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Determination of 2,3,7,8 Chlorine (Cl)-substituted Dibenzo-*p*-Dioxins and Furans at the Part per Trillion Level in United States Beef Fat Using High Resolution Gas Chromatography/High Resolution Mass Spectrometry

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ABSTRACT

As part of the U.S. EPA Dioxin Reassessment Program, the 2,3,7,8-chlorine-substituted dibenzo-*p*-dioxins and furans were measured at part per trillion (ppt) levels in beef fat collected from slaughter facilities in the United States. This is the first statistically designed national survey of these compounds in the U.S. beef supply. Analyte concentrations were determined by high resolution gas chromatography/high resolution mass spectrometry, using isotope dilution methodology. Method limits of detection on a whole weight basis were: 0.05 ppt for TCDD and 0.10 ppt for TCDF, 0.50 ppt for pentas (PeCDDs/PCDFs)/ hexas (HxCDDs/HxCDFs)/ heptas (HpCDDs/HpCDFs), and 3.00 ppt for octas (OCDD/OCDF). Method detection and quantitation limits were established based on demonstrated performance criteria utilizing fortified samples rather than by conventional signal-to-noise or variability of response methods. The background subtraction procedures developed for this study minimized the likelihood of false positives and increased the confidence associated with reported values near the detection limits. Mean and median values for each of the 2,3,7,8-Cl-substituted dioxins and furans are reported along with the supporting information required for their interpretation. The mean toxic equivalence values for the samples are 0.35 ppt (nondetects = 0) and 0.89 ppt (nondetects = $\frac{1}{2}$ LOD).

The United States Environmental Protection Agency (USEPA) is presently undertaking a reassessment of the risk from exposure to chlorinated dibenzo-*p*-dioxins (PCDDs) and chlorinated dioxin-like compounds [e.g. chlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs)]. This reassessment involves both a literature reevaluation of existing studies and new laboratory studies addressing health and ecological risks.¹⁻⁶ The scientific characterization of the

risk from dioxin involves multiple areas of interest, one of which is the determination of the human exposure to chlorinated dioxins through the diet.⁷

The U.S. EPA recently estimated that over 90% of human exposure to dioxins and dioxin-like compounds occurs through the diet, primarily, in the consumption of fatty foods of animal origin.⁸ An attempt to evaluate the literature to determine the concentration of dioxins/furans in beef from the United States revealed that none of the studies were based on a statistically derived sampling plan. In addition, limitations imposed by differences in analytical methodologies and a lack of adequately defined quality assurance/quality control (QA/QC) procedures precluded an objective determination of dioxin/furan concentrations that was both statistically representative and technically defensible. For example, mean values were often listed without an explanation of how "nondetects" were treated in the final calculations, and quite often, no information was provided regarding the frequency of detection, the accuracy, the precision, or the method limits of detection (LODs)/ limits of quantitation (LOQs).

The study described here is a statistically designed survey of the 2,3,7,8-Cl-substituted dibenzo-*p*-dioxins and furans in the back fat of beef slaughtered at federally inspected facilities. All analytical methods employed were validated prior to sample analysis, and rigorous QA/QC procedures were maintained throughout the survey. The LODs/LOQs were developed and verified using the results from the analyses of fortified samples and all of the required information (i.e. precision, accuracy) is provided. This practical approach for the determination of LODs/LOQs based on demonstrated performance criteria differs conceptually and in practice from the conventional methods employing signal-to-noise (S/N) ratios or variability of response. These later methods rely on extrapolations from an instrumental response, most often in the absence of the analyte of interest, and the derived LODs are not verified before being reported.

A performance-based method is more appropriate in that the determinations are based on known amounts of the analytes of interest recovered from a fortified sample and measured within preestablished performance criteria. Target LODs/LOQs are first estimated in this manner and are verified prior to being finalized. Values below these verified LODs are not reported.

Many chlorinated dioxins and furans have become ubiquitous in the environment and are routinely detected in method blanks and control tissue samples. Two important issues, the significance of this background in defining the method LODs and the procedures used for addressing the presence of this background in ultra trace analysis, are discussed in this paper that are often not described when results are presented. The rigorous QA/QC procedures implemented, together with the data reduction techniques discussed in this paper, ensure that the results are statistically significant.

EXPERIMENTAL SECTION

Analytical Procedures. Prior to the initiation of the study, a detailed Quality Assurance Project Plan (QAPjP) was compiled and submitted to both the project QA Officer and an independent QA Officer for review and approval. The QAPjP contained detailed sections addressing each of the following critical elements: project description, organizational responsibilities, data quality objectives for measurements, sampling procedures, sample custody, calibration procedures, analytical procedures, data reduction, validation and reporting, internal QA checks, audits, preventive maintenance, corrective actions, and QA reports to management.

Many of the sample preparation procedures, analytical techniques, and quality control strategies described in this paper parallel those defined in the U.S. EPA Method 1613.⁹ Specific details common to both methods that are adequately explained in U.S. EPA Method 1613 will not be readdressed here.

Sampling Procedures. The sampling frame for this study was constructed based on 1993 United States slaughter information. In 1993, over 32 million beef animals were slaughtered in 925 federally inspected establishments. Establishments that slaughtered an average of one or more beef animals per week were included in this sampling frame. There are 741 establishments in this category, and they account for more than 99.9% of all beef animals slaughtered in the United States. To ensure that each animal in the population had an approximately equal chance of being selected, establishments were randomly selected with a probability in proportion to the total number of bulls, steers, heifers, cows, and dairy cows slaughtered.⁸ The number of samples taken per animal class was based on the proportion of each animal class slaughtered to the total, with a minimum of two animals per animal class (i.e., the samples were approximately self-weighting). Due to practical considerations, a total of 65 samples were selected for this study. The number of samples per animal class was as follows: two bulls, 33 steers, 18 heifers, six dairy cows, and six beef cows. Due to the improper sampling, two dairy cow samples were not analyzed. Therefore, the actual number of study samples were 63.

Approximately 200-300 g of back fat was obtained by USDA/FSIS (Food Safety & Inspection Service) personnel from randomly selected beef carcasses taken at USDA/FSIS inspected slaughter facilities. The samples were placed in precleaned glass jars equipped with Teflon-lined screw caps and shipped frozen to the U.S. EPA/Environmental Chemistry Section (ECS). Upon receipt at EPA/ECS, the samples were logged in and inspected, and their condition was recorded. They were then immediately stored in a subzero freezer at -40°C.

Sample chain-of-custody was maintained at all times. Before removal from storage, a sample set was compiled which consisted of nine samples, one method blank, one matrix blank, and one laboratory spiked control sample. After removal from storage, samples were tracked through preparation, extraction, clean-up, and analysis.

Safety. The potential health hazards associated with chlorinated dioxins/furans require that the laboratories involved in their analyses follow prudent laboratory practices for handling toxic materials. U.S. EPA Method 1613 specifically addresses the various aspects involved in personnel protection and the safe handling of these materials (i.e., protective clothing, personal hygiene, periodic wipe testing, waste disposal, etc.). Additionally, numerous publications are available which address these specific issues.¹⁰

Sample Preparation. One hundred grams of beef fat was homogenized and a 10-g subsample taken for analysis. The sample was fortified at 10 ppt with ¹³C analogs of the various 2,3,7,8-Cl-substituted congeners prior to extraction. The crude extract was cleaned up using acid/base modified silica gel, alumina, and graphitized carbon column chromatography. The eluent was then concentrated, fortified with ¹³C internal standards, and analyzed by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS).

Though the methodology used in this study followed U.S. EPA Method 1613 closely, there were several significant changes which should be noted:

(1) Standard solutions were prepared at lower concentrations than 1613. Method 1613's lowest calibration standard contains 500 fg/μl TCDD and 100 pg/μl of ¹³C-labeled surrogates. This study's lowest calibration standard contained 50 fg/μl TCDD and 5 pg/μl of ¹³C-labeled

surrogates. The samples in this study were fortified with ^{13}C -labeled surrogates to deliver 5 pg/ μL , where Method 1613 delivers 100 pg/ μL from the same 20- μL final volume. This lower ^{13}C surrogate fortification level at 10 ppt allowed for a more realistic approximation of the actual recovery of native analytes at low part per trillion (ppt) levels and better approximates the behavior of trace levels of natives during extraction, concentration, and chromatography.

(2) A DB-5ms column was used in place of the DB-5 specified by Method 1613. The DB-5ms has a superior separation of the 2,3,7,8-TCDD from the other tetra isomers and better resolves the 2,3,7,8-Cl-substituted dioxins and furans. It should be noted that the elution order of the 1,2,3,7,8,9,-HxCDF and the 2,3,4,6,7,8-HxCDF is reversed on the DB-5ms relative to the DB-5.

(3) Method 1613 uses a AX21 and Celite 545 mixture for the graphitized carbon column cleanup; this study used a mixture consisting of 9.5g of BioSil A Silica Gel and 0.5 g of Amoco PX-21 carbon. The eluting solvents were also different: the column was conditioned with 10.0 mL of 50% dichloromethane/benzene solution, 10 mL of toluene, and 2 mL of hexane. The sample extract was then added with 1 mL of hexane, followed by 5 mL of dichloromethane and 10 mL of 50% dichloromethane/benzene solution. The eluents added up to this point were discarded. The column was inverted, and the analytes were eluted with 12 mL of toluene. Two microliters of tetradecane was added and the sample concentrated to <10 μL . The sample was then stored in a refrigerator until HRGC/HRMS analysis.

Instrumental Analysis. All analyses were performed on a Kratos Concept high-resolution mass spectrometer using isotope dilution. The HRMS was operated in the electron impact ionization mode using selected ion monitoring. Chromatographic separations were achieved using a Hewlett Packard 5890 Series II high resolution gas chromatograph, utilizing a 60-m x 0.32-mm (0.25- μm film thickness) DB-5ms capillary column. The GC conditions were optimized to completely separate the various 2,3,7,8-Cl-substituted dioxins/furans: initial oven temperature, 130°C; injector temperature, 270°C; interface temperature, 300°C; temperature programming, time 1, 1.0 min, rate 1, 5°C/min, time 2, 15.0 min, rate 2, 6°C/min; temperature 3, 295°C; injector, splitless, 1.0 min; split flow, 30-40 mL/min; purge flow, 1-2 mL/min; and temperature equilibration time, 2 min.

The mass spectrometer was tuned and calibrated prior to all analyses. It was tuned to a minimum resolution of 10 000 ppm (10% valley) using $m/z = 330.9792$ (or any suitable reference peak) at full accelerating voltage of 8000 V. Pertinent MS parameters were as follows: cycle time for each congener group, ≈ 1.0 s; ESA sweep (analytes), 10 ppm, native ion dwell, ≈ 100 ms; ^{13}C -labeled ion dwell, ≈ 35 ms; lock mass sweep, 200 ppm; lock mass dwell, 50 ms; ionization voltage, ≈ 35 eV; source temperature, 250°C; accelerating voltage, 8000 V; and trap current, 500 μA .

Quality Control and Calibration. Between four and six calibration standards with native analyte concentrations bracketing the expected analyte concentrations were analyzed prior to analyzing samples. Calibration solutions contained all ^{13}C 2,3,7,8-Cl-substituted recovery surrogates (5 pg/ μL) and two internal standards (10 pg/ μL) each. Native analyte concentrations varied depending on the calibration standard number. The native analyte concentration in calibration standard 1 were 100 fg/ μL for the tetras (TCDD/TCDF); 500 fg/ μL for the pentas (PeCDDs/PeCDFs), hexas (HxCDDs/HxCDFs), and heptas (HpCDDs/HpCDFs); and 1000 fg/ μL for the octas (OCDD/OCDF). Native analyte quantities in other calibration standards were multiples of the calibration standard 1 as follows: 0.5x, 2x, 4x, 8x, and 16x. The analyses of these

calibration standards permitted the response factors to be determined as a function of concentration using linear regression. The response factor (RF) for each native analyte at each concentration was calculated relative to its ^{13}C -labeled analog. The relative standard deviation (RSD) for the average response factor for each of the native analytes had to be <20%. Similarly, the RF for each ^{13}C recovery surrogate relative to the appropriate internal standard was also calculated. The RSD for the average RF for each labeled surrogate had to be <35%. The calibration curves were considered linear under these conditions, and the analytical system was considered calibrated when these conditions had been satisfied. If these conditions could be not satisfied, corrective actions were taken. The average RFs were used for subsequent quantitations.

Prior to sample analysis, the linearity of the calibration curve was verified by analyzing calibration solution 2 (200 fg of TCDD) and calculating the RF as described previously. The percent difference between the new RF and the average had to be <20% for the native analytes and <35% for the ^{13}C recovery surrogates. The mass chromatogram was also examined to ensure that all the 2,3,7,8-Cl-substituted congeners were clearly separated. If the S/N values were ≥ 10 , the ion abundance ratios were $\pm 15\%$ of the theoretical, and the RF and isomer separations were within specified limits, then sample analyses proceeded. Corrective actions were initiated if specified control limits were exceeded.

On the days that samples were analyzed, 10 μl of the internal standard solution (20 $\text{pg}/\mu\text{l}$) was added to each sample, and the final volume adjusted to 20 μl . Once all QA/QC parameters had been verified to be within specified limits, sample analyses proceeded. The mass spectrometer was operated in a mass drift correction mode using PFK to provide lock masses.

The selected ion current profile (SICP) areas for the characteristic ions for each native and labeled analyte were measured. Native analyte concentrations were determined by isotope dilution. Peak areas from the characteristic ions for each native analyte and its ^{13}C -labeled analog were used in conjunction with RFs from the internal calibration data to determine concentrations directly. Labeled surrogate concentrations (expressed as % recovery) were similarly calculated using an internal standard method.

Samples were organized and analyzed in sets: method blank, matrix blank, laboratory control spike (LCS), and the nine samples. Peak identification criteria were as follows: $\text{S/N} \geq 3.5$; the isotope ratio of the two characteristic ions for each congener class within 15% of the theoretical value; the peak maxima for the molecular cluster ions coincide within 2 s; and native analytes elute within ± 3 s of their corresponding ^{13}C -labeled analogs. Method blanks were examined for the presence of interfering background, which, if present, was subtracted from the sample amount prior to reporting. For furans, an ion for the appropriate chlorinated diphenyl ether was monitored and the ion chromatogram examined to ensure the absence of chlorinated diphenyl ether contamination.

The amount of any native analyte detected was listed on the quantitation report, along with the recovery of its labeled analog. Recoveries of ^{13}C -labeled analogs for the samples were between 30 and 150%. Sample sets were reviewed by the QA/QC officer to ensure compliance with QA/QC guidelines/criteria.

RESULTS AND DISCUSSION

The mean concentrations of the analyses of the beef fat samples for the 2,3,7,8-Cl-substituted dioxins and furans are listed in Table 1. All values were adjusted to the lipid content of the sample by dividing the whole weight concentration by the percent lipid in each sample.

The percent lipid was determined according to Method 1613. Mean concentrations were initially calculated in three ways: with nondetects = 0, with nondetects = ½ LOD, and by ignoring nondetects and calculating a mean of the detected values. The first two means are weighted to take into account the total number of animals slaughtered per year in each class.¹¹ That is, they are stratified means, where the strata are the five slaughter classes. Even though the original samples were approximately self-weighting, the means were still weighted to compensate for the oversampling of bulls and the loss of two dairy cow samples. These weighing procedures resulted in means which are estimates of the true national population mean, since the relative proportion of each slaughter class's contribution to the calculation of the mean actually reflects the percentage of each animal class slaughtered. The third mean in Table 1, along with the median, minimum and maximum value are unweighted. They are descriptive summary statistics of the analytical data, and the mean does not represent an estimate of a population mean.

This type of information should always be provided in order to objectively evaluate the results and/or make meaningful comparisons to data in the literature. Values reported without this supporting information are confusing and often lead to misinterpretation of the data. For example, means are often listed without an explanation of how the "nondetects" were treated in the final calculations. Quite often no information is provided regarding the frequency of detection, the accuracy, the precision, or the method LOD/LOQ. As is evident from Table 1, the values for the means can be different, and the magnitude of the differences increases as the frequency of detection decreases.

The first two estimates of population means were also converted to the TCDD toxic equivalence (TEQ) using the International toxic equivalence factor scheme.¹² When the nondetects were set to zero, the mean concentration was 0.35 ppt (TEQ); when nondetects were set to ½LOD, the value was 0.89 ppt (TEQ). These are estimates of the true national mean TEQ concentration in back fat of virtually all the beef animals slaughtered at federally inspected establishments in the United States.

Due to significant differences in study design, the results of this survey cannot be directly compared to literature values without making assumptions as to how those values were derived. A complete discussion of the results of this survey in terms of human exposure and the effects of age and feeding patterns on the levels found in the individual slaughter classes is presented elsewhere.⁸

The analytical method described here proved to be quite effective for determining 2,3,7,8-TCDD concentrations down to a detection limit of 0.05 ppt. These are the lowest validated detection limits for a study of this type. This being the case, how they were derived and how our method of background determination and subtraction affected the final results are important issues to consider.

Initially, the results of a demonstration of capability phase were used to estimate target LODs/LOQs that were subsequently verified by fortifying replicate subsamples at the specified levels and measuring the precision and accuracy. Prior to analyzing study samples, an initial demonstration of the method's capabilities was done by fortifying five subsamples of beef fat homogenate at 5 times the targeted LOQs. The results demonstrated that the accuracy and precision were excellent with observed results within 20% of the actual values and a %RSD for all analytes <20%. Recoveries ranged from 48 to 139%. A laboratory control spike also fortified at 5 times the LOQ was prepared and analyzed with each set of samples. A total of 94% of the values of the analytes (16 of the 17 congeners) had to agree within 20% of the fortified amounts

and recoveries had to be within specified limits, or the preparation and analyses of the entire set were repeated.

Target LODs and LOQs were initially based on a level of performance we thought could be maintained for the duration of the project while satisfying the requirements of the stringent QA/QC program. These target LOQs, primarily based on instrument sensitivity, background, and the results of the demonstration phase, were tentatively set at 0.2 ppt for the tetras (TCDD, TCDF), 1.0 ppt for the pentas, hexas, heptas (PCDDs/PCDFs, HxCDDs/HxCDFs, HpCDDs/HpCDFs), and 6.0 ppt for the octas (OCDD/OCDF). The accuracy and precision at these target levels were verified by analyzing five replicates of the beef fat homogenate fortified at the specified limits. The accuracy for all replicates was within 20% of the fortified amounts and the RSDs \leq 20%. Considering the S/N from the results of these analyses at the LOQs, the LODs were estimated to be half the value of the target LOQs. These LODs/LOQs were to be reconsidered after completion of the project. We then verified the target LODs by fortifying replicate subsamples at the appropriate level (i.e., 0.1 ppt for 2,3,7,8-TCDD). These QA samples were then extracted at ECS and analyzed at both the ECS and the Atmospheric Research and Exposure Assessment Laboratory (AREAL). For the majority of the analytes that were free from matrix or method blank background, the interlaboratory precision (\pm 20%) and accuracy (ranging from 56.0 to 96.0% of actual values) were quite good. For a few of the analytes (1,2,3,6,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD, and OCDD), the demonstrable accuracy declined to between 10 and 50%. This apparent decline was due to the fact that these analytes were present in the matrix at concentrations 2-5 times the fortification level and were routinely detected in the method blanks. It is extremely difficult to accurately measure such a relatively small "spike" added to samples that contain background levels. Nonetheless, the results obtained from the analytes that did not have background strongly suggested that the LODs could be achieved for all the analytes of interest, but it cannot be demonstrated when significant background exists. For the tetras at fortification levels lower than the LOD, the S/N and ion abundance ratio thresholds established in the QA/QC project plan could not be reliably reproduced. Therefore, this level functionally defined the LODs for the tetras.

The manner in which we determined our LODs/LOQs was unique in that we chose to determine the LODs/LOQs for each analyte in the method based on demonstrated performance criteria rather than calculate an individual LOD for each nondetected analyte based on S/N or on the variability of response in a particular sample¹³. Even though these other methods of determining LODs are widespread and generally accepted, we have found that, in most cases, calculated LODs based on S/N or on the variability of response result in LODs that are unrealistic. Often, LODs calculated by these methods require the detection of amounts that are far below the instrument's demonstrated detection limits. LOD values that would require the detection of amounts below those that can actually be extracted from a fortified sample and detected on the basis of established performance thresholds were not and should not be reported since they cannot be validated. LODs calculated as such imply an absence of the analyte of interest at a concentration much lower than can actually be verified by analyses and are therefore misleading.

Background contamination at ppt detection limits is an important consideration when analyzing samples for compounds that are ubiquitously distributed in the environment. Background contamination did, in fact, define the lower limits of detection for several compounds in this study. In cases where background contamination is routinely present, the critical issue to

be resolved is the level above background that can be reliably determined to be "real" (i.e., contributed from the sample matrix). One must have some mechanism to define the level of background contamination and its variability over the course of the study. This can only be done retrospectively by examining the method blanks.

Background levels for each of the native penta to hepta congeners were determined by calculating the mean concentration for each native analyte from the method blanks done with each sample set ($n = 14$). Many of the analytes were often not detected in all of the method blanks. Therefore, we determined the average background of only those detected amounts. This resulted in a mean with a calculated value larger than had we substituted zeros or $\frac{1}{2}$ LOD for nondetects (NDs) in the calculation of the mean. When subtracting background from an analyte in a particular sample, we subtracted either the average amount for that analyte or the amount found in the blank from that particular sample set, whichever was the greater value. No value for any analyte was reported unless it was ≥ 2 times the mean level of the blank after background subtraction. This procedure defined the method LOD in cases where background contamination was present for a particular analyte.

This method of background determination and subtraction is quite conservative and increases the possibility of false negatives for values close to the detection limits. It also tends to increase method LODs/LOQs. However, it also increases the confidence associated with reported values near the LOD and minimizes the likelihood of false positives. Upon completion of the study, we revised the method LODs/LOQs based on an evaluation of the method's overall performance for the duration of the study. Final LODs/LOQs were based on results from fortified samples done in replicate, the replicate analyses of real samples with analytes present at the specified limits, and the calculation of background levels detected in method blanks. As a result of these procedures, the method LOD/LOQ were 0.1/0.2 ppt for TCDF, 0.5/1.0 ppt for the pentas, hexas, heptas, and 3.0/6.0 ppt for the OCDD and OCDF.

The LOD for 2,3,7,8-TCDD was not determined in the manner just described. No background levels were subtracted from TCDD values, since we did not routinely detect TCDD in the blanks. We had no detectable TCDD background at the onset of the project and therefore did not feel we had to initiate the procedure described above. However, we did sporadically detect TCDD at approximately the detection limit in some subsequent blanks. Since the frequency of detection of TCDD in both the blanks and the samples was low (17%), we decided to reprocess and reanalyze all samples that were positive for TCDD.

We analyzed 10 samples in triplicate (replicate analyses were performed on different days) and several others in duplicate to ensure that positive results for 2,3,7,8-TCDD were not due to contamination and to define the precision of the measurement at the low levels. All values reported for TCDD were the average of the replicate analyses (Table 2). These analyses also provided a means to verify the precision associated with reported values of other congeners. As a result of these replicate analyses, the LOD/LOQ for 2,3,7,8-TCDD was found to be 0.05/0.1 ppt. A positive response for TCDD below the detection limit could not be verified within the guidelines established in the QA/QC plan. Therefore, no value was assigned.

This method of reporting data results in values falling into one of two categories: values at or above the LOQs which have a defined and demonstrated precision and accuracy of within 20% or values that lie between the LOQ and LOD which are flagged denoting their status. It should be understood that the uncertainty associated with reported values below the LOQ increases as they approach the method detection limit, at which point no value should be reported. This

relationship is graphically depicted in Figure 1. As is evident in Figure 1A, the S/N and ion abundance ratios fall outside of the established thresholds; therefore, a qualitative identification cannot be made (i.e., not detected). As the signal improves (Figures 1B,C), the S/N exceeds 3.5 and ion abundance ratios are within the guidelines established in the QA plan. Even though a qualitative identification can be made, the accuracy at this fortification level is <20% (i.e., below the LOQ). At and above the LOQ (Figure 1D), the signal is quite good (S/N > 10), and ion abundance ratios are consistently within the specification. The accuracy and precision at this fortification level are within 20%. The methods defined herein for background subtraction and defining LODs/LOQs result in the reporting of technically defensible values.

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Table 1. Lipid Adjusted Mean and Median Concentrations of PCDDs and PCDFs in Beef Fat (picogram/gram, ppt)

compound	detects	mean 1	mean 2	mean 3	minimum	median	maximum
2,3,7,8-TCDD	11	0.025	0.052	0.173	0.069	0.104	0.736
1,2,3,7,8-PeCDD	2	0.042	0.348	1.862	0.689	1.862	3.035
1,2,3,4,7,8-HxCDD	8	0.184	0.464	1.728	0.728	1.144	4.691
1,2,3,6,7,8-HxCDD	21	1.208	1.424	3.929	0.701	3.935	12.459
1,2,3,7,8,9-HxCDD	9	0.259	0.533	1.981	0.784	1.787	3.679
1,2,3,4,6,7,8-HpCDD	45	4.388	4.475	6.673	0.775	2.576	47.557
1,2,3,4,6,7,8-OCDD	13	3.256	4.782	18.309	4.221	11.733	71.839
2,3,7,8-TCDF	0	0.000	0.031	ND	ND	ND	ND
1,2,3,7,8-PeCDF	0	0.000	0.314	ND	ND	ND	ND
2,3,4,7,8-PeCDF	4	0.061	0.356	0.968	0.860	0.960	1.094
1,2,3,4,7,8-HxCDF	8	0.269	0.546	2.226	0.894	1.812	4.291
1,2,3,6,7,8-HxCDF	7	0.120	0.401	1.086	0.710	1.039	1.964
1,2,3,7,8,9-HxCDF	0	0.000	0.314	ND	ND	ND	ND
2,3,4,6,7,8-HxCDF	5	0.096	0.387	1.224	0.710	1.158	1.753
1,2,3,4,6,7,8-HpCDF	14	0.745	0.995	3.763	0.770	2.990	10.115
1,2,3,4,7,8,9-HpCDF	0	0.000	0.314	ND	ND	ND	ND
1,2,3,4,6,7,8,9-OCDF	0	0.000	1.884	ND	ND	ND	ND

There were 63 samples analyzed.

Mean 1 was obtained using 0 for non-detects (weighted means), mean 2 was obtained using ½ LOD for nondetects (weighted means), mean 3, median, minimum, maximum were obtained using detects (unweighted). nd, not detected

Table 2. Lipid Adjusted Mean Concentrations (pg/ μ l) of PCDDs and PCDFs in a Beef Fat Sample, Triplicate Analysis

Compound	exptl concn			Mean	Std Dev	%RSD
	1	2	3			
2,3,7,8-TCDD	0.047	0.035	0.039	0.040	0.006	15.0
1,2,3,7,8-PeCDD	nd	nd	nd	-	-	-
1,2,3,4,7,8-HxCDD	0.508	0.387	0.403	0.433	0.066	15.2
1,2,3,6,7,8-HxCDD	2.244	1.863	1.940	2.016	0.201	10.0
1,2,3,7,8,9-HxCDD	1.044	0.863	0.982	0.963	0.092	9.6
1,2,3,4,6,7,8-HpCDD	7.206	5.347	5.846	6.133	0.962	15.7
1,2,3,4,6,7,8-OCDD	3.436	3.341	3.561	3.446	0.110	3.2
2,3,7,8-TCDF	nd	nd	nd	-	-	-
1,2,3,7,8-PeCDF	nd	nd	nd	-	-	-
2,3,4,7,8-PeCDF	nd	nd	nd	-	-	-
1,2,3,4,7,8-HxCDF	1.946	1.454	1.492	1.630	0.273	16.7
1,2,3,6,7,8-HxCDF	0.430	0.323	0.357	0.370	0.055	14.8
1,2,3,7,8,9-HxCDF	nd	nd	nd	-	-	-
2,3,4,6,7,8-HxCDF	nd	nd	nd	-	-	-
1,2,3,4,6,7,8-HpCDF	2.046	1.692	1.472	1.737	0.290	16.6
1,2,3,4,7,8,9-HpCDF	nd	nd	nd	-	-	-
1,2,3,4,6,7,8,9-OCDF	nd	nd	nd	-	-	-

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Figure 1. Ion Chromatograms Depicting Instrumental Response for 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) at Quantitation & Detection Limits.

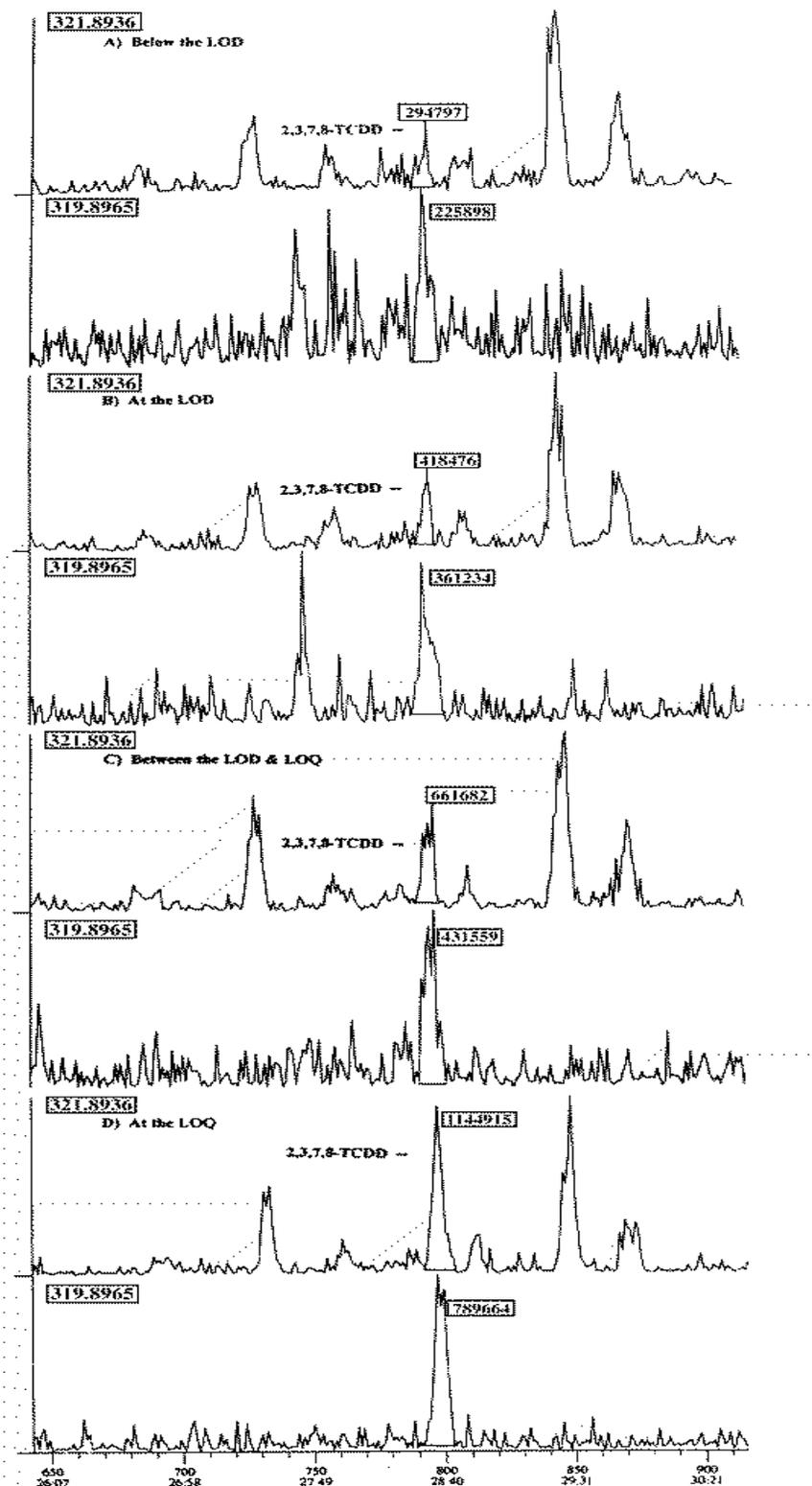


Figure 1. Ion chromatograms depicting instrumental response for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) at quantitation and detection limits.