DISTRIBUTION OF DIOXINS INTO BEEF LIPID STORES: ANOTHER LOOK AT LEVELS IN TYPICAL SAMPLING MATRICES AND RETAIL MEAT CUTS

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Introduction

The U.S. EPA and USDA conducted a statistically-based survey of U.S. beef in the mid-1990's using back fat samples from cattle to determine dioxin, furan, and PCB levels[1]. In a geographical survey conducted by our research group at the USDA, perirenal fat was used as a sampling matrix to report dioxin levels in beef cattle from various regions of the U.S.[2] It is assumed that like other lipophilic compounds, dioxin-like compounds distribute evenly into lipid compartments[3], and therefore, the lipids in edible beef cuts will contain amounts of dioxins similar to the adipose tissues sampled in the surveys. A few studies have been done to investigate this assumption. Ferrario and Bryne analyzed breast and thigh meat from chickens and found dioxin levels equivalent to adipose tissue on a lipid-adjusted basis[4]. Data from our geographical survey and from a dioxin dosing experiment have previously been used to compare lipid compartments in beef animals[5,6]. For animals near steady-state, the lipid-adjusted levels of dioxins and furans appeared to be equal in back fat, perirenal fat, and ribeye. One critical exception was TCDD which showed twice the levels in ribeye than in either fat matrix in dosed animals. We have since discovered a discrepancy which may have caused an error in the quantitation of TCDD for some of the matrices in this dosing study. In this study we have, therefore, reanalyzed back fat, perirenal fat, and ribeye samples and also analyzed tenderloin samples to compare lipid-weight concentrations of the PCDD/Fs in each of these matrices.

Methods and Materials

All samples were from a dosing experiment in which four steers received an identical dose of nine 2,3,7,8-substituted dioxins and furans in their feed for 120 days[6]. Tissue samples were stored at –60°C until analyzed. A modification of the previously described method (based on EPA Method 8290A)[6] was used for sample purification and analysis. Samples were spiked with fifteen isotopically-labeled dioxins and furans. Tissues were ground with Celite and extracted on an Accelerated Solvent Extractor (Dionex, Sunnyvale, CA) with methylene chloride/hexane, 50/50, at 100°C. Lipid extracts or dissolved fats were purified on a Dioxin-Prep™ system (Fluid Management Systems, Waltham, MA) utilizing tri-phasic silica, alumina, and carbon columns for chromatography. High resolution GC-high resolution MS was performed on a Micromass Ultima Autospec instrument coupled to an Agilent 6890 chromatograph. Non-detects are reported as zero.

Results and Discussion

After 120 days of feeding, serum concentrations in the dosed steers had plateaued for the dosed tetra- and penta-CDD/Fs, indicating that these congeners had reached steady states in the animals. Serum concentrations of higher chlorinated congeners were still rising after 120 days and did not

appear to reach steady states either because the length of the feeding interval was too short or because a secondary dioxin source (pentachlorophenol-treated wood) was found to contribute additional unknown amounts to the exposure.
Initial data from the study[6] showed that five of the dosed congeners, TCDF, 2,3,7,8-PeCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,7,8-PeCDD, and 1,2,3,6,7,8-HxCDD, were distributed equally in back fat, perirenal fat, and ribeye fat. OCDF, 1,2,3,4,6,7,8-HpCDD, and OCDD, however, were two- to four-times more concentrated in ribeye fat than in back or perirenal fat, perhaps due to the lack of steady state equilibria. These same conclusions are supported by the most recent analyses (Table 1 and Figure 1). In addition, tenderloin was found to be identical to ribeye on an average lipid-weight basis for dosed and non-dosed congeners. Figure 1 shows that the total TEQ for these samples was also statistically equivalent in the four fat matrices.

Originally TCDD levels in ribeye were reported as twice that of back or perirenal fat[6]. Figure 2 shows the concentrations of TCDD on an individual and average basis determined by new analyses. Aside from the ribeye in steer #353 and the tenderloin in steer #419, all lipid-weight concentrations of TCDD were equivalent within the standard error expected of the method. Higher levels in two of the muscle samples are most likely due to the much lower lipid percentages in these samples. The low lipid content resulted in lower amounts of dioxins for quantitation (closer to background), and also small errors in lipid weight determination would magnify the lipid-weight concentrations. For example, tenderloin from steer #419 had a lipid percent of 1.2 compared to the other tenderloin samples which ranged from 2.4 to 3.5%. This tenderloin was the most outlying value.

The average ratios of ribeye to back fat concentrations on a lipid-weight basis were calculated and are shown in Table 1. Values for the congeners near equilibria (i.e. tetras and pentas) were close to unity, ranging from 1.17 – 1.42. The ratio for TCDD is closer to unity than previously reported and is in accord with ratios found in animals from a geographical survey, 0.9 – 1.4[5]. Based on these recent results it appears that TCDD along with other toxic dioxins and furans are evenly distributed into lipid matrices at steady state. However, prior to reaching a steady state, muscle lipids may have higher concentrations of dioxins and furans than adipose tissues. Thorpe et al. also observed high muscle to subcutaneous fat ratios (5-6) after a short-term feeding study in cattle[7]. One possible explanation for this may be the greater perfusion of blood to muscle tissues causing equilibrium to be reached sooner in muscle than in adipose.

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References

1. Winters, D., Cleverly, D., Meier, K., Dupuy, A., Byrne, C.; Deyrup, C., Ellis, R., Ferrario, J., Harless, R., Leese, W., Lorber, M., McDaniel, D., Schaum, J., and Walcott, J. (1996) Chemosphere 32, 469-478.
2. Feil, V.J., Davison, K.L., Larsen, G.L., and Tiernan, T.O. (1995) Organohalogen Compounds 26, 117-119.
3. Fries, G.F. (1995) J. Animal Sci. 73, 1639-1650.
4. Ferrario, J. and Bryne, C. (2000) Chemosphere 40, 221-224.

5. Lorber, M., Feil, V.J., Winters, D., and Ferrario, J. (1997) Organohalogen Compounds 32, 327-334.
6. Feil, V.J., Huwe, J.K., Zaylskie, R.G., Davison, K.L., Anderson, V.L., Marchello, M., and Tiernan, T.O. (2000) J. Agric. Food Chem. 48, 6163-6173.
7. Thorpe, S., Kelly, M., Startin J., Harrison N., and Rose M. (1999) Organohalogen Compounds 43, 405-408

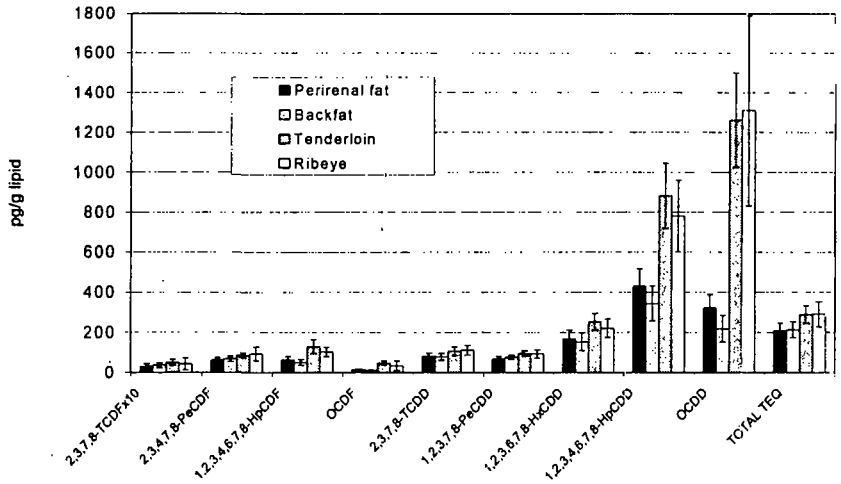

Figure 1. Comparison of nine dosed congeners and total TEQ levels in four beef matrices. Error bars show the standard deviations for the analyses, n=4.

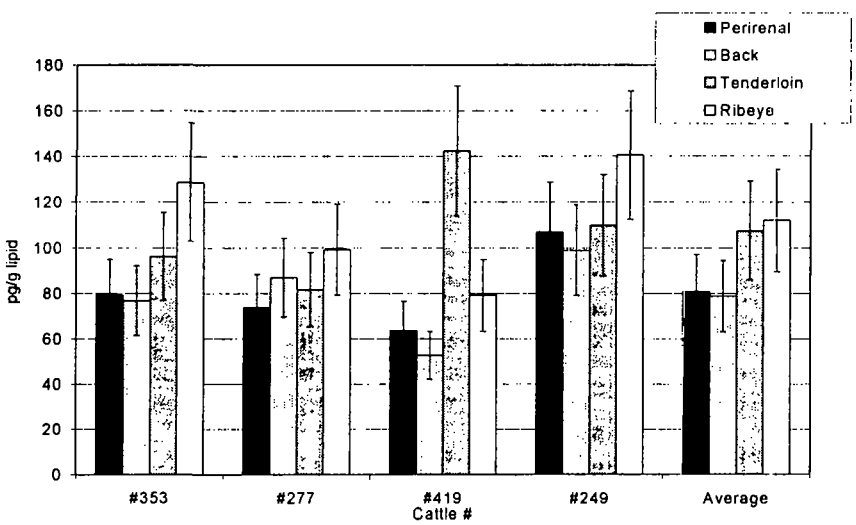

Figure 2. TCDD concentrations in individual beef cattle (pg/g lipid). Error bars indicate the accepted error limits of the method, ± 20%.

Table 1. Summary of lipid concentrations in beef matrices (ppt) and the ratio of average ribeye concentrations to average back fat concentrations.

	BACK		PERIRENAL		RIBEYE		TENDERLOIN		RATIO
Congener	Average	SD	Average	SD	Average	SD	Average	SD	Ribeye/back
2,3,7,8-TCDF*	3.76	1.29	3.15	1.30	4.40	3.00	5.25	1.48	1.17
1,2,3,7,8-PeCDF	0.00	0.00	0.00	0.00	0.63	0.76	0.89	0.81	Nd
2,3,4,7,8-PeCDF*	68.03	13.17	62.68	15.94	94.61	34.58	85.87	10.41	1.39
1,2,3,4,7,8-HxCDF	8.37	3.05	8.54	2.52	16.78	4.74	16.73	4.14	2.01
1,2,3,6,7,8-HxCDF	5.91	2.33	5.91	1.63	10.73	2.56	11.14	3.89	1.81
2,3,4,6,7,8-HxCDF	6.70	2.29	6.79	1.70	13.10	2.78	12.10	3.36	1.96
1,2,3,7,8,9-HxCDF	0.02	0.01	0.01	0.02	0.29	0.37	1.00	0.71	18.33
1,2,3,4,6,7,8-HpCDF*	51.86	14.57	65.30	14.75	103.42	23.98	130.48	35.33	1.99
1,2,3,4,7,8,9-HpCDF	1.82	0.69	2.49	0.75	0.00	0.00	2.10	2.22	0.00
OCDF*	12.20	2.04	16.93	3.49	34.92	24.16	48.84	8.83	2.86
2,3,7,8-TCDD*	78.94	17.04	81.09	16.18	112.01	24.20	107.65	22.45	1.42
1,2,3,7,8-PeCDD*	76.70	10.81	69.06	12.43	93.17	21.61	95.38	13.82	1.21
1,2,3,4,7,8-HxCDD	9.00	3.09	9.64	2.62	10.24	6.48	16.42	4.30	1.14
1,2,3,6,7,8-HxCDD*	153.45	44.90	167.57	36.99	220.92	45.06	253.43	40.73	1.44
1,2,3,7,8,9-HxCDD	13.65	5.23	14.96	4.54	20.63	4.95	24.62	6.34	1.51
1,2,3,4,6,7,8-HpCDD*	343.68	88.64	429.87	89.54	781.48	180.33	881.76	162.94	2.27
OCDD*	218.07	66.01	323.06	56.76	1310.07	480.09	1261.72	236.93	6.01
TOTAL TEQ	213.73	38.33	208.15	37.35	291.21	63.20	290.36	43.90	1.36
Average % lipid	82.91	4.22	94.15	14.05	1.13	0.18	2.44	0.8	

*Indicates dosed congeners. Nd = not determined.