 (0.00)
other minor, $n = 13$	0.22			0.55	0.05		0.05	0.27			0.23 (0.31)
TMR, PCB TEQ, ppt dry	0.01	0.01	<0.01	0.02	NA	0.02	0.01	<0.01	0.01	0.01	
TMR, D/F TEQ, ppt dry	0.04	0.03	0.02	0.07	NA	0.04	0.02	0.03	0.06	0.05	
TMR, TOT TEQ ppt dry	0.05	0.04	0.02	0.09	NA	0.06	0.03	0.04	0.07	0.06	

and beets. The feed-type averages and standard deviations (SD) are shown in **Table 2**. The average concentration of the 36 leafy components was 0.13 ppt TEQ dwt (0.11 ppt CDD/F TEQ dwt,0.02 ppt PCB TEQ dwt, SD = 0.14 ppt), while the 42 nonleafy components averaged 0.04 ppt TEQ dwt (0.03 ppt CDD/F dwt, 0.01 ppt PCB dwt, SD = 0.04 ppt). A comparison of the means of the two groups was performed using the two-sample *t*-test. The *p* value obtained (0.0005) provides evidence of a significant difference in the means of the two data sets (derived using the Excel spreadsheet *t*-test function, assuming one tail and unequal variances in the two populations). This supports the hypothesis that leafy vegetation is more highly impacted as compared to nonleafy vegetation.

The possibility that oven drying resulted in lower concentrations in the feed is discussed below. If oven drying results in lower concentrations, the likely mechanism is thru volatile loss of dioxin associated with evaporated water lost by drying. The difference in moisture contents of leafy and nonleafy feeds was examined by retrieving records of the drying process that were maintained by the WDFRC. Weight measurements before and after oven drying allowed for an estimate of the percent of total weight that was dry matter. If nonleafy feed had higher moisture contents than leafy feed and the hypothesis that dioxin loss was associated with moisture loss, then oven drying may have caused more dioxin loss in the nonleafy compared to leafy feeds. In fact, nonleafy feeds were much drier than leafy feeds. The average percent dry matter content of nonleafy feeds was 89%, while it was 58% for the leafy feeds. Of the leafy feeds, corn silage was actually a relatively wet feed, with an average dry matter content of 34%. The hays and grasses, within the leafy vegetation category, had higher dry matter contents at 80−90%. Corn silage did have a lower concentration than these hays—corn silage had an average concentration of 0.09 ppt dwt, while the hays and grasses had concentrations more like 0.15 ppt dwt (see **Table 2**). This could be an oven-drying phenomena or a surface area phenomena: silages include the more bulky stalks of plants that are not present in hays and grasses. In any case, this suggests that nonleafy feeds did not have lower concentrations than leafy feeds because they lost more moisture through oven drying. The air-to-leaf hypothesis remains the most likely reason for the difference in the dioxin contents of the feed types.

3. Evaluation of Minor Feed Components. As seen in **Table 1**, minor components typically comprise no more than 5% of the mass of feeds, with any individual component usually less than 1%. Therefore, unless the TEQ concentrations were unusually high, then overall feed quality would not be influenced by minor components. **Table 2** shows that the TEQ concentration of most minor components is less than 0.05 ppt TEQ dwt, with a few components a bit higher at around 0.5 ppt TEQ dwt. The one glaring exception to this was a minor component from the New York (NY) research facility termed "Flobond", which had a concentration of 38.5 ppt TEQ dwt. According to the manufacturer of this product, Brookside Agra L.C., Flobond is a "select, high affinity sorbent Hydrated Sodium Calcium Aluminosilicate (HSCAS) which is used in animal feeds and ingredients. Its use has been proven world wide when molds, caking, and flowability are problems" (source: http://www.qjbrookside-agra.com/Flobnd.html).

The high TEQ concentration found is reminiscent of ball clay, which had been added to poultry feed in the 1990s as a flowability agent as well, although the concentration is much lower in Flobond as compared to ball clay. High TEQ concentrations found in poultry samples during a national joint survey between the USDA and EPA in the mid-1990s was traced back to the ball clay in the poultry feed. With TEQ concentrations well into the hundreds of parts per trillion and several samples with concentrations above 1000 ppt TEQ, this minor component influenced the quality of the feed and subsequently the quality of the poultry meat (*2*).

Not only is the TEQ concentration of Flobond unusually high as a minor component, in fact the congener profiles of Flobond and ball clay are similar. **Figure 1** shows the congener profiles

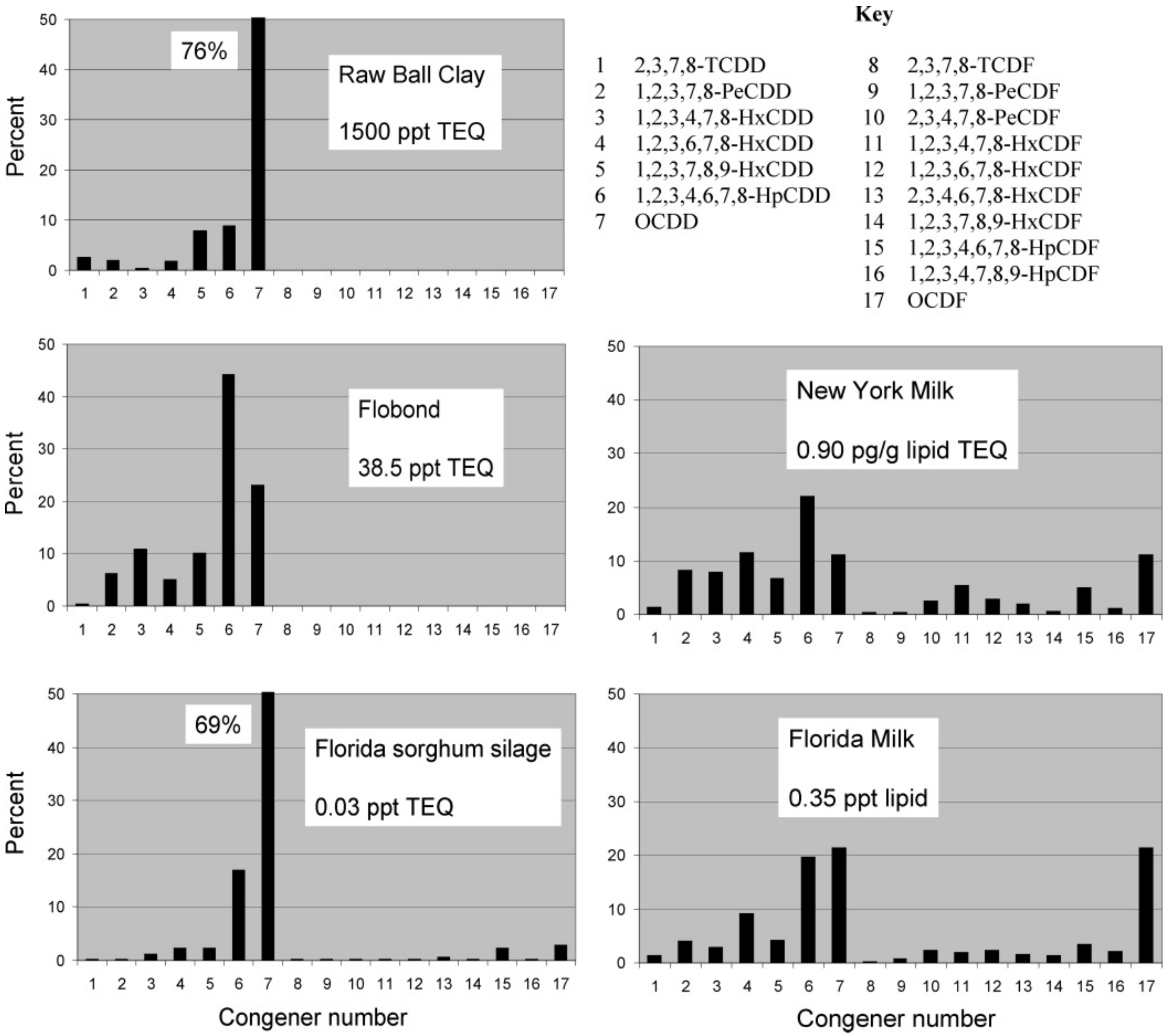

Figure 1. Comparison of the dioxin and furan congener profile of raw ball clay to the “Flobond” minor component and the Michigan corn silage profiles.

of ball clay and Flobond and, for comparison, the profile of sorghum silage found in the Florida (FL) research facility. Common to both Flobond and ball clay are the overwhelming predominance of dioxin congeners and the virtual absence of furan congeners. The PCB congeners in Flobond were also not remarkable; they were not found at elevated levels in ball clay and were found in Flobond at similar levels as in other minor and major components of this survey. The highest congener found in Flobound is the hepta-dioxin congener, 1,2,3,4,6,7,8-HpCDD, while the highest congener typically found in the ball clay was OCDD. The sorghum silage profile is typical of all the mixed feeds in this survey and essentially the archetype background profile of dioxins in furans found in soil, air, vegetation, and food products of terrestrial origin. Four congeners dominate this archetype background profile: the hepta- and octa-dioxins and the hepta- and octa-furans congeners. There is some suggestion that the profile of milk from the NY facility was influenced by the Flobond, in contrast to the milk from the FL facility. Congener profiles of milk samples from both facilities are also shown in **Figure 1**. As seen there, the NY milk sample generally had higher percentages of dioxin congeners as compared to the FL sample, and the hepta-dioxin congener dominated the NY milk sample, while the hepta- and octa-congeners were similar and highest in the FL milk sample.

4. Predicting TMR Concentration from Feed Components. In theory, one should be able to predict the TMR concentration by measuring the concentrations of the components and then deriving a weighted average concentration. The procedure for doing so was outlined in the Materials and Methods section above. A weighted average concentration was determined and compared to a measured concentration of TMR for 9 of the 10 sites which had a TMR sample; a TMR sample was not taken for the research facility in Wisconsin. **Table 3** demonstrates this calculation by showing the concentrations of the major and minor feed components from one of the sites, Michigan, along with the measured TMR concentrations and finally concentrations that could be calculated as weighted averages from the components.

As seen in the last two columns of **Table 3**, there was a clear correlation between measured and predicted concentrations. The highest measured concentrations in the TMR—the PCB 118, PCB 105, and OCDD at 16.9, 5.9, and 7.3 ppt, respectively—

Table 3. Congener-Specific Concentrations in Michigan of Major and Minor Feed Components, and a Comparison of Measured and Predicted Concentrations (units are pg/g dwt)[a]

	minor feed components		major feed components								TMR	
congener	M/V	O	CS1	CS2	AH1	AH2	AH3	CG	SM	CW	measd	calcd
PCB 77	0.25	11.52	1.34	1.68	2.56	6.88	5.14	1.65	1.97	0.37	1.55	3.08
PCB 81	0.02	0.82	–	–	0.12	0.29	0.24	0.10	0.17	0.02	–	–
PCB 105	1.05	45.04	6.34	12.82	10.14	25.29	22.65	15.78	7.79	5.05	5.87	14.91
PCB 114	0.11	4.68	–	–	0.41	1.54	1.09	0.60	0.44	0.10	–	–
PCB 118	2.25	118.43	15.77	30.37	19.41	59.49	50.55	29.77	17.01	8.42	16.87	33.74
PCB 123	0.03	2.39	–	–	0.31	1.16	0.68	0.45	0.29	0.09	–	–
PCB 126	0.01	0.36	0.19	0.35	0.20	0.55	0.48	0.05	0.06	0.02	0.25	0.27
PCB 156	0.40	13.24	0.77	3.75	1.80	3.57	3.78	4.35	3.35	2.01	0.61	3.27
PCB 157	0.09	2.81	0.25	0.97	0.47	0.89	0.97	0.89	0.70	0.43	0.17	0.78
PCB 167	0.10	4.39	–	–	0.51	1.37	1.39	1.08	0.78	0.44	–	–
PCB 169	<0.01	0.06	0.25	0.97	0.47	0.89	0.04	0.01	0.01	0.01	0.02	0.02
PCB 189	0.02	0.69	–	–	0.09	0.24	0.25	0.07	0.11	0.06	–	–
2378-D	<0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
12378-D	<0.01	0.25	0.04	0.08	0.01	0.04	0.02	0.01	0.01	0.01	0.01	0.04
123478-D	<0.01	0.24	0.04	0.12	0.01	0.04	0.04	0.01	0.01	0.01	0.04	0.05
123678-D	<0.01	0.56	0.08	0.24	0.04	0.07	0.08	0.01	0.01	0.01	0.08	0.10
123789-D	<0.01	0.47	0.08	0.24	0.01	0.04	0.06	0.01	0.01	0.01	0.07	0.08
1234678 -D	0.03	4.26	1.05	4.13	0.20	0.53	0.88	0.02	0.02	0.14	1.11	1.26
OCDD	0.25	17.07	5.10	19.69	0.83	2.63	4.57	0.54	1.07	1.29	7.27	6.21
2378-F	<0.01	0.01	0.02	0.03	0.03	0.03	0.04	0.01	0.03	0.06	0.01	0.03
12378-F	<0.01	0.01	0.01	0.04	0.01	0.02	0.03	0.01	0.01	0.05	0.01	0.02
23478-F	<0.01	0.03	0.03	0.06	0.01	0.04	0.04	0.01	0.02	0.05	0.02	0.03
123478-F	<0.01	0.12	0.02	0.11	0.01	0.03	0.04	0.01	0.01	0.01	0.03	0.04
123678-F	<0.01	0.07	0.04	0.20	0.01	0.04	0.04	0.01	0.01	0.01	0.03	0.06
234678-F	<0.01	0.08	0.05	0.20	0.06	0.03	0.11	0.01	0.01	0.01	0.04	0.08
123789-F	<0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	<0.01	0.01
1234678-F	0.01	0.59	0.48	3.07	0.06	0.20	0.24	0.01	0.01	0.03	0.51	0.71
1234789-F	<0.01	0.07	0.03	0.10	0.02	0.02	0.03	0.01	0.01	0.02	0.03	0.04
OCDF	0.05	0.62	0.48	2.65	0.11	0.13	0.26	0.01	0.01	0.01	0.58	0.65
PCB-TEQ	<0.01	0.07	0.02	0.04	0.02	0.07	0.06	0.01	0.01	<0.01	0.02	0.03
D/F-TEQ	0.02	0.49	0.10	0.31	0.03	0.11	0.10	0.02	0.03	0.05	0.07	0.12

[a] Notes: (a) "—" means congener not measured in this sample. (b) Minor feed components: M/V = minerals/vitamins; O = other, which included "Energy Booster" and "16M7 Ult Ext 24/36". (c) Major feed components: CS1 = corn silage from silo 1 (17%), CS2 = corn silage from silo 2 (17%), AH1 = alfalfa hay from silo 1 (6%), AH2 = alfalfa hay from silo 2 (9%), AH3 = alfalfa hay from silo 3 (19%) CG = corn, ground (11%), SM = soybean meal (6%), CW = cottonseed, whole (6%). (d) TMR = total mixed ration; "measd" = measured, "calcd" = weighted average calculated TMR concentration.

were also the highest predicted concentrations at 33.8, 14.9, and 6.2 ppt, respectively. All measured concentrations in the TMR at less than 0.10 ppt were also predicted to be less than (or equal to in one case) 0.10 ppt. The simple correlation coefficient was greater than 0.99 when looking at the entire set of 24 pairs of measured and predicted concentrations (17 CDD/Fs + 7 PCBs; there were 12 PCBs measured in most samples; the weighted average concentration could only be developed for seven of them because only seven PCBs were measured for the two corn silage components), as well as when looking at the CDD/Fs separate from the PCBs.

For the nine research facilities, comparison between predicted and measured CDD/F/PCB TEQ, in ppt, was as follows: MI, 0.12 and 0.07 (predicted and measured); NY, 0.09 and 0.05; VA, 0.04 and 0.04; FL, 0.03 and 0.02; OK, 0.09 and 0.06; NE, 0.05 and 0.03; UT, 0.28 and 0.04; WA, 0.11 and 0.07; and OR, 0.12 and 0.06. It is important to note that in each case the predicted TMR TEQ concentration is higher than the measured TMR concentration, and in the case of Utah, the disparity is striking at 0.28 ppt predicted and 0.04 ppt measured. Further analysis of this Utah result is included in the oven-drying section below. It was noted above that the predicted concentrations of the PCBs, while following the trend for the measured PCBs, were also consistently higher, almost by a factor of 2.

Generally, it can be concluded that mixed feed concentration can be reasonably predicted from the concentration of the components, with an occasional exception such as in Utah. Given this finding, additional analyses related to the influence of leafy components vs nonleafy components and the influence of minor components were undertaken.

Leafy components comprise about 68% of the total dry weight of the TMR in Michigan, but these components contribute 85% of the TEQ to TMR. This is a finding easily derived by combining the fraction by weight each component contributes to the total mass of the mixed feed along with the concentrations of the congeners on each component. Similar calculations for the other nine locations are as follows: NY, 77% (percent of TMR mass attributed to leafy feeds) and 31% (percent of TMR TEQ attributed to leafy feeds); VA, 81% and 87%; FL, 66% and 68%; OK, 48% and 80%; NE, 48% and 57%; UT, 37% and 65%; WA, 48% and 60%; OR, 57% and 88%; WI, 80% and 87%. In only one site, NY, did the leafy feeds contribute more proportionally to weight (77%) than to TEQ (31%). For all other sites, leafy feeds contributed proportionally more to TEQ as compared to TMR weight, some by nearly a factor of 2. Over all 10 locations, leafy feeds made up, on average, 62% of TMR by weight and 71% of TEQ.

The NY results are interesting not only in the disparity of this result from the other locations but also in the cause for this result. One major reason is clearly the presence of the minor component, Flobond, which had a TEQ concentration of 38.5 ppt, while contributing 0.1% of the total mass of the mixed feed. With this minor component, the predicted TEQ measurement of the mixed feed is 0.093 ppt; without it the predicted concentration is 0.051 ppt. Therefore, while comprising a

Table 4. Summary of Results of Pilot Study Samples Taken To Evaluate the Impact of Preparing Samples at the Wisconsin Dairy Facility Research Center (WDFRC) (results in pg/g dwt total, not TEQ, concentration)

description	sum of 7 PCB congeners	sum of 17 CDD/F congeners
high-moisture corn, EPA sample 1	70.6	0.7
high-moisture corn, EPA sample 2	63.0	0.8
high-moisture corn, WDFRC sample 1	37.7	0.8
high-moisture corn, WDFRC sample 2	31.8	0.8
alfalfa silage, EPA sample 1	157.3	3.4
alfalfa silage, EPA sample 2	138.1	13.4
alfalfa silage, WDFRC sample 1	73.8	3.5
alfalfa silage, WDFRC sample 2	108.5	4.9
soymeal, EPA sample 1	33.7	0.3
soymeal, EPA sample 2	43.1	0.6
soymeal, WDFRC sample 1	51.6	1.1
soymeal, WDFRC sample 2	27.0	0.8
average of 6 EPA samples	84.3	3.2
average of 6 WDFRC samples	55.0	2.0

miniscule portion of the overall feed mixture by weight, this calculation suggests that it would nearly double the concentration. Also, when redoing the calculation for percent of TEQ contributed by leafy vegetation without Flobond, the result is 56%, and if this is included in the average over all 10 locations, the leafy vegetation makes up about 74% of dioxin TEQ instead of 71%.

5. Evaluation of the Possibility that Oven Drying Resulted in Loss of Dioxins and PCBs. As noted in several places above, a concern has been raised that the concentrations measured in this survey are lower than expected and that this could be due to the use of ovens to dry the feed samples. The possible influence of oven drying was, in fact, investigated in three different ways: (1) with a presurvey pilot study evaluating the practices of the WDFRC which cooperated with the EPA in this survey by drying and grinding all samples in preparation for analysis at the EPA lab, (2) with a postsurvey analysis of milk samples to see if they were similarly low in concentrations, and (3) with a second postsurvey sampling, preparation, and analysis of a limited number of TMR samples with the sample preparation and analysis conducted entirely at the EPA laboratory.

In this pilot survey the WDFRC collected eight bags each of high-moisture corn, alfalfa silage, and soymeal from their stocks of dairy feed (24 bags in all). The kept bags 1, 3, 5, and 7 of each feed type and sent bags 2, 4, 6, and 8 of that feed type to the EPA laboratory. The WDFRC then mixed bags 1 and 3 of each feed type to get one of two samples of that feed type; they mixed bags 5 and 7 to get the second sample. Likewise, the EPA mixed bags 2 and 4 and then bags 6 and 8 for their two samples of that feed type. Then both facilities used available equipment and standard procedures to dry and grind the feed samples. WDFRC shipped their ground samples to the EPA, where they were analyzed. The final analysis included 12 samples: six from WDFRC (two each of corn, silage, and soymeal) and six from EPA.

The results are summarized in **Table 4**. The seven PCB congeners are summed as are the 17 CDD/Fs without converting to TEQs. As seen in **Table 4**, the samples prepared at the EPA had higher concentrations of PCBs than the samples prepared at the WDFRC for high-moisture corn and alfalfa silage, by about a factor of 1.5, but the PCB concentrations of soybean meal were comparable whether prepared at the WDFRC or EPA. These PCB total concentrations ranged from about 25 to 150 ppt dwt. In contrast, the CDD/Fs were comparable for all three feed types, in the low ppt to sub-ppt range. If anything appears out of the ordinary, it would be the one CDD/F analysis of 13.4 ppt dwt for one of the two EPA alfalfa hay samples. The other EPA alfalfa hay sample was 3.4 ppt dwt, and the two WDFRC alfalfa hay samples were 3.5 and 4.9 ppt dwt. On a TEQ basis, all measurements were low and typical of the full survey data: 11 of 12 samples were less ≤0.09 ppt CDD/F/PCB TEQ dwt, and 8 of those 11 were ≤0.03 ppt CDD/F/PCB TEQ dwt. It would appear this prestudy analysis, where the feeds were prepared at both the EPA lab and the WDFRC, found generally the same low concentrations as found for the overall study. Although some influence of the WDFRC preparation methods was suggested for PCBs, it was decided to undertake the study as planned, particularly since the WDFRC, as a forage research center, was well equipped to prepare samples for analysis.

Still, when the full study samples were analyzed and the results examined, the concentrations were lower than expected. The mass balance study, conducted just a few years earlier, with samples prepared and analyzed at the EPA laboratory using similar drying and grinding procedures, had CDD/F TEQ concentrations of mixed feed in the range of 0.13−0.22 ppt, with an average of 0.17 ppt, while the samples in this study were all less than 0.10 ppt CDD/F/PCB TEQ dwt, with an average of 0.05 ppt dwt. When the analysis was completed, it was decided that a portion of the milk samples that were collected during the survey (collected in case feed was unusually high and it was desired to see if the milk was also high) would be measured to see if they also had lower concentrations. In the interest of time and expense, only six samples were measured, and they were measured only for CDD/Fs.

Unlike the concentrations in the feed, however, the concentrations of CDD/Fs in the milk were more typical for the United States. The average concentration over the six samples was 0.93 ppt TEQ lwt, with a range of 0.28−2.4 ppt TEQ lwt. This compares to a national average of about 0.71 ppt TEQ lwt (*7*). The average of the three milk samples taken in the mass balance study was the same as the national average, 0.71 ppt TEQ lwt (*10*).

To further characterize the disparity in feed and milk samples, bioconcentration factors, BCFs, were applied to congener concentrations from the TMR feed sample originating from the locations where the milk samples were taken. A set of congener-specific BCFs was developed as part of the EPA mass balance study (*10*). These BCFs are defined as the ratio of congener-specific milk concentrations, in units of pg/g lipid basis, divided by the average congener-specific concentration in the feed, in units of pg/g dry weight. Therefore, multiplication of a congener-specific average feed concentration times a congener-specific BCF would yield a prediction of a lipid-based congener-specific milk concentration.

The results for this exercise showed that there was only one station in six where the TEQ concentration of the milk was reasonably well predicted from the feed concentration. In Washington, the predicted milk concentration was 0.29 ppt TEQ lwt while the observed concentration was 0.28 ppt TEQ lwt. Individual congener predictions of milk at this site were usually within a factor of 2−5 of the observed milk concentrations, either higher or lower, which is also a good match. In all other sites, however, the predicted milk concentrations were much lower than observed, with a range of factors of 2−15 times lower. Over all six samples, the average predicted concentration was 0.18 ppt TEQ lwt. This compares with the observed 0.93 ppt TEQ lwt from the six sites and also with the national average of 0.71 ppt TEQ lwt. With the exception of the Washington site, there was a clear trend in this postsurvey examination that the low concentration feeds would not explain the more average concentration milk.

A final limited postsurvey sampling and analysis included a new collection of six TMR samples from six different facilities in 2006 and preparing and analyzing them entirely at the EPA laboratory. Similar to the practice at the WFDRC, the samples were dried for 48 h in an oven but at a temperature of 60 °C, slightly higher than the 55 °C at the WFDRC ovens. Again, in the interest of time and expense, only the CDD/Fs were analyzed. The results for this second postsurvey analysis showed CDD/F concentrations that were comparable to the original CDD/F analyses, where the preparation was done entirely at the WDFRC. The original site CDD/F TEQ concentration, in ppt dwt, compared with the postsurvey analysis for the six sites are as follows: WA, 0.06 (original study) and 0.03 (postsurvey); MI, 0.07 and 0.03; OR, 0.05 and 0.03; VA, 0.03 and 0.04; NY, 0.04 and 0.05; and FL, 0.02 and 0.03.

In general, the reasons for low feed concentrations in this study are not known. Pilot and postsurvey analyses described above were attempts at providing confidence that the WDFRC sample preparation—drying and grinding—was not resulting in some off-gasing of the dioxins. Since the earlier mass balance study results, with feed samples prepared fully at the EPA laboratory, were about four times higher than these, the speculation was that somehow the WDFRC procedures could be at fault. The pilot study did suggest some possible influence of the WDFRC with regard to PCBs, but the CDD/Fs in the feeds were similar whether prepared at the WFDRC or the EPA. Further, the postsurvey analysis of the six samples, prepared entirely at the EPA lab, also showed low concentrations of CDD/Fs, similar to the full survey samples prepared at the WDFRC. Thus, while the earlier mass balance samples were higher than the full dairy survey samples, the evidence does not immediately point to the WDFRC as having unique sample preparation techniques that would drive off, at least, CDD/Fs.

The reality could simply be that the feeds are truly that low at this time, not influenced by the sample preparation methods. However, even this possibility has to be questioned in light of the postsurvey analysis of milk samples. If the feed concentrations were truly that low, then the more average milk concentrations are being affected by something external to the feed, such as by contact of the lactating cow with PCP-treated wood. Another possible explanation is that the animals at the research facilities were allowed to graze, thus coming in contact with soils that have higher concentrations than feeds. In the mass balance study, the cattle were carefully sequestered and well into a milking cycle when samples were taken. Thus, the somewhat higher feed samples fully explained the average milk concentrations, while the average milk concentrations of this study may have been due to exposures other than just their feeds.

Even with all of this analysis, there is still the finding that a weighted average concentration of TMR tends to be higher than the TMR measurement itself. This was discussed earlier in the section above on predicting a TMR concentration from the feed components. In examining **Table 3**, showing this calculation for one site, it is seen that the predicted PCB concentrations seem consistently higher than the measured PCB concentrations, whereas the predicted CDD/F concentrations seem more nearly equal to the measured CDD/Fs concentrations. Generally, the predicted PCB congener concentrations were higher in the components by about a factor of 2 in the Michigan feed, as seen in the final two columns of **Table 3**. In fact, the slope of the best-fit line between predicted and measured PCBs in the Michigan example was 2.00; the predicted concentration was twice the measured concentration. The best-fit line between predicted and measured CDD/Fs at the Michigan site had a slope of 0.85; the predicted CDD/F was slightly less than the measured CDD/F.

This best-fit analysis between predicted and measured CDD/F/PCB congeners was conducted for the full data set. There are a total of 63 paired sets (a pair includes a predicted and an observed concentration) of PCB congeners (9 facilities × 7 PCB congener pairs per facility = 63). The correlation between predicted and observed PCB concentrations was 0.84, and the best-fit slope between predicted and observed was 1.24 (predicted was 1.24 times higher than observed). For dioxins and furans, there were 153 paired sets (9 facilities × 17 CDD/Fs). The correlation between predicted and observed was 0.74, and the best-fit slope was 1.18. Overall, the trend is that the weighted average concentration is higher than the measured concentration for both PCBs and CDD/Fs. In the mass balance study discussed in the background section, also conducted by the EPA with analysis at the same EPA laboratory as in this study, it was similarly observed that the concentrations of the CDD/F congeners in the feed components were higher than in the mixed feed (*11*).

The disparity in Utah, 0.28 ppt TEQ predicted versus 0.04 ppt TEQ measured, is of particular note. Upon examination of the data it was found that the measurement of 2,3,7,8-TCDD on alfalfa hay, which comprised 22% of the Utah feed mixture, was 0.76 ppt. Subsequently, the mixed feed predicted concentration of 2,3,7,8-TCDD was high at 0.23 ppt. With a toxic equivalency factor of 1.0 it is clear why the predicted concentration came out as high as 0.28 ppt TEQ, but it is not known why this was not reflected in the feed mixture measurement. The likely explanation is that the sample of alfalfa hay was not representative of the alfalfa hay in the feed mixture (although records were double-checked, no errors found).

However, perhaps another explanation could circle right back to the issue of oven drying. The sample preparation records were retrieved, including sample wet weights prior to drying and then the dry weight after drying. It was found that all nine TMR samples weighed between 40% and 60% of their initial wet weight after drying—they lost between 40% and 60% of their weight as evaporated water. In contrast, many of the individual leafy and nonleafy vegetation major components lost only a fraction of their weight in evaporated water. As discussed earlier in the section on major components above, the average dry matter content of the nonleafy vegetation was 89%, meaning that these components lost only 11% of their weight by oven drying. The Utah alfalfa hay measured at 86% of its original weight after drying. Like other feed components, it was essentially dry when put in the oven. However, when part of the Utah TMR, perhaps this dry alfalfa hay took on some moisture. Perhaps some dioxins volatilized along with evaporating water in the drying process for the generally wet TMR samples. If this is a true hypothesis, then it would be most relevant for the lighter, more volatile, dioxin molecules, such as 2,3,7,8-TCDD. Maybe the higher concentration of 0.76 ppt of 2,3,7,8-TCDD in the alfalfa hay, while not evaporating when the already dry alfalfa hay sample itself was oven dried, did evaporate as part of the more wet TMR. More experiments would need to be conducted to evaluate this possibility. Still, it is a plausible explanation generally and could account for why predicted concentrations of CDD/F/PCBs in TMR were consistently higher than measured CDD/F/PCB.

6. Discussion of Primary Findings. In summary, the following key findings can be stated. (1) The air-to-leaf pathway appears to be the predominant pathway through which dairy cattle feed gets impacted by dioxin-like compounds, as evidenced by higher concentrations in leafy feed components (by a factor of 2 or more), as compared to nonleafy feed components. It was also found that leafy feed components make up the majority of the mass of mixed feed, 62%, and combining this mass fraction with concentration data, that leafy components deliver the majority of TEQ dose to the animal by animal feed, 71% of the feed-delivered dose. (2) Minor components mostly do not affect feed quality, but in this survey, a component comprising 0.1% of overall feed mixture at one site had a concentration of 38.5 ppt TEQ dwt, and this resulted in a doubling of the concentration of dioxin-like compounds in the overall mixed feed as compared to what the mixed feed concentration would be without this component. (3) In dairy feed, dioxins and furans contribute about four times as much dioxin-like TEQ as do PCBs. (4) Total mixed feed concentrations can be predicted reasonably well by measuring the individual components of the feed.

The concentrations of dioxin-like compounds found in this study, generally low at less than 0.10 ppt CDD/F/PCB TEQ, are not characterized as a key finding as they appear, for an as-yet undetermined reason, to be lower than other analyzed dairy feeds. There was a hypothesis offered that possibly oven drying may result in some loss of dioxins. However, the evidence was not conclusive, and more study would be required to test that hypothesis.

If in fact the feed concentrations were truly that low as fed to the dairy cows, then this would cast doubt that feeds were supplying the dioxins to the dairy cattle in these facilities. This is because a limited postsurvey analysis of milk suggested average milk concentrations that could not be explained by the low dairy feed concentrations. For that reason, it also cannot be concluded that the air-to-leaf pathway is a primary route of exposure of dairy cattle to dioxins. Note that the primary finding above was specific only to the quality of the dairy feed—that this feed was mostly influenced by an air-to-leaf pathway. This contrasts the finding of the earlier EPA mass balance study, where in fact dioxin concentrations in milk corresponded more appropriately to levels in the feed.

Future work and thought may lead to better characterization of the concentrations in dairy feeds nationally and the role feeds play in delivering dioxins to lactating cows. Questions remain as to the influence of sample preparation on final sample concentrations. In addition, future work may provide insights as to the concentrations of dioxin-like compounds in other terrestrial or aquatic animal feeds, the role of the air-to-leaf pathway, and the importance of minor components of these feeds.

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