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Brominated Dioxins and Furans in Human Adipose Tissue

Final Report

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PREFACE

This report provides a summary of the analyses of the FY1987 NHATS composited human adipose tissue samples for polybrominated dibenzo-*p*-dioxins (PBDDs) and polybrominated dibenzofurans (PBDFs) completed for all sample batches under EPA Contract No. 68-02-4252, Work Assignment 27, "Analysis of Human Adipose Tissue for Dioxins and Furans." These adipose tissue samples were analyzed for specific 2,3,7,8-substituted PBDDs and PBDFs according to the analytical protocol identified in the Quality Assurance Program Plan (QAPP) for this work assignment. This document presents the first reported effort to determine the presence of PBDDs and PBDFs in the human population. These data and reporting activities were generated by Midwest Research Institute under the direction of Mr. Paul H. Cramer and Dr. John S. Stanley for EPA's Office of Toxic Substances Field Studies Branch. Mr. Michael McGrath and Mr. Paul Cramer were responsible for preparation of the composite samples, Mr. Kelly Thornburg and Mr. Gene O'Donnell conducted the HRGC/HRMS analyses, and Mr. Randy Ayling was responsible for standards preparation and the data reduction efforts. The study design was provided by Battelle Columbus Laboratories. Mr. John Schwemberger of the Design Development Branch of OTS and Ms. Karin Bauer of MRI provided the statistical evaluation of the quality control data for assessment of overall method performance.

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This study on the determination of polybrominated dibenzo-*p*-dioxins (PBDDs) and dibenzofurans (PBDFs) in human adipose tissue is one part of the ongoing Human Adipose Tissue Survey. The work reported herein was conducted as a collaborative effort among EPA's Office of Toxic Substances, Midwest Research Institute, and Battelle Columbus Division.

EPA participation was within the Office of Toxic Substances (OTS) Exposure Evaluation Division, the Field Studies Branch (Ms. Janet Remmers, Work Assignment Manager, and Dr. Joseph Breen, Project Officer) and the Design and Development Branch (Mr. John Schwemberger, Work Assignment Manager, and Ms. Edith Sterrett, Project Officer). Contract support to OTS included MRI for the conduct of the method evaluation, sample analysis, and reporting (Mr. Paul Cramer and Dr. John Stanley, Co-work Assignment Managers, and Mr. Paul Constant, Program Manager); Battelle Columbus Division for processing of patient summary reports (Ms. Tamara Collins and Ms. Jan Clark) and composite design (Ms. Barbara Leczynski); and Mr. Robert Heath for design of the approach to the quality control program.

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EXECUTIVE SUMMARY

As part of the U.S. Environmental Protection Agency's efforts to determine human exposure to potentially toxic compounds from commercial products and environmental routes, a study has been conducted to determine human adipose tissue levels of polybrominated dibenzo-*p*-dioxins (PBDDs) and dibenzofurans (PBDFs). The study included the analysis of adipose tissues collected from the general U.S. population in fiscal year 1987 through the National Human Adipose Tissue Survey (NHATS). The 2,3,7,8-substituted analytes (tetra- through hexabrominated dioxins and furans) targeted for analysis were not detected in any of the study samples. However, the analytical techniques and the data generated provide a basis for application and comparison in future human exposure studies. Preliminary data were generated for another class of compounds, polybrominated diphenyl ethers (PBDPEs), which were tentatively identified as interferences in the analytical procedure. This report describes the development and evaluation of the analytical protocol and the results of the analysis efforts.

Regulations recently promulgated under Sections 4 and 8 of the Toxic Substances Control Act (TSCA) have focused attention on the possible exposures to halogenated (brominated and chlorinated) dibenzo-*p*-dioxins and dibenzofurans from commercial products. The purpose of the regulations is to limit the potential releases of these compounds to the environment. While considerable information has been generated on the chlorinated compounds (PCDDs and PCDFs) in commercial products and environmental and biological matrices, few or no data have been generated regarding the incidence of the PBDDs and PBDFs.

Since no methods existed for measurement of PBDDs and PBDFs, this study required modification and evaluation of existing sample preparation techniques and HRGC/HRMS analysis methods that were originally developed for determination of PCDDs and PCDFs. The preliminary method evaluation studies focused on the development of HRGC/HRMS acquisition parameters, establishing linearity of the calibration curves, determining method sensitivity, and conducting the analysis of a series of spiked samples. The results of the preliminary method evaluation studies demonstrated that the PBDDs and PBDFs could be determined at low picogram/gram (pg/g, ppt) levels.

Following the evaluation of the analytical method, a total of 48 composite samples (prepared from 865 individual tissue specimens) and 20 quality control (QC) samples were analyzed for this study. The composite samples represented the nine U.S. census divisions and three age groups (0-14, 15-44, and 45 plus). The targeted 2,3,7,8-substituted PBDDs and PBDFs were not detected in any of the composite samples. Detection limits for each congener were calculated for each sample. The average detection limits calculated from the 48 composite samples were approximately 1 pg/g for TBDD and TBDF, 10 pg/g for the PeBDD, PeBDF, and HxBDD, and 40 pg/g for the HxBDF.

Data on the precision and accuracy of the method for three PBDDs and three PBDFs were generated from the analysis of the QC samples. These samples included five unspiked and 10 spiked (five replicates at two spike levels) adipose tissue (lipid) samples. Statistical treatment of the QC data

demonstrated that with the exception of 1,2,3,4,7,8-HxBDF, the analytical method is unbiased in measuring sample concentrations. The bias determined, though not statistically significant, is larger for the hexabromodioxin than for the tetra- and pentabrominated compounds. For the hexabromofuran, the method has a significant negative bias believed to be due to either the lack of the appropriate internal quantitation standard, the difference in the HRMS sensitivity, or both. These limitations are reflected in the higher detection limits reported for the HxBDF. The method provides precise results for the tetra- and pentabrominated compounds, while providing poor precision for the hexabromo compounds.

While PBDDs and PBDFs were not detected in the study samples, there was evidence of the presence of other brominated compounds in the adipose tissues. Specifically, responses were noted that correspond to the qualitative criteria for PBDPEs (hexa- through octabromo). It is recommended that these responses should be confirmed as brominated diphenyl ethers through additional analysis efforts in order to expand the data base on documented human exposures.

The overall program effort has provided a basis for evaluating the potential exposure to PBDDs and PBDFs. Although the data generated at this time do not provide an indication of the presence of these compounds in human adipose tissues, this study will serve as a landmark effort for future comparison studies.

I. INTRODUCTION

The National Human Adipose Tissue Survey (NHATS) operated through the U.S. Environmental Protection Agency's (EPA's) Office of Toxic Substances/ Exposure Evaluation Division (OTS/EED) has served as the Agency's primary mechanism for monitoring human exposure to potentially toxic materials since the early 1970s. The NHATS program, as operated through the 1970s, served primarily as a monitor of the exposure to organochlorine pesticides and PCBs. However, over the last several years, OTS has implemented a strategy for a broader view of the number of toxic substances present in adipose tissues that provide evidence of exposure either through environmental routes or as a result of consumer products use.

Samples that were collected in the FY 1982 NHATS collection were analyzed for a broad range of compounds that included volatile organics (chlorinated alkanes, chlorinated aromatics, and aromatics), semivolatile organics (organochlorine pesticides, PCBs by homolog, polynuclear aromatic hydrocarbons, chlorinated benzenes, phthalates and phosphate esters), polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), and trace metals.[1-4] This required development of procedures based on state-of-the-art instrumentation, high-resolution gas chromatography/mass spectrometry (HRGC/MS).

The samples collected from the FY 1984 NHATS program were analyzed for the broad range of semivolatile organic compounds using the same preparation procedures as used for the FY 1982 samples and HRGC/MS and packed column gas chromatography/electron capture detection (PGC/ECD) to demonstrate the comparability of the techniques for specific organochlorine pesticides. The broad scan effort for semivolatile organics based on the HRGC/MS procedure has been repeated for the samples collected in the FY 1986 NHATS program, although the emphasis on the analysis has been expanded to include the determination of compounds that are classified under other federal regulations, such as SARA Title III.

In addition to these specific NHATS programs, OTS has been involved in two additional studies to determine human body burden levels of PCDDs and PCDFs. These studies include a collaborative effort with the Veterans Administration to determine the levels of specific 2,3,7,8-substituted congeners in adipose tissues collected from adult males between 1972 and 1982. The second study focused on developing national estimates of body burdens of these compounds using samples that were collected in the FY 1987 program.[5-8]

As a result of regulations promulgated during 1987 under Sections 4 and 8 of the Toxic Substances Control Act (TSCA), EPA is responsible for ensuring that specific commercial products do not present a route for release of halogenated (chlorinated or brominated) dibenzo-*p*-dioxins and dibenzofurans to the environment. As a result of the focus of this regulation, there is a need for developing data on the exposure of humans to these compounds.

A. Work Assignment Objectives

The overall objective for this work assignment was to provide data on the body burden levels of polybrominated dibenzo-*p*-dioxins (PBDDs) and

dibenzofurans (PBDFs) using adipose tissue specimens that were collected in the FY 1987 NHATS collection effort. Data quality objectives for this program effort are presented below:

Measurement	Data quality objective
Initial Relative Response Factor (RRF) calibration	Relative standard deviation (≤ 20% for tetra isomers, ≤ 30% for all others)
Daily RRF	Within 20% of initial RRF determined for tetra isomers, within 30% for all others
IQS recovery from samples	40%-150%
Recovery of compound from spiked control samples	40%-150%

Data were also generated for the PCDD and PCDF residues, although the results of this study have been provided under separate cover.[7-8]

B. Organization of Report

The remainder of this report provides details on the sources of the samples, the procedures used to composite individual specimens into composites representative of three different age groups from nine different census divisions, and the sample preparation and analysis procedures (Section II). Section III of this report provides a summary of the analytical results for this program effort. Summaries of the specific quality control data that were generated in support of this analysis effort are presented in Section IV. Statistical treatment of the quality control data and an assessment of the overall method performance for PBDDs and PBDFs are presented in Section V. References which have been cited in the report are presented in Section VI.

II. EXPERIMENTAL APPROACH

This section describes the procedures that were used for preparing composited samples from the FY 1987 NHATS collection, the methods used for preparing the composited samples for analysis, and the instrumental conditions that were used to conduct the high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) analysis of the sample extracts. Additional details are presented on quality assurance/quality control practices and criteria that were implemented to support overall data quality.

A. Composite Design for the FY 1987 NHATS Samples

The composite design for the FY 1987 NHATS samples was prepared by Battelle Columbus Division (BCD).[5] In developing the compositing design, BCD

considered five criteria: maintaining similarity with the FY 1982 NHATS composite design; maintaining equal weighting of the specimens within the composites; specifying an increased number of samples that were pure sex composites versus the FY 1982 study; controlling metropolitan statistical area (MSA) effects; and providing the best range of group percentages across the composite samples. After considering these criteria, BCD developed a study design that consisted of 48 composited samples prepared from 865 individual specimens that were collected from 41 MSAs.

In addition to developing the composite design, BCD provided a design for order of analysis to be followed for preparation and analysis of the composite samples along with quality control samples such that the analysts were blinded to the characteristics of each sample and that the samples were sufficiently randomized to avoid any potential biases due to age, gender, or geographic region. The FY 1987 NHATS composite design has been summarized as a separate report.[5]

B. Laboratory Compositing Procedures

NHATS FY 1987 specimens from nine census divisions and three age groups were divided into 48 composite samples as identified in the composite design provided by BCD. BCD provided MRI with data sheets for each composite that identified the specific individual specimens that were to be included in each composite. Each composite consisted of 3 to 32 specimens. The composite sample data sheets provided sufficient information (EPA ID number, package number, sample weight, hospital code, etc.) such that the individual specimens could be cross-checked with the study design. The data sheets provided by BCD were also used to record the actual laboratory compositing procedures.

Initially, the samples were grouped into composites, and any samples of questionable weights were noted. The mass of each specimen required for a composite was determined by dividing the targeted weight of the final composite (10 g) by the number of individual samples to be included. For example, a composite to be prepared from 20 individual specimens required 0.5 g of each specimen. Three specimens of the available 865 specimens (identified below) did not have the weight required by the composite design. These results were relayed to the EPA work assignment manager (WAM) by telephone on June 23, 1988. After consultation with the Design Development Branch, the EPA WAM forwarded to MRI the following responses regarding the problem samples on June 24, 1988.

Composite number	Sample number	Problem	Response
ACD8700023	8706954	Low weight, ~ 0.1 g need 0.5 g	Include as is
ACD8700032	8701765	No sample remaining	Omit
ACD8700201	8703464	Low weight, ~ 1.3 g need 2.0 g	Include as is

The samples in a composite were placed on dry ice during the compositing procedure to ensure that thawing would not occur. An electronic 4-place balance was used to weigh the samples. The calibration of the balance was checked before any weighing was begun and once during the sample weighings with a Class P set of weights (laboratory-grade, tolerance 1/25,000).

To weigh the samples, a clean culture tube was labeled with the composite number and placed on the balance and the weight tared. A sample was removed from the storage area, the jar opened, and a portion of the frozen adipose removed with a clean stainless steel spatula. The adipose was placed in the culture tube and the weight recorded to three decimal places on the compositing sheets. Additional adipose was added if necessary. A goal of ±10% of the desired weight (0.30 to 3.0 g depending on the number of specimens specified to achieve 10 g) was attempted where possible. The weight of the individual specimens were recorded on the data sheets provided by BCD.

The weight of the culture, beaker, and adipose tissue was rezeroed and the next specimen in the composite was weighed. A clean spatula was used for each specimen. This procedure was repeated for each specimen in the composite. When the composite was completed, it was sealed and stored in a sample freezer at -10°C. All data on the actual compositing procedures were recorded on the data sheets provided by BCD. All data sheets were submitted as a separate interim report to document the compositing activity.[6]

C. Analytical Procedures

Analytical procedures included the extraction and cleanup of the composite tissue samples and the analysis by HRGC/HRMS. These procedures are described below in detail.

1. Sample Preparation

a. Extraction

After compositing, the adipose tissue composites (~ 10 g) were stored at -10°C in 50-mL culture tubes sealed with aluminum foil. To begin the sample extraction procedure, the samples were allowed to come to room temperature and then fortified with 100 µL of the chlorinated internal quantitation standards (IQS) spiking solution and 100 µL of the brominated IQS spiking solution (Table 1). Ten milliliters of methylene chloride was added and the sample homogenized for 1 min with a Tekmar Tissuemizer. The mixture was allowed to separate and the methylene chloride was decanted through a funnel of sodium sulfate into a 100-mL volumetric flask. The homogenization was repeated with a fresh 10-mL portion of methylene chloride. The culture tube was rinsed with additional methylene chloride and the remaining contents of the tube transferred to the funnel. Finally, the funnel was rinsed with additional methylene chloride until the volumetric was brought up to volume (100 mL).

At this point the flask was stoppered and inverted several times to mix the extract, and 1.0 mL was removed with a disposable pipet and placed into a preweighed 1-dram (measured to 0.0001-g) glass vial. The

Table 1. Internal Standard Spiking Solutions for Chlorinated and Brominated Species[a]

Compound	Concentration (pg/μL)
Chlorinated Internal Quantitation Standards[b]	
$^{13}C_{12}$-2,3,7,8-TCDD	5
$^{13}C_{12}$-2,3,7,8-TCDF	5
$^{13}C_{12}$-1,2,3,7,8-PeCDD	5
$^{13}C_{12}$-1,2,3,7,8-PeCDF	5
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	12.5
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	12.5
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	12.5
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	12.5
$^{13}C_{12}$-OCDD	25
Brominated Internal Quantitation Standards[b]	
$^{13}C_{12}$-2,3,7,8-TBDD	5
$^{13}C_{12}$-2,3,7,8-TBDF	5
$^{13}C_{12}$-1,2,3,7,8-PeBDF	5
Internal Recovery Standard[c]	
$^{13}C_{12}$-1,2,3,4-TCDD	50
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	125

[a]All internal quantitation and recovery stanards were obtained as solutions from Cambridge Isotope Laboratories, Woburn, Massachusetts.
[b]Prepared in isooctane. One hundred microliters spiked. Separate solutions were used for chlorinated and brominated species.
[c]Prepared in tridecane. Used for both chloro and bromo analyses. Ten microliters were added to each final extract.

methylene chloride in the vial was reduced under nitrogen until a constant weight (measured to 0.0001 g) of extractable lipid was obtained. The weight of the lipid was obtained by difference and the percent lipid for the composite calculated.

The remaining portion of the extract (99 mL) was quantitatively transferred with a 20- to 30-mL rinse to a 500-mL round-bottomed flask. The extract was concentrated to an oily residue (extractable lipid) using rotary evaporation.

b. Bulk Lipid Removal

An acidic silica gel slurry cleanup of the extract was conducted by adding 200 mL of hexane and a Teflon-coated stirring bar to the lipid in the round-bottomed flask. Then, while stirring the extract on a magnetic stir plate, 100 g of 40% w/w sulfuric acid-impregnated silica gel was slowly added to the extract. The mixture was stirred for 2 h. During the 2-h slurry period, acid/neutral silica gel columns (4 g 40% H_2SO_4/silica gel, 1 g silica gel) (E. Merck, W. Germany) were prepared. After the 2-h period, the slurry mixture was allowed to settle and the hexane was decanted off the acid-impregnated silica gel through a funnel of sodium sulfate into the acid/neutral silica gel column. Two 50-mL aliquots of hexane were added to the slurry mixture, and the mixture was stirred for 15 min each time. The rinses were added to the silica gel column through the sodium sulfate funnel. The eluate of the column was collected in a 500-mL Kuderna evaporation flask. An additional 50 mL of hexane was placed onto the column when the solvent level had reached the level of the chromatographic packing. The extract was then reduced in volume over a steam bath, and the final volume was adjusted to approximately 1 mL using nitrogen blow down.

c. Separation of Chemical Interferences

A layered, neutral alumina (ICN Adsorbentien, W. Germany) column was prepared containing 1 g sodium sulfate, 1 g neutral alumina, and 1 g sodium sulfate. The extract from the acid/neutral silica gel column was transferred to the alumina column, followed by two 1-mL portions of hexane and 10 mL of 8% (v/v) methylene chloride in hexane. These eluents were archived. The dioxins and furans (chloro and bromo) were eluted from the column with 15 mL of 60% (v/v) methylene chloride in hexane. The eluent was concentrated to approximately 2 mL under a stream of nitrogen.

A disposable column of AX-21 carbon (Anderson Development Corporation) on silica gel was prepared using approximately 1 g of the mixed adsorbent (1 g AX-21 and 19 g silica gel) and preeluted with 4 mL of toluene, 2 mL of 75:20:5 methylene chloride/methanol/benzene, and 2 mL of 1:1 cyclohexane/methylene chloride. The concentrated eluate from the alumina column was added to the AX-21/silica gel column followed by two 1-mL hexane rinses. The column was eluted sequentially with two 0.5-mL aliquots of hexane, 10 mL of 1:1 cyclohexane/methylene chloride, and 5 mL of 75:20:5 methylene chloride/methanol/benzene. These eluents were combined and archived. The columns were then turned upside down and the dioxins and furans eluted with 20 mL of toluene. The extract was then reduced in volume to approximately 100 µL at

which time 10 µL of recovery standard was added (Table 1) and the volume further reduced to 10 µL under nitrogen. The extract was stored in a freezer until HRGC/HRMS analysis.

2. HRGC/HRMS Analysis

All sample analyses were completed using a Kratos MS-50TC double-focusing HRMS. Initial calibration of the GC/MS system was conducted by making duplicate 1-µL injections of the standards listed in Table 2. A CS5 (10 pg/µL of TBDD, TBDF to 25 pg/µL HxBDD, HxBDF) standard was analyzed on a daily basis to ensure adherence to the initial calibration curve.

Sample analysis by HRGC/HRMS was conducted after initial and routine calibration criteria were met. Prior to the injection of the first sample, an injection of tridecane was analyzed to document system cleanliness. Corrective action was taken by analyzing another tridecane blank if any evidence of system contamination was found. A typical daily sequence of injections is shown in Table 3.

A 1-µL aliquot of the extracts was injected into the GC/MS system, operated under the conditions previously used to produce acceptable results with the daily calibration standard.

Selected ion monitoring (SIM) data were acquired according to the same acquisition and MS operating conditions previously used to determine the relative response factors (Tables 4 and 5). Instrument performance was monitored by examining and recording the peak areas for the recovery standard, $^{13}C_{12}$-1,2,3,4-TCDD.

D. QC for Chemical Analyses

The following QC criteria were targeted for the chemical analysis portion of this program.

1. Instrument Performance

The instrument performance was characterized primarily by three criteria: a mass resolution ≥ 3,000, relative response factors (i.e., adherence to the initial RRFs) and instrument sensitivity.

a. Mass Calibration and Resolution

The mass spectrometer was tuned on a daily basis to yield optimum sensitivity and peak shape using an ion peak (m/z 542.9664) from perfluorokerosene (PFK). The resolution was visually monitored and maintained at ≥ 3,000 (10% valley definition) to provide adequate noise rejection while maintaining good ion transmission. A visual check of the static resolution was made by using the peak matching unit before and after each analysis.

Table 2. Concentration Calibration Solutions for PBDD/PBDF

Compound	Concentration in calibration solutions in pg/µL							
	CS1	CS2	CS3	CS4	CS5	CS6	CS7	CS8
2,3,7,8-TBDD	200	100	50	25	10	5	2.5	1
2,3,7,8-TBDF	200	100	50	25	10	5	2.5	1
1,2,3,7,8-PeBDD	200	100	50	25	10	5	2.5	1
1,2,3,7,8-PeBDF	200	100	50	25	10	5	2.5	1
1,2,3,4,7,8-HxBDD	500	250	125	62.5	25	12.5	6.25	2.5
1,2,3,4,7,8-HxBDF	500	250	125	62.5	25	12.5	6.25	2.5
Internal Quantitation Standards								
$^{13}C_{12}$-2,3,7,8-TBDD	50	50	50	50	50	50	50	50
$^{13}C_{12}$-2,3,7,8-TBDF	50	50	50	50	50	50	50	50
$^{13}C_{12}$-1,2,3,7,8-PeBDF	50	50	50	50	50	50	50	50
Internal Recovery Standard								
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	125	125	125	125	125	125	125	125

Note: The unlabeled and labeled brominated standard were obtained from Cambridge Isotope Laboratories, Woburn, Massachusetts. All standards were prepared in tridecane.

Table 3. Typical Daily Sequence for PBDD/PBDF Analysis

1. Tune and calibrate mass scale versus perfluorokerosene (PFK).
2. Inject concentration calibration solution 10 to 25 pg/µL (CS-5) solution.
3. Inject blank (tridecane).
4. Inject samples 1 through "N."
5. Inject concentration calibration solution 10 to 25 pg/µL (CS-5) solution or other concentration calibration solutions CS1 to CS8 to bracket observed sample concentration.

Table 4. Ions Monitored for PBDD/PBDF

Descriptor	ID	Mass	Nominal dwell time (s)
B1	-COBr (TBDF)	374.784	0.036
	TBDF	481.697	0.056
	TBDF	483.695	0.056
	$^{13}C_{12}$-TBDF	493.737	0.056
	$^{13}C_{12}$-TBDF	495.735	0.056
	-COBr (TBDD)	390.779	0.034
	TBDD	497.692	0.052
	TBDD	499.690	0.056
	$^{13}C_{12}$-TBDD	509.732	0.026
	$^{13}C_{12}$-TBDD	511.730	0.026
	$^{13}C_{12}$-HxCDD	401.856	0.033
	$^{13}C_{12}$-HxCDD	403.853	0.034
	HxBDPE	643.530	0.043
	PFK lock mass	480.970	0.056
B2	-COBr (PeBDF)	454.693	0.059
	PeBDF	561.606	0.049
	PeBDF	563.604	0.049
	$^{13}C_{12}$-PeBDF	573.646	0.092
	$^{13}C_{12}$-PeBDF	575.644	0.092
	-COBr (PeBDD)	470.688	0.056
	PeBDD	577.601	0.092
	PeBDD	579.599	0.092
	HpBDPE	721.441	0.092
	PFK lock mass	580.963	0.072
B3	-COBr (HxBDF)	532.603	0.098
	HxBDF	641.514	0.085
	HxBDF	643.512	0.085
	-COBr (HxBDD)	548.598	0.098
	HxBDD	657.509	0.079
	HxBDD	659.507	0.085
	OBDPE	801.349	0.131
	PFK lock mass	580.963	0.092

Table 5. HRGC/HRMS Operating Conditions for PBDD/PBDF Analysis

Mass spectrometer:	Kratos MS50-TC
Accelerating voltage:	8,000 V maximum
Trap current:	500 μA
Electron energy:	70 eV
Electron multiplier voltage:	-2,000 V
Source temperature:	280°C
Resolution:	> 3,000
Overall SIM cycle time:	1 s
Gas chromatograph:	Carlo-Erba MFC-500
Column coating:	DB-5
Film thickness:	0.25 μ
Column dimensions:	30 m x 0.253 mm
He linear velocity:	~ 35 cm/s
He head pressure:	0.75 kg/cm^2
Injection type:	Splitless
Split flow:	30 mL/min
Purge flow:	3 mL/min
Injector temperature:	300°C
Interface temperature:	290°C
Injection size:	1 μL
Initial temperature:	200°C
Initial time:	2 min
Temperature program:	5°/min to 330°C
Final hold time:	6 min

Mass calibration of the mass spectrometer for the HRGC/MS analysis of PBDD/PBDF was carried out on a daily basis. The magnetic field was adjusted to pass m/z 368 at full accelerating voltage. PFK was admitted to the MS and an accelerating voltage scan from 8,000 to 800 V was acquired by the data system. This corresponded to an effective mass range of 369 to 843 amu. Upon completion of a successful calibration step, the ion descriptors shown in Table 5 were updated to reflect the new mass calibration.

b. Relative Response Factor and Instrumental Sensitivities

As part of the initial and routine instrument performance checks, calibration standards were analyzed and RRF values of the respective analytes were compared to specific internal standards. The initial and routine calibration criteria required that the precision (percent difference) of the RRF measurements were within ±20% for the tetrabromo congeners and within ±30% for the other compounds.

Sensitivity of the HRMS was documented through the responses noted for the first calibration standard analyzed for each analysis day. The method required the analysis of a low level standard (CS5) to document sufficient instrumental response to support instrumental detection limits of 10 pg/µL for TBDD.

Routine checks on the instrumental sensitivity was achieved by monitoring the response for the internal recovery standard ($^{13}C_{12}$-1,2,3,7,8,9-HxCDD) from injection to injection and was documented in the MS log book.

2. QC Samples

Samples included for QC purposes are summarized in Table 6. Each of the five sample batches included four quality control samples: a method blank, an unspiked control lipid sample, and two spiked lipid samples. The method blank was included to detect background contributions introduced by the laboratory procedures. The unspiked and spiked controls were included as a means of assessing method precision and bias (or accuracy).

Table 6. Quality Control Samples

Type	Frequency
Method blank	One per batch
Spiked control adipose tissue sample	Two per batch (two different spike levels)
Unspiked control adipose tissue sample	One per batch

a. Method Blanks

One method blank was generated with each batch of samples. A method blank was generated by performing all steps detailed in the analytical procedure using all reagents, standards, equipment, apparatus, glassware, and solvents that were used for a sample analysis, but omitting the addition of the adipose tissue. The method blank contained the same amounts of ^{13}C-labeled internal quantitation standards that were added to samples before sample extraction.

b. Unspiked Control Samples

Control samples were prepared from a bulk sample of human adipose tissue. This material was prepared by blending the tissue with methylene chloride, drying the extract by eluting through anhydrous sodium sulfate, and removing the methylene chloride using rotoevaporation at elevated temperatures (80°C). The evaporation process was extended to ensure all traces of the extraction solvent had been removed. The resulting oily matrix (lipid) was subdivided into 10-g aliquots which were analyzed with each sample batch.

c. Spiked Control Samples

Spiked lipid samples were prepared using a portion of the homogenized control lipid. Sufficient spiked lipid matrix was prepared to provide a minimum of two spiked samples per sample batch. A low and high spike was prepared with each batch. The native spiking solution concentration is presented in Table 7. The spiking solutions were checked for accuracy prior to spiking the adipose composites with the native isomers. The results of this spike check are shown in Table 8. The results in Table 8 demonstrate that the spiking solution was prepared correctly. The differences in measured concentration can be attributed to variance in the HRMS procedure, specifically the variability in RRF values from initial calculation to the evaluating of the spike check.

The low spiked sample was prepared such that each aliquot was fortified at concentrations equivalent to 25 pg/g for tetra- and pentabrominated compounds and 62.5 pg/g for the hexabrominated compounds. The high spikes were prepared such that the nominal concentrations of 50 pg/g for tetra- and pentabrominated compounds and 125 pg/g of the hexabrominated compounds were achieved.

d. Order of Analysis

Each of the QC samples and NHATS composite samples was assigned to a specific analysis batch and a specific analysis order within a batch. The method blank was the first sample analyzed within each batch. The lipid unspiked control and spiked control samples were randomly assigned analysis orders within the batch. The QC samples and NHATS composites were labeled with barcodes such that the analyst was blinded to the exact nature of a specific sample.

Table 7. Spiking Solutions for PBDDs and PBDFs

Compound	Concentration (pg/µL)
Internal Quantitation Standards	
$^{13}C_{12}$-2,3,7,8-TBDD	5
$^{13}C_{12}$-2,3,7,8-TBDF	5
$^{13}C_{12}$-1,2,3,7,8-PeBDF	5
Natives[a]	
2,3,7,8-TBDD	5
2,3,7,8-TBDF	5
1,2,3,7,8-PeBDD	5
1,2,3,7,8-PeBDF	5
1,2,3,4,7,8-HxBDD	12.5
1,2,3,4,7,8-HxBDF	12.5

[a]Low spiked samples were prepared by adding 50 µL of the native solution to each 10-g control lipid aliquot. High spiked samples were prepared by the addition of 100 µL. All spike solutions were prepared in isooctane.

Table 8. Brominated Spike Check Results[a]

Compound	Spike level (pg/μL)	Br spike check 1 (pg/μL)	Br spike check 1 % Recovery	Br spike check 2 (pg/μL)	Br spike check 2 % Recovery	Br spike check 3 (pg/μL)	Br spike check 3 % Recovery	Average recovery (% recovery)	% RPD[b]
^{13}C-TBDF	50	42	85	41	82	43	86	84	4.8
^{13}C-TBDD	50	41	82	41	83	42	83	83	1.2
^{13}C-PeBDF	50	43	86	45	90	44	88	88	4.5
TBDF	25	27	110	28	110	27	110	110	0
TBDD	25	27	110	27	110	28	110	110	0
PeBDF	25	26	100	26	100	30	120	110	18
PeBDD	25	25	100	24	97	23	92	96	8.3
HxBDF	62.5	50	80	58	93	55	88	87	15
HxBDD	62.5	44	70	56	89	58	93	84	27

[a]The evaluation of the spiking solution was achieved by adding 100 μL of the internal quantitation standards solution (Tables 1 and 7), 50 μL of the unlabeled standards solution (Tables 1 and 7), and 10 μL of the internal recovery standard (Table 1). The solution was concentrated to a final volume of 10 μL.

[b]Relative percent difference = $\frac{\text{(High recovery - low recovery)}}{\text{Average recovery}} \times 100\%$

III. RESULTS

The analytical efforts conducted as part of this study included a preliminary method evaluation as well as analysis of the 48 composite samples and associated QC samples. This section presents the results of the preliminary method evaluation and summarizes data for the composite samples. Data from the analysis of the quality control samples are presented in Section IV.

A. Preliminary Method Evaluation

Preliminary method evaluation studies focused on developing the necessary HRGC/HRMS acquisition parameters, establishing linearity for the calibration curves, determining limitations of the instrument sensitivity, and conducting the analysis of a series of spiked samples that were taken through the analytical procedures described in the experimental approach.

1. Evaluation of Instrument Variables and Establishing Calibration Criteria

Initial investigations into the response relationship between the chlorinated recovery standard ($^{13}C_{12}$-1,2,3,7,8,9-HxCDD), the three brominated IQS, and the six native brominated dioxins and furans were conducted by analyzing the brominated dioxin and furan calibration standards (Table 2) in triplicate. Although all eight levels of the standards were analyzed, response was only observed for the tetra- and pentabrominated dioxins and furans at the CS7 level (2.5 pg) and higher. For the hexabrominated dioxin and furan, response at 2.5 times noise was observed at the CS5 level (10 pg) and above. The result of this effort is shown in Table 9.

The 2,3,7,8-TBDF was paired with the ^{13}C-TBDF, the 2,3,7,8-TBDD was paired with the ^{13}C-TBDD, and the remaining unlabeled brominated dioxins and furans were paired with the ^{13}C-PeBDF for the determination of relative response factors. The three internal quantitation standards were paired with the recovery standard, $^{13}C_{12}$-1,2,3,7,8,9-HxCDD, for the calculation of relative response factors.

2. Recovery of Brominated Dioxins and Furans From Adipose Tissue--Preliminary Evaluation Study

Six samples of the control adipose tissue (lipid) were spiked with specified levels of brominated dioxins and furans by preparing three spike levels in duplicate. These levels were 10, 25, and 50 pg/g for the tetra and penta isomers; and 25, 62.5, and 125 pg/g for the hexa isomers. Two unspiked samples were also included as well as a method blank.

The results of the extraction and analysis of the brominated species from adipose tissue are given in Table 10. The average recoveries of the internal standard quantitation standards ranged from 59% to 140%. These recoveries were calculated versus the ^{13}C-HxCDD recovery standard.

Table 9. Mean RRFs for PBDDs and PBDFs From Initial HRGC/HRMS Calibration

Compound	n[a]	Mean RRF	RSD (%)	Calibration range (pg/µL)
^{13}C-2,3,7,8-TBDF	24	0.415	7.84	50
^{13}C-2,3,7,8-TBDD	24	0.374	7.32	50
^{13}C-1,2,3,7,8-PeBDF	24	0.127	13.7	50
2,3,7,8-TBDF	21	1.005	7.51	2.5-200
2,3,7,8-TBDD	21	0.953	10.2	2.5-200
1,2,3,7,8-PeBDF	19	0.930	8.55	5-200
1,2,3,7,8-PeBDD	20	0.750	11.1	5-200
1,2,3,4,7,8-HxBDF	15	0.110	14.4	10-500
1,2,3,4,7,8-HxBDD	15	0.105	19.3	10-500

[a]n = Number of calibration standards for which all qualitative criteria were met. A total of eight calibration standards (CS1 to CS8) were analyzed in triplicate.

Table 10. Recovery of Brominated Dioxins and Furans From Adipose Tissue

		Compound								
		^{13}C-TBDF	^{13}C-TBDD	^{13}C-PeBDF	TBDF	TBDD	PeBDF	PeBDD	HxBDF	HxBDD
	Spike level (pg/g)	50	50	50	-	-	-	-	-	-
Unspiked Adipose	AD MX A (pg/g)	31	21	32	ND (< 5)[a]	ND (< 5)	ND (< 5)	ND (< 10)	ND (< 25)	ND (< 25)
	AD MX B (pg/g)	44	38	77	ND (< 5)	ND (< 5)	ND (< 5)	ND (< 10)	ND (< 25)	ND (< 25)
	Average recovery (%)	76	59	110	-	-	-	-	-	-
	Spike level (pg/g)	50	50	50	10	10	10	10	25	25
Low Spike Level	AD-1-A (pg/g)	35	28	35	11	11	11	ND (< 10)	ND (< 25)	ND (< 25)
	AD-1-B (pg/g)	39	31	37	11	11	11	ND (< 10)	ND (< 25)	ND (< 25)
	Average recovery (%)	74	60	72	110	110	110	-	-	-
	Spike level (pg/g)	50	50	50	25	25	25	25	62.5	62.5
Medium Spike Level	AD-1-C (pg/g)	51	35	56	26	27	26	27	ND (< 25)	16
	AD-2-A (pg/g)	b	b	b	b	b	31	29	30	29
	Average recovery (%)	100	70	110	110	110	110	110	49	37
	Spike level (pg/g)	50	50	50	50	50	50	50	125	125
High Spike Level	AD-2-B (pg/g)	55	46	65	48	53	51	57	35	34
	AD-2-C (pg/g)	62	46	79	52	58	52	48	37	47
	Average recovery (%)	120	92	140	100	110	100	100	29	32

[a]Not detected. Estimated detection limit in parentheses.
[b]^{13}C-HxCDD recovery standard, ^{13}C-TBDD, $^{13}C_{12}$-TBDF, TBDD, and TBDF not observed.

The average recovery of the TBDD, TBDF, and PeBDF isomers ranged from 105% to 110% with a relative standard deviation of less than 7% per compound, not including sample AD-2-A. The average recovery of the native pentabromodioxin over the medium and high spiked levels was 109% with a relative standard deviation of 9%. The hexa isomers showed low recoveries at all levels. It is expected that this is a function of their high molecular weight, solubility, low volatility, and low RRF (lack of appropriate IQS).

In one sample spiked at the medium (25/62.5 pg/g) spike level, sample AD-2-A, the recovery standard, the tetrabromodioxin and furan IQSs, and the native tetrabromodioxins and furans were not observed. The reason for this was unclear. The penta and hexa native isomers were observed, and recoveries were calculated versus the pentabromofuran IQS.

B. Analysis Results for the FY1987 NHATS Composites

A total of 48 composite samples from the FY1987 NHATS specimens and 20 quality control samples were analyzed for PBDDs and PBDFs. The targeted 2,3,7,8-substituted PBDDs and PBDFs were not detected in any of the samples except those prepared as spiked QC materials. The detection limits calculated for the tetrabromo congeners in the samples ranged from 0.4 to 8.9 pg/g. The detection limits for the higher brominated compounds were typically greater than that observed for the tetrabrominated compounds. Table 11 provides a summary of the detection limits determined for each of the targeted PBDDs and PBDFs. Although the data are reported for selected isomers (which were the only available standards), there were no indications of the presence of other PBDDs or PBDFs of the same degree of bromination.

Although PBDDs and PBDFs were not detected, there were several instances in which a response for both characteristic ions for 2,3,7,8-TBDF were noted at greater than 2.5 times the observed background noise. In several of these instances the ion ratios for the integrated peaks were within the qualitative criteria specified in the QAPP for acceptance as a positive identification. However, upon closer evaluation of the data, it was apparent that in some cases, the integration for the characteristic ions was not coincident, and in other cases the peak shapes indicated potential interference to the determination of the 2,3,7,8-TBDF. For these reasons these responses were reported as not detected values, and the measured responses were used to calculate the reported limit of detection.

Figure 1 provides an example of a possible 2,3,7,8-TBDF response noted for a specific sample, NHATS Composite No. ACD8700265. In this sample, three characteristic ions for TBDF have been plotted along with one characteristic ion for the $^{13}C_{12}$-2,3,7,8-TBDF internal quantitation standard. The three characteristic ions for TBDF include m/z 482 and m/z 484 from the molecular cluster, and m/z 375 which reflects the loss of COBr from the molecular ion. The ion m/z 494 is characteristic of the $^{13}C_{12}$-2,3,7,8-TBDF.

Table 11. Detection Limit Summary

		DETECTION LIMIT (pg/g)					
MRI ID	Field ID	2,3,7,8-TBDF	2,3,7,8-TBDD	1,2,3,7,8-PeBDF	1,2,3,7,8-PeBDD	1,2,3,4,7,8-HxBDF	1,2,3,4,7,8-HxBDD
16254	ACD8700247	1.7	0.46	4.3	4.9	31	5.1
16255	ACD8700425	1	0.69	3.3	3	19	4.6
16257	ACD8700023	2.1	0.72	7.5	2.3	40	8.4
16258	ACD8700318	2.2	0.51	3.1	3.2	30	3.9
16259	ACD8700112	0.73	0.5	5.8	4.7	53	7
16260	ACD8700069	1.16	0.39	2.9	1.5	27	7.1
16261	ACD8700461	2.9	0.58	6.9	6.5	94	28
16262	ACD8700194	1.5	0.82	5.1	6.2	25	14
16263	ACD8700390	1.1	0.94	1.7	2.5	7.6	3.8
16264	ACD8700176	1.1	1.2	3	2.2	27	7.2
16267	ACD8700256	0.74	1.3	2.4	3	7.6	3.8
16268	ACD8700470	1.33	0.91	3	3.3	27	6.1
16269	ACD8700283	1	0.95	7.6	6.3	29	12
16270	ACD8700087	0.86	0.79	2.7	1.2	22	4
16272	ACD8700489	1.7	1.5	2.1	3.1	18	4.3
16274	ACD8700096	1.2	1.2	5.3	6.3	22	8.8
16275	ACD8700416	3.9	0.94	4.3	3.4	30	6.3
16276	ACD8700014	1.4	0.9	6.7	11	54	18
16277	ACD8700274	0.95	0.87	2.6	3.6	37	14
16279	ACD8700185	1.4	0.8	17	21	49	33
16280	ACD8700292	1.1	1.4	8.7	5.9	35	14
16281	ACD8700354	1.7	1.1	11	12	80	30
16282	ACD8700434	1.6	1.1	13	6.6	92	29
16283	ACD8700309	2.1	1.7	13	15	37	34
16284	ACD8700238	0.95	0.66	9.5	12	68	37
16286	ACD8700381	1.7	0.8	4.1	8.4	24	9.5
16288	ACD8700210	1.5	0.89	4.8	7.1	42	9
16289	ACD8700167	8.9	1.1	16	8.9	86	13
16291	ACD8700078	3.3	1.1	6.6	6.7	33	13
16293	ACD8700201	2.5	1.6	30	21	63	30
16294	ACD8700130	4	0.88	12	12	63	15
16295	ACD8700103	1.9	1.4	5.8	16	36	13
16297	ACD8700158	2.8	1.6	12	9.5	52	17

Table 11 (Concluded)

MRI ID	Field ID	DETECTION LIMIT (pg/g)					
		2,3,7,8-TBDF	2,3,7,8-TBDD	1,2,3,7,8-PeBDF	1,2,3,7,8-PeBDD	1,2,3,4,7,8-HxBDF	1,2,3,4,7,8-HxBDD
16299	ACD8700345	3.6	1.1	15	8.5	120	15
16300	ACD8700149	5.1	1.7	24	3.6	97	54
16301	ACD8700032	4.8	4.2	14	9.9	90	24
16302	ACD8700041	2.1	0.75	10	11	97	19
16304	ACD8700229	2.5	1.3	0.83	12	2.5	1.2
16306	ACD8700363	1.2	0.7	4.7	3.7	44	6.3
16308	ACD8700121	3.2	0.79	5.7	6.3	33	13
16309	ACD8700265	3.8	0.54	13	11	53	28
16310	ACD8700372	1.8	1.1	14	8.8	27	9
16311	ACD8700050	1.9	0.67	4.7	1.7	38	6.1
16313	ACD8700452	1.3	1.1	4.8	5.4	20	12
16314	ACD8700327	1.6	0.81	5.3	4	37	9.4
16315	ACD8700443	3	1.1	14	11	35	11
16316	ACD8700336	0.86	0.59	7.6	6.2	39	6.7
16317	ACD8700407	1.9	1.2	4.8	4.7	44	8.3

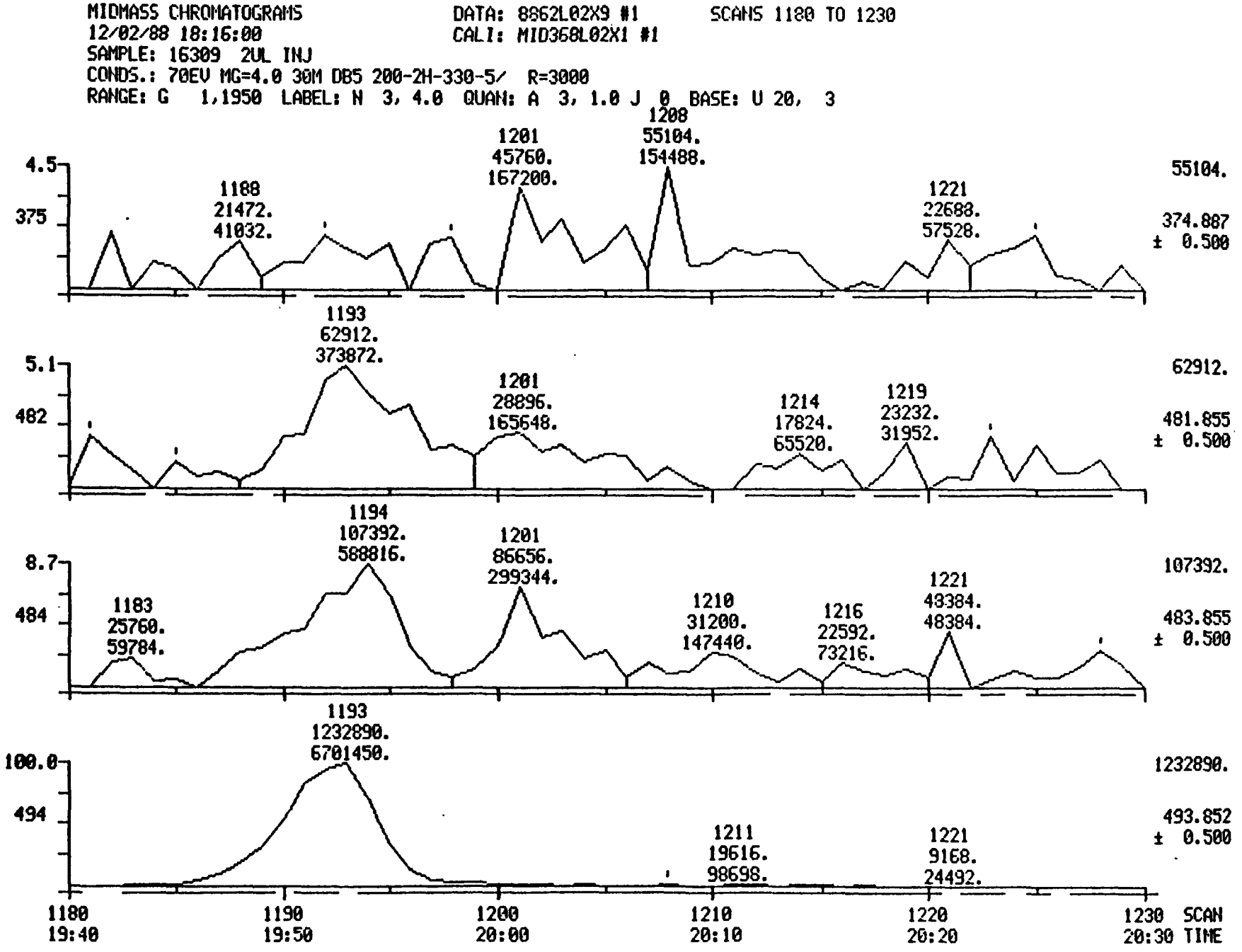

Figure 1. Ion plots from the analysis of sample 16309 (ACD8700265) for 2,3,7,8-TBDF. Ions monitored include: the molecular cluster was m/z 482 and 484; the M-COBr fragment of m/z 375; and a characteristics ion (m/z 494) from the $^{13}C_{12}$-2,3,7,8-TBDF internal standard.

Although the ratios of m/z 482/484 by both peak area and peak height meet the qualitative criteria (0.54 to 0.82) for identification as the TBDF, the peak shapes do not maximize at the same scan, the peaks are obviously not integrated over an equivalent window, and there is a lack of response at m/z 375 to support confirmation. Therefore, 2,3,7,8-TBDF was determined to be not detected in the sample, and the limit of detection was calculated at 3.8 pg/g. Other samples for which this type of response was noted included:

MRI bar code	NHATS code
16260	ACD8700069
16261	ACD8700461
16275	ACD8700416
16276	ACD8700014
16283	ACD8700309
16289	ACD8700167
16308	ACD8700121
16309	ACD8700265
16310	ACD8700372
16311	ACD8700050

Figures 2 through 5 provide examples of the total reconstructed ion chromatogram (RIC) and the ion plots from the analysis of tetra- through hexabrominated dioxins and furans for a specific composite sample.

The data presented in these figures are typical of the responses noted for the other composite adipose tissue samples. Although PBDDs and PBDFs were not detected, several peaks that are shaded in Figures 3 through 5 warrant further consideration. These responses appear to arise from hexabromo- (m/z 644, Figure 3), heptabromo- (m/z 722, Figure 4), and octabromo- (m/z 802, Figure 5) diphenyl ethers. Fragment losses of two bromines from the brominated diphenyl ethers would yield an ion species with exact masses consistent with the corresponding brominated furans. In each case there is also considerable response to an ion that corresponds to the fragment losses of COBr from each of the potential brominated diphenyl ether (PBDPE) responses.

Table 12 lists the estimated concentrations of the PBDPE in the samples. These concentrations were determined by using the furan ion responses and RRFs to calculate the DPE concentration when there was a coeluting ion at 162 amu higher than the furan masses. These are estimates to one significant figure only. Estimates are given for the composite samples only, although a similar pattern of PBDPEs was observed in the control lipid and for spikes and controls. No PBDPEs were observed in the method blanks.

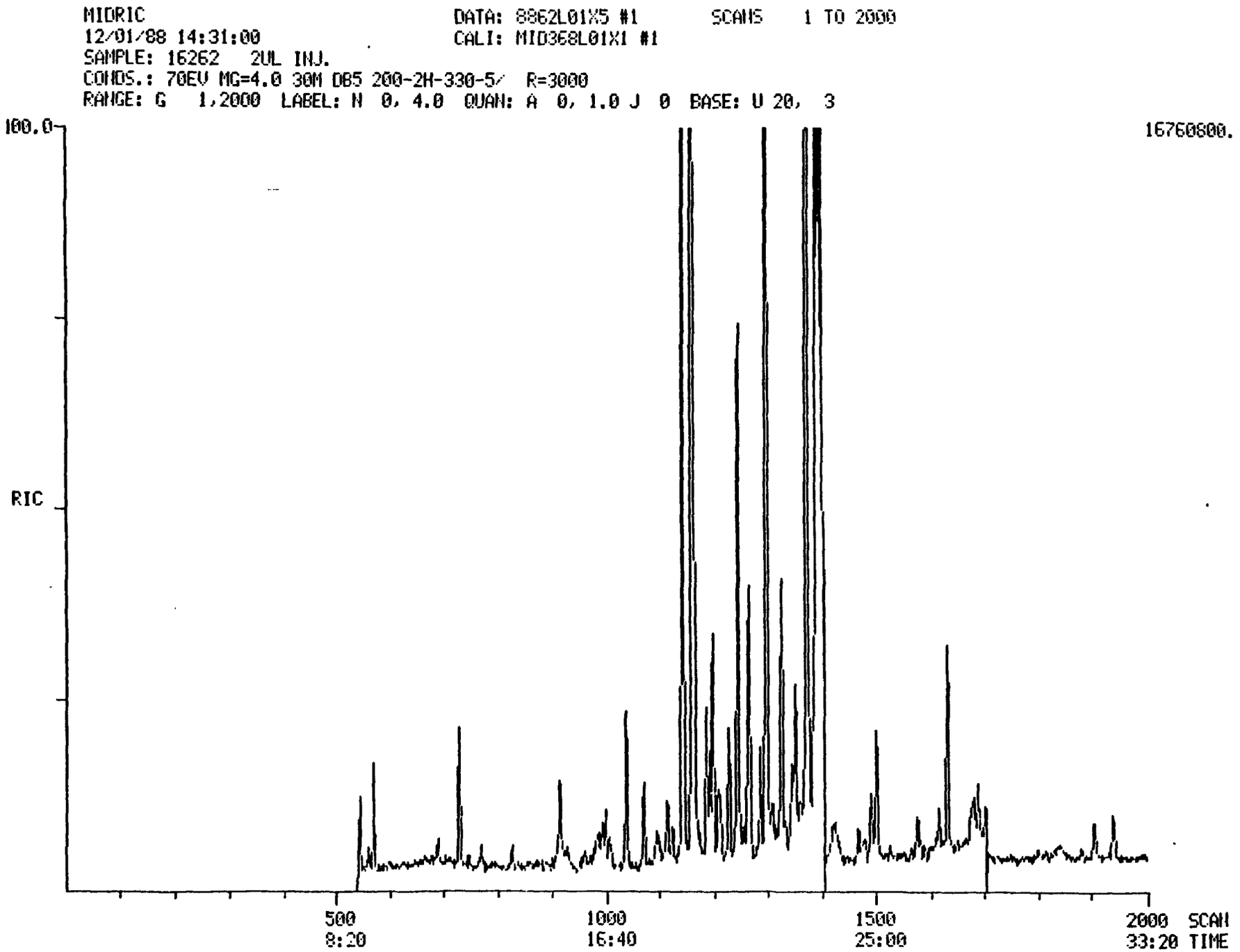

Figure 2. Reconstructed ion chromatogram (RIC) from the analysis of sample 16262 (ACD8700194) for tetra- through hexabromo dioxins and furans.

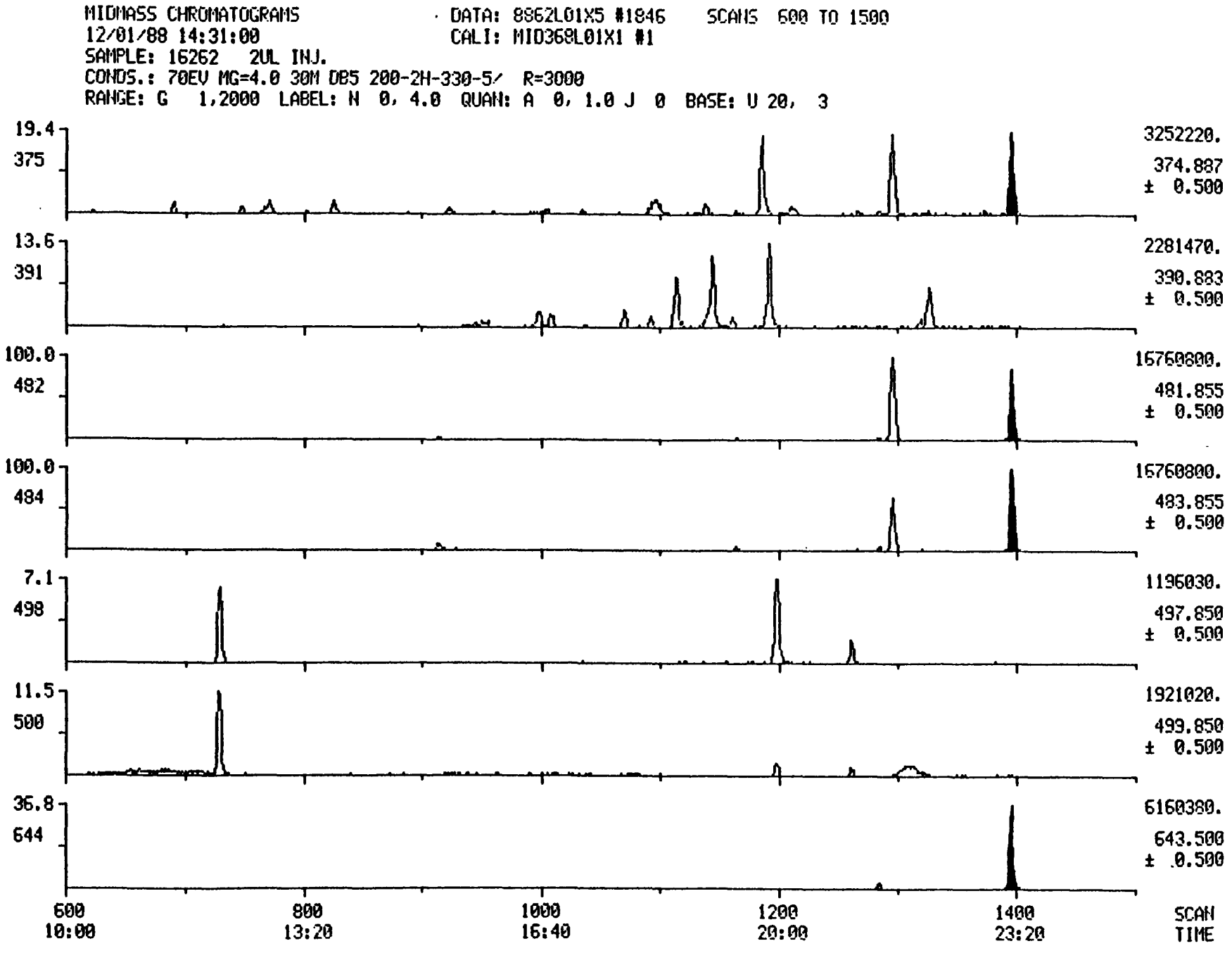

Figure 3. Ion plots from the analysis of sample 16269 (ACD8700194) for tetrabromo dioxins and furans. Ions monitored include m/z 375, 482, and 484 for TBDF; m/z 391, 498, and 500 for TBDD; and m/z 644 for HxBDPE. The shaded peaks represent response due to a suspected HxBDPE.

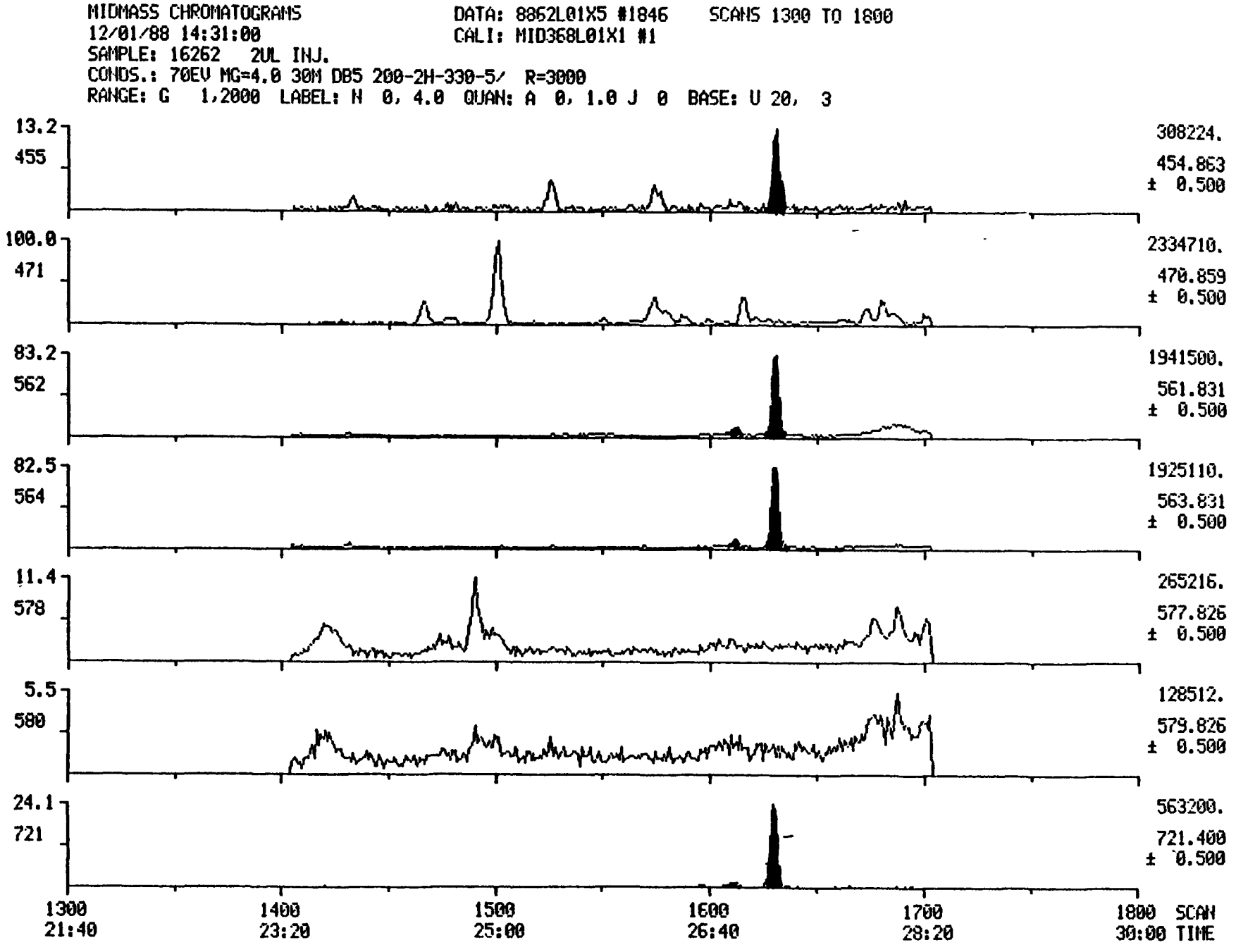

Figure 4. Ion plots from the analysis of sample 16262 (ACD8700194) for pentabromo dioxins and furans. Ions monitored include m/z 455, 562, and 564 for PeBDF; m/z 471, 578, and 580 for PeBDD; and m/z 721 for HpBDPE. The shaded peaks represent response due to a suspected HpBDPEs.

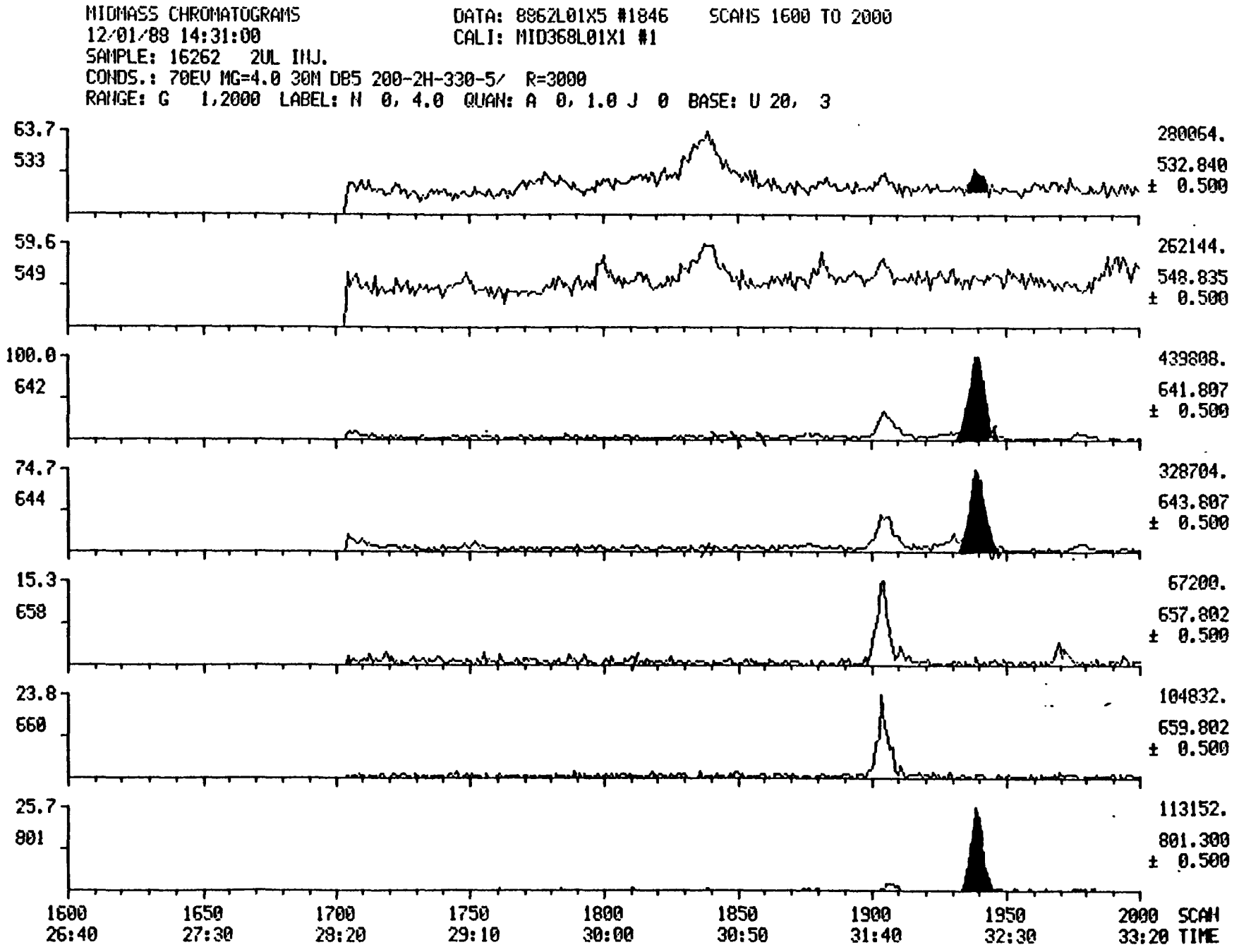

Figure 5. Ion plots from the analysis of sample 16262 (ACD8700194) for hexabromo dioxins and furans. Ions monitored include m/z 533, 642, and 644 for HxBDF; m/z 549, 658, and 660 for HxBDD; and m/z 801 for OBDPE. The shaded peaks represent response due to a suspected OBDPE.

Table 12. Estimated Bromodiphenyl Ether Concentrations (pg/g) in NHATS FY87 Composites

MRI ID	Field ID	HxBDPE	HpBDPE	OBDPE
16254	ACD8700247	200	200	ND
16255	ACD8700425	ND (a)	100	400
16257	ACD8700023	700	200	800
16258	ACD8700318	300	200	800
16259	ACD8700112	8	200	ND
16260	ACD8700069	200	90	200
16261	ACD8700461	20	200	100
16262	ACD8700194	NA (b)	NA	NA
16263	ACD8700390	200	40	200
16264	ACD8700176	400	300	2000
16267	ACD8700256	ND	50	ND
16268	ACD8700470	5	100	600
16269	ACD8700283	ND	80	ND
16270	ACD8700087	300	200	600
16272	ACD8700489	700	100	400
16274	ACD8700096	500	200	400
16275	ACD8700416	600	90	100
16276	ACD8700014	4	300	8000
16277	ACD8700274	2	100	ND
16279	ACD8700185	300	50	ND
16280	ACD8700292	200	40	200
16281	ACD8700354	7	100	600
16282	ACD8700434	10	400	ND
16283	ACD8700309	ND	100	ND
16284	ACD8700238	ND	30	ND
16286	ACD8700381	300	100	400
16288	ACD8700210	ND	100	400
16289	ACD8700167	600	300	3000
16291	ACD8700078	1000	60	ND
16293	ACD8700201	100	1	ND
16294	ACD8700130	900	400	3000
16295	ACD8700103	200	200	400
16297	ACD8700158	20	200	ND
16299	ACD8700345	ND	300	ND
16300	ACD8700149	ND	50	300
16301	ACD8700032	10	70	ND
16302	ACD8700041	ND	100	700
16304	ACD8700229	ND	3	ND
16306	ACD8700363	9	200	100
16308	ACD8700121	30	200	ND
16309	ACD8700265	500	2000	3000
16310	ACD8700372	ND	200	ND
16311	ACD8700050	ND	200	600
16313	ACD8700452	ND	200	ND
16314	ACD8700327	10	70	ND
16315	ACD8700443	900	30	70
16316	ACD8700336	500	400	2000
16317	ACD8700407	600	100	1000

(a) - ND= Not Detected.
(b) - NA= Data Not Available.

The values presented in Table 12 are considered estimates and most likely lower concentration estimates since: (1) the sample preparation procedures were developed to remove gross interferences such as diphenyl ethers, and the extent of sample cleanup is not known; and (2) the calculation of the concentrations were based on the recovery of the carbon-13 PBDFs which are expected to recover at a different level. The effort to provide an estimate of the concentration, however, provides some indication of the range of concentration that these compounds are present.

Future sample analysis efforts for the determination of PBDPEs should include experiments that exclude the carbon column cleanup from sample preparation. This should provide a better indication of actual PBDPE concentrations in human tissues, especially if carbon-13-labeled PBDPEs are available as internal quantitation standards.

Although no PBDDs and PBDFs were detected in these analysis efforts, further studies regarding background levels of these compounds warrant some changes in the HRMS monitoring techniques. Specifically, these differences are based on recent reports in the literature[9,10] that demonstrate that there is considerable potential for the overlap of PBDPEs which have one bromine more than PBDFs which elute within the same retention window. Since there are potentially 209 PBDPE isomers, it may be possible that there is some overlap of homologs. Although the COBr fragment provides valuable confirmational information, its relative response to the molecular ion clusters is small and may not be detected unless the compound is present at very high concentration levels. Given these considerations, it may be more practical to monitor ions which represent PBDPEs which are one and two bromines greater than the target PBDFs. Having raised these considerations, however, the authors of this report must stress that there was no evidence in the raw data to invalidate the results reported.

IV. QUALITY CONTROL

This section provides a summary of the quality control data that were generated as part of the analysis effort for the FY 1987 NHATS composites. Summary data are provided for calibration efforts, internal quantitation standard recoveries, control lipid analyses, and spiked sample analyses.

A. Internal Quantitation Standard Recoveries

The recoveries of the IQS compounds are considered as a measure of method performance from sample to sample.

In the total of the 68 samples, controls, spikes, and method blanks, the ^{13}C-2,3,7,8-TBDF IQS was out of the targeted data quality objective (DQO) of 40% to 150% recovery in six samples. Recovery of the ^{13}C-2,3,7,8-TBDD IQS was out of the targeted DQO in 18 of the samples and the ^{13}C-1,2,3,7,8-PeBDF IQS was out of the targeted DQOs in 16 of the samples.

The recoveries of the ^{13}C-TBDF and ^{13}C-TBDD, which were outside the DQOs, were always less than 40%. While the recoveries for the $^{13}C_{12}$-PeBDF

outside of the DQOs were both greater than 150% in some samples and less than 40% in others. These recoveries may be due in part to recovery through the analytical procedure or as a result of pairing the IQS versus the recovery standard.

The calculated recovery of the IQS for the brominated species were affected by the responses observed for the recovery standard (RS, 1,2,3,7,8,9-HxCDD). Responses for the recovery standard were often saturated in the adipose tissue extracts although the daily standards consistently showed normal response.

Previous method development work conducted to assess the recovery of the brominated species from adipose tissue also showed a consistently high response of the RS in the sample extract compared to the daily standard. Reanalysis of these samples showed identical results. The cause of this phenomenon was not determined. No reanalyses were conducted since there were no apparent responses to the PBDDs and PBDFs. Samples for which recoveries were less than 40% would have required recompositing and preparation of the samples. Further method evaluation is necessary to address the difficulties encountered in pairing of the IQS compounds versus a more appropriate recovery standard.

Detailed plots of the recoveries of each internal quantitation standard versus an index number corresponding to the laboratory identification number (Table 13) are presented as Figures 6 through 8.

B. Calibration

Although calibration curves were established prior to the method evaluation study, it was necessary to conduct additional verification of the RRF values prior to analysis of the composites.

The RRFs from these calibrations are given in Table 14 in the order of analysis. Upon reviewing the data in Table 14 versus the initial evaluation (Table 9), it is noted that there are considerable differences in the mean RRF values for the ^{13}C internal standards and the heptabromo compounds between the two sets of calculations. However, the RRF values for the tetra- and pentabrominated compounds are within ±10% of the two calibration events. This difference is possibly due to the pairing of the standards versus the appropriate recovery or internal quantitation standard. The variability in the measured RRFs appears to be a function of the relative retention time (RRT) of the standards versus the corresponding internal quantitation or recovery standards. For example, PeBDD, PeBDF, HxBDD, and HxBDF are each measured relative to the $^{13}C_{12}$-PeBDF. The % RSD for the pentabrominated compounds are less than that observed for the hexabrominated compounds. The $^{13}C_{12}$-PeBDF elutes coincidentally with the unlabeled PeBDF, and the % RSD of the RRF is less than 10% across the calibration range. Likewise, the PeBDD elutes very close to this IQS. However, both the HxBDD and HxBDF elute several minutes from the IQS. This lower sensitivity of the HRMS analysis and the differences in RRT lead to greater variability in the measured RRF values.

Table 13. Index to Figures 6, 7, and 8 and Order of Sample Analysis

Index	MRI ID	Field ID	Mass Spec. File
1	16251	Method Blank	8862K30X2
2	16252	S1	8862K30X3
3	16269	ACD8700283	8862K30X4
4	16310	ACD8700372	8862K30X5
5	16267	ACD8700256	8862K30X6
6	16313	ACD8700452	8862K30X7
7	16304	ACD8700229	8862K30X8
8	16283	ACD8700309	8862K30X9
9	16260	ACD8700069	8862L01X2
10	16256	C	8862L01X3
11	16253	S2	8862L01X4
12	16262	ACD8700194	8862L01X5
13	16317	ACD8700407	8862L01X6
14	16280	ACD8700292	8862L01X7
15	16265	Method Blank	8862L01X8
16	16279	ACD8700185	8862L01X9
17	16291	ACD8700078	8862L01X10
18	16271	S1	8862L01X11
19	16274	ACD8700096	8862L01X12
20	16315	ACD8700443	8862L01X13
21	16294	ACD8700130	8862L01X14
22	16273	S2	8862L01X15
23	16270	ACD8700087	8862L02X2
24	16272	ACD8700489	8862L02X3
25	16295	ACD8700103	8862L02X4
26	16266	C	8862L02X5
27	16286	ACD8700381	8862L02X6
28	16263	ACD8700390	8862L02X7
29	16278	Method Blank	8862L02X8
30	16309	ACD8700265	8862L02X9
31	16290	S2	8862L02X10
32	16287	C	8862L02X11
33	16264	ACD8700176	8862L02X12
34	16275	ACD8700416	8862L02X13
35	16300	ACD8700149	8862L05X2
36	16311	ACD8700050	8862L05X3
37	16268	ACD8700470	8862L05X4
38	16288	ACD8700210	8862L05X5
39	16255	ACD8700425	8862L05X6
40	16306	ACD8700363	8862L05X7
41	16285	S1	8862L05X8
42	16254	ACD8700247	8862L05X9
43	16292	Method Blank	8862L05X10
44	16299	ACD8700345	8862L05X11
45	16314	ACD8700327	8862L05X12
46	16259	ACD8700112	8862L05X13
47	16301	ACD8700032	8862L05X14
48	16297	ACD8700158	8862L05X15
49	16298	S2	8862L06X2
50	16296	C	8862L06X3
51	16303	S1	8862L06X4
52	16276	ACD8700014	8862L06X5
53	16302	ACD8700041	8862L06X6
54	16281	ACD8700354	8862L06X7
55	16284	ACD8700238	8862L06X8
56	16305	Method Blank	8862L06X9
57	16261	ACD8700461	8862L06X10
58	16318	S1	8862L06X11
59	16277	ACD8700274	8862L06X12
60	16282	ACD8700434	8862L06X13
61	16312	S2	8862L06X14
62	16308	ACD8700121	8862L06X15
63	16307	C	8862L06X16
64	16257	ACD8700023	8862L07X2
65	16293	ACD8700201	8862L07X3
66	16316	ACD8700336	8862L07X4
67	16258	ACD8700318	8862L07X5
68	16289	ACD8700167	8862L07X6

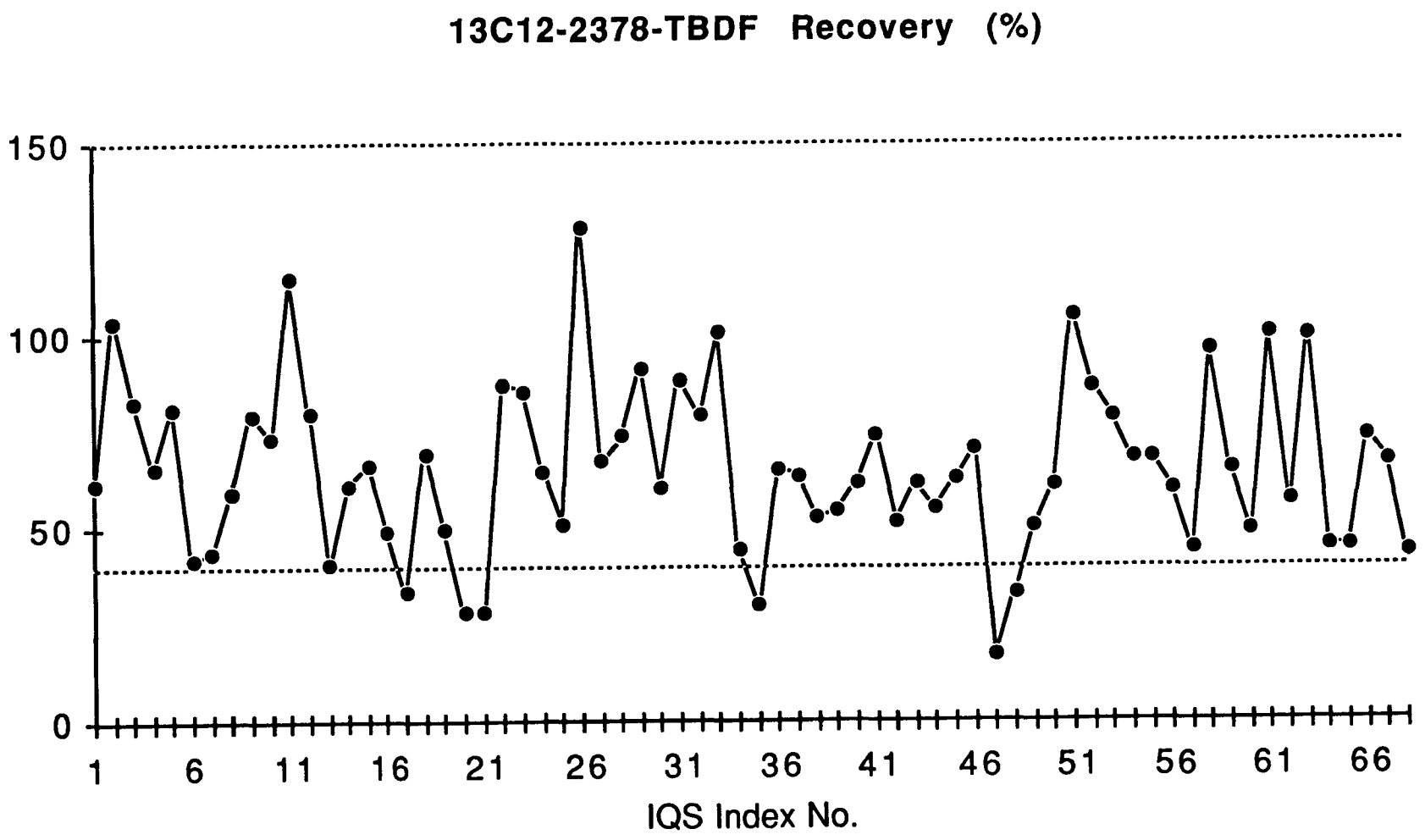

Figure 6. Recoveries of $^{13}C_{12}$-TBDF from the 48 NHATS composites and 20 QC samples. Table 13 provides a cross-reference for samples vs. index no.

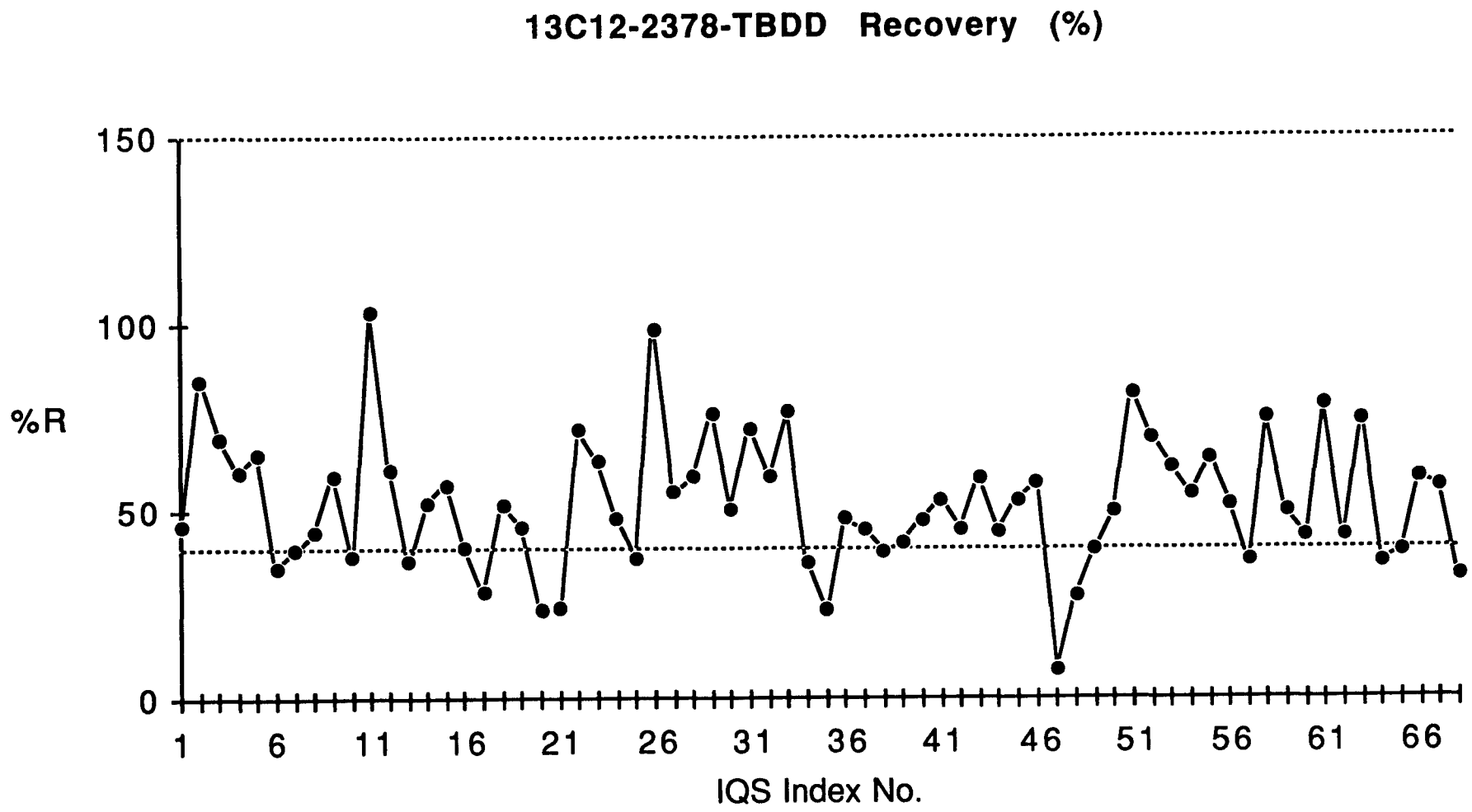

Figure 7. Recoveries of $^{13}C_{12}$-2,3,7,8-TBDF from the 48 NHATS composites and 20 QC samples. Table 13 provides a cross-reference for samples vs. index no.

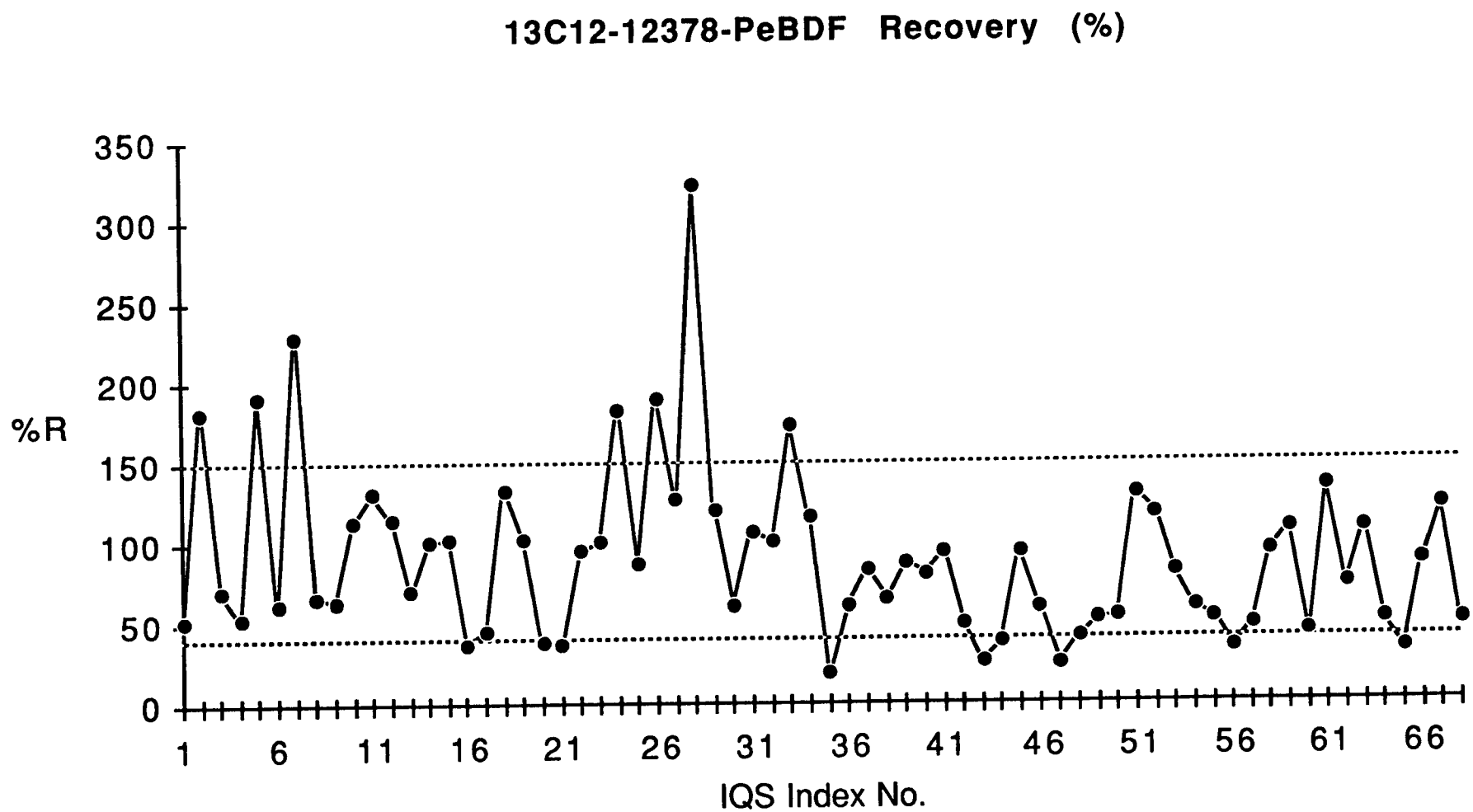

Figure 8. Recoveries of $^{13}C_{12}$-1,2,3,7,8-PeBDF from the 48 NHATS composites and 20 QC samples. Table 13 provides a cross-reference for samples vs. index no.

Table 14. Calibration Data--Relative Response Factor[a]

	RELATIVE RESPONSE FACTORS													
COMPOUND	CS6	CS5	CS4	CS3	CS2	CS1	CS6	CS5	CS4	CS3	CS2	CS1	MEAN	%RSD
13C-2,3,7,8-TBDF	0.217	0.231	0.241	0.266	0.283	0.301	0.249	0.277	0.277	0.271	0.278	0.304	**0.266**	10.1
13C-2,3,7,8-TBDD	0.185	0.204	0.206	0.212	0.245	0.257	0.217	0.219	0.216	0.230	0.230	0.267	**0.224**	10.4
13C-1,2,3,7,8-PeBDF	0.045	0.059	0.069	0.053	0.066	0.079	0.056	0.061	0.060	0.064	0.059	0.068	**0.062**	14.0
2,3,7,8-TBDF	0.977	0.898	1.103	1.023	1.109	1.133	1.023	0.959	0.981	1.104	1.111	1.138	**1.047**	7.6
2,3,7,8-TBDD	0.878	0.929	0.952	1.094	1.069	1.087	0.997	0.968	0.973	1.013	1.107	1.065	**1.011**	7.3
1,2,3,7,8-PeBDF	0.974	0.945	0.934	1.029	1.008	1.081	0.853	0.814	0.913	0.958	1.008	1.064	**0.965**	8.3
1,2,3,7,8-PeBDD	0.812	0.711	0.704	0.745	0.756	0.805	0.722	0.662	0.783	0.660	0.724	0.810	**0.741**	7.2
1,2,3,4,7,8-HxBDF	0.075	0.080	0.093	0.094	0.073	0.111	0.069	0.086	0.064	0.079	0.085	0.100	**0.084**	16.2
1,2,3,4,7,8-HxBDD	0.054	0.065	0.084	0.089	0.073	0.117	0.042	0.087	0.070	0.066	0.080	0.105	**0.078**	26.8

(a) - Calibration data generated prior to the analysis of the FY87 NHATS composite samples.

Other calibration criteria that were considered were mass calibration of the HRMS instrumentation used in the demonstration of mass resolution as greater than 3,000. These criteria were demonstrated on a daily basis during sample analysis.

The daily analysis of a CS5 calibration standard was conducted to demonstrate the QA objectives of maintaining variability with ±20% of the means for 2,3,7,8-TBDD and 2,3,7,8-TBDF and ±30% of the means for all other compounds. A summary of calibration events conducted during the sample analyses is given in Table 15. The analysis of the daily standards showed that the RRFs of the internal quantitation standards and the hexabromo compounds were difficult to keep within limits (±30%) which is associated with the pairing of IQS versus RS. It is anticipated that the difference in retention time between the recovery standard ($^{13}C_{12}$-HxCDD) and the penta- and hexa-substituted IQS standards contributes to greater variability in the measured RRF values.

C. Spiked Internal QC Samples

Each sample batch contains two lipid samples that were fortified with tetra- through hexabrominated PBDDs and PBDFs. These data were included as a means to demonstrate the accuracy of the method for determining the target compounds in adipose tissues.

The results of the analysis of the spiked control samples indicate that the accuracy of the method met the targeted DQOs of 40% to 150% in all but 8 out of 60 cases. These instances included only the hexa-substituted isomers. These data were considered outside the DQOs because of incorrect ion ratios (3 samples) and less than 40% but greater than 30% (3 samples). Two of the data points could not be calculated because of nonrecovery. The specifics of each case are summarized in Table 16. The results of the analysis of the spiked control samples (spiked lipid) are given in Tables 17 through 21. The native spike recoveries are graphically shown in Figures 9 and 10.

In general, the low recoveries experienced for the hexa-isomers may be attributed to the limited solubility and low volatility of the hexa-isomers. In addition, the RRFs for these compounds (calculated versus the ^{13}C-PeBDF) are extremely small, less than 0.1, which adversely contributes to the accuracy and precision measured for these higher brominated isomers.

D. Control QC Samples

The results of the analysis of the control samples (unspiked lipid) are given in Table 22 which provides a summary of the results from the analysis of the control lipid. PBDDs and PBDFs were not detected in any of the control samples.

E. Method Blanks

The method blanks for batches 1 through 5 did not contain any detectable PBDDs or PBDFs.

Table 15. Calibration Event Summary

File name	Date	Time	Calibration standard	Sample analysis batch	Comments	Corrective action
8862K30XQ3	11/30/88	11:52	CS5	1	^{13}C-1,2,3,7,8-PeBDF is -40%. 1,2,3,4,7,8-HxBDD is -39%.	
8862K30XQ4	11/30/88	21:39	CS2	1	RRFs good.	
8862L01XQ2	12/01/88	09:39	CS5	1/2	1,2,3,4,7,8-HxBDD is -46%.	
8862L01XQ3	12/01/88	21:48	CS2	1/2	^{13}C-1,2,3,7,8-PeBDF is +86%.	Rerun standard.
8862L01XQ4	12/01/88	22:25	CS2	1/2	RRFs good.	
8862L02XQ3	12/02/88	10:37	CS5	2/3		Retune m/z 631 for maximum sensitivity.
8862L02XQ4	12/02/88	11:51	CS5	2/3	RRFs good.	
8862L02XQ5	12/02/88	21:54	CS2	2/3	^{13}C-2,3,7,8-TBDF is +51%. ^{13}C-2,3,7,8-TBDD is +60%.	Rerun standard.
8862L02XQ6	12/02/88	22:34	CS2	2/3	^{13}C-2,3,7,8-TBDF is +62%. ^{13}C-2,3,7,8-TBDD is +71%. ^{13}C-1,2,3,7,8-PeBDF is +138%. 1,2,3,4,7,8-HxBDF is +67%. 1,2,3,4,7,8-HxBDD is +81%.	
8862L05XQ2	12/05/88	10:34	CS5	3/4	1,2,3,4,7,8-HxBDD is +70%.	
8862L05XQ3	12/05/88	22:15	CS2	3/4	^{13}C-1,2,3,7,8-PeBDF is +83%.	Rerun standard.

Table 15 (Concluded)

File name	Date	Time	Calibration standard	Sample analysis batch	Comments	Corrective action
8862L05XQ4	12/05/88	22:58	CS2	3/4	^{13}C-1,2,3,7,8-PeBDF is +41%.	
8862L06XQ2	12/06/88	09:27	CS5	4/5	RRFs good.	
8862L06XQ3	12/06/88	21:29	CS2	4/5	^{13}C-2,3,7,8-TBDF is +56%. ^{13}C-2,3,7,8-TBDD is +60%. ^{13}C-1,2,3,7,8-PeBDF is +195%. 1,2,3,4,7,8-HxBDF is +35%. 1,2,3,4,7,8-HxBDD is +55%.	Rerun standard.
8862L06XQ4	12/06/88	22:09	CS2	4/5	^{13}C-1,2,3,7,8-TBDF is +45%. ^{13}C-1,2,3,7,8-TBDD is +42%. 1,2,3,4,7,8-HxBDD is +38%.	
8862L07XQ2	12/07/88	07:25	CS5	5	1,2,3,4,7,8-HxBDF is -36%.	
8862L07XQ3	12/07/88	14:13	CS2	5	^{13}C-2,3,7,8-TBDF is +33%. ^{13}C-2,3,7,8-TBDD is +38%. ^{13}C-1,2,3,7,8-PeBDF is +68%.	

Table 16. Spike Recovery Summary for Samples Outside DQOs

Sample	Compound	Recovery	Explanation
S2--batch 2[a]	1,2,3,4,7,8-HxBDF	Not calculated	Ion ratio outside criteria
S1--batch 3	1,2,3,4,7,8-HxBDF	Not calculated	Ion ratio outside criteria
S2--batch 3	1,2,3,4,7,8-HxBDF	Not calculated	Ion ratio outside criteria
S1--batch 4	1,2,3,4,7,8-HxBDF	34%	Low recovery
	1,2,3,4,7,8-HxBDD	30%	Low recovery
S2--batch 4	1,2,3,4,7,8-HxBDD	39%	Low recovery
S1--batch 5	1,2,3,4,7,8-HxBDF	Not detected	-
	1,2,3,4,7,8-HxBDD	Not detected	-

[a]S1 = low level spike.
[b]S2 = high level spike.

Table 17. Batch 1 Spiked Sample Results

Compound	Control Amount Found (pg/g)	S1 Amount Found (pg/g)	S2 Amount Found (pg/g)	Spike Level 1 (pg/g)	Spike Level 2 (pg/g)	S1 % Recovery	S2 % Recovery
2378TBDF	ND (0.65)	27.8	51.5	25	50	111	103
2378TBDD	ND (1.0)	25.0	51.0	25	50	100	102
12378PeBDF	ND (1.4)	19.5	53.4	25	50	78	107
12378PeBDD	ND(2.5)	21.5	58.2	25	50	86	116
123478HxBDF	ND (7.6)	38.0	121	62.5	125	61	97
123478HxBDD	ND (4.5)	44.7	167	62.5	125	72	134

Note: Method accuracy, as % recovery, was calculated as follows:

$$\% \text{ Recovery} = \frac{\text{Amount found in spike} - \text{Amount found in control}}{\text{Spike level}} \times 100\%$$

Table 18. Batch 2 Spiked Sample Results

Compound	Control Amount Found (pg/g)	S1 Amount Found (pg/g)	S2 Amount Found (pg/g)	Spike Level 1 (pg/g)	Spike Level 2 (pg/g)	S1 % Recovery	S2 % Recovery
2378TBDF	ND (0.37)	25.2	48.0	25	50	101	96
2378TBDD	ND (0.58)	27.4	53.8	25	50	110	108
12378PeBDF	ND (3.2)	24.2	53.7	25	50	97	107
12378PeBDD	ND(4.7)	32.0	54.2	25	50	128	108
123478HxBDF	ND (10)	79.1	ND (69.0)	62.5	125	127	(a)
123478HxBDD	ND (5.2)	85.0	76.6	62.5	125	136	61

(a) - The ion ratio criteria in the S2 spike were not met for 123478HxBDF.

Note: Method accuracy, as % recovery, was calculated as follows:

$$\% \text{ Recovery} = \frac{\text{Amount found in spike} - \text{Amount found in control}}{\text{Spike level}} \times 100\%$$

Table 19. Batch 3 Spiked Sample Results

Compound	Control Amount Found (pg/g)	S1 Amount Found (pg/g)	S2 Amount Found (pg/g)	Spike Level 1 (pg/g)	Spike Level 2 (pg/g)	S1 % Recovery	S2 % Recovery
2378TBDF	ND (0.52)	25.1	49.1	25	50	100	98
2378TBDD	ND (0.69)	28.0	52.3	25	50	112	105
12378PeBDF	ND (3.2)	17.9	51.0	25	50	72	102
12378PeBDD	ND(3.0)	19.2	59.2	25	50	77	118
123478HxBDF	ND (20)	ND (29)	ND (98)	62.5	125	(a)	(a)
123478HxBDD	ND (17)	25.8	112	62.5	125	41	90

(a) - The ion ratio criteria in the S1 and S2 spikes were not met for 123478HxBDF.

Note: Method accuracy, as % recovery, was calculated as follows:

$$\% \text{ Recovery} = \frac{\text{Amount found in spike} - \text{Amount found in control}}{\text{Spike level}} \times 100\%$$

Table 20. Batch 4 Spiked Sample Results

Compound	Control Amount Found (pg/g)	S1 Amount Found (pg/g)	S2 Amount Found (pg/g)	Spike Level 1 (pg/g)	Spike Level 2 (pg/g)	S1 % Recovery	S2 % Recovery
2378TBDF	ND (0.87)	23.4	50.8	25	50	94	102
2378TBDD	ND (1.4)	27.2	47.4	25	50	109	95
12378PeBDF	ND (7.5)	23.1	59.1	25	50	92	118
12378PeBDD	ND(13)	26.8	54.1	25	50	107	108
123478HxBDF	ND (22)	TR (21.2)	TR (59.7)	62.5	125	34 (a)	48
123478HxBDD	ND (9.5)	TR (18.5)	TR (49.2)	62.5	125	30 (a)	39 (a)

(a) - Outside the targeted data quality objectives of 40 to 150% recovery.

Note: Method accuracy, as % recovery, was calculated as follows:

$$\% \text{ Recovery} = \frac{\text{Amount found in spike} - \text{Amount found in control}}{\text{Spike level}} \times 100\%$$

Table 21. Batch 5 Spiked Sample Results

Compound	Control Amount Found (pg/g)	S1 Amount Found (pg/g)	S2 Amount Found (pg/g)	Spike Level 1 (pg/g)	Spike Level 2 (pg/g)	S1 % Recovery	S2 % Recovery
2378TBDF	ND (0.59)	24.1	47.9	25	50	96	96
2378TBDD	ND (0.77)	25.8	51.5	25	50	103	103
12378PeBDF	ND (3.9)	23.5	47.8	25	50	94	96
12378PeBDD	ND(5.6)	22.6	52.6	25	50	90	105
123478HxBDF	ND (49)	ND (32)	98.2	62.5	125	NR (a)	79
123478HxBDD	ND (13)	ND (17)	129	62.5	125	NR (a)	103

(a) - No recovery. Outside the targeted data quality objectives of 40 to 150% recovery.

Note: Method accuracy, as % recovery, was calculated as follows:

$$\% \text{ Recovery} = \frac{\text{Amount found in spike} - \text{Amount found in control}}{\text{Spike level}} \times 100\%$$

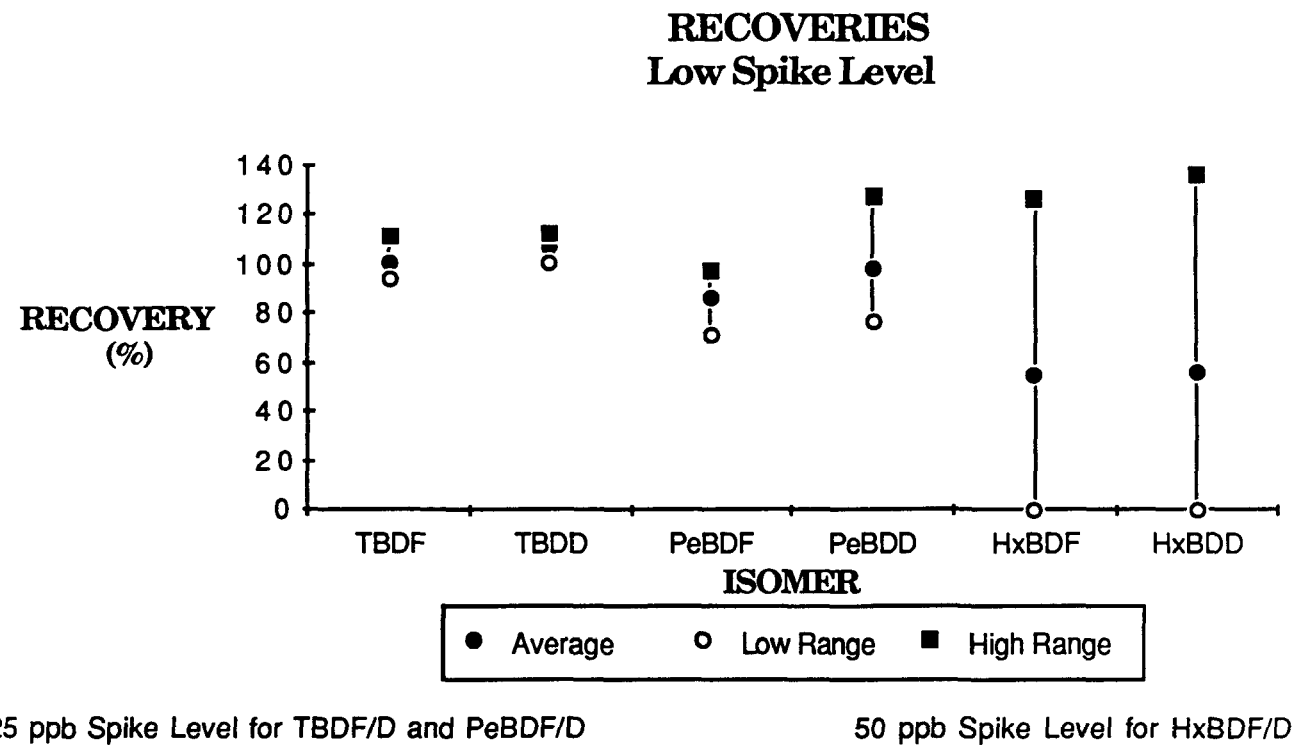

Figure 9. PBDD/PBDF recoveries from quality control samples (low spike level).

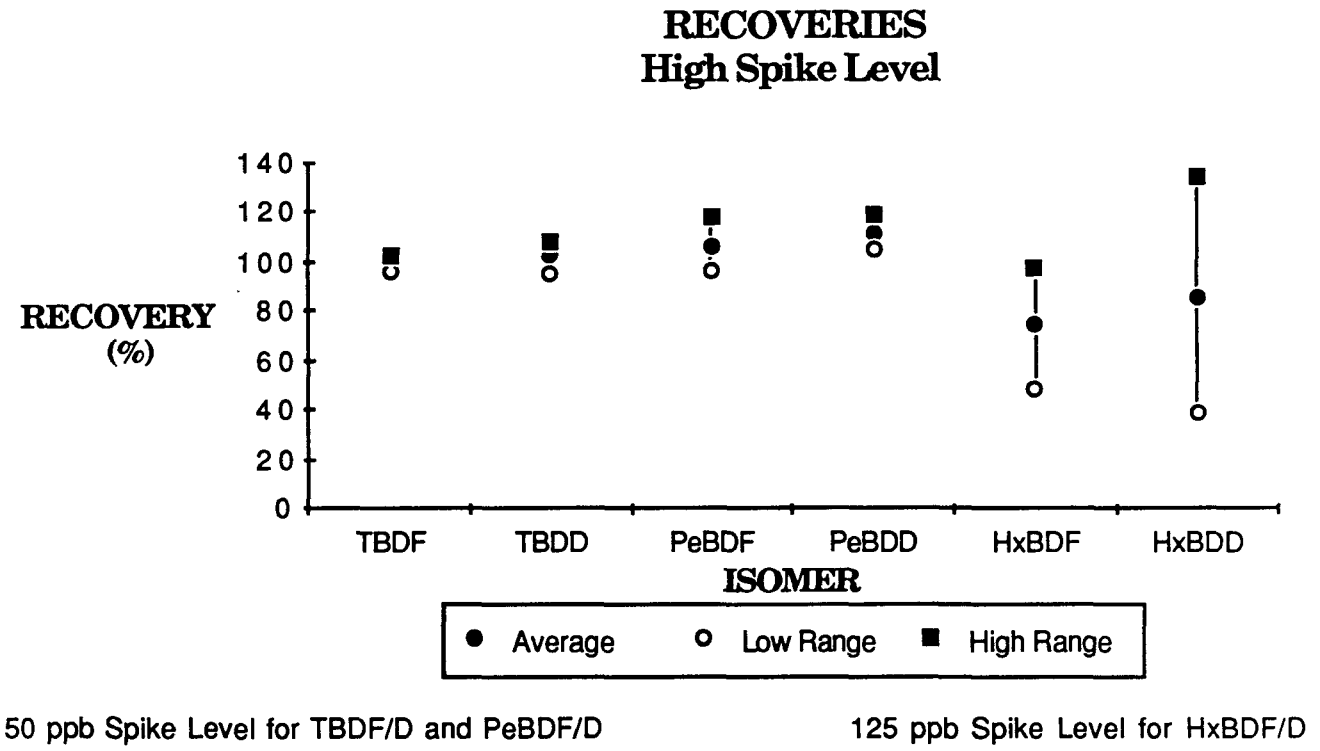

Figure 10. PBDD/PBDF recoveries from quality control samples (high spike level).

Table 22. Control Sample Results

Compound	Batch1	Batch 2	Batch 3	Batch 4	Batch 5
	Amount Found (pg/g)	Amount Found (pg/g)	Amount Found (pg/g)	Amount Found (pg/g)	Amount Found (pg/g)
2378TBDF	ND (0.65)	ND (0.37)	ND (0.52)	ND (0.87)	ND (0.59)
2378TBDD	ND (1.0)	ND (0.58)	ND (0.69)	ND (1.4)	ND (0.77)
12378PeBDF	ND (1.4)	ND (3.2)	ND (3.2)	ND (7.5)	ND (3.9)
12378PeBDD	ND(2.5)	ND(4.7)	ND(3.0)	ND(13)	ND(5.6)
123478HxBDF	ND (7.6)	ND (10)	ND (20)	ND (22)	ND (49)
123478HxBDD	ND (4.5)	ND (5.2)	ND (17)	ND (9.5)	ND (13)

F. Tridecane Blanks

Tridecane blanks were analyzed daily to confirm that carryover from the injection of standards was not a problem and to demonstrate a clean analytical system prior to the analysis of samples. These analyses demonstrated no response to the PBDDs, PBDFs, or the internal quantitation standards.

V. ASSESSMENT OF METHOD PERFORMANCE

Control lipid and spiked lipid samples were included in each of the five batches to assess the method accuracy and precision. This assessment has been conducted through plots of measured vs. spiked concentrations, calculation of descriptive statistics, and regression analysis.

A. Assignment of Data Values

For each target chemical, 15 data points were generated. These data points were obtained from the analysis of one control and two spiked lipid QC samples within each of the five sample batches. The analytical results were treated as follows in subsequent computations.

1. For observations where all qualitative identification criteria were met and the response exceeded the limit of detection, the reported concentration was used as the measured concentration (i.e., positive quantifiable and trace values).

2. For data points where the response was below the limit of detection (i.e., not detected values), one-half of the reported limit of detection was used as the value in the regression analysis.

3. For observations where the response exceeded the limit of detection but all the qualitative identification criteria were not met, the data points were treated as nonresponses and were not included in the analysis. (For example, ion ratio criteria not met.) This occurred in three cases for hexabromo furan--once at the low spike level and twice at the high spike level.

B. Descriptive Statistics

Descriptive statistics for the six PBDD and PBDF compounds are presented in Tables 23 and 24, showing control and spiked lipid sample results, respectively. No PBDDs or PBDFs were detected in the control lipid sample. Table 23 presents the average limit of detection, the standard deviation of the limits of detection, and the relative standard deviation based on the five control lipid samples. The relative standard deviation, calculated as 100 x standard deviation/mean is a measure of the variability of the five measurements around their mean. The average detection limits increase as the degree of bromination increases. The same pattern holds for the standard deviations; however, it is less pronounced when comparing the relative standard deviations. This general pattern is reflective of the relative sensitivities of HRMS to each degree of bromination.

Table 23. Descriptive Statistics for Control Lipid Samples

Compound	Number of Samples	Mean LOD (pg/g)	Standard Deviation of LODs (pg/g)	Relative Standard Deviation (%)
2378-TBDF	5	0.600	0.184	30.7
2378-TBDD	5	0.888	0.325	36.6
12378-PeBDF	5	3.84	2.25	58.5
12378-PeBDD	5	5.76	4.24	73.6
123478-HxBDF	5	21.7	16.5	75.8
123478-HxBDD	5	9.84	5.28	53.6

Table 24. Descriptive Statistics for Spiked Lipid QC Samples

Compound	Number of Samples	Spiked Concentration (pg/g)	Mean of Measured Concentrations (pg/g)	Relative Error *1 (%)	Standard Deviation of Measured Concentrations (pg/g)	Relative Standard Deviation (%)
Low Spike Samples						
2378-TBDF	5	25	25.1	0.4	1.67	6.7
2378-TBDD	5	25	26.7	6.8	1.24	4.6
12378-PeBDF	5	25	21.6	-13.6	2.77	12.8
12378-PeBDD	5	25	24.4	-2.4	5.05	20.7
123478-HxBDF *2	4	62.5	38.6	-38.2	28.6	74.1
123478-HxBDD	5	62.5	36.5	-41.6	30.2	82.7
High Spike Samples						
2378-TBDF	5	50	49.5	-1.0	1.63	3.3
2378-TBDD	5	50	51.2	2.4	2.37	4.6
12378-PeBDF	5	50	53.0	6.0	4.15	7.8
12378-PeBDD	5	50	55.7	11.4	2.87	5.2
123478-HxBDF *3	3	125	93.0	-25.6	31.0	33.3
123478-HxBDD	5	125	107	-14.4	45.8	42.8

1: Relative Error (%) = 100(mean of measured concentrations - spike level)/spike level
*2: Ion ratio identification criteria not met for one sample; data point deleted
*3: Ion ratio identification criteria not met for two samples; data points deleted

Table 24 presents these same statistics for the measured concentrations at the low and high spike levels. In addition, the average relative error, calculated as 100 x (mean of measured concentration-spike level)/spike level, is shown here as a measure of relative accuracy. Measured tetra and penta compound concentrations are relatively accurate as shown by the relative errors which vary between -13.6% and 11.4%. On the other hand, the concentrations for the hexa compounds exhibit a much larger inaccuracy with relative errors ranging between -41.6% and -14.4%. The concentrations of these compounds are consistently underestimated. As described previously in the results section, the data for the hexabromo compounds are affected by the lack of a corresponding carbon-13 labeled internal quantitation standard.

Similar to the pattern seen for control samples, the standard deviations for the spiked samples tend to increase with increasing degree of bromination. This is reflected both in the standard deviations and the relative standard deviations.

Using the relative standard deviation as a measure of imprecision at a particular control or spike level, Table 24 shows that the precision is the same (tetrabromo dioxins) or better for the higher spike levels than for the lower levels for all compounds. However, based on only two spike levels, overall conclusions as to an increasing trend in precision with increasing concentration cannot be drawn. Overall, the precision as measured in the spiked samples for the tetra and penta compounds is considerably better than that for the hexa compounds.

C. Regression Analysis

Regression analysis was carried out to assess the overall accuracy and precision of the analytical method. This analysis was performed for each compound separately. One-half the detection limit was used for all concentrations below the detection limit. Each regression analysis provides an estimate of the slope and intercept of the line that best fits the 15 data points.

The slope of the straight line can be interpreted as a measure of the accuracy of the method, simply by multiplying the slope by 100. If the measurements were accurate, the line would have a slope of one. Thus, a statistical comparison of the slope with 1 provides a test as to whether the accuracy is significantly different from 100%. An estimate of the bias of the method can be obtained by subtracting 100% from the accuracy.

The intercept of the line with the vertical axis provides an estimate of the potential background contribution by the lipid samples. A statistical comparison of this intercept to zero provides a test as to whether the background is statistically negligible.

In addition to the slope and intercept, two basic statistics are relevant in describing the scatter of the data points around the fitted line. One is the proportion of variability in the data explained by the line. This is the square of the correlation between the predicted and observed measurements. The second statistic is the mean squared error (MSE),

an estimate of the overall performance of the method. Mathematically, this statistic is the average squared difference between measured and "true" concentrations. By subtraction, one can obtain an estimate of the overall method precision, or regression standard deviation, as the square root of [MSE - $(\text{Slope}-1)^2$].

Figures 11 through 16 depict the regression lines and their 95% confidence limits as fitted to the data by the least squares method. Figures 11 through 16 also show 95% confidence limits for the prediction of concentration based on the regression results. These confidence bands are always wider than those for the mean found concentrations. Table 25 presents the regression analysis results for the six compounds. The performance parameters of the method for each compound are discussed below.

1. Overall Fit

The proportion of variability (third column in Table 25) in the data that can be explained by the estimated linear relationship between measured and spiked concentrations is high for tetra (slightly above 99%) and pentabromo (slightly above 96%) compounds. For the hexabromo compounds, a large proportion of the variation remains unexplained, making the line in each case a poor predictor of measured from spiked concentrations.

2. Accuracy

The estimated slope for each regression line is shown in column 5 of Table 25. Multiplied by 100, these slopes provide estimates of the method accuracy for each compound. The accuracy is close to 100% for tetras and pentas, and for the hexa dioxin. In fact, as shown in columns 6 and 7, these figures are not statistically different from 100% at the 5% significance level. It should be noted that the confidence interval for the accuracy for the hexa dioxin is wide (47% to 116%) due to the relatively high variability in the measured concentrations (see also Figure 16). On the other hand, the estimates for accuracy for tetras and pentas have narrow confidence intervals associated with them. This is also reflected in Figures 11 through 14 with tetras showing by far the best results overall.

3. Precision

As discussed earlier, the overall method precision was estimated by the regression standard deviation. These results, in pg/g, are shown in column 4 of Table 25. It is clear from these figures that the overall precision decreases with increasing degree of bromination, with an excellent precision (1.30 to 4.22 pg/g) for tetras and pentas, but much poorer precision (22.6 to 32.0 pg/g) for hexas.

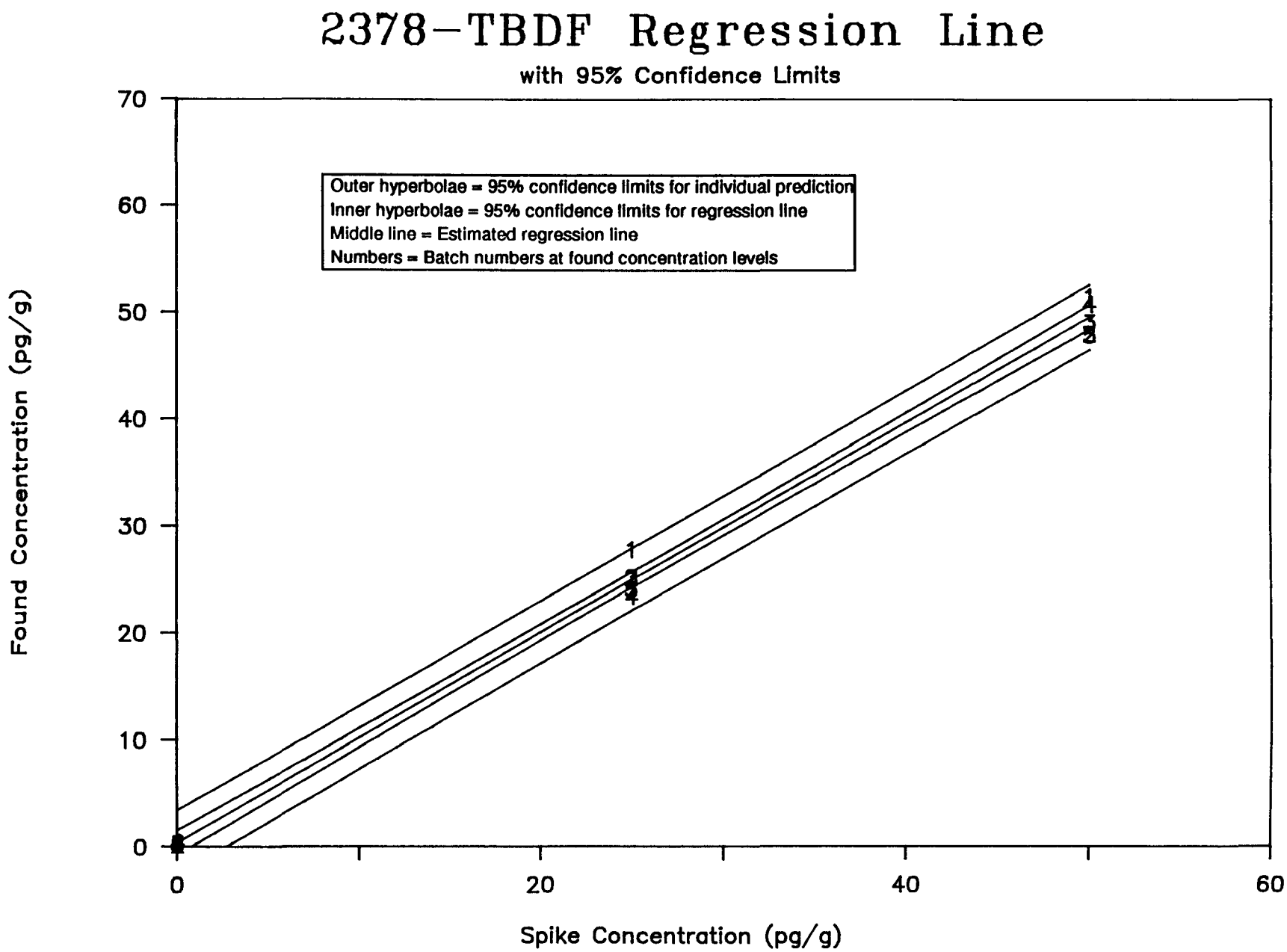

Figure 11. Regression analysis and 95% confidence intervals for QC data on 2,3,7,8-TBDF (codes 1-5 refer to batch number, slope = 0.983, y-intercept = 0.380 pg/g).

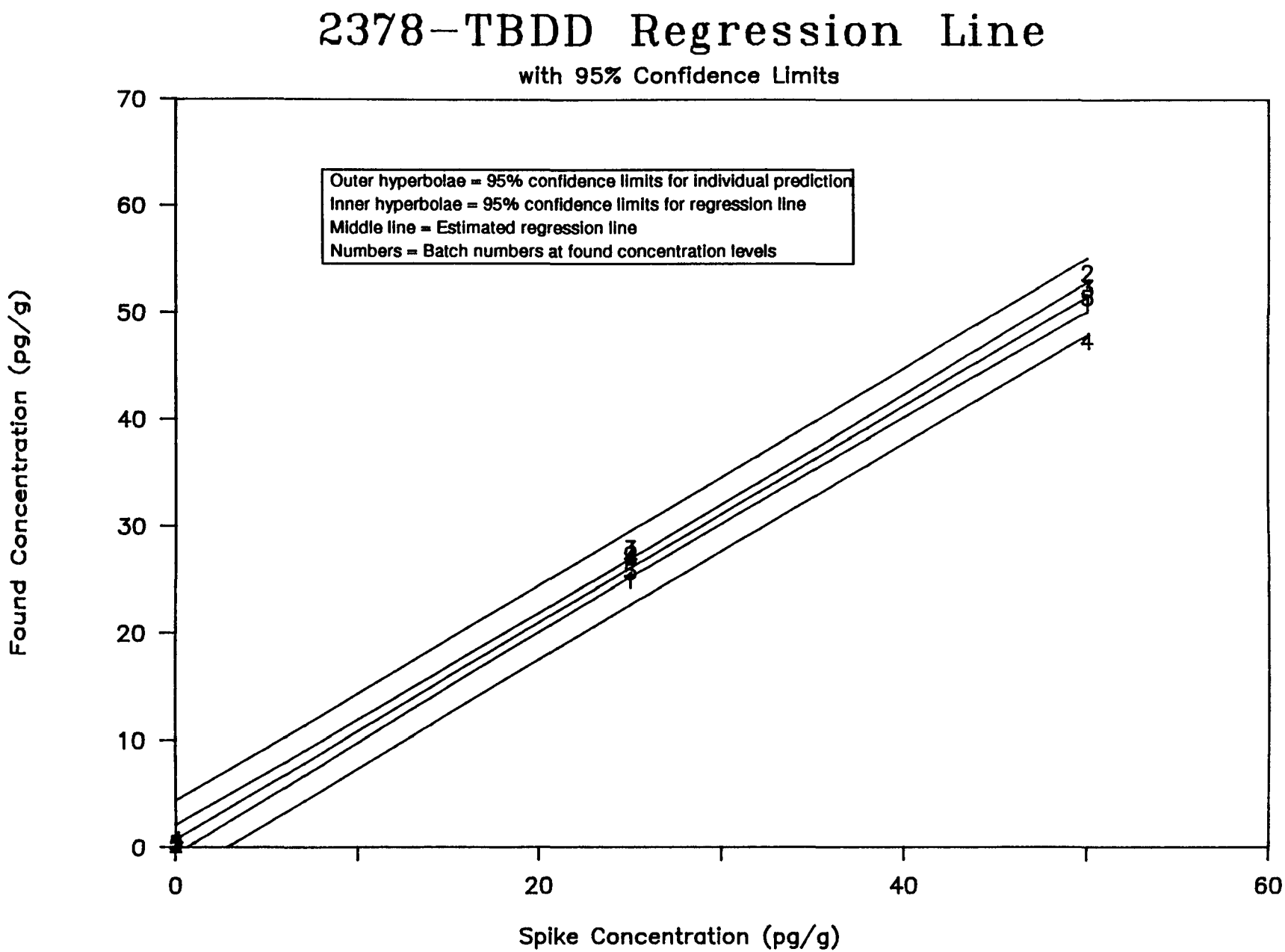

Figure 12. Regression analysis and 95% confidence intervals for QC data on 2,3,7,8-TBDD (codes 1-5 refer to batch number, slope = 1.02, y-intercept = 0.730 pg/g).

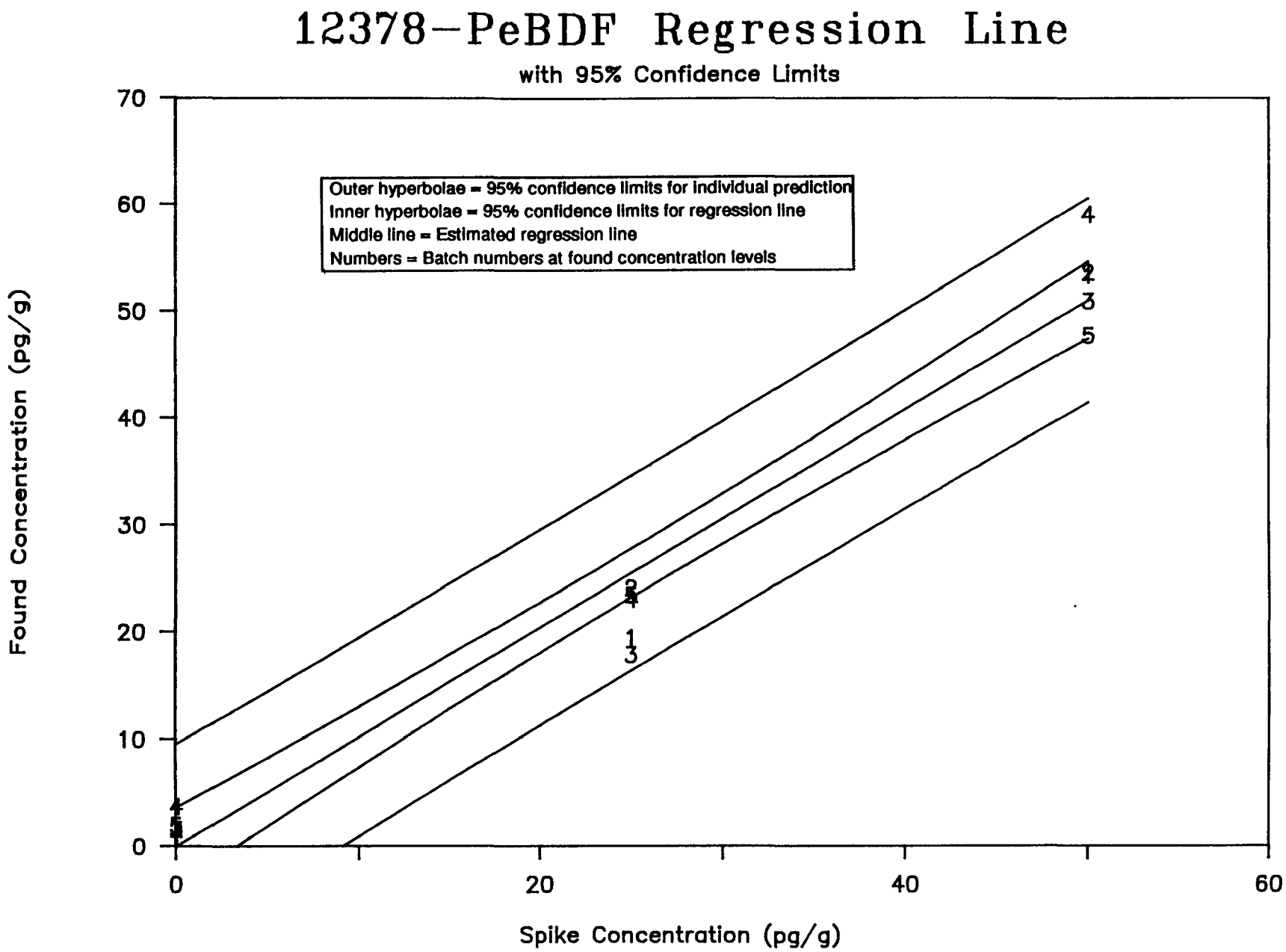

Figure 13. Regression analysis and 95% confidence intervals for QC data on 1,2,3,7,8-PeBDF (codes 1-5 refer to batch number, slope = 1.02, y-intercept = -0.020 pg/g).

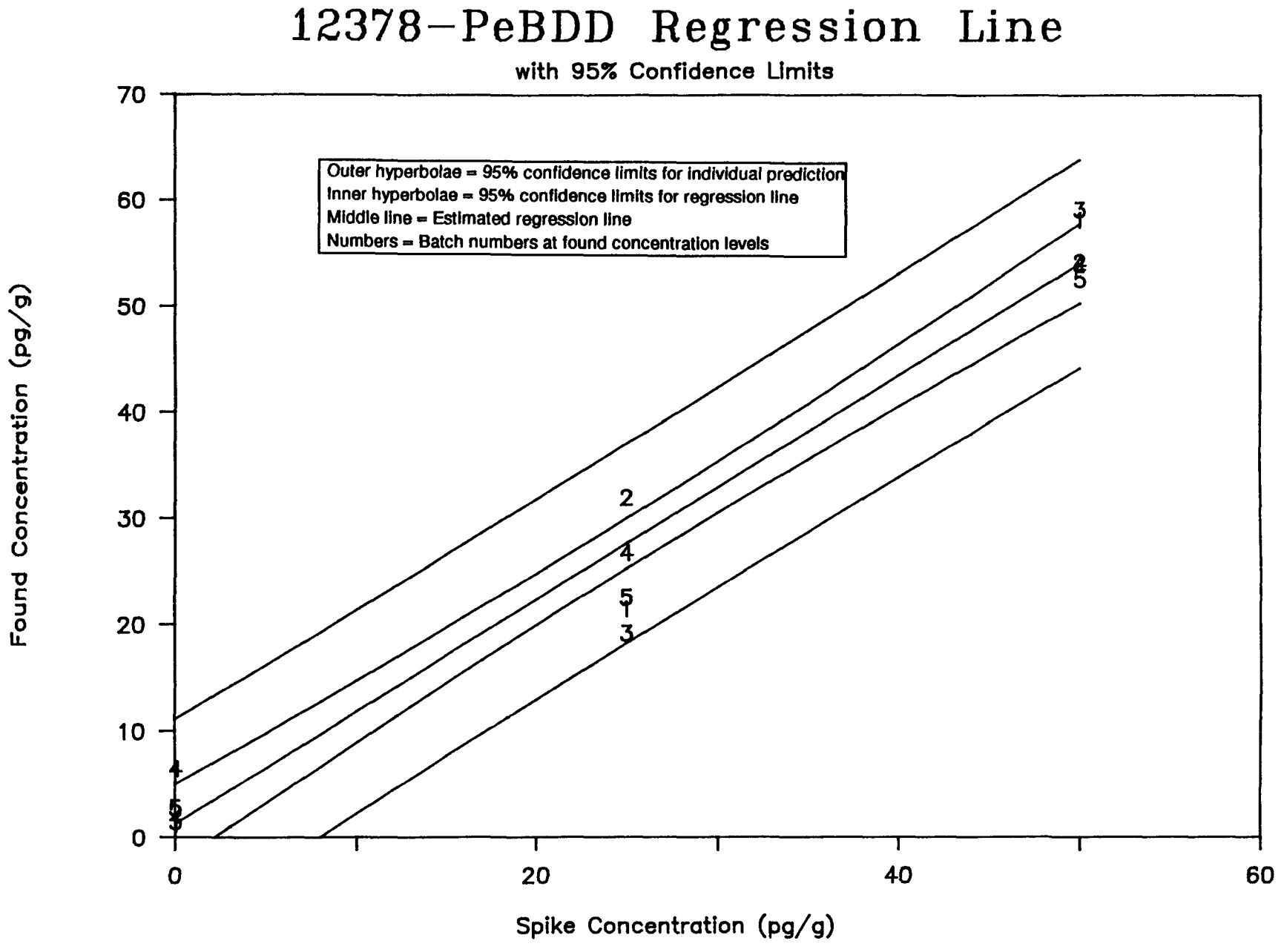

Figure 14. Regression analysis and 95% confidence intervals for QC data on 1,2,3,7,8-PeBDD (codes 1-5 refer to batch number, slope = 1.06, y-intercept = 1.26 pg/g).

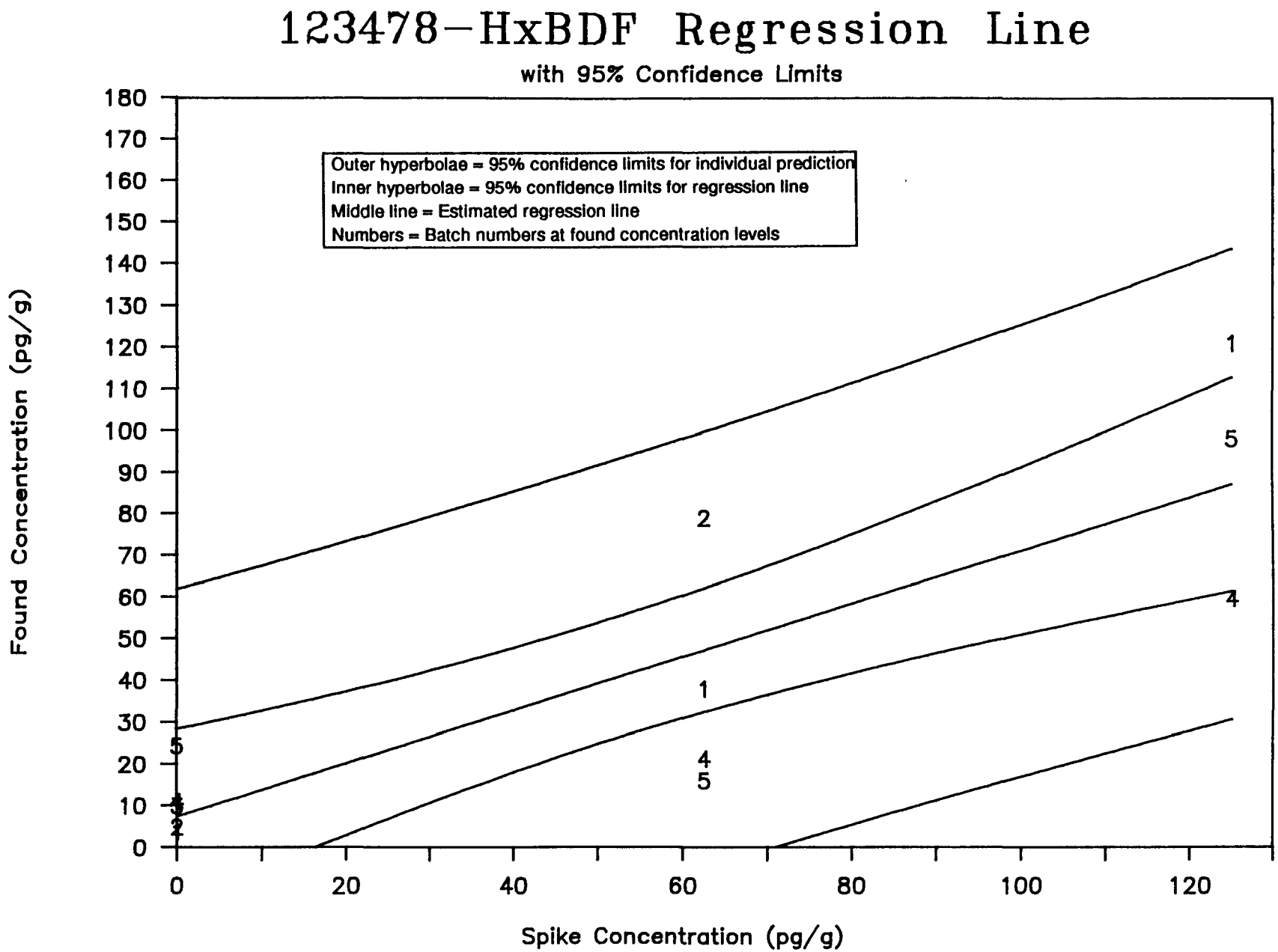

Figure 15. Regression analysis and 95% confidence intervals for QC data on 1,2,3,4,7,8-HxBDF (codes 1-5 refer to batch number, slope = 0.638, y-intercept = 7.38 pg/g).

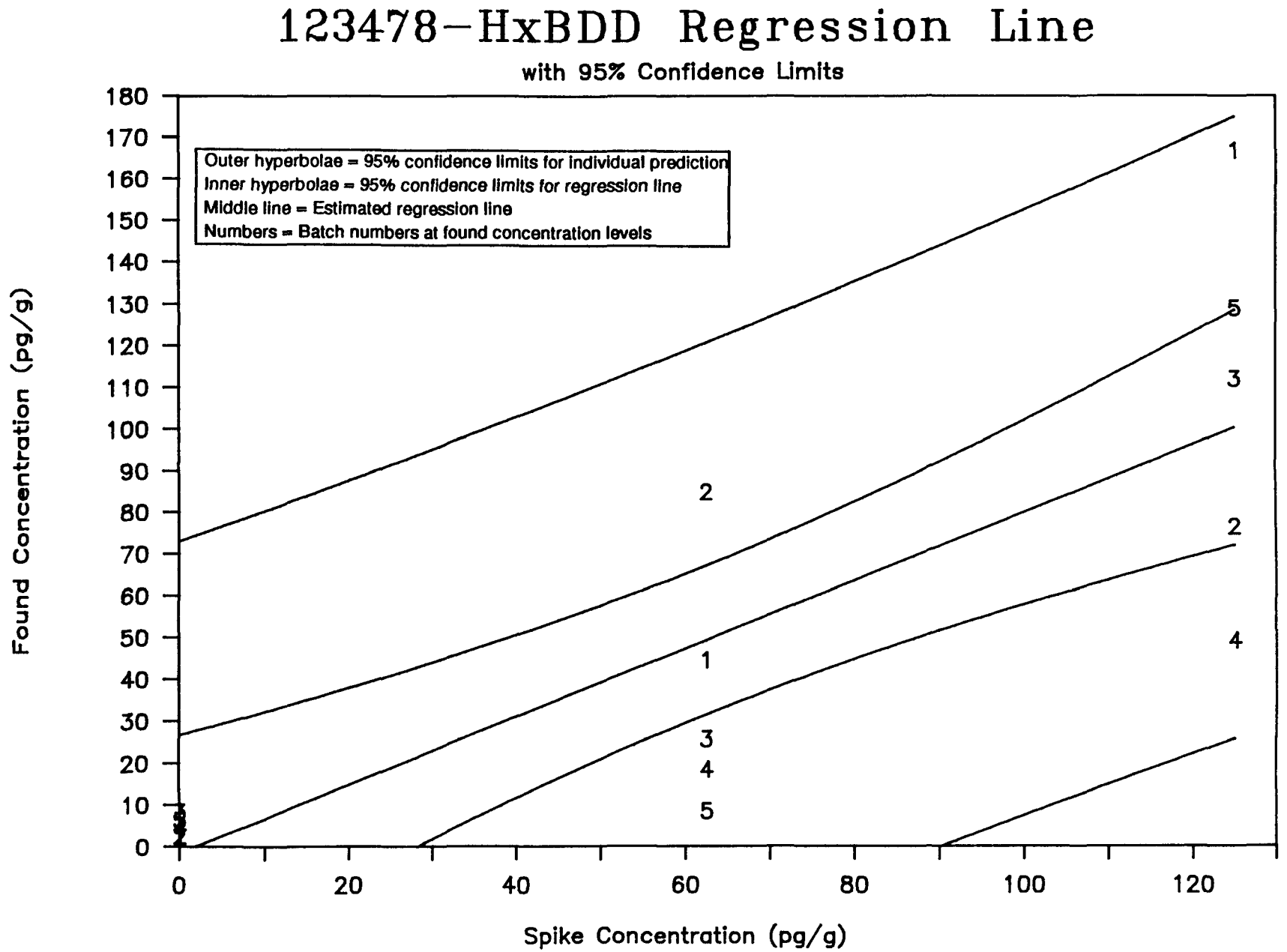

Figure 16. Regression analysis and 95% confidence intervals for QC data on 1,2,3,4,7,8-HxBDD (codes 1-5 refer to batch number, slope = 0.815, y-intercept = -1.53 pg/g).

Table 25. Estimates from Regression of Measured vs. Spiked Concentrations

Compound	Number of Samples	Proportion of Variation Explained by Line (%)	Precision Estimate *1 (pg/g)	Slope Estimate	95% Confidence Interval of Slope		Intercept Estimate (pg/g)	95% Confidence Interval of Intercept	
					Lower Limit	Upper Limit		Lower Limit (pg/g)	Upper Limit (pg/g)
2378-TBDF	15	99.6	1.30	0.983	0.948	1.02	0.380	-0.769	1.53
2378-TBDD	15	99.5	1.55	1.02	0.973	1.06	0.730	-0.637	2.10
12378-PeBDF	15	96.5	4.09	1.02	0.910	1.13	-0.020	-3.63	3.59
12378-PeBDD	15	96.5	4.22	1.06	0.940	1.17	1.26	-2.46	4.98
123478-HxBDF *2	12	67.5	22.6	0.638	0.347	0.929	7.38	-13.6	28.4
123478-HxBDD	15	63.5	32.0	0.815	0.465	1.16	-1.53	-29.7	26.7

*1: Precision estimate = Regression standard deviation

*2: Ion ratio identification criteria not met for three samples; data points deleted

Notes: The confidence interval for the slope including 1 indicates that the slope is not statistically different from 1 at the 5% level, i.e., the accuracy is not statistically different from 100%

The confidence interval for the intercept including 0 indicates that the intercept is not statistically different from 0 at the 5% level, i.e., the background is not statistically different from 0

4. Background

Estimates of background levels for PBDD and PBDF are provided by the intercept of each regression line and are shown in column 8 of Table 25. Lower and upper 95% confidence limits to the background are listed in the last two columns of Table 25. All six confidence intervals include 0, demonstrating that all the estimated backgrounds are statistically negligible. The width of the intervals is a measure of the precision of the background estimates. The width of these intervals increases drastically from the tetras and pentas (less than 7.44 pg/g) to the hexas (above 42.0 pg/g).

D. Conclusions

On the basis of the statistical treatment of the quality control data, one can conclude that except for 1,2,3,4,7,8-HxBDF, the analytical method is unbiased in measuring sample concentrations. The bias, though not statistically significant, is larger for the hexa dioxin than for tetras and pentas. For the hexa furan, the method has a significant bias due to either the lack of the appropriate internal quantitation standards, the difference in the HRMS sensitivities to the higher brominated compounds, or both. Figure 17 provides a summary of the percent bias (calculated as 100 x [slope - 1]) for each of the target analytes, along with the upper and lower 95% confidence intervals. Overall, the method provides very precise results for tetras and pentas, while providing poor precision for the hexa concentrations. In all cases, no significant background contribution from the control lipid samples was found. In addition, limit of detections increase with increasing degree of bromination.

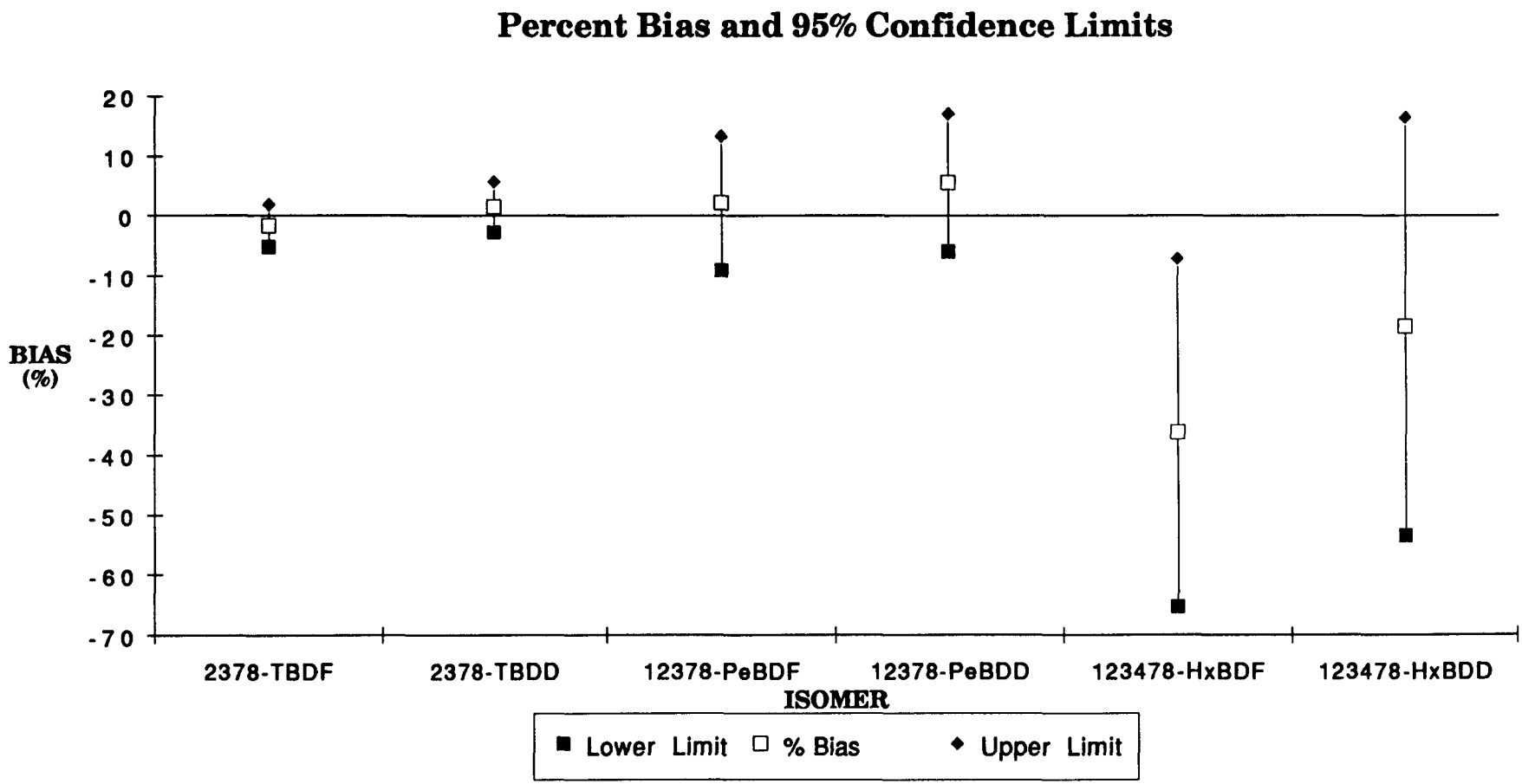

Figure 17. Percent bias and 95% confidence limits for individual PBDD and PBDF isomers.

VI. REFERENCES

1. U.S. Environmental Protection Agency. 1986. Broad scan analysis of the FY82 National Human Adipose Tissue Survey specimens, Vol. II: Volatile organic compounds. EPA-560/5-86-036.

2. U.S. Environmental Protection Agency. 1986. Broad scan analysis of the FY82 National Human Adipose Tissue Survey specimens, Vol. III: Semi-volatile organic compounds. EPA-560/5-86-037.

3. U.S. Environmental Protection Agency. 1986. Broad scan analysis of the FY82 National Human Adipose Tissue Survey specimens, Vol. IV: Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). EPA-560/5-86-038.

4. U.S. Environmental Protection Agency. 1986. Broad scan analysis of the FY82 National Human Adipose Tissue Survey specimens, Vol. V: Trace elements. EPA-560/5-86-039.

5. Leczynski, B, Collins, T, Sasso, N, Clark, J. 1988. FY87 NHATS composite design. Prepared for EPA Exposure Evaluation Division, Office of Toxic Substances. EPA Contract No. 68-02-4294.

6. Cramer, P, Stanley, J. 1988. Analysis of adipose tissue for dioxins and furans: Adipose tissue sample compositing. Prepared for EPA Exposure Evaluation Division, Office of Toxic Substances. EPA Contract No. 68-02-4252.

7. Cramer, P, Ayling, R, Stanley, J. 1989. Determination of PCDDs and PCDFs in human adipose tissue: data report batches 1 and 2, Revision 2. March 7, 1989.

8. Cramer, P, Ayling, R, Stanley, J. 1989. Determination of PCDDs and PCDFs in human adipose tissue: data report batches 3, 4, and 5, Revision 1. March 7, 1989.

9. Donnelly, J R, Munslow, W D, Vonnahme, et al. 1989. The chemistry and mass spectrometry of brominated dibenzo-p-dioxins and dibenzofurans. Biomedical and Environmental Mass Spectrometry, 14, 465-474.

10. Donnelly, J R, Grange, A H, Nunn, N J, Sovocool, G W, Brumley, W C, Mitchum, R K. 1989. Analysis of thermoplastic resins for brominated dibenzofurans. Biomedical Environmental Mass Spectrometry, 181, 884-896.

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16. Abstract (Limit: 200 words)

This report describes the analytical efforts for the determination of polybrominated dioxins (PBDDs) and furans (PBDFs) in human adipose tissues. Data on the precision and accuracy of the method for three tetra- through hexabrominated dioxins and three tetra- through hexabrominated furans (specific 2,3,7,8-substituted isomers) were generated from the analysis of 5 unspiked and 10 spiked (5 replicates at 2 spike levels) adipose tissue samples that were included with the analysis of the FY 1987 samples. In addition, data are presented on the results of the analysis of 48 composite samples for the six specific PBDD and PBDF compounds.

The targeted 2,3,7,8-substituted PBDDs and PBDFs were not detected in any of the samples except those prepared as spiked QC materials. The detection limits calculated for the tetrabromo congeners in the samples ranged from 0.46 to 8.9 pg/g (lipid basis). The detection limits for the higher brominated congeners were typically greater than that observed for the tetrabrominated compounds.

There is some evidence for the presence of other brominated compounds present in the adipose tissue samples. Specifically, responses were noted that correspond to the qualitative criteria for polybrominated diphenyl ethers (hexa through octabromo). It is recommended that these responses should be confirmed as brominated diphenyl ethers through additional analysis efforts in order to expand the data base on documented human exposure.

17. Document Analysis a. Descriptors

Polybrominated dibenzo-p-dioxins Polybrominated dibenzofurans
High resolution gas chromatography/mass spectrometry
Human adipose tissue
National Human Adipose Tissue Survey (NHATS)

b. Identifiers/Open-Ended Terms

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