Distribution of Dioxins, Furans, and Coplanar PCBs in Different Fat Matrices in Cattle

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Introduction The United States Environmental Protection Agency (EPA) and the United States Department of Agriculture (USDA) recently collaborated on a statistically-based, national survey of dioxin-like compounds, including dioxins, furans, and coplanar PCBs, in the back fat from slaughtered cattle[1,2,3]. Back fat was selected because it was a matrix that could easily be sampled by the veterinarians at the slaughter establishments. Also, since it was a matrix that was very high in fat content (in the range of 60-90% lipid), the ability to measure the dioxin-like compounds with a given sample volume was maximized. A principal use of the results of the national beef survey is to evaluate the exposure of individuals in the United States to these compounds through consumption of beef. In order to use the data for this purpose, an assumption needs to be made regarding the relationship between lipid concentrations of these compounds in back fat compared to the concentrations in meat products. However, data on the concentrations of these compounds in different cattle fat reservoirs to derive the proper assumption are sparse. There is some information on compounds with similar properties (lipophilic, persistent), including residues of HCB[4], PBB[5], and DDT[6], and these data do suggest that their lipid-based concentrations in various fat reservoirs in cattle are similar. In order to evaluate whether the same can be said of the dioxin-like compounds, the EPA and USDA collaborated on a second effort to measure these compounds in various cattle fat reservoirs. This abstract provides an overview of this effort.

Description of Data Three data sets were analyzed in this study. In each data set, four animal tissue matrices were evaluated: back fat, perirenal (kidney) fat, muscle tissue, and liver. All concentrations in tissue samples are express on a lipid-adjusted basis. In general, the lipid contents of the four matrices were: back fat 60-90%, perirenal fat 70-90%, muscle tissue <5%, and liver <5 %. The three data sets are:

1. In 1995, Feil[7] reported on the analysis of perirenal fat samples from 20 animals located in 12 research facilities around the U.S. Feil[7] also noted that other tissue samples, including back fat, muscle tissue (specifically, from the ribeye), liver, and serum samples were taken. For the study reported in this abstract, samples of back fat, muscle tissue, perirenal fat, and liver were obtained from 5 selected animals from 3 of the 12 research facilities: 3 animals from Pennsylvania State University (abbreviated PSU hereafter), and 1 each from North Dakota State University (NDSU) and Oregon State University (OSU). The 5 animals selected had the highest TEQ concentrations in the

perirenal fat, therefore maximizing the possibility of measuring all congeners. In a later study of the research facilities, it was found that the wood in some of the housing and feeding structures contained pentachlorophenol (PCP), and this was speculated to be the cause of high concentrations found in some of the slaughtered animals[8]. Animals in these research facilities were raised in a manner similar to feedlot operations of the U.S. The slaughter age of the 5 animals was about 1.5 years. All samples were analyzed for the 17 dioxin and furan congeners, and for 6 coplanar PCBs. All analyses were performed by high resolution GC/MS. Samples were solvent extracted and the extracts cleaned up using silica gel, alumina and carbon column procedures described elsewhere[3,8].

2. In 1996, Feil[10] reported on a dosing study with four animals which had been fed high amounts of several, but not all, of the dioxin and furan congeners. The dosed animals also experienced unexpected exposure to some higher chlorinated congeners that exceeded the administered dose levels. The source of this exposure was subsequently traced to PCP-treated wood used in construction of the feeding facility. The animals were slaughtered 17 weeks after dosing began. Samples of back fat, perirenal fat, muscle tissue (ribeye), serum, and liver were taken at slaughter. Feil[10] reported on the concentrations of the homologue groups in these tissue types for the dosed animals. Feil had also generated information on the 17 individual dioxin-like CDD/F congeners (unpublished), and this data was obtained for the four tissue types for this analysis.

3. In 1995, Startin[11] reported on a depletion study of CDD/Fs in five animals from a herd near Bolsover, Derbyshire in England, that was shown to have very high concentrations in milk. The high concentrations were traced to local contamination of feed. After switching to clean feed, animals were slaughtered at various intervals up to 202 days, and their tissues analyzed for CDD/Fs. The concentrations of the 17 CDD/F congeners were supplied for the four tissue types for one animal slaughtered after 59 days[10], and these results were used in the analysis reported in this abstract.

Analysis Procedure Analysis of the relationships between the four tissue types was accomplished by evaluating:

1) *Clusters of samples*: The 5 animals from the three research facilities were not clustered, but rather evaluated as five individual animals. The dosed animals were clustered as a group of 4 for analysis, and the Bolsover animal was considered an individual animal. Therefore, there were five research animals, identified by their location as, "PSU" (n=3), "NDSU" (n=1), and "OSU" (n=1), the cluster of 4 "dosed" animals, and the single "Bolsover" animal.

2) *Total and TEQ concentrations*: The lipid-based concentrations of the 17 congeners were summed and reported as a "total" concentration. The TEQ concentration of a tissue type was calculated using the international TEQ scheme[12] for CDD/Fs and the WHO recommendations[13] for the coplanar PCBs. All averages were derived assuming non-detected values were equal to 0.0, rather than assuming that non-detects were equal to 1/2 detection limit, since results originated from different laboratories and had different detection limits.

3) *Congener profiles*: The fraction that each of the 17 congeners contributes to the total concentration was calculated by that congener's concentration divided by the total concentration.

4) *Muscle tissue to back fat ratios*: The lipid-based congener concentrations of the muscle tissue were divided by the lipid-based back fat congener concentrations for each animal individually. A ratio of 1.0 would mean that muscle and back fat concentrations are equal; a ratio less than 1.0 means that muscle fat concentrations are lower than back fat, and a ratio greater than 1.0 means that muscle fat concentrations are higher. The ratio was not derived when either the muscle fat or the back fat congener was not detected. For the dosed animals, the ratio was developed as the average of the individual animal ratios.

Results Table 1 shows the total and TEQ concentrations of the four tissue types for 5 research herd cattle, the dosed cluster, and the Bolsover cattle. Table 2 shows the muscle to back fat ratios for the cluster of dosed animals and the 6 other individual animals. Figure 1 shows the muscle to back fat ratios for congeners grouped by degree of chlorination, and also by these groupings: the 5 research animals (as one group), the 4 dosed animals, and the Bolsover animal. Figures 2 and 3 address the comparison of results for the four tissue types obtained for analysis.

The relationship between back fat concentrations and concentrations in cattle products in general is best investigated by examination of the 5 research animals, rather than the dosed animal or the Bolsover animal. This is because the 5 animals from research herds were raised in feedlot-like conditions and slaughtered after about 1.5 years. In contrast, the dosed cluster was not in steady state as the tissues were analyzed after 17 weeks on a high dose, and the depletion animal from Bolsover also was not in steady state as it was slaughtered after 59 days after switching to clean feeds.

Based on the 5 research animals, the authors conclude that lipid-based concentrations of CDD/Fs in back fat are an acceptable surrogate for lipid-based concentrations of CDD/Fs in intramuscular fat. Total and TEQ concentrations comparable between back fat and intramuscular fat (Table 1). For one animal, intramuscular fat concentrations were higher than back fat by about 50%, in three animals they were lower by 10-40%, and in the fifth animal, they were similar. On an individual congener basis, the ratios of intramuscular fat to back fat concentrations mostly ranged between 0.5 and 1.5 for the CDD/F congeners (Table 2). On a TEQ basis, the ratio ranged between 0.6 and 1.7, with an average of 0.9 (Table 2).

The data does not show as clear a relationship for PCBs. In all 5 animals, total and TEQ intramuscular concentrations were higher than back fat concentrations, and for 2 animals, the muscle fat/back ratio for total concentration was about 3.0 (from Table 1). As seen in Table 2 by examining the muscle to back fat ratios, these overall higher ratios were due mainly to concentrations of PCBs 77, 118, and 105, which were higher in muscle than back fat by up to 16 times. The other ratios for PCBs 126, 156, 157, and 169 indicate that back fat would appear to be an acceptable surrogate for intramuscular fat as ratios ranged between 0.3 and 1.5.

Other observations from these table and figures include:

1) After analysis of the tissues from the 5 research animals, it appears that the 3 animals from PSU generally had higher concentrations than the NDSU and OSU animals. The PSU animals had higher concentrations: the TEQ (CDD/Fs only) concentrations in the back fat from these three animals were 8, 15, and 34 pg TEQ/g lipid (ppt), and the total concentrations were 107, 183, and 290 pg/g lipid. The NDSU and OSU animals, in contrast, had back fat concentrations were 3 and 4 pg TEQ/g lipid, and 33 and 83 pg total/g lipid.

2) The 5 research herd animals all had substantially higher CDD/F tissue concentrations than the national EPA/USDA survey concentrations[1,2,3], which are shown in Tables 1 and 2 for the sake of comparison. These animals had between 0.5 to 3 orders of magnitude higher concentrations than the national survey concentrations. As noted above, these elevated concentrations may have been due to contact with PCP-treated wood.

3) As noted above, the dosed and Bolsover animals were further from steady state than the research animals. This may have implications regarding the delivery of CDD/Fs to various tissues as well as the depletion of dioxins from those tissues. In terms of depletion, the speculation is that the dioxins are depleted more rapidly from muscle tissue than back fat. This possible trend is seen in the Bolsover animal, where the muscle fat to back fat ratios are generally less than 1.0, except for the HpCDD and the OCDD congeners, which had ratios of 1.8 and 6.1, respectively. Startin[10] similarly noted that there was a general tendency in his results for the concentrations in muscle fat to be lower

than in the back fat. In the same way that muscle tissue may deplete before back fat, it may also become enriched in CDD/Fs more rapidly than back fat upon initiation of an exposure. This relations is seen in the dosed animals, which had ratios of muscle to back fat greater than 1.00 for all congeners, and almost 5.0 for the octa congeners, OCDD and OCDF. The research animals are closer to steady state, and their ratios are closer to 1.00 for all congeners. These trends can be seen in Figure 1.

4) Differential depositions in body tissues may also be a function of chlorination. Schecter[14] examined the partitioning of CDD/Fs between human blood and adipose. His study suggested that on a lipid-basis: 1) blood plasma levels were higher than the adipose among the higher chlorinated compounds, 2) blood plasma and adipose levels were similar among the lower chlorinated compounds and 3) blood plasma and adipose levels were similar for all CDD/Fs on a TEQ basis. Other investigators report these same observations[14]. As shown in Figure 1 and Table 2, the research cattle show a similar partitioning relationship as seen in humans, with OCDD having a ratio of 1.8, indicating preferential deposition in muscle as compared to back fat, with the other congeners having a ratio closer to 1.0. This pattern of partitioning is more pronounced in the dosed and Bolsover animals, with ratios for both hepta and octa congeners at around 2.0 and higher.

5) In all cases, the congener profile in liver is easily distinguished from the other three matrices in two ways: the total and TEQ concentrations were substantially higher and there seems to be a disproportionate fraction of OCDD and 1234678-HpCDD in the liver matrix. The higher concentrations are seen in Table 1, and the different profiles are seen in Figures 2 and 3.

6) Within all animals individually, the tissue concentration profiles in the back fat, kidney fat, and intramuscular fat seem substantially similar. However, there are differences among the animals. As an example, differences can be seen in the dosed cluster as compared to the Bolsover animal in Figures 2 and 3. The Bolsover animal (Figure 3) shows an abundance of 123678-HxCDD, while the dosed animal (Figure 2) profiles are dominated by 1234678-HpCDD and OCDD. Other, more subtle differences show up in the profile characteristics for 2378-TCDD, OCDF, and others. Differences in profile characteristics are also seen among the 5 research animals.

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Table 1. Relationship between back fat concentrations and the concentrations in kidney fat, muscle tissue, and liver (all concentrations on pg/g (ppt) lipid basis).

Description	Back fat		Kidney fat		Muscle		Liver	
	Total	TEQ	Total	TEQ	Total	TEQ	Total	TEQ
I. CDD/Fs								
USDA/EPA	11	0.4						
OSU	33	3.3	38	3.3	28	2.0	204	0.6
NDSU	83	3.8	57	2.0	58	2.5	2,430	12.4
PSU	106	8.3	117	8.1	103	7.1	1,639	21.6
PSU	289	34.8	370	37.0	258	24.7	16,811	272
PSU	182	14.6	268	24.1	364	25.1	10,265	26.3
Bolsover (n=1)	321	7.7	295	7.0	256	4.4	848	100
dosed (n=4)	1,330	270	1,850	29	3,330	440	152,300	2,350
II. Coplanar PCBs								
USDA/EPA	609	0.5						
OSU	1,122	1.0	1,208	1.1	1,986	1.0	2,776	0.5
NDSU	1,472	1.3	1,047	1.0	1,749	1.7	3,028	2.8
PSU	1,701	1.1	1,760	1.2	3,237	1.4	2,153	1.1
PSU	4,684	3.5	6,799	3.6	14,640	4.0	4,022	3.1
PSU	4,526	2.4	5,170	2.7	12,280	3.6	4,344	4.6

Table 2. Concentrations in back fat (pg/g lipid, or ppt lipid) and ratio (muscle/back fat; pg/g lipid basis) for the five animals from the research herds and the two other groupings of animals.

Congener (D = dioxin; F = furan)	EPA/ USDA back fat	OSU back fat | ratio	NDSU back fat | ratio	PSU back fat | ratio	PSU back fat | ratio	PSU back fat | ratio	Bolsover back fat | ratio (n=1)	dosed back fat | ratio (n=4)
2378-D	0.03	0.3 | 1.2	0.3 | 0.9	0.2 | 1.2	0.6 | 1.4	0.9 | 1.1	34.3 | 0.5	139.5 | 2.1
12378-D	0.04	2.3 | 0.5	1.6 | 0.6	5.1 | 0.9	9.9 | 1.7	31.5 | 0.6	32.0 | 0.5	90.8 | 1.2
123478-D	0.18	2.2 | 0.6	2.4 | 0.5	6.4 | 0.9	7.3 | 2.4	18.9 | 0.8	2.0 | 0.5	12.00 | NA
123678-D	1.21	7.7 | 0.8	9.3 | 0.7	24.6 | 1.0	36.1 | 1.8	60.4 | 0.9	171.0 | 0.7	212.4 | 1.4
123789-D	0.26	2.3 | 0.7	2.3 | 0.3	7.3 | 0.8	11.6 | 2.7	25.0 | 0.8	42.2 | 0.7	18.9 | 2.0
1234678-D	4.39	8.0 | 0.8	24.8 | 0.7	41.7 | 0.9	56.7 | 1.7	65.7 | 1.1	6.3 | 1.8	351.1 | 2.4
OCDD	3.26	6.3 | 1.7	33.0 | 0.8	12.8 | 1.6	33.7 | 2.4	19.6 | 1.5	8.0 | 6.1	321.8 | 4.8
2378-F	0	0 | NA	0 | NA	0 | NA	0 | NA	0 | NA	0 | NA	5.3 | NA
12378-F	0	0 | NA	0 | NA	0 | NA	0 | NA	0 | NA	0 | NA	0 | NA
23478-F	0.06	0.6 | NA	1.2 | 0.7	1.4 | 0.6	2.9 | 1.0	6.3 | 0.6	7.7 | 0.6	107.2 | 1.2
123478-F	0.27	0.8 | NA	1.6 | 0.7	1.7 | 0.7	2.7 | 0.5	5.2 | 0.3	3.4 | 0.9	10.6 | NA
123678-F	0.12	0.7 | NA	1.3 | 0.3	1.7 | 0.5	6.5 | 1.7	14.0 | 0.7	5.4 | 0.8	15.8 | NA
123789-F	0	0 | NA	0 | NA	0 | NA	0 | NA	0 | NA	0 | NA	0 | NA
234678-F	0.10	0.9 | NA	1.2 | 0.2	1.2 | 0.3	4.3 | 1.3	16.9 | 0.4	5.8 | 0.8	8.6 | NA
1234678-F	0.75	1.3 | NA	4.3 | 0.4	2.8 | 0.6	10.6 | 3.4	24.2 | 1.0	2.7 | 1.3	77.9 | 2.1
1234789-F	0	0 | NA	0 | NA	0 | NA	0 | NA	0.4 | NA	0 | NA	6.2 | NA
OCDF	0	0 | NA	0 | NA	0 | NA	0.1 | NA	0.6 | NA	0.6 | NA	18.2 | 4.7
PCB 77	0.60	0.7 | 12.6	2.0 | 2.8	1.7 | 7.3	16.5 | 16.7	19.5 | 4.7	Note: Coplanar PCBs data not taken for Bolsover and dosed animals NA means that either (or both) of intramuscular and back fat samples had ND, such that a ratio could not be derived.	
PCB 118	440.5	859 | 1.7	1087 | 1.2	1332 | 1.8	3551 | 2.9	3649 | 2.5		
PCB 105	90.6	145 | 2.8	237 | 1.2	233 | 2.8	612 | 5.7	486 | 5.3		
PCB 126	4.0	8.4 | 0.9	11.0 | 1.3	8.8 | 1.1	27.8 | 0.8	18.1 | 1.2		
PCB 156	58.7	88.4 | 0.9	105 | 1.0	102 | 1.5	390 | 1.5	281 | 1.3		
PCB 157	13.4	20.7 | 1.0	26.3 | 1.0	23.1 | 1.4	83.4 | 1.4	69.7 | 1.1		
PCB 169	0.69	1.3 | 1.2	4.7 | 1.1	1.6 | 1.1	4.5 | 0.7	2.7 | NA		
D/F TEQ	0.35	3.3 | 0.6	3.8 | 0.6	8.3 | 0.9	14.6 | 1.7	34.8 | 0.7	77.2 | 0.6	271.5 | 1.6
PCB TEQ	0.49	1.0 | 1.0	1.3 | 1.2	1.1 | 1.3	3.5 | 1.1	2.4 | 1.5	-----	-----

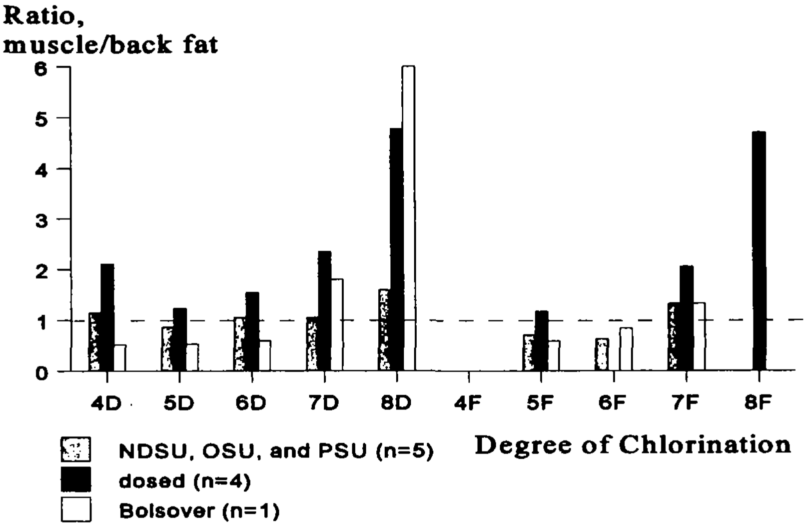

Figure 1. Ratio of muscle to back fat concentrations on a lipid basis, comparing the 5 research herd animals, the 4 dosed animals, and the 1 Bolsover animal, as a function of the degree of chlorination (no bars indicate that muscle and/or back fat concentrations were non-detected so no ratio could be derived; e.g. no ratios were possible for 2378-TCDF in any animal).

Key for Figures 2 and 3 :

Dioxins	D1	2378-TCDD	Furans	F1	2378-TCDF
	D2	12378-PCDD		F2	12378-PCDF
	D3	123478-HxCDD		F3	23478-PCDF
	D4	123678-HxCDD		F4	123478-HxCDF
	D5	123789-HxCDD		F5	123678-HxCDF
	D6	1234678-HpCDD		F6	123789-HxCDF
	D7	OCDD		F7	234678-HxCDF
				F8	1234678-HpCDF
				F9	1234789-HpCDF
				F10	OCDF

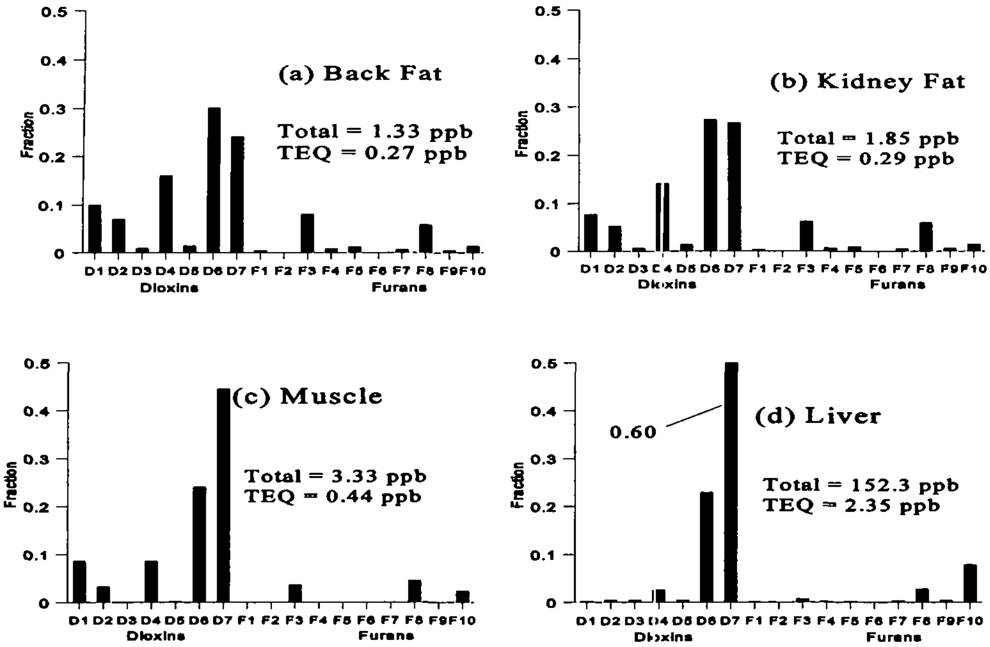

Figure 2. Lipid-based congener profiles and concentrations for the dosed animal matrices.

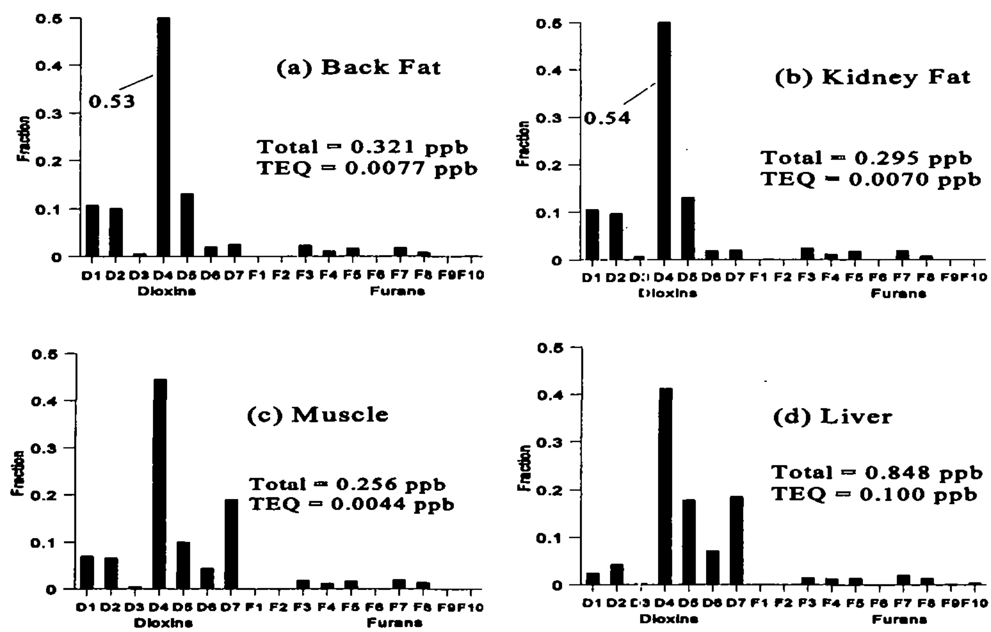

Figure 3. Lipid-based congener profiles and concentrations for the Bolsover cattle matrices.