

A STATISTICAL SURVEY OF DIOXIN-LIKE COMPOUNDS IN THE UNITED STATES BEEF SUPPLY

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1. INTRODUCTION

The United States Environmental Protection Agency (EPA) has conducted an exposure assessment for dioxin-like compounds¹. In this assessment, EPA has estimated that over 90% of the exposure to dioxin-like compounds occurs via food ingestion, primarily beef, milk, dairy products, and fish. The 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 exposure assessment¹ estimated that beef contributes about 37 pg TEQ/day of this total. The average TEQ concentration in beef estimated in EPA's exposure assessment¹, 0.48 ng/kg, is not significantly different than concentrations estimated in pork - 0.26 ng/kg (15% fat), chicken - 0.19 ng/kg (15% fat), and dairy - 0.36 ng/kg (various products and fat contents). This estimated beef concentration is consistent with data for beef from Germany, where a beef fat concentration of 1.69 ng/kg was reported²; assuming 19% fat would yield a whole beef concentration of 0.32 ng/kg. It is also consistent with beef fat concentrations reported in the Netherlands of 1.75 ng/kg TEQ³, and with whole beef concentration of 0.29 ng/kg reported in Canada⁴. However, the EPA's exposure assessment¹ also concludes that very few beef samples have been taken worldwide for measuring the occurrence of specific dioxin congeners. Although consistent with European data, none of the U.S. studies were based on a statistically random sampling plan that would permit statistically representative estimates for the U.S. population. In order to further understand the occurrence and concentrations of dioxin-like compounds in the United States beef supply, a statistical survey jointly

sponsored by the United States Environmental Protection Agency and the United States Department of Agriculture is underway. This paper reports on the design, analytical methods, and plans for data reporting for this survey.

2. SURVEY DESIGN

The design of the survey, and the collection and shipping of samples is the primary responsibility of the United States Department of Agriculture (USDA). The primary objective of the study is to assess the national incidence and amount of dioxin-like compounds in beef animals (steer, heifer, dairy cow, beef cow, and bull carcasses) from federally inspected slaughterhouse establishments in the United States (hereafter referred to as establishments).

In 1993, over 32 million beef animals were slaughtered in 925 federally inspected establishments. Of these animals, approximately 16.9 million (52.0%) were steers, 9.1 million (28.0%) were heifers, 2.9 million (9%) were dairy cows, 2.8 million (9%) were beef cows, and 0.7 million (2.0%) were bulls. 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.

All establishments that slaughtered an average of 1 or more bovine animals per week (52 or more per year) for the specified cattle class (steer, heifer, dairy cow, beef cow, or bull) have been included in the sample frame. There are 741 establishments in this category, and they account for more than 99.9 percent of all beef animals slaughtered. Including all establishments in the frame would add significant logistical difficulty without appreciable additional information.

A sample size of 65 was determined based on the current availability of funds for laboratory analyses, and was also deemed sufficient to meet the objectives of the study. The precision of the national average estimate will depend on this sample size and on the variability of the concentrations of dioxin-like compounds in the sampled cattle.

Establishments were selected with probability proportional to total production (slaughter) of steers, heifers, dairy cows, beef cows, and bulls. They were selected systematically from the frame, stratified by USDA Food Safety and Inspection "Regions", (geographic regions developed for programmatic needs of USDA) and sorted by production for each cattle class. This method ensures that each animal in the population has an approximately equal chance of being selected and that they will be distributed across these USDA regions based on production.

The 65 samples requested specify the establishment and the cattle class, steer, heifer, dairy cow, beef cow, or bull, so that the proportion of these groups requested in the sample match the proportion of the groups in the total beef population (total production), with a minimum of 2 samples per group. Two bulls were requested rather than the expected 1 (2 percent of 65), and the remaining 63 samples were allocated across the other cattle classes. The result was the selection of 33 steers, 18 heifers, 6 dairy cows, 6 beef cows, and 2 bulls.

The requested samples required participation from 43 establishments. Inspectors at each of these establishments randomly selected a carcass for each sample requested. Approximately 230 g of back fat was taken from each carcass. Back fat was selected since back fat is high in lipid content, 50-70%, thereby maximizing the potential to measure and quantify dioxin-like compounds if they are present. In using this data for exposure evaluations, it will be assumed that the concentrations found in back fat are similar to the concentrations in the fat of beef which is consumed. The samples were collected during the month of May, 1994.

3. LABORATORY ANALYSIS

The analysis of samples is the primary responsibility of the United States Environmental Protection Agency (EPA). Samples will be analyzed using a modified version of USEPA Method 1613: Tetra- through Octa-chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS⁸. Samples will be analyzed for fifteen different PCDD and PCDF isomers, and octa PCDD and PCDFs.

Samples will be ground and homogenized, fortified with ¹³C recovery surrogates, and solvent extracted. The extracts will be cleaned using a combination of acidified and basic silica gel, alumina, and carbon column chromatography. The final extracts will be reduced to volume and spiked with an internal standard prior to analysis by high resolution mass spectrometry (HRMS).

Prior to initiation of the survey, the methods to be used for analysis of beef back fat samples were validated. A preliminary set of back fat samples, not of the 65 statistical samples, were sent to EPA laboratories. Replicates of the beef adipose matrix were spiked at approximately the lowest expected method quantitation limits for the 17 2,3,7,8 substituted dioxins and furans. 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. From these data, the target Limits of Quantitation (LOQs) were calculated. The target Limits of Detection (LODs) were estimated to be one half of the target Limits of Quantitation (LOQs). The method validation study was conducted at five times the LOQs. The target Limits of Detection (LODs) and Limits of Quantitation (LOQs) for dioxins and furans in beef fat are:

	LOD (ppt)	LOQ (ppt)
tetras	0.1	0.2
pentas	0.5	1.0
hexas	0.5	1.0
heptas	0.5	1.0
octas	6.0	12.0

These LODs and LOQs pertain to the whole back fat sample. If the congener is found to occur at or above the LOD but below the LOQ, a value for the congener will be reported and "flagged" to denote its status. The uncertainty associated with the values below the LOQ increases as they approach the LOD. It is emphasized that these LODs and LOQs may change, increase or decrease, depending on the background levels of the analytes in the method blanks.

Preceding this initiative, the USEPA surveyed the scientific literature of attempts to measure dioxin-like compounds in beef raised in the U.S. Table 1 is derived from the literature review, and combines the results of three studies^{5,6,7} that could be identified. The lipid concentrations are arithmetic averages from a total of only 14 samples^{5,6,7} of U.S. beef. In all these references, researchers reported lipid-adjusted concentrations of the compounds. The beef samples were taken from grocery stores in various locations in the United States - one article went no further than to describe the sample locations as "Midwest", "Southeast", and so on. Table 1 includes the average lipid-based congener concentration assuming non-detects were one-half the detection limit and that non-detects were 0.0. Table 1 also includes the range of lipid-based detection limits as reported in the citations. It should be understood that a direct comparison of the limits of detection in Table 1 with the expected limits of quantification listed above is not possible. At the very least, it is not clear that these other researchers distinguished

between a limit of detection and a limit of quantification as is being done here. It is also noted that the publications did not report quality assurance and quality control procedures. Therefore, the USEPA cannot account for the accuracy and validity of these data.

3. RESULTS AND REPORTING

The maintenance of a data base for these results, interpretation of the data, and all reporting of the data will be the joint responsibility of the EPA and the USDA. All sixty-five samples have been collected and are being analyzed at EPA Laboratories. Results of that analysis and the statistical interpretation of the results will be available by the Fall of 1994.

4. REFERENCES

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Table 1. Concentrations of dioxin-like compounds in beef fat in the United States, and range of reported Limits of Detection (LODs) as reported in the scientific literature^{5,6,7}

Compound	Average lipid concentrations with non-detects equal:		Range of Reported LOD
	1/2 LOD	0.0	
	----- ng/kg (ppt) -----		
2378-TCDD	0.13	0.06	0.2-0.4
12378-PCDD	1.17	0.13	0.4-17.5
123478-HxCDD	1.38	0.74	0.9-3.2
123678-HxCDD	4.40	4.40	<0.16
123789-HxCDD	1.06	0.34	0.7-4.1
1234678-HpCDD	10.13	9.99	3.2
OctaCDD	15.32	14.84	6.4
2378-TCDF	0.30	0.25	0.02-0.6
12378-PCDF	0.23	0.01	<0.01-1.4
23478-PCDF	1.11	0.70	0.2-0.8
123478-HxCDF	2.66	2.44	0.4-1.2
123678-HxCDF	0.33	0.10	0.02-1.1
123789-HxCDF	0.30	0.10	0.01-1.5
234678-HxCDF	0.36	0.11	0.4-1.4
1234678-HpCDF	2.08	1.74	1.4-2.3
1234789-HpCDF	0.68	0.07	0.02-3.2
OctaCDF	1.18	0.55	0.5-5.3
TOTAL FAT CONCENTRATION	42.82	36.57	
TEQ FAT CONCENTRATION	2.51	1.53	
WHOLE BEEF TEQ CONCENTRATION (19% fat)	0.48	0.29	