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A STATISTICAL SURVEY OF DIOXIN-LIKE COMPOUNDS IN UNITED STATES BEEF: A PROGRESS REPORT

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Abstract

The USEPA and the USDA have completed the first statistically designed survey of the occurrence and concentration of CDDs and CDFs in the fat of beef animals raised for human consumption in the United States. Back fat was sampled from 63 carcasses at federally inspected slaughter establishments nationwide. The sample design called for sampling beef animal classes in proportion to national annual slaughter statistics. All samples were analyzed using a modification of EPA method 1613, using isotope dilution, High Resolution GC/MS to determine the rate of occurrence of 2,3,7,8-substituted CDDs/CDFs. The method detection limits ranged from 0.05 ng kg⁻¹ for TCDD to 3 ng kg⁻¹ for OCDD. The results of this survey showed a mean concentration (reported as I-TEQ, lipid adjusted) in U.S. beef animals of 0.35 ng kg⁻¹ and 0.89 ng kg⁻¹ when either non-detects are treated as 0 value or assigned a value of 1/2 the detection limit, respectively.

1. INTRODUCTION

The purpose of this paper is to report on the progress of a joint effort of the United States Department of Agriculture (USDA) and the United States Environmental Protection Agency (EPA) to survey the rate of occurrence and concentration of chlorinated dibenzo-p-dioxins (CDDs) and chlorinated dibenzofurans (CDFs) in U.S. beef animals. This survey is the first statistically designed national survey of levels of CDDs/CDFs in beef animals in the U.S. It was prompted by EPA's dioxin

reassessment¹ which revealed the lack of reliable data in this area. In the draft reassessment document, EPA estimated that over 90% of the average individual's exposure to dioxin-like compounds occurs via food ingestion, primarily beef, poultry, pork, milk, dairy products, eggs, and fish. The average total daily dose of dioxin toxic equivalents (International TEQs) is estimated at 120 pg/day. Based on limited available U.S. data, the EPA's reassessment estimated that beef contributes about 37 pg TEQ/day of this total, with the average TEQ concentration in beef estimated at 0.48 ng/kg (whole weight basis, assuming 19% fat). This estimated beef concentration is consistent with data for beef from Germany² (whole beef concentration of 0.32 ng/kg, assuming 19% fat), the Netherlands³ (whole beef concentration of 0.33 ng/kg TEQ, assuming 19% fat), and Canada⁴ (whole beef concentration of 0.29 ng/kg, assuming 19% fat). Although consistent with European and Canadian data, none of the U.S. studies cited were based on a statistically designed sampling plan.

This study used state-of-the-art laboratory procedures to quantify CDDs/CDFs in the samples. These analytical protocols were fully validated prior to sample analysis, and appropriate quality assurance/control procedures were employed throughout the study. This report has been developed and reviewed by representatives from both EPA and USDA, but has not been externally peer reviewed. A full study report is under development and will undergo external peer review prior to final publication. When reviewing these results, it is strongly suggested that the reader note the uncertainties listed in section 6 of this paper.

2. SURVEY DESIGN

The primary objective of this study was to estimate the rate of occurrence and concentration of 2,3,7,8-substituted congeners of CDDs and CDFs in the back fat of beef animals sampled from federally inspected slaughter establishments. The first step in designing a study to meet this objective was to characterize the beef industry. In the U.S., the major bovine classes slaughtered for beef are bulls, steers, heifers, beef cows, and dairy cows. In this study, these five animal classes are referred to as beef animals. In 1993 (the latest reporting year), over 32 million beef animals were slaughtered in 925 federally inspected establishments. Table 1 displays the total number of animals slaughtered by animal class in 1993. Slaughter information from this time period was used to construct a sampling frame (i.e., a list of establishments eligible for participation in the survey) and then to randomly select establishments to participate in the survey.

This was a statistically-based survey. All establishments that slaughtered an average of 1 or more beef animals per week (52 or more per year) for the specified animal class were included in the sampling frame. There are 741 establishments in this category, and they accounted for more than 99.9 percent of all beef animals slaughtered in the U.S. Establishments were randomly selected with probability in proportion to the total number of bulls, steers, heifers, beef cows, and dairy cows slaughtered. This method ensured that each animal in the population had an approximately equal chance of being selected.

The initial sample size was limited to 65 individual animals based on funding constraints. The number of individual carcasses sampled per animal class was based on the proportion of each individual animal class to the total beef production in the U.S. in 1993. This resulted in the collection of the following samples: 2 bulls, 33 steers, 18 heifers, 6 dairy cows, and 6 beef cows. Although a total of 65 back fat samples were obtained from the slaughter establishments, two dairy cow samples were rejected because the samples were mostly comprised of sinewy (i.e. connective) tissue and not back fat. It was decided that sinewy tissue did not meet the operational definition of adipose tissue in that it only contained approximately 1% (by weight) lipid. The results are adjusted (weighted) to reflect both the reduction in the dairy cow samples and the addition of the one bull sample (to meet the design requirement of at least two animals per class).

At each selected slaughter establishment, approximately 230 g of back fat was taken from a randomly selected animal carcass that had passed federal inspection. The samples were collected during May and June, 1994. Each sample was taken by cutting a portion of back fat off the carcass. The sample was placed in a pre-cleaned glass jar with a Teflon-coated lid, and carefully packaged and shipped frozen (overnight) to the EPA laboratory in Bay St. Louis, Mississippi for sample extraction, preparation and chemical analysis. Individuals responsible for sampling each animal carcass at each slaughter establishment were given a questionnaire to be filled out and enclosed in the sample package shipped to EPA. The questionnaire was designed to provide information on the animal type, the approximate age and weight of the animal, and information regarding identification of previous owners, including the rate of occurrence of ear tags, brands and markings.

3. LABORATORY ANALYSIS

Samples were analyzed by EPA using a modified version of EPA Method 1613, using isotope

dilution, High Resolution Gas Chromatography (HRGC) coupled with High Resolution Mass Spectrometry (HRMS) to determine the rate of occurrence of 2,3,7,8-substituted CDD/CDF compounds. Samples were ground and homogenized, fortified with ^{13}C recovery surrogates, and solvent extracted. The extracts were cleaned-up using a combination of acidified and basic silica gel, alumina, and carbon column chromatography. The final extracts were reduced to volume and spiked with an internal standard prior to analysis by HRMS. The laboratory methods used for analysis of beef back fat samples were initially validated using a preliminary set of back fat samples, not of the 65 statistical samples. Replicates of the beef adipose matrix were spiked at approximately the lowest expected method quantitation limits for the 2,3,7,8 substituted CDDs/CDFs. From an examination of the resulting data, the mean recoveries, standard deviations, and the percent relative standard deviation (% RSD) were confirmed. The % RSD was less than 25% for all the analytes, and the mean value for 94% of the replicates was within 25% of the true value. The Limits of Detection (LODs) and Limits of Quantitation (LOQs) for dioxins and furans in the beef fat were:

	LOD (ng kg⁻¹)	LOQ (ng kg⁻¹)
tetras	0.05	0.1
pentas	0.5	1.0
hexas	0.5	1.0
heptas	0.5	1.0
octas	3.0	6.0

These LODs and LOQs pertain to the whole back fat sample.

4. RESULTS

The results of the analysis of the 2,3,7,8-substituted CDDs and CDFs are summarized in two ways: 1) non-detects (ND) are assigned zero values, and 2) non-detects (ND) are assigned a value by dividing the LOD by two. All results were adjusted to the lipid content of the sample by dividing the whole weight concentration (ng kg⁻¹) in the sample by the lipid fraction in each sample. The lipid-adjusted ng kg⁻¹ concentrations were then converted to the 2,3,7,8-TCDD toxic equivalence (TEQ) using the International-Toxic Equivalence Factor (I-TEFs) scheme⁵. The summaries presented here are preliminary; additional statistical analyses have not been completed.

Table 2 shows the overall mean TEQ concentration of all the back fat samples. When non-detects (ND) are set to a zero value, the mean concentration is 0.35 ng kg⁻¹ (TEQ). When ND is set to

equal 1/2 the limit of detection (LOD), the mean TEQ increases to 0.89 ng kg⁻¹. The results in Table 2 are estimates of the national mean TEQ concentration (ng kg⁻¹) in back fat of virtually all of the beef animals slaughtered at federally-inspected establishments in the United States. The difference between means calculated by these two approaches is due to the fact that not all the CDD/CDF congeners were detected in each beef fat sample. Table 3 displays the frequency at which at least one toxic CDD/F congener was detected (in a back fat sample) by class of animal.

Table 4 displays the rate of occurrence and mean concentration (ng kg⁻¹) of the various congeners across all samples when the ND is set equal to zero and 1/2 the LOD, respectively. The most frequently detected congener was 1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin, which was detected in nearly 3/4 of all samples. It is interesting to note that whenever other congeners were detected in a sample, the 1,2,3,4,6,7,8-HpCDD congener was always present in that sample. The following is the rate of occurrence of the HpCDD congener by animal class: bull=100% (2/2); dairy cow=100% (4/4); steer=73% (24/33); heifer=66.7% (12/18); beef cow=50% (3/6). The most toxic congener, 2,3,7,8-TCDD, had a rate of occurrence of 16 % of all samples. The following is the prevalence of TCDD by sampled animal: bull=100% (2/2); steer=15% (5/33); heifer=16.7% (3/18); beef cow=16.7% (1/6); dairy cow=not detected. A surprising result is the low frequency of detection of OCDD. The relatively high LOD (3 ng kg⁻¹) for OCDD probably resulted in a reduced frequency of detection in all samples. The following congeners were below the limit of detection (<LOD) in all samples: 2,3,7,8-TCDF; 1,2,3,7,8-PCDF; 1,2,3,7,8,9-HxCDF; 1,2,3,4,7,8,9-HpCDF; and OCDF.

5. DISCUSSION OF RESULTS AND CONCLUSIONS

As summarized in Table 3, the results show noticeable differences in mean TEQ concentration among the animal classes surveyed. For example, the mean TEQ concentration of the two bulls sampled was 2.9 ng kg⁻¹ and 3.3 ng kg⁻¹, using ND=0 and ND=1/2 LOD, respectively. This is approximately 3 to 10 times higher than the means of the four other animal classes. The mean TEQ concentration was lowest in the four dairy cows sampled (i.e. 0.02 ng kg⁻¹ when ND=0), reflective of the fact that the only congener detected in any of the samples was 1,2,3,4,6,7,8-HpCDD. The result in dairy cows is consistent with the general observation that lactation can be a principal mechanism of elimination for CDDs/CDFs. The greatest difference in mean TEQ concentration is between the bull and other classes of animals. Although a detailed statistical analysis of these data has not yet been

conducted, some observations on the results so far may prove enlightening. In particular, differences in TEQ concentrations between animal classes may be explained by differences in age or feeding patterns. For example, bulls are generally slaughtered at an older age than other classes, and spend the majority of their life-spans feeding on pasture, fodder, ensilage, and hay. On the other hand, steer and heifers generally are slaughtered at a younger age, and spend a large fraction of their life-spans housed in commercial feed lots where they are fattened with feed grain prior to slaughter. Definite conclusions on the existence and cause of these apparent differences can only be resolved through further study.

Due to significant differences in study design, the results of this survey and the literature values cited in EPA's exposure assessment of dioxin-like compounds⁶⁻⁸ cannot be directly compared. The authors recognize, however, that many readers will be interested in whether this survey implies different human exposure levels than suggested by the earlier study. Therefore, we have converted the results of both reports into common units of mean, lipid-adjusted, ng kg^{-1} concentration by assuming an average lipid content of 19% in whole beef. The literature values cited in the Dioxin Reassessment give a mean TEQ of 1.47 ng kg^{-1} (lipid-based), whereas this beef survey gives a mean TEQ of 0.35 ng kg^{-1} , when ND is counted as a zero value. If ND is set to equal 1/2 the LOD, then the mean TEQs are 2.5 and 0.89 ng kg^{-1} for the literature values and this study, respectively. It appears that the literature studies cited in the Dioxin Reassessment produce a mean result that is about four times higher than the mean concentration reported in this survey. Extreme caution must be used in evaluating these differences, because these studies have very different study designs. The USDA/EPA study was a statistically-designed survey of CDDs/CDFs in back fat from beef animals slaughtered at federally inspected establishments, whereas the literature data were derived from grab samples of different types and cuts of beef. In the USDA/EPA study, 63 samples were randomly selected from establishments across the entire U.S.; the literature data consists of 14 samples obtained from grocery stores in only a few locations. Therefore differences could be due to: 1) lack of representativeness in the literature studies, 2) inequalities in analytical procedures; 3) increasing dioxin levels which occurred during commercial meat preparation and packaging after shipping from the slaughter establishment; and 4) it is possible that the general levels of CDDs/Fs in beef have changed during the period of time between the studies.

6. UNCERTAINTIES

When interpreting the results of this national survey of dioxin-in-beef fat, the following uncertainties should be regarded:

1. Although internally reviewed by USEPA and the USDA, this study has not yet undergone external peer review. Alternate interpretations of these data may be suggested by external reviewers.
2. Concentrations reported as non-detects do not necessarily imply that the levels are truly zero. The actual level is somewhere within the range from zero to the LOD. In order to reflect the influence that this uncertainty could have on the calculation of means, the authors chose to calculate means by assuming both ND=0 and ND=1/2 the LOD.
3. The survey measured CDD and CDF levels in back fat samples collected from slaughter establishments. Levels of CDDs/CDFs may decrease or increase after the beef or beef products leave the slaughter establishment. These changes could occur as a result of commercial operations such as packaging, processing, shipping, and handling, or consumer practices such as handling, trimming and cooking. Therefore, the results of this survey may not be representative of CDD/CDF levels in beef or beef products that are purchased at grocery stores or when consumed.
4. This was a survey of the CDD/CDF levels in beef back fat. It is presently uncertain if CDDs and CDFs partition equally to all fat compartments in cattle and dairy cows.
5. Since this study did not analyze for the presence of coplanar PCBs (substances which exhibit dioxin-like toxicity) the actual TEQ levels for all the samples could be higher than those reported in this paper.

Table 1. Total number of animals slaughtered, by animal class, in federally inspected establishments in 1993

Animal Class	Total Animals Slaughtered	Percent of Total Slaughtered
Bull	662,211	2%
Steer	16,912,566	52%
Heifer	9,174,380	28%
Beef Cow	2,797,834	9%
Dairy Cow	2,900,250	9%
Total	32,447,241	100%

Table 2. Estimates of national I-TEQ rate of occurrence and mean I-TEQ concentration (lipid adjusted ng kg⁻¹) using two different values for non-detects.

	Number of Samples	Rate of Occurrence¹ (percent)	Mean¹ (ng kg⁻¹)	Standard Error of Mean¹	95% Confidence Interval for the mean
ND³ = 0	63	72%	0.35	0.08	(0.19, 0.50)
ND=1/2 LOD⁴	63	72%	0.89	0.07	(0.75, 1.03)

¹ All estimates are weighted to take into account the total number of animals slaughtered per year in each animal class.

² ND= non-detect at the stated limit of detection (LOD) of the analytical method.

³ LOD = Limit of Detection of the analytical method. All values are reported as lipid adjusted for each sample.

Table 3. Mean I-TEQ concentrations (lipid adjusted ng kg⁻¹) by animal class, using non-detect=1/2 LOD, or (ND=0).

Animal Class	Number Animals	Number Samples with Detects¹	Mean (ng kg⁻¹)	Min (ng kg⁻¹)	Max (ng kg⁻¹)
Bull	2	2	3.30 (2.90)	2.52 (2.02)	4.10 (3.80)
Steer	33	24	0.80 (0.45)	0.52 (0.00)	2.20 (1.80)
Heifer	18	12	1.01 (0.45)	0.55 (0.00)	3.40 (3.30)
Beef Cow	6	3	0.81 (0.26)	0.52 (0.00)	2.00 (1.50)
Dairy Cow	4	4	0.60 (0.02)	0.59 (0.01)	0.61 (0.03)
Total	63	45	0.89 (0.35)		

¹Number of samples with at least one CDD, CDF toxic congener present.

Table 4. Estimates of the rate of occurrence and mean concentration of CDDs/CDFs over all 63 samples, and using ND=0.

CDD/CDF Congener	Frequency detected (>LOD)	Rate of Occurrence (%)¹	Mean (ng kg⁻¹)¹	Standard Error (ng kg⁻¹)¹
2,3,7,8-TCDD	11	16.1	(0.03) 0.05	(0.01) 0.01
1,2,3,7,8-PeCDD	2	2.6	(0.04) 0.35	(0.03) 0.03
1,2,3,4,7,8-HxCDD	8	11.4	(0.18) 0.64	(0.07) 0.06
1,2,3,6,7,8-HxCDD	21	31.7	(1.21) 1.42	(0.29) 0.28
1,2,3,7,8,9-HxCDD	9	12.9	(0.26) 0.53	(0.09) 0.08
1,2,3,4,6,7,8-HpCDD	45	72.0	(4.39) 4.48	(0.91) 0.91
1,2,3,4,6,7,8,9-OCDD	13	19.2	(3.26) 4.78	(1.01) 0.95
2,3,7,8-TCDF	0	0	(0.00) 0.03	(0.00) 0.00
1,2,3,7,8-PeCDF	0	0	(0.00) 0.31	(0.00) 0.00
2,3,4,7,8-PeCDF	4	6.3	(0.06) 0.36	(0.03) 0.02
1,2,3,4,7,8-HxCDF	8	12.1	(0.27) 0.55	(0.11) 0.10
1,2,3,6,7,8-HxCDF	7	11.0	(0.12) 0.40	(0.05) 0.03
1,2,3,7,8,9-HxCDF	0	0	(0.00) 0.31	(0.00) 0.00
2,3,4,6,7,8-HxCDF	5	7.9	(0.10) 0.39	(0.04) 0.03
1,2,3,4,6,7,8-HpCDF	14	20.8	(0.75) 1.00	(0.21) 0.19
1,2,3,4,7,8,9-HpCDF	0	0	(0.00) 0.31	(0.00) 0.00
1,2,3,4,6,7,8,9-OCDF	0	0	(0.00) 1.88	(0.00) 0.03

¹ All estimates are weighted to take into account the total number of animals slaughtered per year in each animal class.

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