A Statistical Survey of Dioxin-Like Compounds in United States Pork Fat

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1. INTRODUCTION

The purpose of this paper is to report on the results of a joint survey of the United States Department of Agriculture (USDA) and the United States Environmental Protection Agency (EPA) on the rate of occurrence and concentration of chlorinated dibenzo-p-dioxins (CDDs), chlorinated dibenzofurans (CDFs), and coplanar polychlorinated biphenyls (PCBs) in the fat of U.S. pork animals. This survey is the first statistically designed national survey of levels of CDDs/CDFs/PCBs in pork animals in the U.S. It was prompted by EPA's Reassessment of Dioxin-Like Compounds[1] and funded from EPA's Dioxin Exposure Initiative[2]. It is the second joint USDA/EPA effort of its kind, the first being a survey of beef back fat.[3]

This report has been developed and reviewed by representatives from both EPA and USDA, but has not been externally peer reviewed.

2. SURVEY DESIGN

The primary objective of this survey was to assess the national prevalence and concentrations of polychlorinated dibenzo-p-dioxins (abbreviated CDDs), polychlorinated dibenzofurans (CDFs), and dioxin-like coplanar polychlorinated biphenyls (coplanar PCBs, or just PCBs) in the skinless belly fat of swine slaughtered in federally inspected establishments in 1995. The first step in meeting this objective was to characterize the swine industry in the United States. This information was used as the basis for developing a sampling frame of establishments slaughtering swine, and for designing a statistically based (probability) sampling plan.

The three swine classes for pork production are: barrows/gilts, sows, and boars/stags. These classes will be referred to by their common names, market hogs, sows, and boars, respectively. In 1994, USDA reported that over 93 million swine were slaughtered in approximately 800 federally inspected establishments. Table 1 provides a breakdown of the total number of swine slaughtered at federally inspected establishments by slaughter class. This table also shows the number of samples obtained from each slaughter class to comprise the final sample size of 78.

Establishment specific slaughter information from the Food Safety and Inspection Services's

(FSIS) Animal Disposition Reporting System (ADRS) was used to construct sampling frames for each of the three slaughter classes, and to randomly select establishments to participate in the survey. There are approximately 737, 580, and 319 federally inspected establishments currently slaughtering market hogs, sows, and boars, respectively. The majority of these establishments slaughter more than one type of swine. For each slaughter class, the sampling frame included only the largest establishments which account for 99.5% of all animals slaughtered annually. The smallest establishments were excluded since they slaughter only a small number of head per week and may not have had an animal available for sampling during the short time period of the sampling.

The original survey design called for subcutaneous belly fat samples from 80 individual randomly selected swine, including 56 market hogs, 12 sows, and 12 boars. The latter two classes were oversampled in order to optimize the ability to distinguish concentration patterns among the three classes, and to allow for an estimate of the variability of the slaughter class estimates. The majority of samples, 56, covered the market hogs which account for almost 95% of total pork production The final sample had 78 samples, as one boar and one sow sample were compromised (ie., jar broken in delivery, incorrect animal sampled). The sample size was determined by resource constraints on laboratory analyses of CDDs, CDFs, and coplanar PCBs. Belly fat was selected because it was a matrix that was very high in fat content (in the range of 60-90% lipid), and therefore, the ability to measure the dioxin-like compounds was maximized.

In order to achieve a random sample, the selection was performed in two stages. The first stage was to randomly select a federally inspected slaughter establishment from the sampling frame, and the second was to have the USDA safety inspector at the establishment randomly select the animal. For each animal class, establishments were selected from a sampling frame with probabilities in proportion to the total number of those animals slaughtered at the establishment as provided in the ADRS. The final sample set of 78 originated from 46 establishments. Approximately one half of a pound of subcutaneous belly fat was taken from each carcass half. The samples were collected on a randomly selected day and time within a short (3-4 weeks) time period in August/September of 1995.

3. LABORATORY ANALYSIS

EPA's Environmental Chemistry Laboratory (ECL) in Bay St. Louis, Missouri, extracted, prepared, and analyzed the samples. The procedures to analyze for these compounds in beef fat, a very similar matrix to the pork belly fat of these samples, are described in Ferrario, et al.[4] for CDDs/CDFs, and in Ferrario, et al.[5] for the coplanar PCBs. Sample analysis was based on a modified version of USEPA Method 1613: Tetra- through Octa-chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, April 1990. The percentage of lipids in the tissue samples was determined by lipid determination procedures described in EPA Method 8290: Polychlorinated Dibenzodioxins (PCDDS) and Polychlorinated Dibenzofurans (PCDFS) by High-Resolution Gas Chromatography/High-Resolution Mass Spectrometry (HRGC/HRMS). The belly fat samples averaged 60% lipids (standard error = 12%). Samples were analyzed for the seventeen CDD/CDF compounds which have toxicity equivalency to 2,3,7,8-TCDD. Also analyzed were coplanar PCBs which are dioxin-like, including PCBs 77, 105, 118, 126, 156, 157, and 169. PCBs 77, 105, 118, 126, 156, and 157 were analyzed in the fraction from the carbon column and PCB 169 together with the dioxins and furans were analyzed in the fraction from the carbon column that had been through the alumina column cleanup.

Samples were ground and homogenized, fortified with ^{13}C recovery surrogates, and solvent extracted. The extracts were cleaned 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.

Replicates of the pork adipose matrix were spiked at approximately the lowest expected method quantitation limits for the seventeen 2,3,7,8 substituted dioxins and furans. From an examination of the resulting data, the mean recoveries, standard deviations, the percent relative standard deviation (% RSD) were confirmed, and the target Limits of Quantitation (LOQS) were calculated. The target Limits of Detection (LODs) were estimated to be one half of the target LOQs. The target LODs/LOQs are for CDDs and CDFs in the pork fat matrix, which averaged 60% lipid. Therefore, to calculate a lipid-based LOD/LOQ, these limits need to be divided by the lipid fraction of 0.60:

	TCDD/F	PCDD/F	HxCDD/F	HpCDD/F	OCDD/F
LOD, ppt	0.1	0.5	0.5	0.5	1.0
LOQ, ppt	0.2	1.0	1.0	1.0	2.0

The LODs and LOQs for the coplanar PCBs were determined based on background laboratory levels and background levels in the pork matrix determined through preliminary testing. A further discussion of the issue of background laboratory levels of the coplanar PCBs, and the application of that information to the measurement of these compounds in beef fat, can be found in Ferrario, et al.[5]. The final reported levels in the pork were determined by first analyzing the pork and subtracting the following background concentrations from the concentrations measured in the matrix:

	PCB 77	PCB 118	PCB 105	PCB 126	PCB 156	PCB 157	PCB 169
LOD, ppt	1.5	50.0	26.0	0.2	10.0	2.5	0.1
LOQ, ppt	1.5	50.0	26.0	0.2	10.0	2.5	0.2

4. RESULTS

The results of the analysis of the 2,3,7,8-substituted CDDs and CDFs, and coplanar PCBs, are summarized in two ways: 1) non-detects (NDs) are assigned zero values, and 2) NDs 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 (ppt) in the sample by the lipid fraction in each sample. Since the LOD/LOQs were developed on a sample basis and not on a lipid basis, the precise value used for the 1/2 detection limit assumption for ND varied for each sample since each sample had a different lipid content. The lipid-adjusted ppt concentrations were then converted to the 2,3,7,8-TCDD toxic equivalence (TEQ) using the International-Toxic Equivalence Factor (I-TEFs) scheme[6] for CDD/CDFs and the WHO recommendations for coplanar PCBs[7]. The summaries presented here are preliminary; additional statistical analyses have not been completed.

Table 2 shows the overall mean TEQ concentration of all the swine belly fat samples, for the United States as a whole and for the individual swine classes. Another delineation made for purposes of this paper was to divide the "boar" class, defined strictly as uncastrated male swine animals, into two classes based on age and weight of the animal. Since the USDA samplers collected ancillary information on all samples, including the age and weight of the animal at slaughter, it became possible to define "young" boars as uncastrated males <= 1 year old and < 300 lbs, whereas "old" boars were uncastrated males >= 2 yrs old and > 300 lbs. Table 3 shows a TEQ summary of results of these two subclasses. Tables 4 and 5 show the congener specific results for all swine for CDD/CDFs and coplanar PCBs, respectively.

Some observations from these tables and otherwise include:

1) The overall mean CDD/CDF TEQ concentration in belly fat was 1.3 ppt. An earlier estimate of the CDD/CDF TEQ concentration in pork made by EPA[9] was 1.7 ppt. This estimate originated from grocery store grab sampling[9,10,11] comprising 12 samples of pork products. The TEQ concentration of coplanar PCBs was much lower than the CDD/CDF TEQ concentration at 0.06 ppt TEQ.

2) The results for CDD/CDF TEQs from this survey appear to show higher concentrations as compared to pork monitoring recently reported for Europe. Some results from Europe are as follows (all on a TEQ lipid basis using 1/2 detection limit for NDs): Furst[12] reports a pork concentration of 0.43 ppt TEQ for Germany, and in a later publication[13], 0.5 ppt TEQ; Furst[13] reports a concentration of 0.20 ppt for pork in Canada; Theelen[14] reports a concentration of 0.43 ppt in the Netherlands.

3) The concentration of CDD/CDFs in sows appeared higher than market hogs: 1.7 pg TEQ/g for sows versus 1.3 pg TEQ/g for market hogs, although the difference is not striking. What did appear striking, however, were the results for the boar class. While a very small class in terms of exposure (only 1% of the pork food supply), it is clear from Table 3 that older boars are significantly different from all other classes. Their lipid TEQ concentration of 6.5 ppt for CDD/CDFs and 0.54 ppt for coplanar PCBs are about 5 and 10 times higher than the overall averages for CDD/CDFs and coplanar PCBs, respectively. One factor that may contribute to elevated dioxin concentrations in boars as compared to other swine classes is their age. The average age of slaughter for market hogs is less than 1 year, while the old boars lived for greater than 2 years. Sows may be as old as boars, but they have the mechanism of milk excretion, which is thought to reduce the body burden of dioxins. The young boars, also slaughtered very early at roughly the same age as market hogs, have similar body burdens as the market hogs, as seen by the similarity of concentrations in their lipid (Table 3) compared to that of market hogs (Table 2). A second factor which could contribute to elevated levels in the lipids of old boars is the fact that they may spend a large part of their lives outdoors being exposed to soil. In contrast, market hogs are raised in many parts of the country on concrete during their short lives. These are preliminary speculations. USDA and EPA are researching the issue further.

5. REFERENCES

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Table 1. Overview of sampling frame (circa 1994), and number of samples from each category in the final survey (information from USDA's FSIS Animal Disposition Reporting System).

Animal Class	Number in survey	Total animals slaughtered	Percent of total slaughtered
market hogs	56	88,615,000	94.8
sows	11	3,917,000	4.2
boars	11	904,000	1.0
TOTAL	78	93,435,000	100.0

Table 2. TEQ summary of nationally exptrapolated results on a lipid basis assuming non-detects (ND) equal 1/2 detection limit (results are in ppt, or pg/g; ND=0 results are in parenthesis).

Description	Dioxins and Furans			Coplanar PCBs		
	Mean	Stan. Dev.	Min/Max	Mean	Stan. Dev.	Min/Max
Overall	1.3 (0.46)	——	0.61/23 (0/23)	0.06 (0.04)	——	0.02/1.7 (0/1.7)
Market Hogs	1.3 (0.42)	1.3 (1.34)	0.62/10 (0/9.6)	0.06 (0.04)	0.07 (0.08)	0.02/0.40 (0/0.11)
Sows	1.7 (0.94)	1.6 (1.8)	0.66/5.8 (0/5.4)	0.06 (0.04)	0.03 (0.04)	0.02/0.11 (0/0.11)
Boars	3.6 (3.0)	6.5 (6.6)	0.61/23 (0/23)	0.27 (0.26)	0.48 (0.48)	0.02/1.7 (0/1.7)

Table 3. TEQ summary of "old" and "young" boars on a lipid basis assuming non-detects (ND) equal 1/2 detection limit (results are in ppt, or pg/g; ND=0 results are in parenthesis).

Description	Dioxins and Furans			Coplanar PCBs		
	Mean	Stan. Dev.	Min/Max	Mean	Stan. Dev.	Min/Max
Old boars (n=5)	6.5 (6.1)	9.2 (9.3)	1.1/23 (0.59/23)	0.54 (0.04)	0.63 (0.63)	0.18/1.7 (0.17/1.7)
Young boars (n=6)	1.2 (0.5)	0.8 (0.9)	0.61/2.8 (0/2.2)	0.06 (0.04)	0.03 (0.05)	0.02/0.11 (0/0.11)

Table 4. Overall national averages of dioxin and furan congeners on a lipid basis, calculated at ND equals 1/2 detection limit (ppt or pg/g; results for ND = 0 in parenthesis).

Congener	Frequency detected (n=78)	Rate of Occurrence (% nationally)	Mean	Standard error	Max
2378-TCDD	3	2	0.10 (0.01)	0.01 (0.01)	1.01
12378-PCDD	3	2	0.45 (0.01)	0.01 (0.01)	3.64
123478-HxCDD	12	7	0.52 (0.10)	0.04 (0.04)	22.04
123678-HxCDD	29	33	1.10 (0.80)	0.23 (0.24)	53.98
123789-HxCDD	5	3	0.47 (0.04)	0.02 (0.02)	8.67
1234678-HpCDD	43	50	10.15 (9.93)	3.09 (3.10)	632.33
OCDD	49	57	52.77 (52.40)	16.94 (16.96)	2405.13
2378-TCDF	1	2	0.09 (0.004)	0.003 (0.004)	0.24
12378-PCDF	0	0	0.45 (0)	0.01 (0)	ND
23478-PCDF	7	6	0.56 (0.14)	0.07 (0.08)	3.57
123478-HxCDF	15	13	0.98 (0.60)	0.38 (0.39)	22.59
123678-HxCDF	12	8	0.58 (0.58)	0.07 (0.09)	5.36
123789-HxCDF	0	0	0.45 (0)	0.01 (0)	ND
234678-HxCDF	9	8	0.57 (0.16)	0.06 (0.08)	5.02
1234678-HpCDF	45	52	3.56 (3.35)	1.19 (1.20)	147.64
1234789-HpCDF	10	10	0.57 (0.17)	0.07 (0.09)	4.11
OCDF	41	49	2.30 (1.85)	0.56 (0.59)	31.02

Notes: "Frequency detected" is the frequency of detection greater than the limit of detection in the actual sample of n =78 animals; "Rate of occurrence (%)" is the nationally extrapolated rate of occurrence.

Table 5. Overall national averages of coplanar PCBs on a lipid basis, calculated at ND equals 1/2 detection limit (ppt or pg/g; results for ND = 0 in parenthesis).

Congener	Frequency detected (*n*=78)	Rate of Occurrence (% nationally)	Mean	Standard error	Max
PCB 77	13	13	1.57 (0.41)	0.09 (0.14)	6.12
PCB 118	24	27	95.45 (62.64)	12.73 (14.79)	3250.10
PCB 105	15	18	33.44 (14.12)	4.41 (5.16)	441.67
PCB 126	24	26	0.33 (0.20)	0.06 (0.07)	5.07
PCB 156	30	32	21.60 (15.51)	3.61 (3.95)	1058.22
PCB 157	32	35	5.12 (3.69)	0.71 (0.80)	349.16
PCB 169	29	29	0.26 (0.19)	0.05 (0.06)	7.50