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Dioxins and Dibenzofurans in Adipose Tissue of U.S. Vietnam Veterans and Controls

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EXECUTIVE SUMMARY

The primary reason for concern about the adverse effects of exposure to Agent Orange is attributable to its toxic contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Because TCDD accumulates preferentially in body fat and has a long half-life in humans, TCDD levels in adipose tissue can serve as a biological marker of exposure to Agent Orange.

The main objectives of the study were to determine if individuals with military service in Vietnam had significantly higher levels of 2,3,7,8-TCDD in adipose tissue than a similar group of non-Vietnam veterans or civilians, and to determine if TCDD levels were associated with specific demographic and military service characteristics. Under an agreement between the Department of Veterans Affairs (VA) and the U.S. Environmental Protection Agency (EPA), the adipose tissue collected for the EPA's National Human Adipose Tissue Survey (NHATS) was made available to the study as the source of tissue specimens. The EPA developed and evaluated all analytical methods for determination of 2,3,7,8-substituted polychlorinated dibenzo-p-dioxin (PCDD) and dibenzofuran (PCDF) levels in human adipose tissue. Since the vast majority of Vietnam veterans were males born between 1936 and 1954, the study focused on tissue specimens from men in this age bracket. Adipose tissue samples from 36 Vietnam veterans, 79 non-Vietnam veterans and 80 civilian men were selected and analyzed for 17 PCDD's and PCDF's including 2,3,7,8-TCDD.

It was found that, with or without adjustment for several demographic variables, the mean level of 2,3,7,8-TCDD in the adipose tissue of the 36 Vietnam veterans was not significantly different from that of the 79 non-Vietnam veterans or the 80 civilian men. The geometric mean TCDD levels for these groups were 11.7, 10.9 and 12.4 parts per trillion (ppt) respectively. Furthermore, the results showed no association between TCDD levels and any estimate of Agent Orange exposure opportunity based on military records. None of the Vietnam veterans in the study had an occupation which involved routine handling or spraying of Agent Orange in Vietnam. The study results suggest that heavy exposure to 2,3,7,8-TCDD for most Vietnam veterans was unlikely and that available military unit records used in the study were inadequate in assessing exposure to Agent Orange for those Vietnam veterans.

I. INTRODUCTION

The use of herbicides to control vegetation has caused one of the most persistent controversies arising from the Vietnam conflict. The U.S. Air Force applied most of these herbicides to dense jungle areas to uncover hidden enemy staging areas, and to clear vegetation from the vicinity of military bases and along lines of communication. The most common defoliant, Agent Orange, was used during the years 1965 to 1970. Agent Orange is the code name for a phenoxyherbicide consisting of a mixture of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxy-acetic acid (2,4,5-T). The 2,4,5-T contained 1-50 parts per million (ppm) of the contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin, also known as TCDD or dioxin[1]. TCDD is extremely toxic to laboratory animals, and many Vietnam veterans believe it is responsible for health problems ranging from skin rash to cancer.[1-3]

TCDD accumulates preferentially in the body fat of animals and man. The TCDD half-life in laboratory animals is estimated to be between 2 and 5 weeks.[4] Data on the TCDD half-life in humans, however, are limited and preliminary in comparison to animal data. Poiger and Schlatter[5] reported that a single dose of ^{3}H-2,3,7,8-TCDD ingested by a human volunteer was absorbed almost completely from the intestine and cleared the body with an estimated half-life of 5.8 years. A recent report by the Centers for Disease Control (CDC)[6] indicated that the median half-life of TCDD estimated from 36 Air Force veterans involved in the "Operation Ranch Hand" spraying missions in Vietnam was 7.1 years based on the difference between two measurements taken 5 years apart. This study indicated that it should be possible to detect elevated levels of TCDD in persons one or even two decades after their exposure if they were exposed to a substantial amount of TCDD.

Since 2,3,7,8-TCDD is a known contaminant of Agent Orange, several studies have suggested using the TCDD levels in adipose tissue as a biological marker of exposure to Agent Orange. Gross et al[7], for example, reported that 2 of 3 Vietnam veterans classified as "heavily exposed veterans", based on military records, had the highest TCDD levels in their adipose tissue among Vietnam veterans. The third veteran had a non-detectable level of TCDD when the detection limit was 3 ppt. Another 17 Vietnam veterans had TCDD levels in their adipose tissue similar to the TCDD levels of 10 veterans who did not serve in Vietnam. Kahn et al[8] found that the average level of 2,3,7,8-TCDD in the adipose tissue of 10 Vietnam veterans who were considered "heavily exposed" to Agent Orange was almost 10 times higher than in the controls (41.7 ppt vs. 4.3 ppt). Nine of the 10 veterans handled herbicides while in Vietnam: 5 Air Force Ranch Handers, 2 Army Chemical Corps personnel, 1 Air Force veteran who handled drums of defoliant and 1 Army helicopter crew chief who participated in the spray missions. In another study of Vietnam veterans[9], a group of 13 veterans who had sought medical

assistance were selected and their adipose tissues were analyzed for 2,3,7,8-TCDD. The TCDD was detected from 5 of the 13 samples at levels ranging from 3.0 to 12.4 ppt. These 13 Vietnam veterans' histories of exposure to Agent Orange and their military characteristics were unknown to the investigators and therefore not reported.

More recently, the CDC[10] reported serum 2,3,7,8-TCDD levels in U.S. Army Vietnam-era veterans. The levels of serum TCDD in 646 Army Vietnam combat troops and 97 Army non-Vietnam veterans were nearly identical (mean values of 4 ppt on a lipid weight basis), and the levels of TCDD did not increase with exposure levels to Agent Orange estimated from military records.

Mean values for 2,3,7,8-TCDD in adipose tissue collected from several American and Canadian populations seldom exceeded 10.0 ppt. Examples include: 9.6 ppt for 35 autopsy cases from Georgia and Utah[11]; 7.0 ppt for 35 autopsy cases from St. Louis, Missouri[12]; 6.4 ppt for 6 cases from New York State[13]; 10.0 ppt for 10 deceased hospital patients in Eastern Ontario[13]; 6.2 ppt for 46 accident victims across Canada.[13]

The studies of veterans and the general population to date, suggest that Vietnam Veterans without known "occupational" exposure to phenoxyherbicides (eg. military personnel who were not Ranch Hand or Chemical Corps personnel) have 2,3,7,8-TCDD levels similar to the general population of U.S. men. However, all of these veteran studies were based on measurements made up to two decades after a veteran left Vietnam, i.e. a passage of an estimated 2 to 3 half lives of TCDD. The study reported herein utilized adipose tissue specimens collected from the general population between 1971 and 1982. For some of the Vietnam veterans included in the study, the time between their departure from Vietnam and the sample collection year was considerably less than the estimated half-life of 2,3,7,8-TCDD in humans. Furthermore the specimens were analyzed not only for 2,3,7,8-TCDD, but for 16 other polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). Because analyses of human adipose tissue from the general population have indicated the presence of a number of PCDDs and PCDFs at ppt levels[12-16], and because 2,3,7,8-TCDD was the only one of these found in Agent Orange as a contaminant, knowing the levels of PCDDs and PCDFs would help determine whether adipose tissue TCDD levels of Vietnam veterans might be the result of Agent Orange exposure in Vietnam or some other exposure to PCDDs. For example, if most PCDDs and PCDFs as well as 2,3,7,8-TCDD levels are found to be elevated among Vietnam veterans, contributions from sources other than Agent Orange are likely.

The purpose of this study was twofold: (1) to determine if a group of individuals with military service in Vietnam have significantly higher levels of 2,3,7,8-TCDD in adipose tissue than either a similar group of non-Vietnam veterans or civilian controls and (2) to determine if TCDD levels in adipose tissue were associated with specific demographic and military service characteristics. In prior studies, more time had passed between

the specimen collection year and the year since departure from Vietnam. Findings of this study, therefore, should complement the results of other studies.

II. METHODS

A. Identification and Selection of Study Subjects

1. Source of Human Adipose Tissue Specimens

The present retrospective study took advantage of the existing specimens that had been collected from the general population by the U.S. Environmental Protection Agency (EPA). The EPA has conducted the National Human Adipose Tissue Survey (NHATS) since 1970 to monitor the human body burden of pesticides and other selected chemicals. Up to 1,000 adipose tissue specimens have been collected annually from pathologists and medical examiners across the country and analyzed by the EPA for the selected chemicals. After analysis, the unused tissue specimens were sent to a central facility to be stored at 0°C to -20°C. There is evidence that the specimens had been exposed to freeze/thaw cycles.

The NHATS sampling scheme provided a representative sample of the Standard Metropolitan Statistical Areas (SMSA) in terms of age, sex, and race. The target population for the NHATS program was all non-institutionalized persons in the conterminous U.S. However, due to the invasive nature of collecting adipose tissue samples, the sampling population was limited to cadavers and surgical patients. Within each SMSA, hospitals or medical examiners were identified and asked to contribute tissue specimens according to the design specifications of age (0-14 years, 15-44 years, 45+ years), sex and race (white, non-white). A detailed description of the NHATS sampling scheme was reported elsewhere.[17,18] Since the vast majority of Vietnam veterans were men born between 1936 and 1954, this study was restricted to specimens from men born in that period.

2. Selection Procedures and Criteria

The NHATS Master File contained information on 21,000 specimens identified by age, race and sex. No personal identifying information, such as name or social security number (SSN), was available. The specimen and data files were examined to determine how many of the 21,000 specimens collected had adequate tissue remaining for further analysis. An Inventory File was created for the 8,000 specimens that were recorded to have an adequate amount of tissue. The Master File was then merged with the Inventory File. It was found that a total of 528 specimens were from males born between 1936 and 1954. The hospitals or medical examiners who originally collected the 528 specimens were recontacted to obtain enough identifying information on the donors to determine their military service status. The collection effort yielded information for 494 or 94% of the 528 specimens. The military service status for these men, including any Vietnam service, was determined by reviewing records archived at the National Personnel Records Center (NPRC) in St. Louis and military records maintained at other locations. From this effort, 134 men were initially found to have served in the military, 40 of whom served in Vietnam. Military personnel

records of these 134 veterans were located and abstracted for items such as enlistment and discharge dates, rank, branch, military occupational specialty codes (MOSC), place of service and educational levels.

The tissue from the 40 Vietnam veterans was utilized for the study. From the 94 remaining veterans, 80 were randomly selected for the non-Vietnam veteran group. Two civilian men were closely matched to each Vietnam veteran by birth year ($\pm$ 2 years) and sample collection year ($\pm$ 2 years). Matching by birth year and sample collection year would provide adjustment of the subjects for age at the time of sample collection and for storage duration of the specimens. Age was considered an important matching variable due to the probable accumulation of TCDD in the body with each year of exposure during the lifetime of the individual.[11,12,19] The storage time of the tissue specimens was also considered important because of the possible degradation of TCDD while being stored in the freezer.[11]

Demographic data were taken from the NHATS file except for occupational information which came from the "usual occupation" listed in the official death certificates. Body mass index (BMI) was calculated from weight and height as follows: BMI = (weight in kg)/(height in m^2)[10]. Age at accession was determined by the difference between the sample collection year and birth year. The location of the participant's hospital was categorized into four U.S. census regions (west, north central, north east and south) to determine geographic residence. The sample storage time was calculated by the difference between the specimen analysis year and the specimen collection year. All military data were taken from military personnel records. All adipose tissue specimens were analyzed during 1987.

B. Determination of Opportunity for Agent Orange Exposure

A precise estimate of the exposure of each Vietnam veteran to Agent Orange is not considered feasible based on either military records or self-reported data. In this study the probable opportunity for exposure was determined from the following: service in the Army or Marine Corps, military occupation specialty code (MOSC), broad geographical location of the individual's unit in Vietnam, and combinations of the above.

It has been suggested that ground troops (Army and Marine) in Vietnam might have had a higher probability of contact with Agent Orange than other Vietnam veterans due to the nature of their military operations through defoliated zones and the practice of base perimeter spraying. Furthermore, it has been suggested that, among ground troops, those engaged in combat were more likely to be placed in herbicide-sprayed areas than individuals who were not in combat. There was no single data element from military personnel records, applicable to all veterans, that would indicate whether they had actually been in combat. As an alternative measure, MOSCs were categorized into combat-related and non combat-related. Combat-related MOSCs were those occupations where primary duties involved direct offensive

and defensive action against an armed hostile force. Examples of combat MOSCs include those of the Infantry, Artillery, Armored and Air Cavalry branches.

As another surrogate measure for herbicide exposure, the broad geographic location of an individual's military unit in reference to recorded herbicide spray missions was also determined. According to the records of military spray missions (U.S. Air Force Ranch Hand Operation),[20] defoliation and crop destruction were most extensive in military region III. A total of 5.3 million gallons of Agent Orange were sprayed in military region III from 1965 to 1970. During the same period, the amounts of Agent Orange sprayed within military regions I,II, and IV were 2.2, 2.5, and 1.2 million gallons, respectively. Army and Marine Vietnam veterans were then classified as occupying military regions I, II, III or IV.

Finally, troop locations were determined on a 100 meter grid map of Vietnam at intervals of 90 days or less. Each company was assumed to occupy the last location for the duration of each interval. Computer matching of troop locations with respect to time and distance from recorded herbicide spray tracts was carried out using the HERBS tape and Services HERBS tape databases.[20,21] The HERBS tape contained information on most of the herbicide spray missions flown by fixed-wing aircraft from 1965 to 1971, and on crop missions flown by helicopter between 1968 and 1971. The tape contained information on the type of herbicide, gallons, dates, and where spray runs started and ended. The U.S. Army and Joint Services Environmental Support Group (ESG) identified and documented an additional 1.6 million gallons of herbicide sprayed mainly by Army personnel around the perimeter of base camps, fire bases, air bases and other fixed military installations. This additional spray data, which was not included in the original HERBS tape, was designated as the "Services HERBS tape".

Based on information from the HERBS and Services HERBS tapes, the opportunity for Agent Orange exposure was determined in two ways: (1) an individual's company was located within 2 kilometers of a recorded Agent Orange spray tract within 3 days of application; and (2) an individual's company was located within 8 kilometers of a recorded Agent Orange spray tract within 90 days of application. When this requirement was fulfilled at least once, an individual was considered to have had an opportunity for exposure.

C. Statistical Methods

The purpose of the statistical analyses was to determine if the mean level of 2,3,7,8-TCDD in adipose tissue of the Vietnam veteran study subjects was different from either the non-Vietnam veteran levels or the civilian levels. The mean levels of the non-Vietnam veterans and the civilian controls were also compared. Multiple comparisons and testing for differences were done by using the F test in one way analysis of variance (ANOVA) and analysis of covariance with adjustments for demographic

variables such as age, collection year, and body mass index.[22] A paired t-test[23] was conducted to compare the means of Vietnam veterans with their matched civilian controls. In all analyses, the TCDD values were transformed to natural logarithmic scale because the TCDD values were found to have approximately log-normal distributions in this study and another study.[19]

A stepwise linear regression model[24] was also used to determine whether TCDD levels were associated with demographic and military service characteristics. Factors considered a priori as covariates were age, sample collection year, race, and body mass index (body weight in kg per height in m^2). A regression model specific to Vietnam veterans included such covariates as military occupation, calendar year of tour in Vietnam, geographic region in Vietnam, number of years since Vietnam service, time of and distance from recorded Agent Orange spray and sample collection year. All statistical tests were conducted at the .05 level of significance.

D. Laboratory Analysis

The analytical protocol of this study provided for the detection and quantitative determination of the seventeen 2,3,7,8-substituted polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) in human adipose tissue. The minimum measurable concentration was estimated to range from 1 picogram per gram (pg/g) for 2,3,7,8-TCDD and 2,3,7,8-TCDF, up to 5 pg/g for OCDD and OCDF based on a 10-g aliquot of human adipose tissue. These detection limits depended on the kinds and concentrations of interfering compounds in the sample matrix and the absolute method recovery. Figure 1 presents a schematic of the analytical procedure. This protocol was evaluated for method performance (accuracy and precision) prior to being used in this study. The results of this method evaluation are found in an EPA report.[25] The measurements were precise to three significant digits. Since the initial method evaluation effort did not provide evidence of potential interference, specific interference studies were not included in this study.

1. Standard Materials

Native 2,3,7,8-TCDD was supplied as a certified standard solution in isooctane from the U.S. EPA QA Reference Materials Branch, Office of Research and Development, Environmental Monitoring Systems Laboratory-Las Vegas. All other native compounds were provided in crystalline form by Cambridge Isotope Laboratories, Woburn, MA. Carbon-13 ($^{13}C_{12}$) labeled internal standards were supplied in n-nonane solution by Cambridge Isotope Laboratories. Table 1 provides a summary of the standards used for this study. Methylene chloride, toluene, benzene, cyclohexane, methanol, acetone and hexane were obtained from Burdick & Jackson distilled in glass quality. Tridecane in reagent grade was also from Burdick & Jackson.

Chromatographic materials were purchased and prepared according to specifications. The acidic alumina (Biorad, AG-4)

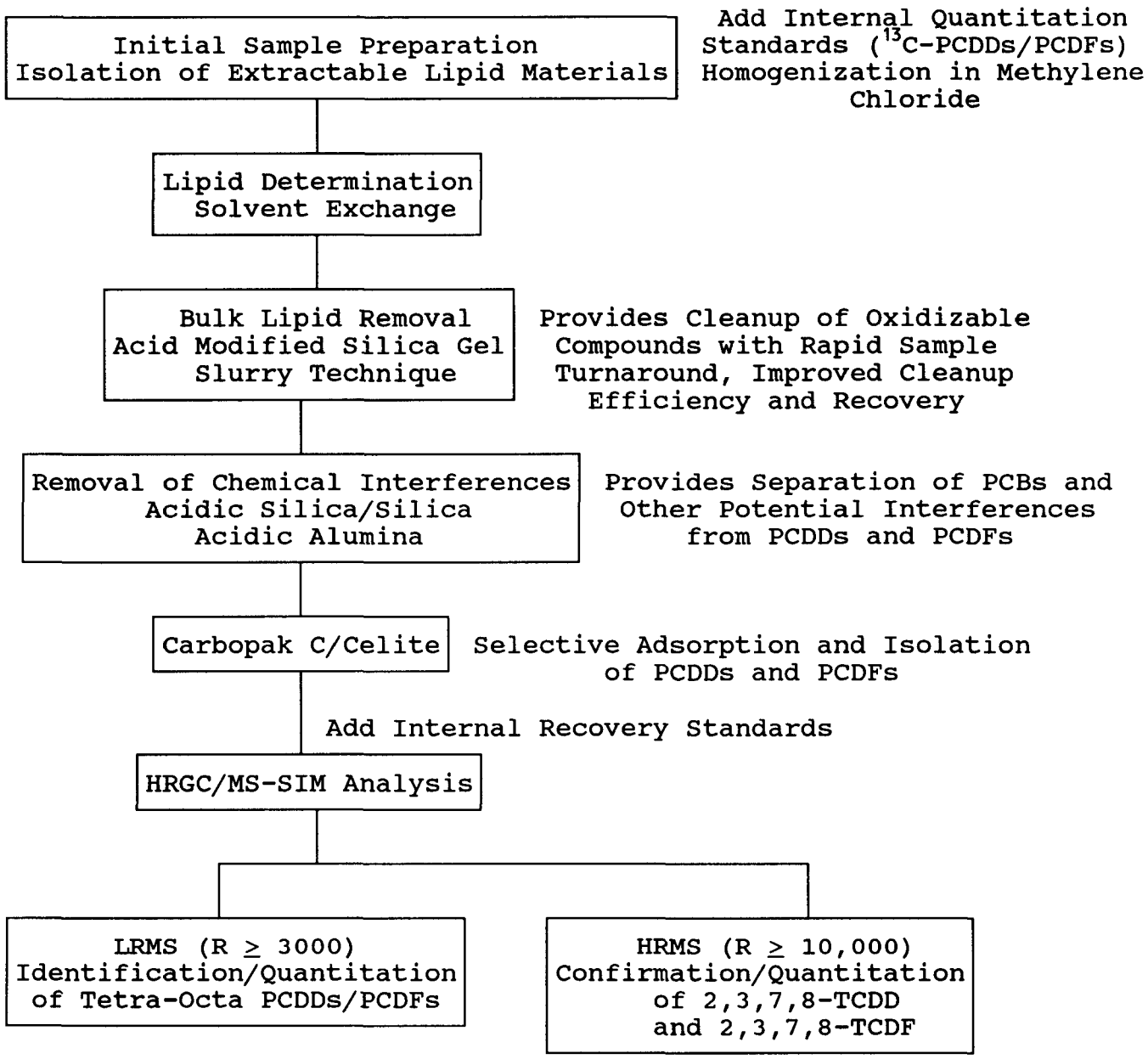


FIGURE 1. Schematic diagram of the sample preparation and instrumental analysis procedures for determination of PCDDs and PCDFs in human adipose tissue

TABLE 1
Analytical Standards Used to Prepare the Calibration Standards

Compound	Source	Lot/Code
Native		
2,3,7,8-TCDD	EPA QA Reference Material Branch	20603
2,3,7,8-TCDF	CIL	AWN 1203-74/EF-903-C
1,2,3,7,8-PeCDD	CIL	MLB-706-53/ED-950-C
1,2,3,7,8-PeCDF	CIL	AWN-729-21/EF-953-C
2,3,4,7,8-PeCDF	CIL	AWN-729-45/EF-956-C
1,2,3,4,7,8-HxCDD	CIL	830244/ED-961-C
1,2,3,6,7,8-HxCDD	CIL	MLB-706-47/ED-960-C
1,2,3,7,8,9-HxCDD	CIL	MLB-706-73/ED-969-C
1,2,3,4,7,8-HxCDF	CIL	AWN-729-20/EF-964-C
1,2,3,6,7,8-HxCDF	CIL	MB 13106-7/EF-962-C
1,2,3,7,8,9-HxCDF	CIL	MB 13106-47/EF-967-C
2,3,4,6,7,8-HxCDF	CIL	MB 13106-3/EF-968-C
1,2,3,4,6,7,8-HpCDD	CIL	MLB-706-21/ED-971-C
1,2,3,4,6,7,8-HpCDF	CIL	AWN-729-22/EF-973-C
1,2,3,4,7,8,9-HpCDF	CIL	MB-13-106-77/EF-975-C
OCDD	CIL	F2832/ED-980-C
OCDF	CIL	8465-F-982-C/EF-982-C
$^{13}C_{12}$-Internal Standards		
1,2,3,4-TCDD	CIL	AWN-1203-93/ED-911
2,3,7,8-TCDD	CIL	R00208/ED-900
2,3,7,8-TCDF	CIL	R00236/EF-904
1,2,3,7,8-PeCDD	CIL	R00241/ED-955
1,2,3,7,8-PeCDF	CIL	R00221/EF-952
1,2,3,6,7,8-HxCDD	CIL	R00249/ED-966
1,2,3,7,8,9-HxCDD	CIL	AWN-729-73/ED-996
1,2,3,4,7,8-HxCDF	CIL	R00234/EF-963
1,2,3,4,6,7,8-HpCDD	CIL	R00248/ED-972
1,2,3,4,6,7,8-HpCDF	CIL	MB13106-73/EF-974
OCDD	CIL	R00263/ED-981

Standard purity documentation was received from the supplier for each of the standards. Additional purity checks of these standards have not been conducted.

Note: CIL stands for Cambridge Isotope Laboratories

was extracted in a Soxhlet apparatus with methylene chloride for 18 hours, air dried and activated by heating in a foil-covered glass container for 24 hours at 190°C. Silica gel (Kieselgel EM Scientific, high purity grade, type 60, 70-230 mesh) was extracted in a Soxhlet apparatus with methylene chloride for 10 hours, air dried, and activated by heating in a foil covered glass container for 24 hours at 130°C.

Sulfuric acid modified silica gel (40% w/w) was prepared by combining two parts (by weight) concentrated sulfuric acid (Taychemco, Taylor Chemical Co., ACS grade) with three parts (by weight) silica gel (extracted and activated) in a glass bottle and tumbled for 6 hours.

Graphitized carbon black (Carbopack C, Supelco, surface of approximately 12 m^2/g, 80-100 mesh) was mixed thoroughly with Celite 545[R] (Fischer Scientific, reagent grade). A total of 3.6 g of Carbopack C and 16.4 g of Celite 545[R] were mixed in a 40-mL vial, activated at 130°C for 6 hours and stored in a desiccator.

Granular anhydrous sodium sulfate was extracted with methylene chloride for 16 hours, air dried, then put into a muffle furnace for at least 4 hours in a shallow tray at 400°C. The substance was then stored in an oven at 130°C. Silanized glass wool (Supelco) was extracted with methylene chloride and hexane and air dried prior to use.

2. Sample Preparation

Ten grams of frozen human adipose tissue (sample size was smaller in some cases depending on availability) were weighed into a culture tube (2.2 X 15 cm). The adipose tissue specimen was allowed to reach room temperature. The tissue was then spiked with known amounts of nine carbon-13 labeled PCDDs and PCDFs as internal quantitation standards (see Table 2). Extraction and homogenization were accomplished using 10 mL methylene chloride and a Tekmar Tissuemizer[R] for 1 minute. The extract was filtered through 5-10 grams of anhydrous sodium sulfate to remove water. The extraction procedure was repeated (three to five times) until the tissue sample was thoroughly homogenized. The filter funnel and contents were rinsed with an additional 20-40 mL of methylene chloride. The final extract was adjusted to 100 mL in a volumetric flask.

The extractable lipid was determined using a minimum of 1% of the final volume. A 1.0 mL aliquot was removed from the final extract. This aliquot was placed in a 2-dram vial preweighed to the nearest 0.0001 g, and the solvent was removed using purified nitrogen and a heated water bath (50-60°C). The vial was reweighed and the lipid content was determined using the weight difference. Nitrogen blow-down was continued until a constant weight was achieved for the vial.

The methylene chloride in the remaining extract was concentrated using rotary evaporation until only an oily residue remained. The residue was diluted with 200 mL of hexane. One hundred grams of sulfuric acid modified silica gel (40% w/w) was stirred into the solution. The mixture was stirred for

TABLE 2
Internal Quantitation Standards

Congener	Spike Level (picograms)
$^{13}C_{12}$-2,3,7,8-TCDF	500
$^{13}C_{12}$-2,3,7,8-TCDD	500
$^{13}C_{12}$-1,2,3,7,8-PeCDF	500
$^{13}C_{12}$-1,2,3,7,8-PeCDD	500
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	1250
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	1250
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	1250
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	1250
$^{13}C_{12}$-OCDD	2500

approximately 2 hours and the supernatant was decanted and filtered through 20 grams of anhydrous sodium sulfate. The silica gel was washed with at least two additional 50 mL aliquots of hexane for 15 minutes, dried by elution through sodium sulfate, and combined with the first hexane extract. The sodium sulfate filter was rinsed with an additional 25 mL of hexane and combined with the two previous hexane extracts.

The combined hexane extracts were eluted through a column consisting of a layer of sulfuric acid modified silica gel, and a layer of unmodified silica gel. The acidic silica column was prepared by plugging a 1 cm X 10 cm chromatographic column with glass wool and adding 1.0 g of silica gel and 4.0 g of 40% w/w sulfuric acid impregnated silica gel. The eluate was concentrated to approximately 1 mL using nitrogen blow-down and added to a column of acidic alumina. The acidic alumina column was prepared by plugging a 1 cm X 30 cm chromatographic column with glass wool and adding 25 mL of hexane and 6.0 g of acidic alumina. Then the acidic alumina was allowed to settle in the column. This was topped with a 1 cm layer of sodium sulfate. The alumina column was washed with 40 mL of 50% v/v methylene chloride/hexane, followed by an additional 100 mL of hexane. The PCDDs and PDCFs were eluted from the alumina using 30 mL of 20% (v/v) methylene chloride/hexane.

The eluate from the alumina column was added to a 500 mg Carbopack C/Celite column as described below. A different carbon column was used for each analysis. The Carbopack C/Celite column was prepared by cutting a 5-mL disposable glass pipet (6 to 7 mm ID) at the 4-mL mark. A glass wool plug was added and pushed to the 2-mL mark. 500 mg of the activated Carbopack C/Celite mixture was added, followed by another glass wool plug. Using two glass rods, the glass wool plugs were pushed simultaneously, gently compressing the Carbopack C/Celite to a length of 3.0 to 3.5 cm. The column was pre-eluted with 2 mL of toluene, followed by 1 mL of 75:20:5 methylene chloride/methanol/benzene, 1 mL of 1:1 cyclohexane in methylene chloride and 2.0 mL of hexane. The flow rate was less than 0.5 mL/minute.

The entire eluate (30mL) from the alumina column was added to the top of the Carbopack C/Celite column. The vial that contained the extract from the alumina column was rinsed twice with 1 mL of hexane and added to the top of the column. The column was eluted sequentially with two 1-mL aliquots of hexane, 1 mL of 1:1 cyclohexane in methylene chloride, and 1 mL of 75:20:5 methylene chloride/methanol/benzene. The column was turned up side down and the PCDDs and PCDFs were eluted from the column using 20 mL of toluene.

The toluene extract was concentrated to less than 1 mL using a stream of nitrogen, transferred to 1-mL conical vials and reduced to a volume of about 200 uL using a stream of nitrogen. The concentrator tube was rinsed 3 times with 500 uL of 10% toluene in methylene chloride and concentrated to 200 uL. Tridecane (10 uL) containing the internal recovery standards (500 pg of $^{13}C_{12}$-1,2,3,4-TCDD and 1250 pg of $^{13}C_{12}$-1,2,3,7,8,9-HxCDD) was

added as a keeper, and the extract was concentrated to final volume of 10 uL.

3. Instrumental Analysis

Instrumental analyses were accomplished by using a Carlo Erba MFC500 high resolution gas chromatograph (HRGC) coupled to a Kratos MS50TC high resolution double-focusing mass spectrometer (MS) operated in the electron impact mode. The HRGC/MS interface was a direct connection of the HRGC column to the ion source of the MS via a heated interface oven. The sample extracts were injected through a Grob-style splitless injector. Separation of PCDD and PCDF analytes was achieved using a 60-meter DB-5 capillary column (J&W Scientific). For the high resolution mass spectrometer analyses of 2,3,7,8-TCDD and 2,3,7,8-TCDF, a Rtx-2330 Capillary Column (Restek Corp.) or an SP-2330 capillary column (Supelco Co.) was used. Data acquisition and processing were controlled by a Finnigan MAT Incos 2300 data system.

Analysis of each sample was accomplished in two HRGC/MS runs. Analysis of the tetrachloro through octachloro 2,3,7,8-congeners was achieved in the low resolution mode ($R \geq 3,000$) on the mass spectrometer (LRMS). Analysis of the tetrachloro 2,3,7,8-congeners was also confirmed in the high resolution mode ($R \geq 10,000$) on the mass spectrometer (HRMS). Data reported for the tetrachloro congeners were taken from the high resolution mass spectrometer run. The 2,3,7,8-TCDD concentrations were very comparable between the two resolutions.

The HRGC/LRMS selective ion monitoring (SIM) analysis of the tetrachloro through octachloro congeners was carried out with the instrumental conditions and parameters listed in Table 3. For each HRGC/LRMS run, five distinctive groups of ions, which correspond to each chlorine level, were sequentially monitored. These ion descriptors are shown in Table 4. Parameters monitored included two characteristic molecular ions and the corresponding carbon-13 labeled internal standard for each PCDD and PCDF homolog. In addition, the masses corresponding to the molecular ions of the hexachloro through decachlorodiphenyl ethers (PCDEs) were monitored to demonstrate that responses for specific PCDF congeners were not due to potential interferences. A lock mass of m/z 381 for perfluorokerosene was monitored throughout each analysis to ensure that proper mass calibration was maintained.

Isomer specific analyses for 2,3,7,8-TCDD and 2,3,7,8-TCDF were carried out under the instrumental conditions and parameters shown in Table 5. In addition to monitoring the masses of the most abundant molecular ions of TCDD and TCDF, the ions corresponding to the loss of a carbon, oxygen, chlorine fragment (COCL) from the molecular ions were monitored for verification purposes.

Ten concentrations of calibration standards containing the 17 native and 11 carbon-13 labeled internal standards were prepared. Table 6 presents a summary of the calibration standards.

TABLE 3
HRGC/LRMS Operating Conditions for PCDD/PCDF Analysis

Low resolution Mass spectrometer	
Accelerating voltage:	8,000 V
Trap current:	500 uA
Electron energy:	70 eV
Electron multiplier voltage:	-1,800 V
Source temperature:	280°C
Resolution:	≥ 3,000 (10% valley definition)
Overall SIM cycle time:	1 s
Gas chromatograph	
Column coating:	DB-5
Film thickness:	0.25 um
Column dimensions:	60 m X 0.25 mm ID
He linear velocity:	≈ 25 cm/sec
He head pressure:	1.75 kg/cm^2 (25 psi)
Injection type:	Splitless, 45 s
Split flow:	30 mL/min
Purge flow:	6 mL/min
Injector temperature:	270°C
Interface temperature:	300°C
Injection size:	1-2 uL
Initial temperature:	200°C
Initial time:	2 min
Temperature program:	200°C to 330°C at 5°C/min

TABLE 4
Ions Monitored for HRGC/MS of PCDD/PCDF

Descriptor	ID	Mass	Nominal dwell time (sec)
A1	TCDF	303.902	0.090
		305.899	0.090
	$^{13}C_{12}$-TCDF	315.942	0.090
		317.939	0.090
	TCDD	319.897	0.090
		321.894	0.090
	$^{13}C_{12}$-TCDD	331.937	0.090
		333.934	0.090
	HxCDPE	373.840	0.090
	PFK(lock mass)	380.976	0.090
A2	TCDF	303.902	0.045
		305.899	0.045
	TCDD	319.897	0.045
		321.894	0.045
	PeCDF	337.863	0.045
		339.860	0.045
	$^{13}C_{12}$-PeCDF	349.903	0.045
		351.900	0.045
	PeCDD	353.858	0.045
		355.855	0.045
	$^{13}C_{12}$-PeCDD	365.898	0.045
		367.895	0.045
	PFK(lock mass)	380.976	0.035
	HpCDPE	407.801	0.035
A3	HxCDF	373.821	0.080
		375.818	0.080
	PFK(lock mass)	380.976	0.080
	$^{13}C_{12}$-HxCDF	385.861	0.080
		387.858	0.080
	HxCDD	389.816	0.080
		391.813	0.080
	$^{13}C_{12}$-HxCDD	401.856	0.080
		403.853	0.080
	OCDPE	443.759	0.080

TABLE 4 (continued)

Descriptor	ID	Mass	Nominal dwell time (sec)
A4	PFK(lock mass)	380.976	0.040
	HxCDD	389.816	0.040
		391.813	0.040
	HpCDF	407.782	0.040
		409.779	0.040
	$^{13}C_{12}$-HpCDF	419.822	0.040
		421.819	0.040
	HpCDD	423.777	0.040
		425.774	0.040
	$^{13}C_{12}$-HpCDD	435.817	0.040
		437.814	0.040
	NCDPE	477.720	0.040
A5	PFK(lock mass)	380.976	0.060
	OCDF	441.743	0.070
		443.740	0.070
	$^{13}C_{12}$-OCDF	453.783	0.070
		455.780	0.070
	OCDD	457.738	0.070
		459.735	0.070
	$^{13}C_{12}$-OCDD	469.779	0.070
		471.776	0.070
	DCDPE	511.681	0.060

TABLE 5
HRGC/HRMS Operating Conditions

Mass spectrometer

Accelerating voltage:	8,000 V
Trap current:	500 uA
Electron energy:	70 eV
Electron multiplier voltage:	2,200 V
Source temperature:	280°C
Resolution:	10,000 (10% valley definition)

SIM Parameters

Identify	Mass	Nominal dwell time(s)
TCDD-COC1	258.930	0.04
TCDD	319.897	0.07
TCDD	321.894	0.07
$^{13}C_{12}$-TCDD	331.937	0.07
$^{13}C_{12}$-TCDD	333.934	0.07
PFK(lock mass)	230.983	0.07
TCDF-COCL	242.935	0.04
TCDF	303.902	0.07
TCDF	305.872	0.07
$^{13}C_{12}$-TCDF	315.942	0.07
$^{13}C_{12}$-TCDF	317.939	0.07

Overall SIM cycle time = 1 s

Gas chromatograph

Column coating:	Rtx-2330 or SP-2330
Film thickness:	0.1 um
Column dimensions:	60 m X 0.25 mm ID
Helium linear velocity:	≈ 25 cm/s
Helium head pressure:	1.75 kg/cm^2 (25 psi)
Injection type:	Spitless, 45 s
Split flow:	30 mL/min
Purge flow:	6 mL/min
Injector temperature:	270°C
Interface temperature:	260°C
Injection size:	2 uL
Initial temperature:	200°C
Initial time:	2 min
Temperature program:	200°C to 270°C at 4°C/min

TABLE 6
Concentration Calibration Solutions

Compound	Concentration in calibration solutions in pg/uL									
Native	CS1	CS2	CS3	CS4	CS5	CS6	CS7	CS8	CS9	CS10
2,3,7,8-TCDD	200	100	50	25	10	5	2.5	1	1000	500
2,3,7,8-TCDF	200	100	50	25	10	5	2.5	1	1000	500
1,2,3,7,8-PeCDD	200	100	50	25	10	5	2.5	1	1000	500
1,2,3,7,8-PeCDF	200	100	50	25	10	5	2.5	1	1000	500
2,3,4,7,8-PeCDF	200	100	50	25	10	5	2.5	1	1000	500
1,2,3,4,7,8-HxCDD	500	250	125	62.5	25	12.5	6.25	2.5	2500	1250
1,2,3,6,7,8-HxCDD	500	250	125	62.5	25	12.5	6.25	2.5	2500	1250
1,2,3,7,8,9-HxCDD	500	250	125	62.5	25	12.5	6.25	2.5	2500	1250
1,2,3,4,7,8-HxCDF	500	250	125	62.5	25	12.5	6.25	2.5	2500	1250
1,2,3,6,7,8-HxCDF	500	250	125	62.5	25	12.5	6.25	2.5	2500	1250
1,2,3,7,8,9-HxCDF	500	250	125	62.5	25	12.5	6.25	2.5	2500	1250
2,3,4,6,7,8-HxCDF	500	250	125	62.5	25	12.5	6.25	2.5	2500	1250
1,2,3,4,6,7,8-HpCDD	500	250	125	62.5	25	12.5	6.25	2.5	2500	1250
1,2,3,4,6,7,8-HpCDF	500	250	125	62.5	25	12.5	6.25	2.5	2500	1250
1,2,3,4,7,8,9-HpCDF	500	250	125	62.5	25	12.5	6.25	2.5	2500	1250
OCDD	1,000	500	250	125	50	25	12.5	5	5000	2500
OCDF	1,000	500	250	125	50	25	12.5	5	5000	2500

TABLE 6 (continued)

Compound	Concentration in calibration solutions in pg/uL									
	CS1	CS2	CS3	CS4	CS5	CS6	CS7	CS8	CS9	CS10
Internal Quantitation Standards										
$^{13}C_{12}$-2,3,7,8-TCDD	50	50	50	50	50	50	50	50	50	50
$^{13}C_{12}$-2,3,7,8-TCDF	50	50	50	50	50	50	50	50	50	50
$^{13}C_{12}$-1,2,3,7,8-PeCDD	50	50	50	50	50	50	50	50	50	50
$^{13}C_{12}$-1,2,3,7,8-PeCDF	50	50	50	50	50	50	50	50	50	50
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	125	125	125	125	125	125	125	125	125	125
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	125	125	125	125	125	125	125	125	125	125
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	125	125	125	125	125	125	125	125	125	125
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	125	125	125	125	125	125	125	125	125	125
$^{13}C_{12}$-OCDD	250	250	250	250	250	250	250	250	250	250
Internal Recovery Standards										
$^{13}C_{12}$-1,2,3,4-TCDD	50	50	50	50	50	50	50	50	50	50
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	125	125	125	125	125	125	125	125	125	125

The original calibration curve was established using 7 of the 10 levels of calibration standards analyzed in triplicate (CS2, CS3, CS5, CS6, CS7, CS8, CS10, see Table 6). The calibration standards ranged from 1-500 pg/uL for the tetrachloro and pentachloro congeners, and 5-2500 pg/uL for the octachloro congeners. The solution concentrations (pg/uL) can be considered equivalent to residue levels in picograms/gram of adipose tissue assuming a 10-gram sample is available for analysis.

The criteria for acceptance of the initial calibration were the percent relative standard deviations for the response factors (RRF) for each triplicate analysis. The single concentration calibration standard for each analyte were less than ± 30%, except for TCDD and TCDF, which were less than ± 20%. In addition, the variation of the mean RRFs for the six concentration calibration standards was less than 30% except for TCDD and TCDF which was less than 20%. All quantitation ions presented a signal-to-noise ratio of ≥ 2.5 and the isotopic ratios were within 20% of the theoretical values.

At the beginning of each day the mass spectrometer was tuned and mass calibrated using perfluorokerosene (PFK). Mass resolution at the beginning of the day met a minimum resolution of 3,000 (10% valley) for the low resolution. For the high resolution mass spectrometer analyses, mass resolution met a minimum resolution of 10,000 (10% valley) both at the beginning of the day and at the end of the day.

Column performance for TCDD was demonstrated daily after the mass resolution check. A solution of eight TCDD isomers was used to document the separation of 2,3,7,8-TCDD from all other isomers. This solution contained TCDD isomers eluting close to 2,3,7,8-TCDD, the first and the last eluting TCDDs and carbon-13 labeled and unlabeled 2,3,7,8-TCDD. For the low resolution mass spectrometer analysis, the chromatographic peak separation between 2,3,7,8-TCDD and the other peaks representing the other TCDD isomers was resolved with the height of the valley less than or equal to 60% of the height of the 2,3,7,8-TCDD peak. For the high resolution mass spectrometer analysis the peak separation was less than or equal to 25%.

Routine calibrations were conducted at the beginning of each analysis day before actual sample analyses were performed and as the last analysis of each day. A calibration standard was also analyzed whenever there was a change in the mass spectrometry (MS) analyst during the day. The CS7 calibration standard was run at the beginning of the analysis day. The levels that were run at other times of the day varied but were within an acceptable range of the original calibration. The criterion for accepting the routine calibration was when the RRFs for all analytes were within ± 30% of the grand mean values established in the initial calibration, except for TCDD and TCDF, which were within ± 20%.

Tridecane blanks were analyzed daily after the routine calibration standard to ensure that there was no carryover of

analytes into the sample runs. Sample analyses followed the tridecane blanks.

Criteria for a positive identification of a PCDD/PCDF isomer were as follows: (1) the ion current response for each mass of a particular PCDD/PCDF analyte were within ± 1 second; (2) the ion current intensity for a particular PCDD/PCDF must be greater than or equal to 2.5 times the noise level; (3) the integrated ion current ratio of the analytical mass for a particular PCDD/PCDF was within ± 20% of the theoretical value.

Compounds that met the criteria for qualitative identification were quantitated. Complete details can be found in the analytical protocol.[25] The target analytes were quantitated using the appropriate internal quantitation standard. The internal quantitation standards were quantitated using the appropriate internal recovery standard. Table 7 shows the pairing of the target analytes, internal quantitation standards, and internal recovery standards. All results were reported on a lipid adjusted basis.

The data were classified to indicate the intensity of the signal response: Not Detected (ND)--Signal-to-noise ratio was less than 2.5; Trace (TR)--Signal-to-noise ratio was greater than or equal to 2.5 but less than 10; Positive Quantifiable (PQ)--Signal-to-noise ratio was greater than or equal to 10.

E. Quality Assurance Program

The Quality Assurance (QA) Program for this study included the analysis of the Quality Control (QC) samples. The QC samples consisted of internally spiked lipid samples, unspiked control lipid samples, method blank samples, externally spiked lipid samples, split samples, and performance audit solutions.

Other facets of the daily QA program were the verification of the following: the relative response factors for each analyte, column performance checks, mass resolution verification, and solvent (tridecane) blanks. Each of these have been described previously in the Instrumental Analysis Section.

Another aspect of the QA program was the determination of the absolute recovery for the internal quantitation standards in each sample. Nine stable isotope labeled PCDDs and PCDFs were added to each sample at the beginning of the sample preparation to quantify the target analytes. Two internal recovery standards were added to the sample extract prior to injection into the HRGC/MS. They were used to quantitate the internal quantitation standards and to determine the percent recovery of each sample.

Three system audits were conducted by the quality control (QC) coordinator during the course of the study (beginning, middle, and end). The system audits involved reviewing, assessing and inspecting various aspects of the study including personnel, facilities, equipment, record keeping, data management, written protocols, standard operating procedures, and the reporting procedures of the project. All procedures were found to be satisfactory.

TABLE 7
Target Analyte/Internal Quantitation Standard and Internal Quantitation Standard/Internal Recovery Standard Pairs

	Internal Standards	
Target analyte	Quantitation	Recovery
2,3,7,8-TCDD	$^{13}C_{12}$-2,3,7,8-TCDD	$^{13}C_{12}$-1,2,3,4-TCDD
2,3,7,8-TCDF	$^{13}C_{12}$-2,3,7,8-TCDF	$^{13}C_{12}$-1,2,3,4-TCDD
1,2,3,7,8-PeCDF	$^{13}C_{12}$-1,2,3,7,8-PeCDF	$^{13}C_{12}$-1,2,3,4-TCDD
2,3,4,7,8-PeCDF	$^{13}C_{12}$-1,2,3,7,8-PeCDF	$^{13}C_{12}$-1,2,3,4-TCDD
1,2,3,7,8-PeCDD	$^{13}C_{12}$-1,2,3,7,8-PeCDD	$^{13}C_{12}$-1,2,3,4-TCDD
1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
1,2,3,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
2,3,4,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
1,2,3,7,8,9-HxCDF	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
1,2,3,4,7,8-HxCDD	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
1,2,3,6,7,8-HxCDD	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
1,2,3,7,8,9-HxCDD	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
1,2,3,4,6,7,8-HpCDF	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
1,2,3,4,7,8,9-HpCDF	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
1,2,3,4,6,7,8-HpCDD	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
OCDF	$^{13}C_{12}$-OCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
OCDD	$^{13}C_{12}$-OCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD

1. Quality Control Samples

A total of 80 Quality Control (QC) samples were analyzed along with the 200 study specimens. The QC samples provided data on method accuracy and precision. The 80 QC samples were broken down as follows: 20 internally spiked lipid samples, 20 unspiked control lipid samples, 20 method blank samples, 7 externally spiked lipid samples, 6 split samples and 7 performance audit solutions (consisting of 2,3,7,8-TCDD only).

The QC lipid material for the internally spiked lipid samples, the unspiked control lipid samples, and the externally spiked lipid samples were prepared from composited human adipose tissue specimens collected through the EPA National Human Adipose Tissue Survey. Enough homogenized lipid material was prepared for use in the method evaluation study and for use as QC samples. A detailed description of the preparation of the homogenized tissue can be found in the method evaluation report.[25] A summary of the QC lipid sample preparation is given below.

The composited adipose tissue specimens were blended with methylene chloride, and the extract was dried by eluting through anhydrous sodium sulfate. The methylene chloride was removed by rotary evaporation in a water bath at 60°C. The homogenized bulk lipid material was stored in the freezer until further preparation.

In order to prepare the unspiked control lipid samples, the homogenized lipid material was brought to room temperature and warmed slightly to achieve an oily state prior to subdividing. Twenty aliquots of approximately 10.0 grams each of the oily material were transferred by pipette to preweighed glass vials, and the actual weight of the lipid was determined to the nearest 0.01 g. These 20 samples were labeled with unique laboratory numbers so that the laboratory personnel could not identify them as unspiked control lipid samples.

The internally spiked lipid samples were prepared by taking 20 aliquots of approximately 10.0 grams each of the oily material as described above. The spiking solution of native PCDD and PCDF congeners was prepared in isooctane. Table 8 specifies the levels of each of the PCDD and PCDF congeners in this solution.

Sample solutions from the spiking solution were evaluated to verify that the latter was prepared correctly. Concentrations of the native PCDDs and PCDFs in the spiking solution were verified by preparing solutions at three spike levels in triplicate. The spike levels were prepared at concentrations equivalent to 10.0, 25.0, and 50.0 pg/uL for the tetra- and pentachloro congeners; 25.0, 62.5, and 125.0 pg/uL for the hexa- and heptachloro congeners; and 50.0, 125.0, and 250.0 pg/uL for the octachloro congeners.

Internally spiked lipid samples at three spiking levels were planned. Five to nine samples were prepared at each spike level. This was achieved by adding 20, 50 or 100 uL of the native spiking solution to the 10-gram aliquots of lipid, to give low, medium and high level spikes. These spike levels, based on a 10.0 g lipid sample, were equivalent to concentrations of 10, 25,

TABLE 8
Native PCDD and PCDF Spiking
Solution in Isooctane

Compound	Concentration (pg/uL)
2,3,7,8-TCDD	5.0
2,3,7,8-TCDF	5.0
1,2,3,7,8-PeCDD	5.0
1,2,3,7,8-PeCDF	5.0
2,3,4,7,8-PeCDF	5.0
1,2,3,4,7,8-HxCDD	12.5
1,2,3,6,7,8-HxCDD	12.5
1,2,3,7,8,9-HxCDD	12.5
1,2,3,4,7,8-HxCDF	12.5
1,2,3,6,7,8-HxCDF	12.5
1,2,3,7,8,9-HxCDF	12.5
2,3,4,6,7,8-HxCDF	12.5
1,2,3,4,6,7,8-HpCDD	12.5
1,2,3,4,6,7,8-HpCDF	12.5
1,2,3,4,7,8,9-HpCDF	12.5
OCDD	25.0
OCDF	25.0

and 50 pg/g of the lipid matrix for the tetra- and pentachloro PCDD and PCDF congeners, up to 50, 125, and 250 pg/g for the OCDD and OCDF (see Table 9). The exact spiking level was calculated based on the amount of lipid material as determined to the nearest 0.01 g. As with the unspiked control lipid samples, these samples were labeled with unique laboratory numbers by the quality control coordinator so that the laboratory personnel could not identify them as internally spiked lipid samples.

Six study specimens were analyzed as split samples. The six specimens were selected without knowledge of which study group the specimens belonged to and were based on the amount of tissue available for analysis. The six study specimens were separated into two aliquots each. All 12 aliquots were placed in specimen jars similar to the jars used for the rest of the study specimens. The split samples were also labeled with unique laboratory numbers by the quality control coordinator so that the laboratory personnel could not identify the split samples. Both members of the split samples were analyzed within the same batch. The six pairs were analyzed in different batches throughout the study.

The performance audit solutions were prepared by the quality control coordinator according to a predetermined schedule throughout the study and submitted to the analyst as blind samples. The performance audit solutions were prepared from a certified solution of 2,3,7,8-TCDD in isooctane obtained from the National Bureau of Standards (NBS Standard Reference Material 1614, dated April 24, 1986).

A method blank sample was included with each batch. The method blank analysis was generated by performing all steps of the analytical procedure, which included use of all reagents, standards, equipment, apparatus, glassware and solvents, but omitted the addition of the adipose tissue.

2. <u>QC Charts</u>

QC Charts were generated to display the QC data and control limits or data quality objectives. Plots were prepared for four types of data during this study: (1) Relative Response Factors, (2) Recovery of Internal Quantitation Standards, (3) Accuracy of Internally Spiked Lipid Samples, and (4) Measurements of Unspiked Control Lipid Samples.

The charts of the relative response factors illustrate the initial calibrations and the daily calibrations. Control limits of 30% variability for all congeners, with the exception of 20% for tetrachlorinated congeners, were calculated from the initial calibration data and indicated on the charts. A chart was prepared for each native congener and each internal quantitation standard. The data were monitored each analysis day and the charts were updated about every 5th batch.

The plots of the percent recovery of the internal quantitation standards for each sample were prepared for each batch. Cumulative plots were prepared and reported every 5th

TABLE 9
Spiking Levels of the Internally Spiked Lipid Samples (based on 10.00 gram lipid samples)

Compound	Spike Level (pg/g)		
	Low	Medium	High
2,3,7,8-TCDD	10	25	50
2,3,7,8-TCDF	10	25	50
1,2,3,7,8-PeCDD	10	25	50
1,2,3,7,8-PeCDF	10	25	50
2,3,4,7,8-PeCDF	10	25	50
1,2,3,4,7,8-HxCDD	25	62.5	125
1,2,3,6,7,8-HxCDD	25	62.5	125
1,2,3,7,8,9-HxCDD	25	62.5	125
1,2,3,4,7,8-HxCDF	25	62.5	125
1,2,3,6,7,8-HxCDF	25	62.5	125
1,2,3,7,8,9-HxCDF	25	62.5	125
2,3,4,6,7,8-HxCDF	25	62.5	125
1,2,3,4,6,7,8-HpCDD	25	62.5	125
1,2,3,4,6,7,8-HpCDF	25	62.5	125
1,2,3,4,7,8,9-HpCDF	25	62.5	125
OCDD	50	125	250
OCDF	50	125	250

batch. The data quality objectives of 50 - 115% recovery were indicated on the plots.

The percent recovery of the native congener measurements in the internally spiked lipid samples were charted every 5th batch. The data were also reported in tabular form after each batch. The data quality objectives of 50 - 130% recovery were indicated on the plots.

The control charts for the unspiked control lipid samples indicated the measurements of the native congeners in each sample after every 5th batch. The data were also reported in tabular form after each batch. The 95% confidence intervals of the measurements established in the Method Evaluation Study[25] were indicated on the plots.

3. Details of the Analytical Run

A total of 200 study specimens and 80 Quality Control samples were analyzed in 20 batches. Each sample batch typically consisted of 10 study specimens (a random selection of 2 Vietnam veterans, 4 non-Vietnam veterans and 4 civilians), 1 internally spiked lipid sample, 1 unspiked control lipid sample, 1 method blank sample, and 1 of the following: a performance audit solution, a split sample, or an externally spiked lipid sample.

All study specimens and QC samples were coded with a unique laboratory number and submitted to the analysts as blind samples by the quality control coordinator. The batch assignment and order of analysis within a batch were also specified by the QC coordinator. The results of the analysis were submitted back to the QC coordinator who decoded the samples and labeled the results with a study number just prior to the reporting procedure.

III. RESULTS

A. Demographic and Military Service Characteristics

Demographic and military service characteristics of the study subjects are summarized in Tables 10 and 11, respectively. There was no significant group difference with respect to any of the 5 demographic variables--specimen collection year, age at accession, race, geographic region and body mass index. Similarly, military service characteristics--branch of service, rank, education, enlistment year, discharge year and military occupational specialty--of the Vietnam veterans and non-Vietnam veterans were comparable. Tables 11 and 12 describe some of the military service characteristics of Vietnam veterans in the study. Distributions of branch, rank, MOSC, calendar year served in Vietnam and length of service in Vietnam for these Vietnam veterans were approximately similar to other groups of Vietnam veterans reported by the Department of Veterans Affairs and other sources.

Using an exposure likelihood criteria of 3 days/2 km time and distance from recorded Agent Orange spray, approximately 1/10 (n = 4) of the Vietnam veteran subjects were categorized as having had an opportunity for exposure to Agent Orange at least once (Table 12). Using a much broader criterion of 90 days/8 km time and distance from recorded Agent Orange spray, approximately one half (n = 19, which includes the 4 mentioned above) of the Vietnam veterans were categorized as having had an opportunity for exposure to Agent Orange. None of the Vietnam veteran study subjects were members of the Air Force Ranch Hand Operation or Army Chemical Corps. All navy personnel on sea duty were classified as having "unlikely" exposure to Agent Orange.

B. 2,3,7,8-TCDD Levels by Demographic Characteristics

Four of the 40 veterans initially classified as having served in Vietnam and one of the 80 veterans initially classified as not having served in Vietnam were excluded from further analyses for the following reasons: two Vietnam veterans' adipose specimens had less than 20% extractable lipid content; one "Vietnam" veteran served in Southeast Asia but only in Thailand and not in Vietnam; one Vietnam veteran did not have enough tissue for analysis; and one "non-Vietnam veteran" was misclassified as a veteran (his military service could not be documented unequivocally).

Table 13 shows the arithmetic mean, geometric mean and various percentile values for 2,3,7,8-TCDD in adipose tissue of the three study groups. Table 14 presents the percentages of samples in the two veteran groups that fall under the 25th, 50th, 75th and 90th civilian control percentiles. A chi-square test determined that there was no group difference in the distribution of TCDD levels. Histograms of the TCDD distribution for the three groups before and after logarithmic transformation are presented in Figures 2 and 3. The distribution of TCDD levels after logarithmic transformation was found to be approximately

TABLE 10
Demographic Characteristics of Study Subjects

Characteristics	Vietnam Veterans (n = 36)		Non-Vietnam Veterans (n = 79)		Civilians (n=80)	
	No.	%	No.	%	No.	%
Specimen Collection						
1971 to 1973	6	17	10	13	11	14
1974 to 1976	5	14	13	16	11	14
1977 to 1979	12	33	23	29	22	27
1980 to 1982	13	36	33	42	36	45
median	1978		1979		1978	
Age at Specimen Collection						
20 to 26	6	17	10	13	14	17
27 to 32	15	42	29	37	31	39
33 to 38	12	33	27	34	24	30
39 to 45	3	8	13	16	11	14
median	32		33		32	
Race						
white	27	75	69	87	60	75
non white	9	25	10	13	20	25
Geographic Region						
North Central	7	19	21	27	11	14
North East	6	17	17	21	27	34
South	15	42	35	44	34	42
West	8	22	6	8	8	10
Body Mass Index (kg/m^2)						
17 to 21	3	8	2	2	7	9
22 to 24	9	25	30	38	15	19
25 to 27	13	36	19	24	25	31
28 to 30	4	11	10	13	8	10
31 to 54	5	14	5	6	11	14
unknown	2	6	13	17	14	17
median	25.4		25.8		25.8	

TABLE 11
Military Service Characteristics of Veterans

Characteristics	Vietnam Veterans (n = 36)		Vietnam Veteran Reference Population	Non-Vietnam Veterans (n = 79)	
	No.	%	%	No.	%
Branch of Service					
Army	20	55	68[a]	42	53
Air Force	1	3	8	10	13
Marines	6	17	17	9	11
Navy	9	25	7	16	20
Coast Guard	0	0		2	3
Rank					
Officers	4	11	7[b]	7	9
Enlisted	32	89	93	72	91
Education (years)					
11 or less	8	22	20[b]	22	28
12 to 15	23	64	72	48	61
16 or more	5	14	8	8	10
unknown	0	0		1	1
Enlistment Year					
1954 to 1959	1	3	2[c]	10	13
1960 to 1965	13	36	31	29	37
1966 to 1971	22	61	67	34	43
1972 to 1976	0	0	0	6	7
Discharge Year					
1958 to 1963	0	0	0[c]	17	21
1964 to 1969	17	47	51	26	33
1970 to 1975	16	45	45	30	38
1976 to 1982	3	8	4	6	8
Military Occupational Specialty					
Non Combat	24	67	68[c]	56	71
Combat	12	33	32	22	28
unknown	0	0		1	1

[a] Percentage from approximately 120,000 Vietnam veterans in the VA Agent Orange Registry
[b] Data from Vietnam era veterans, VA, September 1981
[c] Percentage from approximately 1,500 Vietnam veterans in the VA Patient Treatment File

TABLE 12
Military Service Characteristics
of Veterans Who Served in Vietnam

Characteristics	Vietnam Veterans (n = 36)		Vietnam Veteran Reference Population
	No.	%	%
Last Year in Vietnam			
1965 to 1966	3	8	9*
1967 to 1968	15	42	32
1969 to 1970	12	33	41
1971 to 1973	6	17	18
Place of Service in Vietnam			
MR I	11	31	47*
MR II	4	11	20
MR III	9	25	24
MR IV	3	8	6
Sea Duty	8	22	3
unknown	1	3	
Time and Distance from Recorded Agent Orange Spray			
A. 3 days / 2 KM			
unlikely	31	86	
likely	4	11	
unknown	1	3	
B. 90 days / 8 KM			
unlikely	16	44	
likely	19	53	
unknown	1	3	

* Percentage from approximately 120,000 Vietnam veterans in the VA Agent Orange Registry

TABLE 13
Distribution of 2,3,7,8-TCDD Levels
in Adipose Tissue by Military Service Status,
in pg/g of the Total Extractable Lipid (ppt)

Status	N	Arithmetic Mean ± SD[a]	Geometric Mean	Percentile 25th	50th (median)	75th	90th	95th
Vietnam Veterans	36[b]	13.4 ± 7.4	11.7	7.8	10.0	17.3	26.8	30.4
Non-Vietnam Veterans	79[c]	12.5 ± 7.2	10.9	7.6	11.4	14.8	19.8	25.3
Civilians	80	15.8 ± 14.5[d]	12.4	7.9	11.8	18.0	30.5	43.4

[a] Standard deviation

[b] Four of the 40 men initially classified as having served in Vietnam were excluded from analysis because two veterans' specimens had less than 20% extractable lipid content, one veteran did not have adequate amount of tissue for analysis, and one veteran served only in Thailand.

[c] One of the 80 men initially classified as having served in the military was excluded because his military service could not be documented unequivocally.

[d] The large standard deviation was attributed to an outlier with a value of 106. The value was verified. The occupational history of this individual is unknown. He was listed as a "laborer" on his death certificate. Analyses conducted without this value resulted in an arithmetic mean of 14.7 (± = 10.3) and a geometric mean of 12.2. There was still no statistically significant difference between the groups ($p = 0.49$).

TABLE 14
Percent Distribution of Samples in Each Study Group that Fall Under the 25th, 50th, 75th and 90th Civilian Percentile TCDD Levels, in pg/g of the Total Extractable Lipid (ppt)

Status	TCDD (ppt)			
	≤ 7.9	≤ 11.8	≤ 18.0	≤ 30.5
Vietnam Veterans (N = 36)	28	58	78	97
Non-Vietnam Veterans (N = 79)	25	53	85	97
Civilians (N = 80)	25	50	75	90

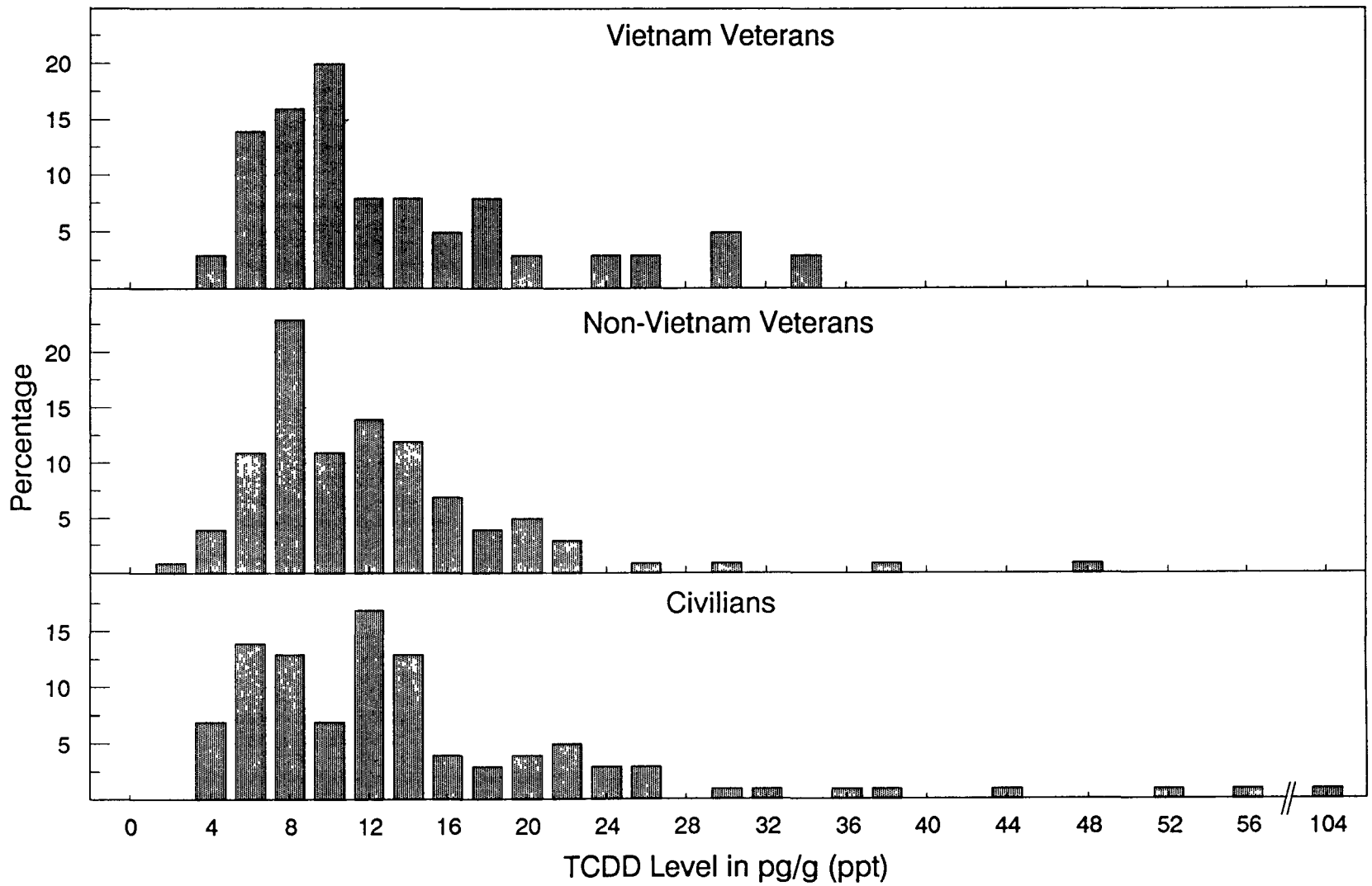


Figure 2. Histogram of 2,3,7,8-TCDD Levels by Study Group Before Log Transformation

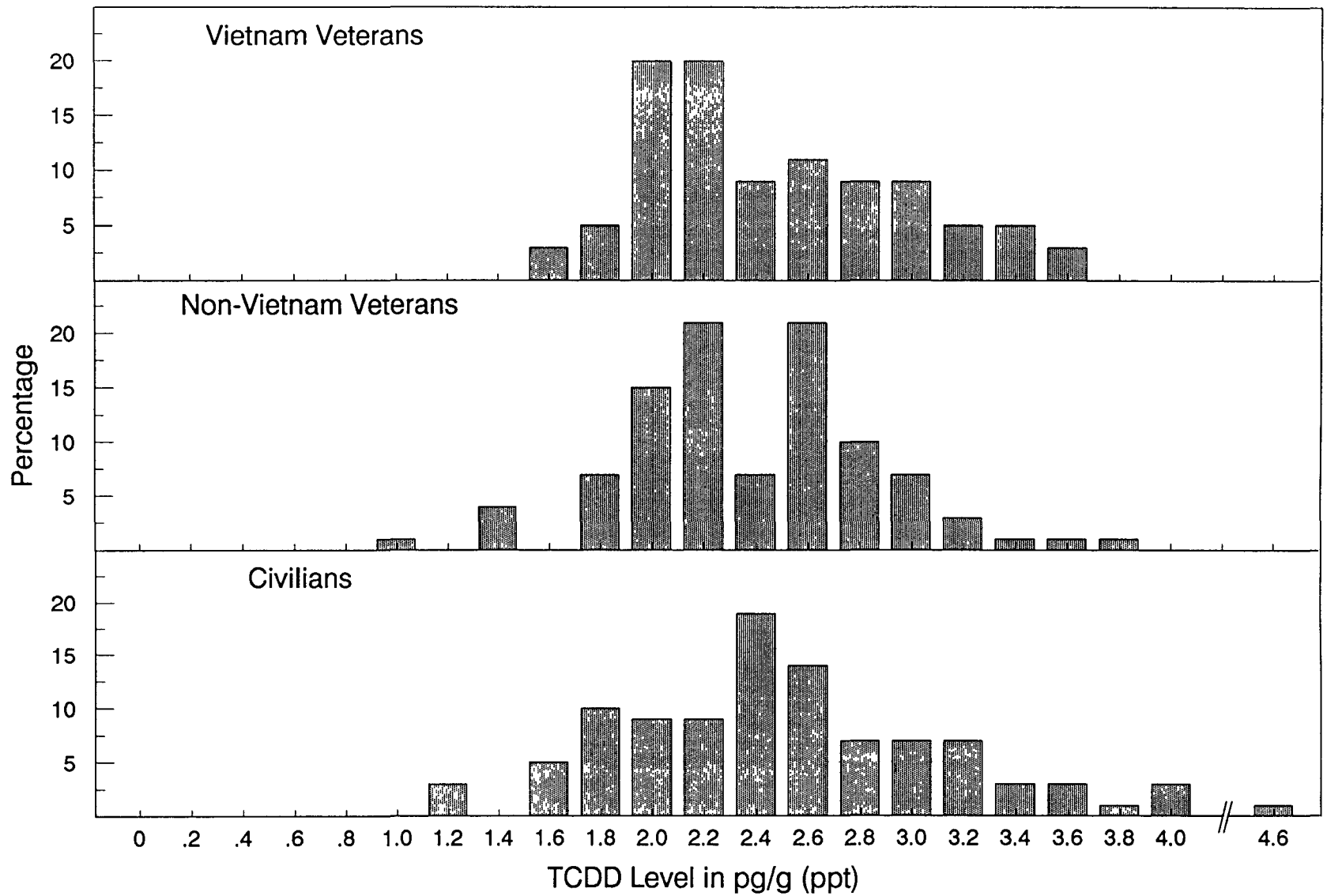


Figure 3. Histogram of 2,3,7,8-TCDD Levels by Study Group After Log_e Transformation

normal as evidenced by plots of the cumulative curve for each group on normal-probability graph paper that resulted in straight lines.

Analysis of variance resulted in no statistically significant difference in the mean TCDD levels between the groups ($p = 0.35$). Analysis of covariance tested the effect of Vietnam service after adjusting for age, sample collection year (or length of storage) or body mass index. The results did not indicate an association between service in Vietnam and TCDD levels. A paired t-test between Vietnam veterans and their matched civilian pairs did not result in significant findings ($p = 0.52$; 95% confidence interval for the difference between two means = -1.32, 1.16).

The geometric mean 2,3,7,8-TCDD levels are presented in Table 15 by age, specimen collection year, storage time, geographic region in the U.S., race and occupational category for each study group. The length of storage time of each specimen was calculated by the difference between the time the specimen was collected and the time it was analyzed in 1987. There does not appear to be any significant differences in TCDD levels by each variable except for specimen collection year or storage time ($p < .0001$). In each group the levels of TCDD tended to be inversely related to the specimen collection year, i.e., the earlier the collection year, the higher the levels of TCDD. In fact, this general time trend was observed for other dioxins (Table 16).

Because the levels of 2,3,7,8-TCDD were adjusted for the amount of extractable lipid in the adipose tissue specimens, the final concentration of TCDD would have increased mathematically for any sample for which there had been degradation of the lipid during storage. However, no significant difference of the percentage of extractable lipid in the tissue specimen by storage time was observed. The mean percent extractable lipid for four categories of storage times ranged from 79.5 to 82.7%.

A stepwise multiple regression was used to determine whether 2,3,7,8-TCDD levels were associated with demographic variables such as age, race, body mass index, and the sample collection year. The TCDD levels in adipose tissue were found to be significantly associated with age ($p < .001$) and sample collection year ($p < .001$). TCDD levels increased approximately 1.5 ppt per 10 years of age and decreased approximately 1.0 ppt per advancing calendar year of sample collection. However, these 4 variables were not good predictors of TCDD levels because they accounted for less than 20% of the variation in tissue TCDD levels ($R^2 = 0.17$).

C. <u>2,3,7,8-TCDD Levels by Military Service Characteristics</u>

TCDD levels for Vietnam veterans and non-Vietnam veterans were evaluated by their branch of service, military occupations and rank. No significant difference was observed between veteran groups in the same military service category or between different military service categories within the same veteran group (Table

TABLE 15
Geometric Mean 2,3,7,8-TCDD Levels in Adipose Tissue
by Demographic Characteristics
in pg/g of the Total Extractable Lipid (ppt)

Variables	Status			
	Vietnam Veterans	Non-Vietnam Veterans	Civilian	Total
Age				
20 to 26	13.87 (6)*	10.38 (10)	15.18 (14)	13.07
27 to 32	12.55 (15)	10.49 (29)	10.70 (31)	10.91
33 to 38	10.91 (12)	11.02 (27)	11.36 (24)	11.13
39 to 45	8.17 (3)	12.43 (13)	18.36 (11)	13.87
Collection Year				
1971 to 1973	16.95 (6)	16.44 (10)	21.12 (11)	18.36
1974 to 1976	16.61 (5)	14.30 (13)	15.33 (11)	15.03
1977 to 1979	10.80 (12)	11.13 (23)	10.18 (22)	10.70
1980 to 1982	9.30 (13)	8.58 (33)	11.36 (36)	9.87
Geographic Region				
North Central	11.25 (7)	12.55 (21)	12.18 (11)	12.18
North East	12.94 (6)	10.80 (17)	12.55 (27)	11.94
South	10.38 (15)	10.80 (35)	13.74 (34)	11.82
West	14.30 (8)	7.85 (6)	8.41 (8)	9.97
Race				
White	10.70 (27)	10.91 (69)	12.30 (60)	11.36
Non White	15.33 (9)	11.36 (10)	13.07 (20)	13.07
Occupational Group[1]				
Non Labor	10.59 (12)	10.91 (28)	13.07 (35)	11.70
Labor	11.59 (9)	10.49 (23)	11.59 (32)	11.13
Agricultural	25.03 (1)	12.94 (1)	(0)	17.99

* the numbers in parentheses represent the cases for that category

[1] 54 cases had unknown occupations; 14 missing for Vietnam veterans; 27 missing for non-Vietnam veterans; 13 missing for civilians

TABLE 16
Selected Arithmetic Mean PCDD Levels in Adipose Tissue by Sample Collection Year, in pg/g of the Total Extractable Lipid (ppt)

Sample Collection Year (by groups)	N	2378-TCDD	12378-PeCDD	123478/ 123678-HxCDD	1234678-HpCDD	OCDD
All Subjects						
1971 to 1973	27	19.8	21.7	173.2	364.4	1530.5
1974 to 1976	29	17.3	23.7	189.9	291.3	1395.2
1977 to 1979	57	11.6	17.9	158.5	267.5	1233.1
1980 to 1982	82	12.6	16.5[1]	148.9	240.9	1126.7
Vietnam Veterans						
1971 to 1973	6	19.3	25.3	205.1	440.3	1754.7
1974 to 1976	5	18.3	30.7	225.6	300.8	1408.2
1977 to 1979	12	11.7	18.6	169.0	271.5	1238.6
1980 to 1982	13	10.3	16.3	134.4	195.2	999.5
Non-Vietnam Veterans						
1971 to 1973	10	17.0	20.0	150.4	288.0	1229.8
1974 to 1976	13	15.7	23.2	184.8	251.5	1097.2
1977 to 1979	23	12.7	19.4	171.9	278.5	1314.4
1980 to 1982	33	9.7	14.9[2]	127.9	204.9	933.6
Civilians						
1971 to 1973	11	22.5	21.3	176.6	392.6	1681.6
1974 to 1976	11	18.7	21.2	179.7	333.8	1741.6
1977 to 1979	22	10.5	15.9	138.7	253.9	1145.2
1980 to 1982	36	16.2	18.0	173.3	290.2	1349.6

[1] N = 81; one Non-Vietnam Veteran had a level that was not detected above the Limit of Detection (LOD)

[2] N = 32; one Non-Vietnam Veteran had a level that was not detected above the Limit of Detection (LOD)

17). For Vietnam veterans, 2,3,7,8-TCDD levels were also analyzed by factors which were assumed to be related to the likelihood of Agent Orange exposure (Table 18). None of the factors, except for number of years between last service date in Vietnam and the date of collection, appeared to be associated with the TCDD levels in adipose tissue. It seemed in general that the shorter the time between last date of service in Vietnam and the date of collection, the higher the TCDD levels in adipose tissue ($p < .01$). However, this observation was confounded by a close relationship between the number of years since last served in Vietnam and the sample collection year ($r^2 = 0.85$, $p < .0001$): the fewer the years since Vietnam, the earlier the sample collection year. As described previously, the earlier the collection year, the higher the levels of TCDD. The TCDD levels for a total of 7 Vietnam veterans, whose number of years since Vietnam service was 4 years or less, were evaluated further in comparison to their non-Vietnam veteran counterparts and also to their matched civilian pairs. For this purpose a total of 19 non-Vietnam Veterans whose tissue specimens were collected on or before 1974, which was the last sample collection year for the above Vietnam veteran group, were selected for a comparison. The geometric mean TCDD levels (± standard deviation) for the Vietnam veteran group ($n = 7$), non-Vietnam veteran group ($n = 19$) and civilian controls ($n = 14$) were at the levels of 16.6 (± 1.6), 15.5 (± 1.5) and 18.4 (± 1.6) ppt, respectively. The difference between the means was not statistically significant at the $p = 0.05$ level for ANOVA. An analysis of covariance, which controlled for sample collection year, supported this conclusion.

The possible contributions of each military factor to the TCDD levels in Vietnam veterans were evaluated by a stepwise linear regression analysis. Factors included in the analysis were surrogate combat status by MOSC, military region, sample collection year, calendar year in Vietnam, number of years between last year served in Vietnam and the sample collection year and Agent Orange exposure likelihood based on time and distance from recorded Agent Orange spray. Regression analysis showed that these Vietnam service characteristics could account for only 14% of the variation in 2,3,7,8-TCDD levels among Vietnam veterans.

Five other 2,3,7,8-substituted PCDDs and 10 other PCDFs were measured and their mean levels calculated from specimens with levels above the limit of detection (Table 19). There were no group differences in the mean level of any of the PCDD congeners. The levels of dioxins increased with an increase in the number of chlorine except for 1,2,3,7,8,9-HxCDD. Levels of dibenzofurans were always lower than their dioxin counterparts.

D. Quality Assurance Program Results

Data were collected on all 17 of the 2,3,7,8-chlorine substituted dioxins and furans. However, since the primary emphasis of this study was on 2,3,7,8-TCDD, the quality assurance

TABLE 17
Geometric Mean 2,3,7,8-TCDD Levels in Adipose Tissue
by Military Service Characteristics,
in pg/g of the Total Extractable Lipid (ppt)

Service Characteristics	Status: Vietnam Veterans	Status: Non-Vietnam Veterans	Total
Branch			
Army	11.59 (20)*	10.38 (42)	10.70
Air Force	6.69 (1)	9.97 (10)	9.58
Marine	12.30 (6)	10.38 (9)	11.13
Navy	12.43 (9)	13.87 (16)	13.33
Coast Guard	(0)	9.87 (2)	9.87
Military Occupation[1]			
Non Combat	11.13 (24)	10.59 (56)	10.80
Combat	13.07 (12)	11.47 (22)	11.94
Rank			
Officer	10.18 (4)	12.43 (7)	11.59
Enlisted	11.94 (32)	10.80 (72)	11.13

* the numbers in parentheses represent the cases for that category

[1] one Non-Vietnam Veteran had a missing Military Occupation

TABLE 18
Geometric Mean 2,3,7,8-TCDD Levels in Adipose Tissue by Vietnam Service Characteristics, in pg/g of the Total Extractable Lipid (ppt)

Service Characteristics	No. of Veterans	2,3,7,8-TCDD
Military Region[1]		
I Corp	11	12.43
II Corp	4	6.89
III Corp	9	11.94
IV Corp	3	14.30
Sea Duty	8	13.33
Last Year in Vietnam		
1965 to 1966	3	8.58
1967 to 1968	15	11.02
1969 to 1970	12	14.59
1971 to 1973	6	10.38
Number of Years Since Last Service in Vietnam[2]		
2 to 4	7	16.61
5 to 7	5	13.87
8 to 10	9	11.82
11 to 14	15	9.39
No. of Months in Vietnam		
6 months or less	2	15.96
7 to 12 months	28	12.06
13 months or more	6	9.12
Agent Orange Exposure[3] Likelihood		
a. 3 days / 2 KM		
no	31	11.47
yes	4	14.30
b. 90 days / 8 KM		
no	16	11.82
yes	19	11.82

[1] one Vietnam Veteran had a missing Military Region
[2] $p < .001$
[3] one Vietnam Veteran has a missing Agent Orange Exposure Likelihood

TABLE 19
Arithmetic Mean Levels of Dioxins and Furans
Detected in Adipose Tissue by Military Service Status,
in pg/g of the Total Extractable Lipid (ppt)

Chemicals	Status		
	Vietnam Veterans	Non-Vietnam Veterans	Civilians
Dioxins			
2378-TCDD	13.35 (36)*	12.48 (79)	15.83 (80)
12378-PeCDD	20.59 (36)	18.26 (78)	18.32 (80)
123478/123678-HxCDD	170.38 (36)	152.97 (79)	165.13 (80)
123789-HxCDD	19.35 (35)	17.23 (79)	17.99 (79)
1234678-HpCDD	276.17 (36)	244.55 (79)	300.30 (80)
OCDD	1261.81 (36)	1108.89 (79)	1392.95 (80)
Furans			
2378-TCDF	2.92 (25)	2.41 (52)	3.30 (51)
12378-PeCDF	1.72 (8)	1.10 (17)	1.94 (16)
23478-PeCDF	23.08 (35)	22.20 (78)	23.31 (80)
123478-HxCDF	21.50 (36)	19.31 (78)	23.22 (79)
123678-HxCDF	10.71 (34)	9.99 (77)	12.02 (79)
234678-HxCDF	3.77 (26)	3.24 (73)	3.64 (78)
123789-HxCDF	1.48 (3)	0.96 (2)	0.90 (4)
1234678-HpCDF	37.39 (36)	32.95 (79)	39.09 (80)
1234789-HpCDF	2.22 (14)	1.91 (35)	2.16 (41)
OCDF	3.61 (27)	4.46 (54)	3.40 (60)

* the number in parentheses represent the number of specimens in that category which were above the limit of detection

data in this section are focused on 2,3,7,8-TCDD. Data on the other congeners can be found in Appendix B.

Method accuracy and precision were measured and evaluated from the internally spiked lipid samples. Precision was also determined from the results of the split samples. Blank samples were run to ensure that no contamination or carryover from sample to sample occurred. Data from the daily mass calibrations, column performance checks and relative response factors provided information on the performance of the instrumentation.

The analytical standards used in this study compared favorably with the following results: (1) the results of the analysis of the National Bureau of Standards Reference Material for 2,3,7,8-TCDD; (2) the results of an interlaboratory study of analytical standards conducted by Cambridge Isotope Laboratories in which Midwest Research Institute participated; and (3) the results and evaluation of the externally spiked QC samples presented in the appendix.

At the onset of the study, protocols, standard operating procedures, data quality objectives (DQOs) and control limits were established in the quality assurance project plan. All data were generated under these procedures. System audits were conducted during the study to verify that the protocols and procedures were present and in use during the study.

Virtually, all of the data were within the DQOs and control limits. However, the several data points that were outside the DQOs, were explicitly noted in the report. These deviations were minor and did not adversely affect the quality of the data.

The overall method accuracy for 2,3,7,8-TCDD was 113% recovery among the spiked lipid samples. The method precision of 10.6% for 2,3,7,8-TCDD was quantified by the coefficient of variation for the unspiked lipid samples.

1. Internally Spiked Lipid Samples

This section on the internally spiked lipid samples includes data from the analysis of the spiking solution used to prepare the samples and the results on the accuracy of the measurements of the PCDDs and PCDFs in the spiked lipid samples.

a. Evaluation of the Standards Spiking Solution

The spiking solution of analytical standards that was used to prepare the internally spiked the lipid samples was analyzed prior to actually spiking the lipid samples. This analysis was conducted to confirm the concentrations of the PCDDs and PCDFs in the spiking solution and to provide data on the potential variability in spiking concentrations that might be expected from preparing the spiked QC samples. Nine check samples comprising three replicate aliquots at each of the three spike levels (x, 2.5x, 5x) were prepared and analyzed. Table 20 presents the data on the percent recovery of each analyte from each analysis. Also provided in the table are the mean percent recovery and the precision (coefficient of variation) at each spike level.

TABLE 20
Results of the Analysis of the Native
PCDD and PCDF Spiking Solution
-Average Percent Recovery (%)

Compound	Spiked Concentration (pg/uL)	% Recovery (3 trials)			Average % Recovery	SD*	CV**
2,3,7,8-TCDF	10	98	125	104	109	14.2	13.0
	25	104	102	104	104	1.2	1.1
	50	105	105	106	105	0.9	0.8
2,3,7,8-TCDD	10	102	131	105	113	15.9	14.2
	25	102	107	107	105	2.7	2.5
	50	112	112	112	112	0.4	0.4
1,2,3,7,8-PeCDF	10	97	126	100	108	15.9	14.8
	25	100	94	98	97	3.2	3.3
	50	93	104	101	99	5.9	6.0
2,3,4,7,8-PeCDF	10	102	136	108	115	18.1	15.7
	25	111	108	106	108	2.7	2.5
	50	106	111	108	108	2.6	2.4
1,2,3,7,8-PeCDD	10	100	127	99	109	15.9	14.6
	25	113	110	98	107	8.1	7.6
	50	103	109	101	104	4.3	4.1
1,2,3,4,7,8-HxCDF	25	110	121	107	113	7.3	6.5
	62.5	112	97	83	97	14.8	15.2
	125	93	106	90	97	8.6	8.9
1,2,3,6,7,8-HxCDF	25	102	120	103	109	10.2	9.4
	62.5	108	95	89	97	9.6	9.9
	125	96	108	102	102	6.0	5.9
2,3,4,6,7,8-HxCDF	25	94	119	99	104	13.2	12.7
	62.5	101	98	96	98	2.8	2.8
	125	100	97	102	99	2.40	2.5
1,2,3,7,8,9-HxCDF	25	102	119	99	107	10.7	10.0
	62.5	98	99	96	98	1.4	1.4
	125	101	104	107	104	3.2	3.1

* Standard Deviation
** Coefficient of Variation

TABLE 20 (continued)

Compound	Spiked Concentration (pg/uL)	% Recovery (3 trials)			Average % Recovery	SD*	CV**
1,2,3,4,7,8-HxCDD	25	95	109	102	102	6.8	6.7
	62.5	96	111	103	103	7.6	7.3
	125	101	98	105	101	3.6	3.6
1,2,3,6,7,8-HxCDD	25	104	124	100	109	13.2	12.1
	62.5	99	99	106	101	3.8	3.7
	125	106	105	106	106	0.8	0.8
1,2,3,7,8,9-HxCDD	25	106	126	115	116	10.4	9.0
	62.5	99	97	101	99	2.4	2.4
	125	99	100	105	101	3.0	3.0
1,2,3,4,6,7,8-HpCDF	25	92	115	92	100	13.4	13.4
	62.5	97	94	91	94	2.9	3.1
	125	94	91	97	94	2.8	3.0
1,2,3,4,7,8,9-HpCDF	25	96	110	89	98	10.4	10.6
	62.5	103	101	95	99	4.3	4.3
	125	98	97	100	98	1.7	1.7
1,2,3,4,6,7,8-HpCDD	25	100	124	101	109	13.8	12.7
	62.5	101	101	102	101	0.2	0.2
	125	104	100	104	103	2.3	2.2
OCDF	50	99	132	103	111	17.6	15.7
	125	112	110	105	109	3.7	3.4
	250	109	108	112	110	2.1	1.9
OCDD	50	90	115	94	100	13.2	13.3
	125	95	92	96	94	2.1	2.2
	250	95	96	96	96	0.8	0.8

* Standard Deviation
** Coefficient of Variation

The average measured recoveries for the analytes in the spiking solution ranged from 94 to 116% of the targeted spiked levels. These results are well within the program objectives of 50 - 130% recovery for spiked QC samples and 70 - 130% recovery for performance audit solutions of standards (i.e. without the matrix). These data verify that the spiking solution was prepared correctly.

The coefficient of variation (CV) of the measurements at each spike level for each analyte ranged from 0.2 to 15.7%. In general, the precision of the measurements on each of the spike levels is better for the highest spike level than for the lowest spike level. This was expected and was consistent with the precision data noted in the preparation of the initial calibration curves for each analyte.

b. Results of the Internally Spiked Lipid Sample Analysis

Twenty internally spiked lipid samples were analyzed during the study. One sample was included in each batch. The spiking levels ranged from 10 pg/g to 50 pg/g for TCDD and from 50 pg/g to 250 pg/g for OCDD. Nine samples were at the low spike level, five samples were at the medium spike level, and six samples were at the high spike level. The levels and spiking procedures were described in detail in the Quality Control Samples section (see Table 9).

Table 21 presents the average percent recovery and coefficient of variation for each analyte for the 20 samples. The average recovery ranged from 87.4% for OCDD to 117.1% for the 1,2,3,4,7,8/1,2,3,6,7,8-HxCDD pair.

Table 22 presents additional details on the accuracy and precision of the measurement of 2,3,7,8-TCDD in these internally spiked lipid samples. The percent recovery for each of the individual analysis is given in this table. The mean recovery for each individual spike level and the overall mean recovery are provided.

The average recovery for the samples at the low level spike (10 pg/g) was 110%, the medium spike level (25 pg/g) was 119%, the high spike level (50 pg/g) was 114%, and the average recovery from the measurements of all 20 samples was 113%. The method accuracy for 2,3,7,8-TCDD that was determined in the Method Evaluation Study[25] conducted prior to these analyses was 113% recovery.

Figure 4 is a plot of the percent recovery of the measurements of 2,3,7,8-TCDD by batch number. As noted in Table 22 and Figure 4, only one sample with a recovery of 135% for 2,3,7,8-TCDD was outside the data quality goal of 50-130% recovery.

Accuracy data on the remaining congeners are presented in Appendix B.

TABLE 21
Percent Recovery and Precision of Measurements for PCDDs and PCDFs From the Internally Spiked Lipid Samples (n = 20)

Compound	Average (%) Recovery	SD*	CV** (%)
2,3,7,8-TCDF	98.7	7.9	8.0
2,3,7,8-TCDD	113.3	10.0	8.8
1,2,3,7,8-PeCDF	105.6	5.3	5.0
2,3,4,7,8-PeCDF	105.9	31.0	29.3
1,2,3,7,8-PeCDD	99.1	7.6	7.6
1,2,3,4,7,8-HxCDF	99.9	11.3	11.4
1,2,3,6,7,8-HxCDF	102.1	8.4	8.2
2,3,4,6,7,8-HxCDF	96.6	6.1	6.3
1,2,3,7,8,9-HxCDF	92.3	5.6	6.0
1,2,3,4,7,8/ 1,2,3,6,7,8-HxCDD	117.1	27.6	23.5
1,2,3,7,8,9-HpCDD	92.8	6.0	6.5
1,2,3,4,6,7,8-HpCDF	94.8	5.9	6.2
1,2,3,4,7,8,9-HpCDF	96.1	12.9	13.4
1,2,3,4,6,7,8-HpCDD	90.3	19.9	22.1
OCDF	90.1	9.4	10.5
OCDD	87.4	25.5	29.2

* Standard Deviation
** Coefficient of variation

TABLE 22
Percent Recovery and Precision of Measurements for 2,3,7,8-TCDD from the Twenty Internally Spiked Lipid Samples (%)

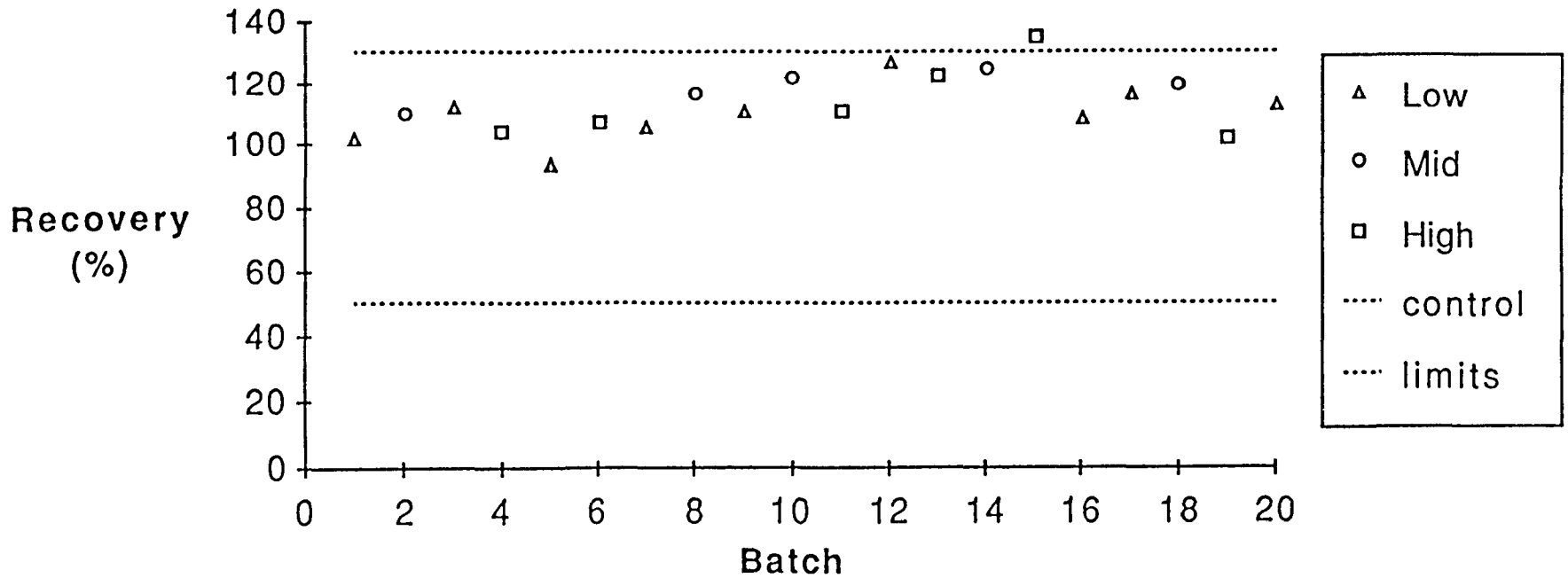
Spike Level*		
Low (10 pg/g) (n = 9)	Medium (25 pg/g) (n = 5)	High (50 pg/g) (n = 6)
102	110	104
112	117	107
93	122	111
106	125	123
111	120	135
127		102
109	Mean = 119	
117	CV** = 4.8%	Mean = 114
113		CV** = 11%
Mean = 110		Overall Mean = 113%
CV** = 8.6%		CV** = 8.8%

* Spike levels based on a 10-gram lipid sample
** Coefficient of variation

$$\% \text{ Recovery} = 100\% \times \frac{\text{Conc. spiked sample} - \text{conc. control sample}}{\text{spike level}}$$

FIGURE 4

2,3,7,8-TCDD: Percent Recovery in Spiked Internal QC Samples
(Spike Levels are Highlighted)
Batches 1-20



2. Split Samples

Six adipose tissue specimens were prepared and analyzed as split samples within single sample batches as part of the original QC program design. No attempt was made to homogenize these samples before the split. Two aliquots of each of these tissue specimens were submitted as "blinds" by an independent QC person to the analytical laboratory for preparation and analysis. The chemists were not informed which samples were the split samples. These samples provide data on within-batch precision.

A seventh adipose tissue specimen was also prepared and analyzed in duplicate. This sample had a high 2,3,7,8-TCDD value (106 pg/g) in the first analysis and was reanalyzed in a later batch during the study to confirm the finding. This sample provides limited data on the between-batch precision.

Data for 2,3,7,8-TCDD in the split samples are given below in Table 23. The relative percent difference (RPD) for 2,3,7,8-TCDD ranged from 1.1% to 10.0%. The between-batch precision was 6.3%.

In general, the precision measurements for all remaining congeners for the split samples were in good agreement. The relative percent difference was typically less than 20% and more often less than 10%. There were only six of 81 measurements that were greater than 20% RPD. The higher RPD values occurred for compounds whose concentrations were typically less than 5 pg/g.

Data on all the congeners in the split samples are in Appendix B.

3. Control Lipid Samples

Information on the precision of the method was also obtained from the analysis of the unspiked control lipid samples. A total of 20 samples from a homogeneous pool of unspiked lipid material were analyzed. One sample was included in each batch.

Table 24 presents the summary data on these samples. The mean and coefficient of variation (CV) are given for each congener. The precision as measured by the CV for 2,3,7,8-TCDD was 10.6%. The precision value of 10.6% does not include the analytical variability of the lipid determination. However, data reported earlier from samples that were analyzed as duplicates are available. The precision for the remaining congeners ranged from 2.9 to 51.3%.

Figure 5 is a plot of the measured values for 2,3,7,8-TCDD in the 20 unspiked control lipid samples. The mean value and the 95% confidence interval for individual analyses which were established from the Method Evaluation Study[25] are shown on the plot. All measurements of 2,3,7,8-TCDD in the unspiked control lipid samples fell within the 95% confidence interval.

Plots of the data on the remaining congeners are presented in Appendix B.

4. Method Blanks

A total of twenty method blank samples were analyzed, one with each sample batch. These samples were taken through all

TABLE 23
Results of Split Sample Analyses for 2,3,7,8-TCDD

Sample Code	Batch #	First Analysis (pg/g)	Second Analysis (pg/g)	Relative Percent Difference (%)
00609	4	8.93	9.09	1.8
29810	7	11.30	11.80	4.3
29805	13	6.99	7.73	10.0
29806	16	6.48	6.41	1.1
18801	19	7.32	6.74	8.3
12823	10	10.90	11.20	2.7
06509*	3, 6	106.00	113.00	6.3

* All split sample pairs were analyzed within single sample batches, except sample code number 06509 which was analyzed in two different batches.

$$\text{Relative Percent Difference (\%)} = \frac{\text{high value - low value}}{\text{average value}} \times 100$$

TABLE 24
Summary of the Results of the Measurements
in the Unspiked Control Lipid Samples (n = 20)

Compound	Mean (pg/g)	SD* (pg/g)	CV** (%)
2,3,7,8-TCDF	1.94	0.20	10.51
2,3,7,8-TCDD	10.06	1.06	10.55
1,2,3,7,8-PeCDF	0.81	0.16	19.50
2,3,4,7,8-PeCDF	27.65	2.79	10.09
1,2,3,7,8-PeCDD	18.46	0.91	4.92
1,2,3,4,7,8-HxCDF	20.24	1.14	5.62
1,2,3,6,7,8-HxCDF	11.11	1.13	10.21
2,3,4,6,7,8-HxCDF	3.76	0.34	9.18
1,2,3,7,8,9-HxCDF	0.39	0.20	50.42
1,2,3,4,7,8/ 1,2,3,6,7,8-HxCDD	155.20	15.11	9.73
1,2,3,7,8,9-HxCDD	15.70	0.97	6.20
1,2,3,4,6,7,8-HpCDF	27.69	1.18	4.25
1,2,3,4,,7,8,9-HpCDF	1.25	0.25	19.65
1,2,3,4,6,7,8-HpCDD	223.70	7.98	3.57
OCDF	2.11	0.37	17.33
OCDD	813.85	23.72	2.92

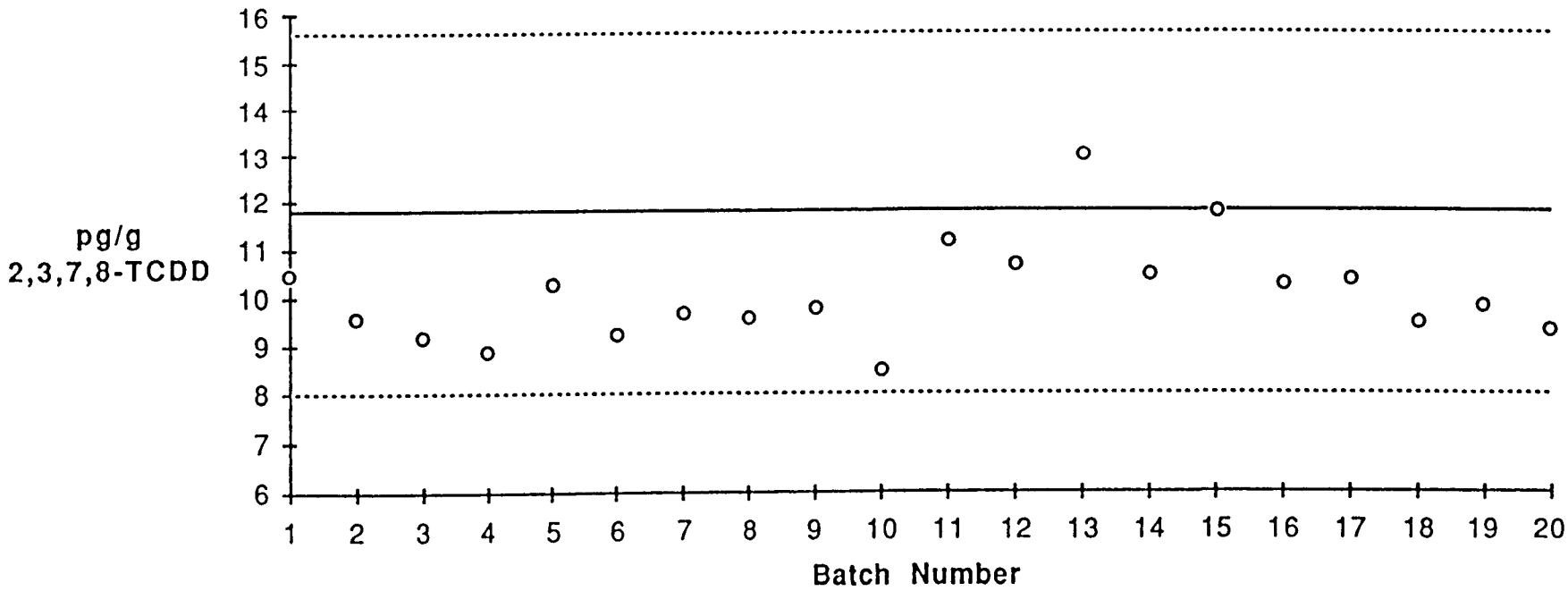
* Standard Deviation
** Coefficient of variation

Calculations included all values. Not detected values were set equal to the level of detection and trace values were used as the level reported.

FIGURE 5

2,3,7,8-TCDD
Concentration in Unspiked Control QC Sample (pg/g)

Control Limits: 95% Confidence Interval for Individual Analyses from Method Evaluation Data



steps of the analytical procedure with the exception of the addition of adipose tissue.

No 2,3,7,8-TCDD was detected in any method blank sample. Only two of the target analytes were seen in any of the method blanks. 1,2,3,4,6,7,8-HpCDD was reported in six samples at concentrations ranging from 0.63 pg/g to 1.29 pg/g (mean = 0.99 pg/g). OCDD was reported in 19 samples at concentrations ranging from 2.00 pg/g to 10.20 pg/g (mean = 4.48 pg/g). The method blank data are shown in Table 25. Summary data on the Limits of Detection for the target compounds which were not detected in the method blank samples are in Table 26.

The amounts of the HpCDD and OCDD found in the method blanks were typically less than 1% of the values measured in the study specimens and were not subtracted from each sample.

The background levels of HpCDD and OCDD apparently arise from general laboratory background.[26] Previous work in the analytical laboratory had demonstrated that the background level is attributed to concentrations of these compounds on the acidic alumina from laboratory air during adsorbent activation.[27] The analytical protocol included procedures for pre-elution of the alumina columns to reduce this background level.

5. Instrument Performance

This section on instrument performance includes information on the daily mass calibrations and column performance checks, the tridecane blanks, and the daily calibration activities.

a. Mass Calibration

Mass calibration was completed as the first function of each day for both the low and high resolution MS analysis. The details are described in the analytical protocol. Figures 6 and 7 are plots of the mass resolution for all batches. All data fall within the quality control objectives of resolution of $\geq$ 3000 for the LRMS and $\geq$ 10,000 for the HRMS.

b. Column Performance

Column performance was demonstrated daily using a mixture of TCDD isomers that elute closely to 2,3,7,8-TCDD. An example of the procedures for calculating column performance (resolution) was presented in the analytical protocol. Figures 8 and 9 show plots of the column performance data for LRMS and HRMS analyses. All analysis events met the quality control objectives for peak separation of $\leq$ 60% for LRMS (DB5 column) and $\leq$ 25% for HRMS (Rtx-2331 or SP-2330 columns).

As noted in Figure 8, there was a column change for the HRMS analyses before batch 8. The change from an Rtx-2331 column to an SP-2330 column resulted in better separation of 2,3,7,8-TCDD from other potentially coeluting TCDD isomers.

c. Tridecane Blanks

Tridecane blanks were analyzed daily to confirm that carryover from the injection of standards was not a problem.

TABLE 25
Measurements of Target Analytes Detected in the Method Blank Samples by Batch Number

Batch #	OCDD (pg/g)	1,2,3,4,6,7,8-HpCDD (pg/g)
1	2.00	
2	4.00	
3	2.70	
4	2.60	
5	4.53	
6	3.32	0.63 trace
7	3.72	
8	5.27	
9	3.55 trace	
10	3.98	
11	2.27	
12	2.87	
13	3.56	
14	10.20	1.29 trace
15	3.72	0.79 trace
16		
17	6.94	1.03 trace
18	6.76	0.99 trace
19	5.84	
20	7.20	1.20 trace
Mean	4.48	0.99
Range	2.0 - 10.2	0.63 - 1.29

OCDD was not detected in the method blank in batch 16. The level of detection for OCDD in that sample was 5.3 pg/g. None of the other target analytes were detected in the method blanks.

TABLE 26
Summary of the Limits of Detection for the Target Compounds which were not Detected in the Method Blank Samples

Congener	Limits of Detection (pg/g)		
	Minimum	Mean	Maximum
2,3,7,8-TCDF	0.1	0.2	0.5
2,3,7,8-TCDD	0.1	0.5	3.7
1,2,3,7,8-PeCDF	0.1	0.2	0.5
2,3,4,7,8-PeCDF	0.1	0.2	0.4
1,2,3,7,8-PeCDD	0.1	0.3	1.0
1,2,3,4,7,8-HxCDF	0.1	0.2	0.6
1,2,3,6,7,8-HxCDF	0.1	0.3	0.6
2,3,4,6,7,8-HxCDF	0.1	0.3	0.6
1,2,3,7,8,9-HxCDF	0.1	0.3	0.7
1,2,3,4,7,8/ 1,2,3,6,7,8-HxCDD	0.1	0.5	1.1
1,2,,3,7,8,9-HxCDD	0.1	0.4	0.9
1,2,3,4,6,7,8-HpCDF	0.1	0.3	0.8
1,2,3,4,7,8,9-HpCDF	0.1	0.4	1.1
1,2,3,4,6,7,8-HpCDD	0.5	0.9	1.6
OCDF	0.1	0.7	2.5
OCDD	5.3	5.3	5.3

FIGURE 6

Mass Resolution: HRMS
Batches 1 to 20

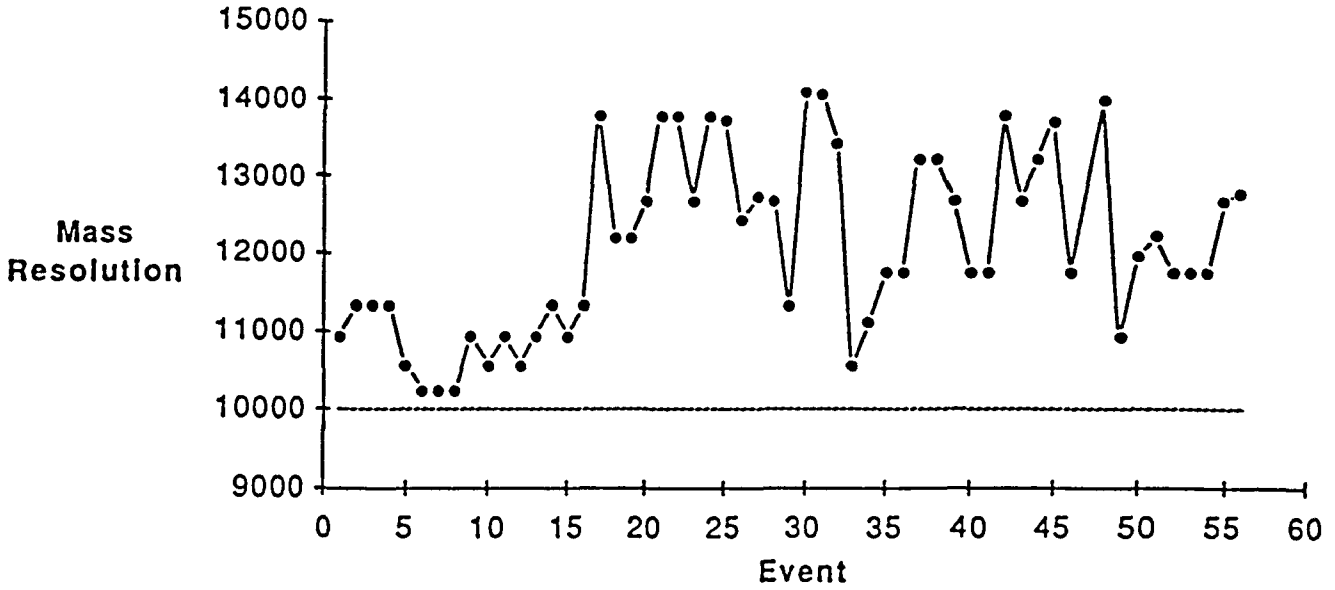


FIGURE 7

Mass Resolution: LRMS
Batches 1 to 20

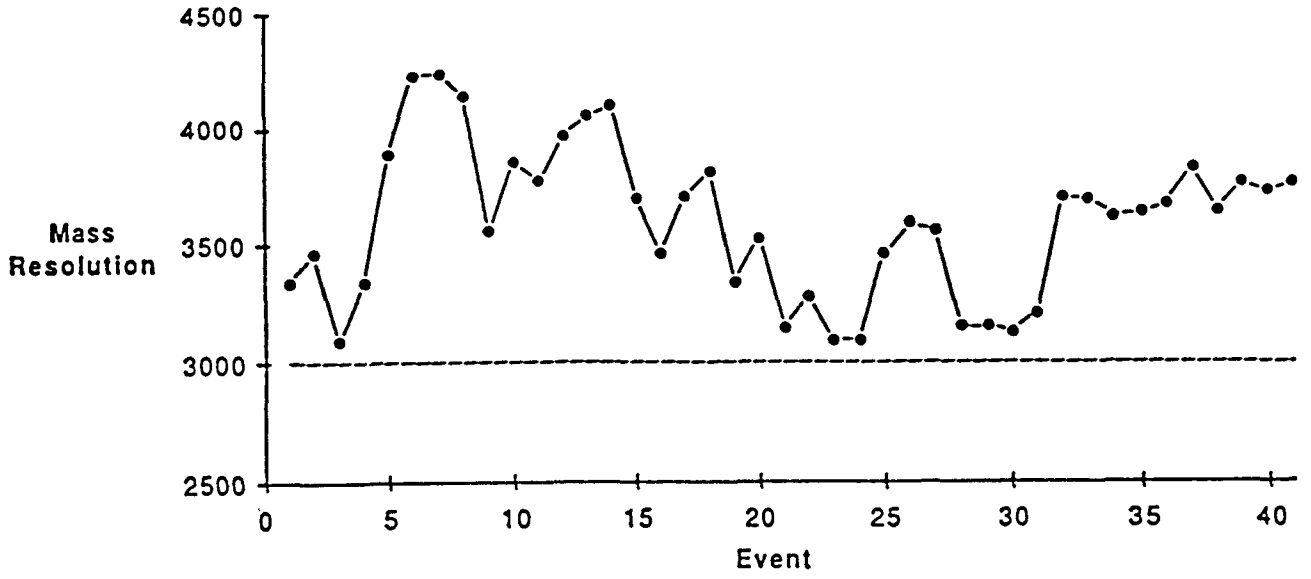


FIGURE 8

Column Resolution (%): HRMS
Batches 1 to 20

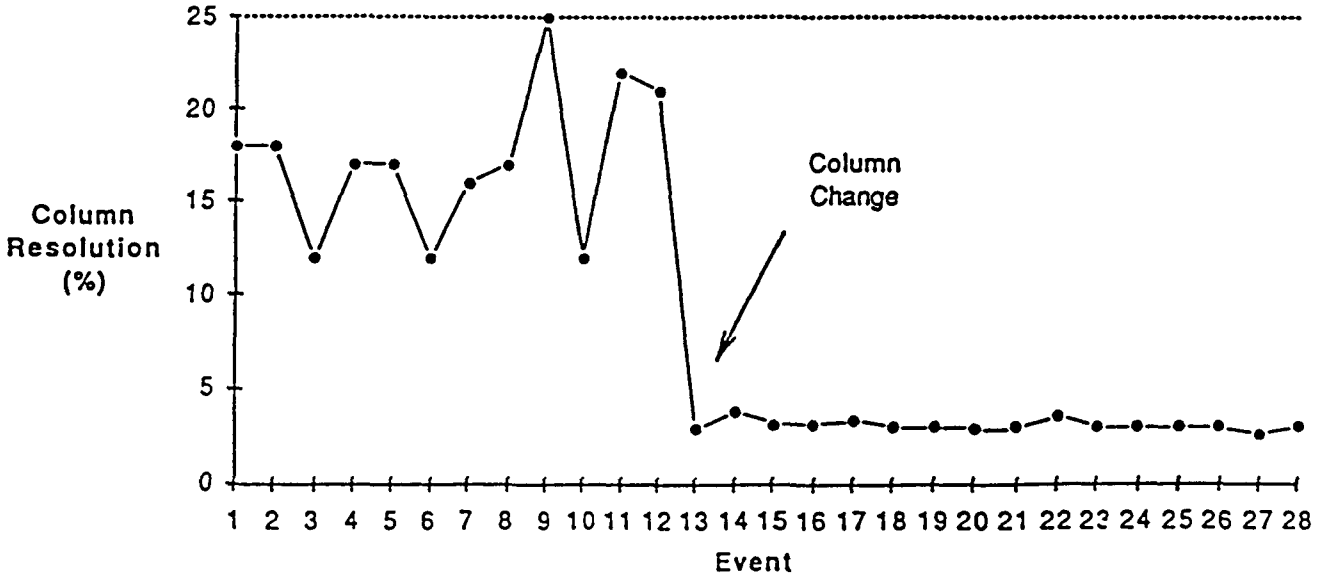
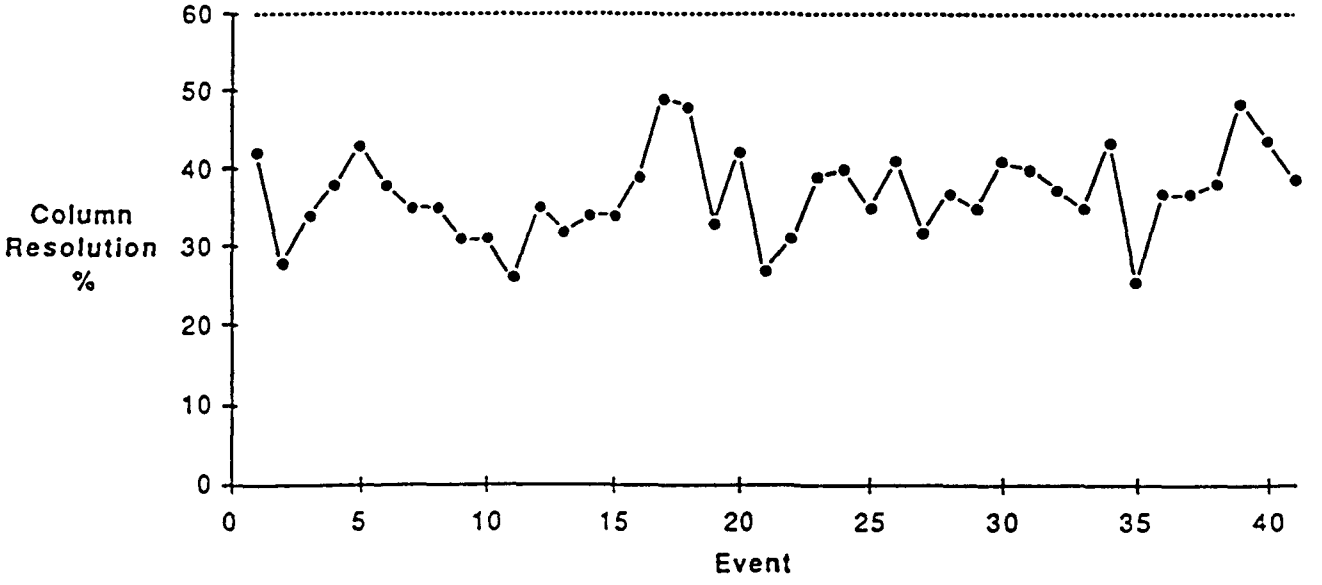


FIGURE 9

Column Resolution (%): LRMS
Batches 1 to 20



These analyses demonstrated no response to the PCDDs and PCDFs and the internal quantitation standards.

d. Calibration Data

The daily analyses of calibration standards bracketing the sample analysis were conducted. The quality control objectives for these analyses were that the relative response factors (RRFs) should be within ± 20% of the means for 2,3,7,8-TCDD and 2,3,7,8-TCDF and ± 30% of the means for all other congeners.

The relative response factor data were plotted in control charts. Figure 10 is a plot of the data for 2,3,7,8-TCDD.

In Figure 10 the RRF control chart for TCDD indicates several data points outside the 20% criteria. Corrections included reevaluation of the mass calibration using PFK, adjustment of the capillary column length, cleaning of the mass spectrometer ion source, and reanalysis of the standard solutions. No sample analyses were conducted following these specific calibration events.

Sample analyses were conducted only after the RRF criteria were met for TCDD. The control charts for the remaining analytes and internal quantitation standards are in Appendix B.

6. Recovery of Internal Quantitation Standards

Nine carbon-13 labeled internal quantitation standards were added to each sample to be used in the quantitation of the native compounds. The absolute recovery of these standards in each sample was calculated. The data quality objectives for the percent recovery of these compounds was 50-115%.

A cumulative plot showing the recoveries of ^{13}C-2,3,7,8-TCDD in each sample analyzed is given in Figure 11. The data points are plotted in the order of analysis. More than 96% of the data points were within the 50-115% recovery objective. The ten points that are outside this range were between 40% and 50% recovery.

Although these recoveries were outside the lower 50% recovery objective, these analyses were not repeated because the recoveries of the other internal quantitation standards were within the data quality objectives and the observed signal-to-noise ratio was greater than 10.

Cumulative plots of the other internal quantitation standards are in Appendix B.

7. National Bureau of Standards

Seven samples consisting of a National Bureau of Standards (NBS) reference material of 2,3,7,8-TCDD were analyzed over the course of the study. The samples were prepared as performance audit samples by the quality control coordinator, using a certified NBS solution (SRM 1614, 67.8 ± 2.3 ng/ml, dated April 24, 1986).

The samples were analyzed by LRMS and HRMS (with the exception of one sample, which was analyzed only by LRMS). A summary of the results is provided in Table 27.

FIGURE 10

CONTROL CHART 2,3,7,8-TCDD

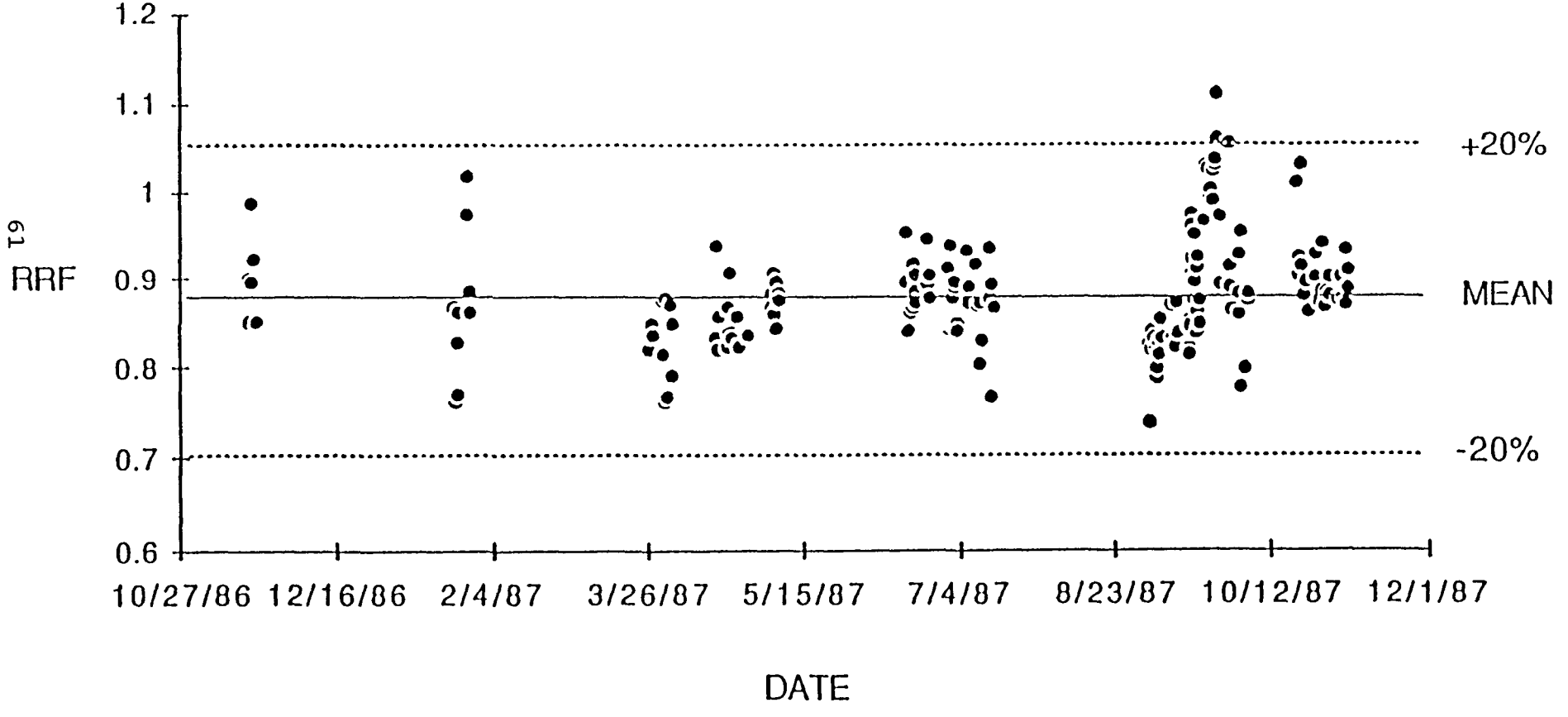


FIGURE 11

13C12-TCDD Recoveries for Batches 1-20

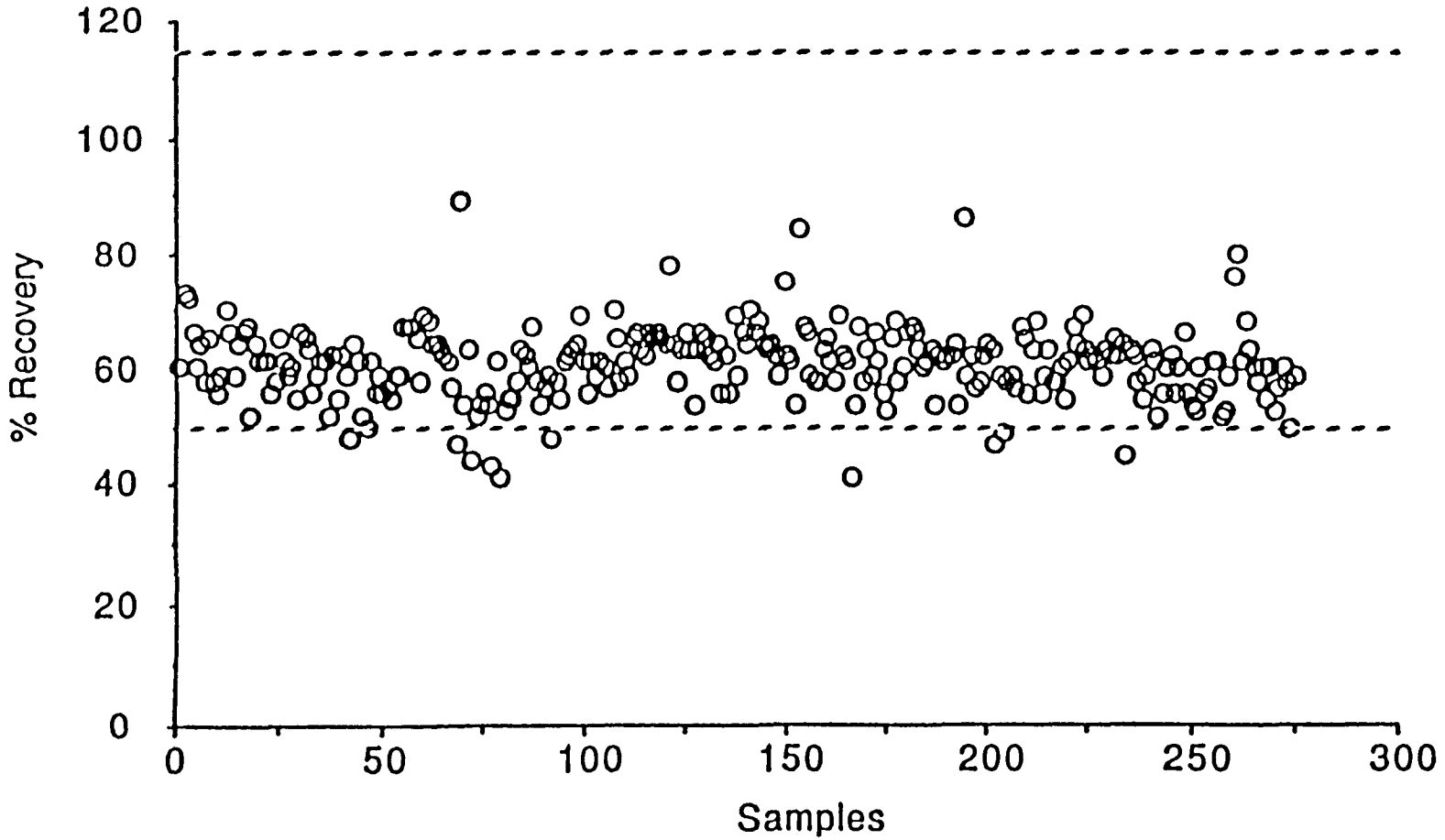


TABLE 27
Results of the Analysis of the National Bureau of Standards Solution of 2,3,7,8-TCDD

Batch #	Found (pg/uL)		% Recovery	
	LRMS	HRMS	LRMS	HRMS
01	79.2	NA[1]	117	NA[1]
03	197.0	209.0	116.0	123.0
06	41.1	42.0	121.0	124.0
09	125.0	120.0	123.0	118.0
12	162.0	196.0	119.0	144.0[2]
15	85.6	104.0	126.0	153.0[2]
18	41.1	40.4	121.0	121.0
		Mean	121.00	130.0
	Standard Deviation		3.50	14.4
		% CV	2.91	11.0

[1] Not analyzed with sample batch
[2] Outside the data quality objectives of 70 to 130%

Prepared from NBS SRM 1614, 67.8 ± 2.3 ng/ml

All but two measurements by HRMS were within the data quality objectives of 70-130% recovery. The mean recovery for the LRMS measurements was 121%, and the mean recovery for the HRMS measurements was 130%.

8. Interlaboratory Study

Midwest Research Institute participated in an interlaboratory study for the comparison of 2,3,7,8-substituted PCDD and PCDF analytical standards, sponsored by Cambridge Isotope Laboratories (Woburn, Massachusetts). The interlaboratory study occurred during the same time as this study. The results of the interlaboratory study are given in Table 28. A total of eight laboratories participated:

Midwest Research Institute (MRI)
Centers for Disease Control
Dow Chemical Company
Monsanto Company
Ontario Ministry of Environment
Triangle Labs, Inc.
Twin City Testing Co.
Environmental Research Center, University of Las Vegas

One objective of this study was to develop consensus values for each of the 2,3,7,8-chlorine substituted PCDD and PCDF standard solutions available from Cambridge Isotope Laboratories (CIL). MRI used the same set of calibration standards and the resulting RRF values that were used for this study to determine the concentrations of the CIL solutions. MRI's data are identified as laboratory 5 in the summary Table 28. The results indicate that the analytical standards used in this study are in good agreement with the standards used by the other participating laboratories.

TABLE 28

Summary of PCDD and PCDF Calibration Standards (µg/mL) - Round Robin Results
(Lab 5 - MRI Results - based on triplicate analyses)

ISOMER	LAB 1	LAB 2	LAB 3	LAB 4	LAB 5	LAB 6	LAB 7	LAB 8
2378 - TCDD	49.8 ± 0.5	46.5 ± 0.75	50.5 ± 4.0	52.2 ± 0.85	47.2 ± 0.6	44.92 ± 0.27	53.6	46.8
12378 - PCDD	49.9 ± 1.4	35.8 ± 0.12	58.6 ± 2.8	49.0 ± 1.4	48.7 ± 0.9	47.3 ± 0.2	----	----
123478 - HxCDD	49.1 ± 0.3	34.8 ± 1.45	54.1 ± 0.9	51.5 ± 1.75	45.5 ± 0.9	48.59 ± 0.34	52.3 ± 3.1	52.0 ± 0.7
123678 - HxCDD	51.4 ± 0.3	49.2 ± 2.05	50.0 ± 1.3	55.5 ± 1.05	46.3 ± 1.9	52.27 ± 0.26	52.9 ± 3.9	49.0 ± 1.7
123789 - HxCDD	49.7 ± 0.5	59.7 ± 1.55	47.1 ± 0.8	48.4 ± 1.3	45.1 ± 0.7	49.85 ± 0.27	----	48.5 ± 1.1
1234678 - HpCDD	53.6 ± 0.5	46.4 ± 1.37	50.7 ± 4.5	52.5 ± 3.25	46.9 ± 1.1	49.88 ± 0.47	----	51.5 ± 0.8
12346789 - OCDD	11.7 ± 0.2	11.4 ± 0.15	11.4 ± 1.0	11.2 ± 1.45	10.8 ± 0.1	11.99 ± 0.15	11.3 ± 0.5	14.9
2378 - TCDF	50.4 ± 0.5	58.9 ± 1.65	49.8 ± 2.1	50.4 ± 1.1	47.5 ± 1.0	51.62 ± 0.18	51.3 ± 1.3	48.7 ± 1.7
12378 - PCDF	50.8 ± 0.2	46.3 ± 1.00	46.5	54.6 ± 1.05	51.8 ± 0.4	48.99 ± 0.55	46.8 ± 0.5	----
23478 - PCDF	51.4 ± 0.5	49.8 ± 0.25	----	54.7 ± 2.85	48.9 ± 0.6	51.55 ± 0.25	50.5 ± 1.4	52.1 ± 0.9
123478 - HxCDF	49.8 ± 0.4	38.5 ± 0.89	51.2 ± 0.7	48.2 ± 1.2	47.0 ± 1.0	52.59 ± 0.37	51.5 ± 1.2	49.7 ± 0.5
123678 - HxCDF	53.3 ± 0.2	46.8 ± 0.76	52.9 ± 2.0	48.0 ± 3.15	49.2 ± 0.5	54.74 ± 0.26	51.9 ± 0.8	50.4 ± 3.2
123789 - HxCDF	48.4 ± 0.5	56.6 ± 1.37	51.7 ± 1.1	43.2 ± 2.45	43.5 ± 0.8	51.64 ± 0.39	48.1 ± 0.9	55.4 ± 2.6
234678 - HxCDF	53.0 ± 0.2	52.2 ± 1.62	52.3 ± 2.8	50.9 ± 1.6	47.4 ± 1.0	54.28 ± 0.37	49.3 ± 0.7	----
1234678 - HpCDF	52.3 ± 0.3	51.7 ± 4.30	56.3 ± 1.7	49.7 ± 1.7	50.7 ± 0.3	48.30 ± 0.39	54.1	48.9 ± 0.2
1234789 - HpCDF	52.0 ± 1.1	51.5 ± 2.65	66.9 ± 2.1	52.1 ± 2.85	47.5 ± 0.8	49.79 ± 0.48	----	52.7 ± 0.5
12346789 - OCDF	53.4 ± 0.8	58.1 ± 1.25	53.3	54.2 ± 0.3	51.9 ± 1.6	51.30 ± 0.30	53.6 ± 0.5	53.5 ± 1.5

Source: J. Bradley (Cambridge Isotope Laboratories), "Interlaboratory Testing Study on 2,3,7,8-Containing Polychlorinated Dibenzo-p-dioxin and Polychlorinated Dibenzofuran Isomer Standard Solutions," presented at the 7th International Symposium on Chlorinated Dioxins and Related Compounds, Las Vegas, Nevada, October 1987.

IV. DISCUSSION

This study did not demonstrate elevated levels of 2,3,7,8-TCDD in the adipose tissue of Vietnam veterans compared to non-Vietnam veterans or civilian controls. Even after adjusting for demographic variables, military service in Vietnam was not associated with elevated TCDD levels in adipose tissue. In addition, no Vietnam service characteristic in the study, measured singularly or in combination, was a good predictor of 2,3,7,8-TCDD levels in adipose tissue. This finding is in accordance with a recent study published by the CDC. The CDC reported that there was no association between serum TCDD levels and indirectly estimated Agent Orange exposure before or after adjustment for other characteristics of the veterans such as age, race, body mass index and self-reported civilian occupational and home herbicide exposure. The results reported herein and the CDC study results are not inconsistent with several studies of Vietnam veterans and their TCDD levels in adipose tissue or blood.

In a study reported by Gross et al[7], 2 of 20 Vietnam veterans showed elevated TCDD levels in adipose tissue compared to non-Vietnam veteran controls. These two Vietnam veterans had a history of direct contact with phenoxyherbicides. Kahn et al[8] reported that levels of TCDD in both blood and adipose tissue of "heavily exposed" Vietnam veterans far exceeded those of 10 other Vietnam era veterans who did not serve in Southeast Asia. Nine of the 10 "heavily exposed" veterans handled herbicide regularly while in Vietnam. The one remaining "heavily exposed" veteran was an "Army light infantry jungle combat soldier" with extensive ground exposure from January 1969 to July 1969. His TCDD levels in both adipose tissue and blood were not significantly different from those of controls. The U.S. Air Force in collaboration with the CDC has measured serum 2,3,7,8-TCDD levels in Air Force Health Study participants. The mean serum TCDD level of the 147 Ranch Hand personnel was 49 ppt, whereas the mean level of the 49 controls was 5 ppt.[28] The Ranch Hand personnel were all enlisted men who were either herbicide loaders or herbicide specialists in Vietnam. The controls were Air Force veterans who served in Southeast Asia but did not participate in the Ranch Hand operation. In all three studies described above, Vietnam veterans with documented direct contact with herbicides have been shown to have elevated 2,3,7,8-TCDD in their blood or adipose tissue almost two decades after their last exposure to herbicide in Vietnam.

It is possible that this study may have failed to detect a small difference in mean TCDD levels because of the relatively small sample size. The study had adequate statistical power (90%) to detect a mean difference of 5 ppt or more between groups. However, for a subgroup of Vietnam veterans (e.g., ground troops), the statistical power to detect the same difference in means decreased to 84%. The study had over a 95% chance of detecting a mean TCDD difference of 8 ppt or more

between Vietnam veteran ground troops and a non-Vietnam veteran comparison group.

Elimination of TCDD from the body since Vietnam service is also an unlikely explanation of failure to observe a difference in TCDD levels. The geometric mean TCDD levels of adipose tissue specimens collected from 7 veterans within 4 years of their return from Vietnam was not significantly different from the control specimens. Furthermore, an analysis of covariance which controlled for the length of time between Vietnam service and the sample collection year supported this conclusion. Unlike other studies in which TCDD levels were measured almost 20 years after a veteran's service in Vietnam, this study included 12 Vietnam veterans whose adipose tissues were sampled within 7 years of their return. One can calculate, however, a theoretical difference in mean TCDD levels that might have existed immediately after the veterans left Vietnam. If one assumes a half-life of TCDD in the body as 7 years, first order elimination kinetics, a 10-year elapsed average since Vietnam service, and a minimum mean TCDD difference of 8 ppt that the study failed to detect, then the mean difference of TCDD levels when veterans left Vietnam as compared to their controls can be extrapolated to approximately 21 ppt (for first order elimination the equations $\log x = \log x_o - [K_e t/2.3]$; and $t_{½} = [0.693/K_e]$ apply; where x denotes total TCDD in the body at time t, x_o the TCDD present at time o, and K_e the rate constant for elimination). A similar extrapolation of the Air Force Health Study data resulted in a mean difference of over 250 ppt at the time of departure from Vietnam.

Although the NHATS sampling scheme was designed to collect a representative sample of the Standard Metropolitan Statistical Areas in terms of age, sex and race, subjects selected for the study may not have represented their respective groups for several reasons. First, over 90% of NHATS samples were collected from deceased persons whose cause of death in most instances was due to traumatic injury. Second, tissue samples from this study were selected from the archived NHATS specimens rather than from the original NHATS samples. Third, 6% of the subjects who were eligible for the study had to be excluded because of missing personal identifiers such as name and social security number. Despite these potential problems, demographic and military characteristics of 36 Vietnam veterans in the study were not substantially different from the overall Vietnam veteran population. They were predominantly white (75%), draft eligible during the Vietnam war (age 18 to 25) and enlisted men (89%); they served in the Army and Marine Corp (72%) with military occupational specialties other than offensive and defensive combat missions (67%).

It is apparent that several military service characteristics examined in the study offered inadequate measures of potential exposure to Agent Orange. The failure to find an association between TCDD levels and an estimate of exposure likelihood based on military records may have resulted either because military

records used in the study were of poor choice for estimating potential exposure or because there was very limited opportunity for exposure to significant amounts of TCDD in Vietnam for most of the U.S. troops.

The extent of Agent Orange exposure among ground troops during their normal course of duties can be approximated under many different assumptions. Gough[29] estimated the amount of dioxin exposure of a soldier standing under a Ranch Hand spray mission. He assumed that Agent Orange sprayed in Vietnam contained 2 ppm of TCDD, and that 3 gallons of Agent Orange were applied per acre of land. He reported that a man in a jungle under this exposure condition would have received 39 picograms, assuming that the efficiency of transfer of dioxin from the environment into a man's body is equal to Steven's estimate of 1/2000.[30] Gough stated that dioxin degrades rapidly in sunlight, binds to soil, and is almost insoluble in water. Therefore, absorption of TCDD from subsequent contacts with the jungle environment would be a tiny fraction of the amount received from a direct spray. If the whole amount of 39 picograms was evenly distributed into the adipose tissue of 80 kg men, the concentration of TCDD adjusted for lipid would be not more than 0.02 ppt. Considering that TCDD levels in adipose tissue range from 5 to 15 ppt in the general population, an episode of direct exposure to Agent Orange for a ground soldier under the conditions described above would have contributed a very small fraction of his total body burden of TCDD.

The mean level of TCDD reported in this study tends to be higher than the levels reported by others including the FY 1982 NHATS samples analyzed in 1984-1985 by the same laboratory that was involved in this study.[16] (Note: There were some minor differences in the analytical protocols and the FY 1982 specimens were analyzed as composites, not as individual specimens as in this study.) The adipose tissue specimens for this study were collected between 1971 and 1982 (median collection year of 1978), whereas samples for other studies had been collected in most instances during the mid 1980's, approximately 7 years later. The observed decline from 1971 to 1982 is consistent with the general trend for chlorinated hydrocarbon chemical compounds in human adipose tissue to decline over time. The U.S. EPA's NHATS program indicates that the median levels of B-BHC, HCB, and PCB had been steadily decreasing over time between 1970 and 1983.[17] In Sweden, the levels of PCDDs and PCDFs in human milk decreased significantly from 1972 to 1985.[31] The Swedish authors attributed the decline to the reduction in use of certain organochlorine compounds such as PCBs, PCP and 2,4,5-T. A study involving a large sample of specimens representative of the U.S. population will be needed to confirm this observation.

In summary, our results indicate that heavy exposure to 2,3,7,8-TCDD in Vietnam for U.S. troops in general was unlikely. These results are consistent with those of CDC[10] and not inconsistent with those of Kahn et al[8], the Air Force Health Study[26] and Gross et a[17] which indicated that those men who

handled or sprayed Agent Orange routinely had much higher levels of 2,3,7,8-TCDD in their tissue. In addition, our results suggest that the levels of PCDD's in U.S. adult males may have decreased significantly between 1971 to 1982.

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APPENDIX A

This section contains the raw data from the Midwest Research Institute analysis of the adipose tissue samples for the PCDDs and PCDFs. The measurements were precise to three significant digits.

LIST OF TABLES

Table A-1. Dioxin Levels in the Adipose Tissue of Vietnam Veterans.

Table A-2. Dioxin Levels in the Adipose Tissue of Non-Vietnam Veterans.

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Table A-4. Six Furan Levels in the Adipose Tissue of Vietnam Veterans (TCDF, PeCDF and HpCDF).

Table A-5. Six Furan Levels in the Adipose Tissue of Non-Vietnam Veterans (TCDF, PeCDF and HpCDF).

Table A-6. Six Furan Levels in the Adipose Tissue of Civilians (TCDF, PeCDF and HpCDF).

Table A-7. Four Furan Levels in the Adipose Tissue of Vietnam Veterans (HxCDF).

Table A-8. Four Furan Levels in the Adipose Tissue of Non-Vietnam Veterans (HxCDF).

Table A-9. Four Furan Levels in the Adipose Tissue of Civilians (HxCDF).

TABLE A-1
Dioxin Levels in the Adipose Tissue of Vietnam Veterans

WID	Batch	2378-TCDD	12378-PeCDD	123478/ 123678-HxCDD	123789-HxCDD	1234678-HpCDD	OCDD
18408	1	8.80	17.00	197.00	18.80	305.00	1230.00
27601	1	18.50	27.20	346.00	21.80	171.00	1350.00
01510	2	6.90	14.60	104.00	14.40	297.00	776.00
17018	2	6.70	10.80	97.70	10.40	92.10	532.00
29607	2	4.70	7.80	63.60	6.10	83.00	313.00
17027	3	13.40	19.40	174.00	19.30	405.00	2030.00
31304	3	8.60	20.70	133.00	20.30	140.00	1580.00
31410	4	7.82	15.60	108.00	0.00	239.00	936.00
28905	5	17.00	20.00	141.00	12.20	192.00	895.00
31403	5	6.99	17.10	151.00	14.50	121.00	619.00
01520	6	9.19	13.50	103.00	18.20	239.00	1040.00
17605	6	14.70	19.20	142.00	21.50	346.00	1640.00
00601	7	9.46	23.30	132.00	15.00	127.00	797.00
31404	7	5.90	11.00	67.70	7.73	77.00	568.00
17006	8	9.84	21.00	195.00	15.30	260.00	1200.00
28906	8	6.10	11.70	104.00	17.50	130.00	707.00
21101	9	7.52	10.60	91.60	12.60	213.00	798.00
28502	9	9.97	18.50	131.00	16.80	309.00	928.00
12802	11	19.40	19.30	136.00	26.60	432.00	1510.00
17306	11	11.30	14.60	124.00	12.90	133.00	591.00
19001	12	10.10	17.20	151.00	23.50	263.00	1080.00
19401	12	25.80	22.50	254.00	17.10	301.00	2130.00
29805	13	7.73	9.33	116.00	9.47	132.00	997.00
28004	14	14.80	28.40	224.00	23.10	140.00	1240.00
31415	14	11.50	15.50	156.00	16.30	226.00	827.00
15504	15	29.90	76.30	413.00	46.80	464.00	1480.00
30601	15	12.30	12.60	129.00	13.40	205.00	793.00

Zero values were assigned to levels that were below the limit of detection.

TABLE A-1 (continued)

WID	Batch	2378-TCDD	12378-PeCDD	123478/ 123678-HxCDD	123789-HxCDD	1234678-HpCDD	OCDD
18804	16	17.40	22.30	164.00	17.00	187.00	1010.00
29610	16	25.00	17.10	141.00	11.60	162.00	1150.00
16702	17	16.40	23.10	198.00	35.00	569.00	2220.00
27204	17	9.28	13.00	119.00	13.80	163.00	694.00
16703	18	33.20	50.40	415.00	40.60	908.00	3230.00
28801	18	18.30	37.10	385.00	57.80	1200.00	4630.00
15301	19	29.20	31.40	239.00	16.80	257.00	1690.00
26601	20	7.80	13.10	125.00	13.50	161.00	984.00
27301	20	9.27	19.00	163.00	19.40	293.00	1230.00

Zero values were assigned to levels that were below the limit of detection.

TABLE A-2
Dioxin Levels in the Adipose Tissue of Non-Vietnam Veterans

WID	Batch	2378-TCDD	12378-PeCDD	123478/ 123678-HxCDD	123789-HxCDD	1234678-HpCDD	OCDD
06510	1	8.61	12.70	122.00	12.50	217.00	1010.00
11601	1	18.60	19.80	201.00	24.60	200.00	1320.00
15505	1	13.10	22.90	219.00	16.60	220.00	861.00
27613	1	21.50	23.90	216.00	16.10	288.00	674.00
00610	2	2.50*	7.60	66.90	5.00	74.90	738.00
04304	2	22.90	23.10	163.00	18.80	313.00	1780.00
17915	2	7.40	11.90	109.00	9.60	174.00	819.00
30418	2	8.50	16.30	135.00	13.70	205.00	544.00
07504	3	15.20	45.50	323.00	31.80	488.00	1080.00
18406	3	8.00	19.10	126.00	17.20	146.00	779.00
28802	3	11.70	20.50	127.00	20.60	149.00	811.00
31101	3	5.50	7.60	66.70	5.70	62.40	289.00
15507	4	9.08	19.60	164.00	21.20	237.00	834.00
23603	4	7.42	14.70	149.00	13.90	276.00	1920.00
28501	4	9.63	22.70	165.00	19.20	174.00	1060.00
29602	4	8.45	14.00	101.00	13.70	139.00	931.00
01508	5	3.72	8.05	98.50	11.80	264.00	775.00
17501	5	18.10	26.00	160.00	29.60	525.00	1460.00
19301	5	9.54	13.70	127.00	17.20	263.00	1460.00
29202	5	10.10	20.30	178.00	23.40	328.00	1380.00
00606	6	5.72	12.50	96.90	10.30	173.00	847.00
10104	6	14.80	20.90	133.00	19.40	184.00	1140.00
21502	6	37.30	22.80	158.00	13.60	154.00	770.00
27206	6	6.72	13.40	137.00	11.80	238.00	1420.00
06508	7	6.40	14.70	128.00	26.40	415.00	1680.00

* Trace amount

Zero values were assigned to levels that were below the limit of detection.

TABLE A-2 (continued)

WID	Batch	2378-TCDD	12378-PeCDD	123478/ 123678-HxCDD	123789-HxCDD	1234678-HpCDD	OCDD
17914	7	9.39	14.00	116.00	13.40	221.00	1040.00
27907	7	12.90	17.80	144.00	13.50	123.00	671.00
28815	7	11.40	21.50	158.00	14.00	90.30	452.00
12814	8	9.86	12.00	104.00	11.60	147.00	658.00
17009	8	17.40	27.50	270.00	27.30	405.00	2340.00
28902	8	19.60	14.90	128.00	16.30	173.00	965.00
31408	8	30.10	57.70	319.00	38.50	472.00	2280.00
16402	9	14.00	21.10	177.00	23.20	275.00	1280.00
23610	9	7.03	10.80	89.40	10.50	145.00	693.00
26701	9	12.80	20.00	163.00	18.80	268.00	1370.00
28805	9	16.70	20.90	162.00	19.00	387.00	1210.00
16201	10	14.50	19.20	166.00	19.60	219.00	732.00
27606	10	5.81	10.20	84.80	10.60	154.00	640.00
27901	10	13.00	24.20	176.00	25.90	375.00	1120.00
27910	10	8.53	18.90	143.00	20.80	267.00	1480.00
31414	10	16.80	0.00	113.00	14.60	171.00	795.00
01512	11	8.59	13.70	174.00	19.60	396.00	1350.00
28810	11	10.30	11.70	117.00	18.10	280.00	1260.00
29808	11	9.87	12.70	96.70	9.73	99.50	797.00
29903	11	11.90	22.30	234.00	23.70	274.00	1480.00
10103	12	14.10	14.40	125.00	12.20	144.00	804.00
12807	12	12.40	12.50	125.00	13.50	194.00	713.00
29204	12	6.70	13.40	134.00	12.40	118.00	670.00
30411	12	6.77	7.07	61.80	10.00	259.00	1180.00
12810	13	14.70	16.50	158.00	17.80	235.00	1080.00
23607	13	4.00**	11.00	88.60	9.85	142.00	628.00

** Half of the limit of detection level.

Zero values were assigned to levels that were below the limit of detection.

TABLE A-2 (continued)

WID	Batch	2378-TCDD	12378-PeCDD	123478/ 123678-HxCDD	123789-HxCDD	1234678-HpCDD	OCDD
18404	13	19.80	19.10	145.00	16.20	201.00	1290.00
27208	13	47.60	64.50	665.00	67.20	1120.00	5370.00
16602	14	13.70	13.50	123.00	16.30	337.00	1370.00
23609	14	8.81	15.30	156.00	21.50	315.00	1240.00
27617	14	6.97	8.27	92.40	9.91	200.00	927.00
28803	14	12.70	23.10	145.00	15.30	110.00	769.00
18405	15	20.50	43.00	291.00	38.80	378.00	1040.00
27610	15	14.30	13.80	159.00	13.30	338.00	1460.00
30410	15	15.30	15.30	136.00	15.10	208.00	1010.00
26702	16	12.60	19.80	190.00	21.30	282.00	1260.00
29608	16	4.28	6.57	61.50	6.81	91.50	385.00
29806	16	6.41	7.02	86.80	7.17	98.90	562.00
31405	16	7.23	11.00	115.00	11.20	165.00	846.00
03502	17	8.48	16.30	180.00	14.30	227.00	1130.00
04303	17	25.30	17.90	127.00	17.40	324.00	902.00
16904	17	19.50	16.60	132.00	14.90	309.00	847.00
18403	17	11.60	22.60	229.00	23.90	257.00	2500.00
04804	18	13.20	16.70	150.00	13.70	262.00	921.00
12817	18	12.30	18.90	172.00	19.20	220.00	1080.00
16902	18	17.00	18.30	133.00	12.70*	280.00	1470.00
01513	19	7.30	18.10	145.00	13.70	115.00	533.00
27202	19	8.31	14.40	157.00	14.20	241.00	1520.00
27806	19	8.34	17.00	124.00	13.50	200.00	882.00
30408	19	7.29*	12.50	106.00	11.90	103.00	515.00
12815	20	9.20	13.00	122.00	14.90	348.00	1120.00
12819	20	7.59	12.80	120.00	13.70	172.00	819.00
14602	20	16.30	34.20	179.00	21.10	275.00	1000.00
29804	20	14.60	14.80	146.00	13.50	201.00	764.00

* Trace amount

Zero values were assigned to levels that were below the limit of detection.

TABLE A-3
Dioxin Levels in the Adipose Tissue of Civilians

WID	Batch	2378-TCDD	12378-PeCDD	123478/ 123678-HxCDD	123789-HxCDD	1234678-HpCDD	OCDD
06506	1	18.10	20.50	275.00	34.40	663.00	2530.00
12806	1	11.00	11.20	135.00	12.20	167.00	726.00
12816	1	8.45	14.90	173.00	9.76	155.00	1090.00
16502	1	32.00	18.60	181.00	16.40	395.00	1720.00
01501	2	4.60	6.60	50.00	8.70	165.00	510.00
18409	2	4.60	11.30	96.80	9.60	67.60	503.00
27210	2	10.90	14.70	128.00	11.10	152.00	998.00
27211	2	13.30	20.30	170.00	18.60	436.00	2140.00
06509	3	106.00	19.80	151.00	20.60	235.00	2500.00
27604	3	13.10	20.40	302.00	33.20	286.00	1150.00
28403	3	12.00	27.80	206.00	20.40	204.00	1270.00
28807	3	6.10	8.80	78.60	9.20	90.50	462.00
00609	4	9.09	20.50	117.00	16.20	177.00	594.00
12828	4	10.90	9.62	94.40	9.02	97.20	401.00
17901	4	14.70	25.80	192.00	26.80	616.00	1760.00
27605	4	3.48	7.67	81.10	9.51	149.00	682.00
01506	5	5.98	12.00	92.80	13.50	372.00	1180.00
06515	5	36.80	50.60	516.00	47.70	624.00	4130.00
27201	5	7.06	11.50	111.00	9.70	213.00	821.00
29802	5	13.60	23.10	169.00	21.00	249.00	2310.00
00607	6	7.83	19.40	130.00	11.30	91.90	449.00
17020	6	6.08	12.10	133.00	7.86	98.30	550.00
27902	6	15.00	15.80	115.00	14.20	293.00	1030.00
30412	6	5.21	9.72	81.20	10.50	162.00	732.00
04802	7	8.66	11.80	90.10	9.97	145.00	555.00
23606	7	11.50	19.40	168.00	20.70	609.00	2760.00
27203	7	14.40	21.30	171.00	17.40	291.00	1510.00

Zero values were assigned to levels that were below the limit of detection.

TABLE A-3 (continued)

WID	Batch	2378-TCDD	12378-PeCDD	123478/ 123678-HxCDD	123789-HxCDD	1234678-HpCDD	OCDD
29810	7	11.80	15.00	127.00	15.70	210.00	868.00
15303	8	19.50	22.70	206.00	15.70	203.00	1290.00
19303	8	12.90	13.60	117.00	18.60	382.00	1110.00
27611	8	5.00	9.10	87.80	8.77	128.00	652.00
31202	8	5.87	6.61	64.10	7.95	183.00	686.00
01507	9	10.70	18.40	138.00	16.60	289.00	953.00
06511	9	23.80	9.49	71.40	10.80	121.00	632.00
12822	9	3.30	6.71	62.00	6.10	114.00	299.00
12823	10	11.20	17.00	156.00	0.00	134.00	822.00
15302	10	25.80	21.30	157.00	14.70	191.00	1010.00
28908	10	7.24	13.00	125.00	11.10	194.00	696.00
29811	10	11.50	15.10	113.00	15.30	121.00	581.00
00602	11	6.34	12.70	111.00	8.14	104.00	529.00
01516	11	6.67	10.70	114.00	12.20	323.00	997.00
23604	11	8.42	13.20	127.00	12.70	162.00	467.00
27222	11	8.86	11.40	139.00	9.63	99.20	814.00
30413	11	27.00	23.40	127.00	10.10	110.00	677.00
02302	12	22.70	19.70	177.00	17.00	297.00	1030.00
11201	12	30.90	19.10	162.00	21.80	402.00	1980.00
27302	12	11.30	20.30	148.00	15.30	146.00	892.00
28002	12	6.49	11.70	98.00	15.80	304.00	1940.00
03501	13	22.00	16.70	161.00	24.90	608.00	2530.00
12818	13	12.40	13.50	124.00	14.20	141.00	677.00
27220	13	51.70	71.20	831.00	102.00	2680.00	8800.00
27602	13	13.60	12.60	114.00	13.00	137.00	715.00
04801	14	20.80	16.50	142.00	16.70	275.00	1540.00
16704	14	15.20	28.10	170.00	21.60	180.00	908.00
17303	14	37.80	35.00	305.00	42.60	870.00	3910.00

Zero values were assigned to levels that were below the limit of detection.

TABLE A-3 (continued)

WID	Batch	2378-TCDD	12378-PeCDD	123478/ 123678-HxCDD	123789-HxCDD	1234678-HpCDD	OCDD
26703	14	11.40	13.30	129.00	15.90	325.00	852.00
03503	15	21.60	11.00	121.00	11.40	253.00	846.00
06512	15	14.30	12.60	105.00	14.90	160.00	1180.00
17005	15	15.70	10.90	89.10	12.60	179.00	894.00
27218	15	43.70	32.90	372.00	35.20	446.00	3560.00
15506	16	11.50	19.30	137.00	12.80	96.40	581.00
17004	16	14.80	13.80	155.00	15.40	304.00	2160.00
17008	16	11.30	14.90	149.00	13.00	215.00	919.00
17007	17	7.00	11.10	114.00	9.16	128.00	685.00
27221	17	23.40	43.10	412.00	34.80	528.00	2450.00
28814	17	17.70	22.20	175.00	18.20	276.00	1310.00
31203	17	7.95	14.00	135.00	14.00	113.00	758.00
06516	18	16.80	13.70	134.00	15.30	242.00	875.00
17002	18	14.30	13.40	114.00	12.60	174.00	1250.00
28903	18	8.58	15.60	210.00	19.20	597.00	1680.00
31204	18	9.66	20.00	149.00	15.00	318.00	841.00
07505	19	55.80	62.60	476.00	53.20	1040.00	4320.00
08803	19	20.90	25.50	231.00	19.30	250.00	1400.00
18801	19	6.74	13.00	113.00	9.93	177.00	1400.00
28804	19	12.10	11.90	95.00	10.40	353.00	1630.00
04803	20	7.43	13.40	104.00	9.14	143.00	709.00
17907	20	22.60	42.20	328.00	57.60	766.00	4780.00
23602	20	12.10	16.40	169.00	19.20	198.00	1070.00
26705	20	11.70	20.10	192.00	16.40	260.00	1660.00
27609	20	6.18	9.62	120.00	7.99	171.00	558.00

Zero values were assigned to levels that were below the limit of detection.

TABLE A-4
Six Furan Levels in the Adipose Tissue of Vietnam Veterans
(TCDF, PeCDF and HpCDF)

WID	Batch	2378-TCDF	12378-PeCDF	23478-PeCDF	1234678-HpCDF	1234789-HpCDF	OCDF
18408	1	1.90	0.00	15.60	32.00	0.00	1.67*
27601	1	0.00	2.46	22.00	40.10	0.00	0.00
01510	2	1.70	0.00	17.60	20.50	1.70*	2.10
17018	2	0.00	0.00	17.70	13.80	0.00	6.30*
29607	2	4.70	3.00	14.00	9.80	0.00	0.80*
17027	3	4.10	1.90	21.40	38.70	1.80*	1.50
31304	3	0.00	0.00	20.50	27.60	0.00	1.20*
31410	4	1.58*	0.00	0.00	27.80	0.00	0.00
28905	5	2.15	0.81*	18.10	43.60	2.08*	2.53
31403	5	0.00	0.46*	13.10	19.80	1.33*	0.00
01520	6	0.00	0.00	14.70	20.80	0.00	16.90
17605	6	0.00	0.00	24.50	28.50	0.00	3.10*
00601	7	0.53*	0.00	14.00	16.60	0.00	2.03
31404	7	0.42*	0.00	12.00	12.60	0.00	1.08*
17006	8	2.23	1.30	22.50	41.40	0.00	14.60
28906	8	1.46	0.00	11.50	36.40	1.29*	2.70*
21101	9	2.63	0.00	10.60	25.40	1.26	2.95
28502	9	3.78	0.00	21.20	52.10	2.31	1.39*
12802	11	5.04	2.83	32.10	68.20	3.05	2.97
17306	11	0.00	0.00	10.90	19.10	0.00	2.54
19001	12	0.00	0.00	18.40	42.20	0.00	1.08*
19401	12	1.11*	0.00	31.70	48.90	0.00	3.05
29805	13	0.00	0.00	14.60	26.70	0.00	0.00
28004	14	16.20	0.00	29.40	30.10	0.00	0.00
31415	14	1.70*	0.00	19.80	18.60	0.00	2.66*

* Trace amount

Zero values were assigned to levels that were below the limit of detection.

TABLE A-4 (continued)

WID	Batch	2378-TCDF	12378-PeCDF	23478-PeCDF	1234678-HpCDF	1234789-HpCDF	OCDF
15504	15	2.78	0.00	29.40	84.50	3.11	2.80
30601	15	1.96	0.00	13.60	21.50	0.00	1.59*
18804	16	1.85	0.00	28.70	18.10	0.00	0.98*
29610	16	0.00	0.00	19.40	56.50	0.00	4.76*
16702	17	2.52	0.00	21.50	58.70	0.00	4.73
27204	17	0.00	0.00	25.20	15.50	0.00	0.00
16703	18	4.67	0.00	56.60	98.80	3.72	5.65
28801	18	1.89	0.98*	82.30	128.00	4.32	3.89
15301	19	1.37	0.00	38.10	46.60	1.93*	0.00
26601	20	1.51	0.00	19.00	25.30	1.26*	0.00
27301	20	3.10	0.00	26.10	31.10	1.87*	0.00

* Trace amount

Zero values were assigned to levels that were below the limit of detection.

TABLE A-5
Six Furan Levels in the Adipose Tissue of Non-Vietnam Veterans
(TCDF, PeCDF and HpCDF)

WID	Batch	2378-TCDF	12378-PeCDF	23478-PeCDF	1234678-HpCDF	1234789-HpCDF	OCDF
06510	1	1.72	0.50*	9.69	21.80	0.00	0.00
11601	1	5.01	0.77*	18.00	55.00	0.00	7.84
15505	1	2.98	0.00	19.00	47.10	0.00	2.29
27613	1	5.97	0.00	25.30	16.10	0.00	0.00
00610	2	0.00	0.00	5.60	19.20	0.00	0.00
04304	2	7.20	2.40	42.10	78.10	2.50*	4.00
17915	2	0.00	0.00	13.60	11.40	0.00	0.00
30418	2	4.20	3.30	22.70	23.30	0.00	0.00*
07504	3	1.60	0.00	23.80	31.60	1.40*	1.60
18406	3	0.00	0.00	21.90	25.20	0.00	3.90
28802	3	0.90	0.00	27.30	24.40	1.30*	1.20
31101	3	2.70	0.70*	13.70	9.20	0.00	0.80*
15507	4	0.00	0.00	23.10	24.70	1.51*	1.55*
23603	4	0.00	0.00	21.30	45.00	0.00	5.67
28501	4	0.00	0.00	28.60	36.60	0.00	0.00
29602	4	1.05*	0.00	52.80	19.10	0.00	1.15*
01508	5	0.59*	0.00	7.57	18.30	0.00	1.70
17501	5	2.80	1.76	29.60	49.20	2.54	15.30
19301	5	2.31	0.00	15.70	36.70	0.00	1.88
29202	5	1.64	0.54	20.90	19.80	1.06*	0.00
00606	6	0.00	0.00	14.10	25.60	0.00	0.00
10104	6	3.49	0.00	38.20	32.60	0.00	1.97*
21502	6	2.87	0.00	43.10	33.50	2.58*	0.00
27206	6	2.80	0.00	22.50	32.00	1.42	2.59
06508	7	0.94*	0.00	11.60	121.00	4.59	28.10

* Trace amount

Zero values were assigned to levels that were below the limit of detection.

TABLE A-5 (continued)

WID	Batch	2378-TCDF	12378-PeCDF	23478-PeCDF	1234678-HpCDF	1234789-HpCDF	OCDF
17914	7	1.28	0.00	10.30	22.90	1.57*	1.70
27907	7	0.00	0.00	21.20	23.10	0.00	0.00
28815	7	0.63*	0.00	14.70	18.00	1.44*	1.61*
12814	8	0.00	0.00	15.40	21.20	0.00	0.00
17009	8	3.55	0.00	34.30	56.60	2.39	4.16
28902	8	0.00	0.00	22.10	20.70	0.00	1.60*
31408	8	1.62	0.00	34.20	45.30	2.42*	3.57*
16402	9	2.08	2.20	32.70	51.00	0.00	7.15
23610	9	1.22	0.00	12.30	17.80	0.00	0.82*
26701	9	1.40	0.00	21.80	35.00	0.00	2.15*
28805	9	3.45	0.00	28.90	23.80	0.00	1.18*
16201	10	2.25	0.76	23.10	44.90	2.10	3.67
27606	10	1.20	0.00	17.20	20.20	0.95*	1.38
27901	10	0.00	0.46*	27.30	32.50	0.90*	0.00
27910	10	0.00	0.00	30.40	40.70	0.00	23.10
31414	10	0.00	0.00	0.00	36.60	2.12*	0.00
01512	11	1.68	0.00	21.30	27.90	1.29	1.81*
28810	11	0.00	0.83	10.80	23.00	0.00	1.30*
29808	11	1.22*	0.53*	20.80	20.50	1.00*	1.03*
29903	11	0.00	0.00	27.40	21.80	1.09*	0.00
10103	12	2.60	0.00	17.40	31.30	0.00	3.11
12807	12	2.98	0.00	24.40	27.30	0.00	2.86
29204	12	0.00	0.00	13.20	12.90	0.00	1.28
30411	12	6.52	1.21*	7.89	14.10	1.12*	0.89*
12810	13	1.60	0.00	23.20	29.00	0.00	2.55*
18404	13	0.00	0.00	15.30	44.10	1.83	2.02
23607	13	0.00	0.00	14.90	21.80	0.00	3.40*

* Trace amount

Zero values were assigned to levels that were below the limit of detection.

TABLE A-5 (continued)

WID	Batch	2378-TCDF	12378-PeCDF	23478-PeCDF	1234678-HpCDF	1234789-HpCDF	OCDF
27208	13	0.00	0.00	91.90	133.00	5.69	8.81
16602	14	5.56*	0.00	19.90	57.80	0.00	0.00
23609	14	1.35*	0.00	17.60	35.30	0.00	0.00
27617	14	1.47*	0.00	11.50	23.70	0.00	0.00
28803	14	2.12	0.00	13.70	13.60	0.00	0.00
18405	15	0.00	0.00	35.00	33.10	0.00	2.06*
27610	15	3.94	0.00	20.40	35.30	0.00	1.67*
30410	15	0.00	0.00	16.50	33.90	0.00	2.07*
26702	16	1.41	0.59*	22.40	43.00	3.15	2.13*
29608	16	0.00	0.46*	9.02	20.80	1.25	0.00
29806	16	0.00	0.00	9.57	15.70	0.00	0.00
31405	16	0.00	0.00	14.20	25.40	1.58	0.00
03502	17	1.33	0.88*	29.20	37.90	1.94	4.60*
04303	17	2.61	0.00	19.40	41.10	2.27*	2.65*
16904	17	3.94	0.00	26.10	27.00	0.00	2.60*
18403	17	0.00	0.00	24.90	58.50	2.96	7.49
04804	18	2.25	0.78*	27.60	30.80	0.00	1.50*
12817	18	1.49	0.00	22.00	33.60	1.51*	1.61*
16902	18	4.50	0.00	27.10	43.50	0.00	25.60
01513	19	1.08*	0.00	17.50	31.80	1.68*	0.00
27202	19	0.95*	0.00	24.20	34.70	0.00	20.40
27806	19	1.85	0.00	16.90	19.60	1.13	0.93*
30408	19	0.00	0.00	17.00	21.10	0.00	0.00
12815	20	1.55	0.00	20.50	32.50	0.00	0.00
12819	20	0.00	0.00	15.70	25.90	1.57*	1.39*
14602	20	1.04	0.00	14.80	24.60	1.44*	0.00
29804	20	1.08	0.00	33.10	29.00	1.54*	1.61*

* Trace amount

Zero values were assigned to levels that were below the limit of detection.

TABLE A-6
Six Furan Levels in the Adipose Tissue of Civilians
(TCDF, PeCDF and HpCDF)

WID	Batch	2378-TCDF	12378-PeCDF	23478-PeCDF	1234678-HpCDF	1234789-HpCDF	OCDF
06506	1	3.86	0.00	20.80	70.70	0.00	0.00
12806	1	2.23	0.58*	17.00	31.00	0.00	4.47
12816	1	2.05	0.73*	16.30	32.80	0.00	0.00
16502	1	3.90	0.83*	20.10	61.90	0.00	1.85*
01501	2	3.50	0.00	9.50	15.70	1.00*	1.40
18409	2	0.00	0.00	11.40	11.60	0.00	1.50
27210	2	1.60	0.00	25.50	23.50	0.00	0.00
27211	2	1.70	0.00	45.20	59.80	1.90*	1.40*
06509	3	0.00	0.00	25.30	42.80	1.80*	3.10
27604	3	1.05	0.00	40.20	99.20	3.00*	1.83*
28403	3	1.20	0.00	56.30	48.30	0.00	2.10*
28807	3	0.60	0.00	11.00	13.60	0.00	2.70
00609	4	2.83	0.00	13.30	10.60	0.69*	3.57
12828	4	1.60	0.00	17.90	16.90	0.75*	1.74
17901	4	2.25	0.00	13.90	53.70	0.00	2.67
27605	4	0.00	0.00	11.10	19.20	0.00	1.69
01506	5	1.50	0.71	18.80	30.10	0.00	0.00
06515	5	6.25	0.00	44.40	93.60	3.03	13.10
27201	5	0.00	0.00	16.60	25.20	1.34	2.41
29802	5	1.80	1.00	41.70	47.70	1.85	2.03
00607	6	0.00	0.00	9.17	13.50	0.55*	0.00
17020	6	1.39*	0.00	16.00	25.00	0.00	2.63
27902	6	2.46	0.00	21.50	38.70	0.00	1.60
30412	6	0.86	0.00	13.80	22.80	0.55*	0.00
04802	7	0.00	0.00	16.00	25.00	0.00	0.00

* Trace amount

Zero values were assigned to levels that were below the limit of detection.

TABLE A-6 (continued)

WID	Batch	2378-TCDF	12378-PeCDF	23478-PeCDF	1234678-HpCDF	1234789-HpCDF	OCDF
23606	7	1.91	0.00	15.40	67.50	3.10	4.86
27203	7	0.85*	0.00	29.20	36.00	1.90	4.20
29810	7	2.29	0.00	24.40	34.10	0.00	1.60*
15303	8	4.58	2.42	29.90	53.10	0.00	5.01
19303	8	3.10	0.00	20.40	52.60	2.48	6.00
27611	8	1.00*	0.00	12.20	22.20	0.00	1.19*
31202	8	4.12	0.00	9.72	13.30	0.00	0.00
01507	9	1.66	0.00	22.30	25.90	2.14*	2.28*
06511	9	1.75	0.00	10.30	26.20	0.00	1.35*
12822	9	2.43	0.00	12.10	11.50	1.00*	0.00
12823	10	0.00	0.00	24.20	22.40	0.00	1.74*
15302	10	3.33	0.00	31.30	36.70	1.70	7.65
28908	10	0.92*	0.00	15.60	38.30	1.73	2.94
29811	10	0.00	0.00	21.20	19.10	0.90	0.00
00602	11	0.00	0.00	13.70	13.90	1.24*	1.24
01516	11	0.00	2.12	11.00	17.20	0.00	1.05*
23604	11	0.00	0.00	16.10	10.40	0.00	1.08*
27222	11	0.00	0.00	16.50	18.30	0.00	1.44*
30413	11	0.00	0.00	12.30	13.10	0.00	2.71
02302	12	1.86	0.00	31.00	32.10	1.50	2.03
11201	12	11.60	7.01	20.50	57.30	2.49	2.81
27302	12	0.00	0.00	24.70	25.70	1.43*	2.44
28002	12	5.30	0.48*	19.20	40.50	0.00	0.00
03501	13	4.86	0.00	26.50	75.80	3.95	7.95
12818	13	1.79	0.00	17.50	25.40	1.29*	1.30*
27220	13	17.50	4.58	92.50	179.00	9.07	9.08
27602	13	0.00	0.00	18.10	17.10	0.00	1.77*

* Trace amount

Zero values were assigned to levels that were below the limit of detection.

TABLE A-6 (continued)

WID	Batch	2378-TCDF	12378-PeCDF	23478-PeCDF	1234678-HpCDF	1234789-HpCDF	OCDF
04801	14	3.33	0.00	28.90	43.80	2.02	2.37
16704	14	0.00	0.00	22.60	22.00	0.00	3.28
17303	14	0.00	0.00	42.70	157.00	5.21	7.77
26703	14	1.63	0.00	17.90	27.60	0.00	0.00
03503	15	0.00	0.00	13.20	32.80	0.00	2.58*
06512	15	0.00	0.00	22.10	25.50	0.00	0.00
17005	15	2.30	0.00	12.40	19.80	0.00	4.91
27218	15	0.00	0.00	33.00	90.90	0.00	6.25*
15506	16	0.00	0.00	18.00	15.50	0.00	0.99*
17004	16	1.11	0.00	17.90	57.10	0.00	5.79
17008	16	0.00	0.00	20.50	26.70	0.00	0.00
17007	17	0.00	0.00	13.00	30.60	0.00	2.87*
27221	17	3.05	1.60*	97.90	56.60	3.16*	3.30*
28814	17	0.00	0.34*	14.60	19.30	0.00	0.00
31203	17	2.23	0.00	17.90	18.90	1.26*	1.22*
06516	18	5.74	0.90*	18.10	37.10	1.61*	5.60
17002	18	0.00	0.00	17.40	41.50	0.00	5.60
28903	18	3.95	0.00	19.50	75.40	3.78	4.72
31204	18	4.04	1.02*	21.70	24.50	0.00	1.49
07505	19	0.00	0.00	45.60	70.00	3.18*	0.00
08803	19	1.68	0.00	39.00	49.90	2.20	3.19*
18801	19	2.77	0.00	20.30	25.10	1.36*	6.38
28804	19	3.34	0.57*	15.40	25.20	1.37*	0.00
04803	20	0.00	0.00	22.50	24.50	1.04*	0.00
17907	20	18.10	6.23	38.60	69.60	4.02	6.57
23602	20	0.00	0.00	26.80	44.20	2.49	0.00
26705	20	0.00	0.00	22.40	61.80	2.61	3.82
27609	20	1.55	0.00	14.40	17.00	1.02*	0.00

* Trace amount

Zero values were assigned to levels that were below the limit of detection.

TABLE A-7
Four Furan Levels in the Adipose Tissue of Vietnam Veterans
(HxCDF)

WID	Batch	123478-HxCDF	123678-HxCDF	234678-HxCDF	123789-HxCDF
18408	1	16.80	9.47	2.74	0.00
27601	1	27.50	10.80	0.00	0.00
01510	2	12.40	7.30	3.50	0.00
17018	2	11.40	7.20	0.00	0.00
29607	2	8.70	4.70	1.80	0.00
17027	3	24.50	11.50	3.00	0.00
31304	3	15.30	9.00	1.60*	0.00
31410	4	14.20	0.00	0.00	0.00
28905	5	19.20	0.00	2.18	0.00
31403	5	11.60	7.12	2.08	0.00
01520	6	10.80	8.05	0.00	0.00
17605	6	16.50	11.40	4.19	0.00
00601	7	9.82	4.91	1.28	0.00
31404	7	7.49	4.00	0.00	0.00
17006	8	23.10	7.40	3.32	0.00
28906	8	9.83	8.33	3.04	0.00
21101	9	13.50	6.32	2.06	0.00
28502	9	25.10	10.30	3.20	0.00
12802	11	31.00	10.80	5.36	0.00
17306	11	11.10	4.41	0.00	0.00
19001	12	25.80	12.10	3.46	0.00
19401	12	29.10	11.30	3.39	0.00
29805	13	12.80	7.67	0.00	0.00
28004	14	19.90	11.80	0.00	0.00
31415	14	15.70	10.20	0.00	0.00

* Trace amount

Zero values were assigned to levels that were below the limit of detection.

TABLE A-7 (continued)

WID	Batch	123478-HxCDF	123678-HxCDF	234678-HxCDF	123789-HxCDF
15504	15	43.20	20.00	6.31	0.00
30601	15	13.30	6.64	2.52	0.00
18804	16	23.00	10.10	2.11	0.33*
29610	16	18.00	9.41	0.00	2.80
16702	17	22.50	12.50	5.20	0.00
27204	17	19.20	10.80	3.04*	0.00
16703	18	69.00	32.00	9.88	1.32*
28801	18	63.30	30.70	13.80	0.00
15301	19	36.30	16.30	3.02	0.00
26601	20	19.70	8.18	1.95	0.00
27301	20	23.30	11.30	4.03	0.00

* Trace amount

Zero values were assigned to levels that were below the limit of detection.

TABLE A-8
Four Furan Levels in the Adipose Tissue of Non-Vietnam Veterans
(HxCDF)

WID	Batch	123478-HxCDF	123678-HxCDF	234678-HxCDF	123789-HxCDF
06510	1	15.50	7.72	2.81	0.00
11601	1	27.80	11.80	2.69	0.00
15505	1	22.50	10.60	2.77	0.00
27613	1	20.00	11.80	0.00	0.00
00610	2	6.70	4.00	1.10*	0.00
04304	2	36.90	13.70	3.80	0.00
17915	2	8.00	0.00	2.00	0.00
30418	2	16.10	10.70	4.60	0.00
07504	3	22.50	10.00	5.10	0.00
18406	3	15.10	6.90	1.80*	0.00
28802	3	18.30	9.60	1.80	0.00
31101	3	8.10	4.90	1.20	0.50*
15507	4	12.50	5.45	0.00	0.00
23603	4	16.40	8.96	3.20	0.00
28501	4	25.00	12.10	2.90*	0.00
29602	4	17.20	9.14	2.98	0.00
01508	5	10.00	4.73	1.72	0.00
17501	5	30.90	16.80	6.52	0.00
19301	5	17.20	8.76	3.65	0.00
29202	5	18.40	9.78	3.69	0.00
00606	6	12.20	6.48	0.00	0.00
10104	6	21.10	14.30	3.14	0.00
21502	6	0.00	0.00	0.00	0.00
27206	6	17.80	9.05	2.67	0.00
06508	7	27.10	13.20	4.94	0.00

* Trace amount

Zero values were assigned to levels that were below the limit of detection.

TABLE A-8 (continued)

WID	Batch	123478-HxCDF	123678-HxCDF	234678-HxCDF	123789-HxCDF
17914	7	10.10	5.70	1.97	0.00
27907	7	18.60	9.82	2.60*	0.00
28815	7	11.20	6.18	1.60	0.00
12814	8	12.20	5.26	2.08	0.00
17009	8	46.60	19.00	3.91	0.00
28902	8	16.00	8.45	2.33	0.00
31408	8	31.00	18.10	0.00	0.00
16402	9	23.80	13.90	4.09	0.00
23610	9	11.90	6.39	1.73	0.00
26701	9	18.90	9.66	4.32	0.00
28805	9	13.90	6.88	4.61	0.00
16201	10	20.80	7.07	2.43	0.00
27606	10	12.00	7.06	1.83	0.00
27901	10	18.80	8.46	6.04	0.00
27910	10	21.20	12.80	3.65	0.00
31414	10	12.60	6.63	2.51	0.00
01512	11	19.40	10.60	4.60	0.00
28810	11	12.20	6.02	2.33	0.00
29808	11	13.40	7.29	1.43	0.00
29903	11	14.50	9.23	2.95	0.00
10103	12	19.10	6.75	2.14	0.00
12807	12	25.40	10.10	2.96	0.00
29204	12	10.50	6.00	1.53	0.00
30411	12	6.72	4.17	2.17	0.00
12810	13	22.10	11.30	3.36	0.00
18404	13	17.70	7.89	1.80	1.42
23607	13	11.20	6.36	2.31*	0.00

* Trace amount

Zero values were assigned to levels that were below the limit of detection.

TABLE A-8 (continued)

WID	Batch	123478-HxCDF	123678-HxCDF	234678-HxCDF	123789-HxCDF
27208	13	98.40	59.00	13.20	0.00
16602	14	21.40	9.77	3.34*	0.00
23609	14	16.70	9.06	4.38	0.00
27617	14	12.80	6.62	2.82	0.00
28803	14	13.30	6.80	0.00	0.00
18405	15	33.90	20.10	5.87	0.00
27610	15	21.00	10.60	4.87	0.00
30410	15	14.00	7.91	2.75	0.00
26702	16	25.90	16.50	6.97	0.00
29608	16	8.91	6.03	1.77	0.00
29806	16	8.40	4.81	1.47*	0.00
31405	16	12.50	7.09	1.79	0.00
03502	17	23.90	12.40	4.17	0.00
04303	17	21.10	9.72	4.90	0.00
16904	17	24.50	10.80	5.50	0.00
18403	17	31.40	14.50	2.57	0.00
04804	18	18.40	10.90	3.72	0.00
12817	18	23.80	11.80	2.82	0.00
16902	18	28.50	10.60	3.65*	0.00
01513	19	14.30	7.56	2.04	0.00
27202	19	17.90	8.79	2.37*	0.00
27806	19	12.70	7.26	2.52	0.00
30408	19	9.19	7.20	2.65*	0.00
12815	20	20.50	9.12	4.91	0.00
12819	20	16.20	8.47	1.66	0.00
14602	20	16.30	8.17	2.77	0.00
29804	20	25.50	9.73	2.57	0.00

* Trace amount

Zero values were assigned to levels that were below the limit of detection.

TABLE A-9
Four Furan Levels in the Adipose Tissue of Civilians
(HxCDF)

WID	Batch	123478-HxCDF	123678-HxCDF	234678-HxCDF	123789-HxCDF
06506	1	32.90	16.00	4.59	0.00
12806	1	25.70	11.10	2.74	0.00
12816	1	26.90	9.57	1.25	0.00
16502	1	34.10	14.40	3.80	0.00
01501	2	8.60	4.60	0.00	0.00
18409	2	6.10	3.90	1.20	0.00
27210	2	17.40	9.50	3.00	0.00
27211	2	29.80	16.30	5.90	0.00
06509	3	28.80	11.70	2.60*	0.00
27604	3	32.80	20.00	5.25	0.00
28403	3	35.10	22.90	3.30*	0.00
28807	3	8.90	4.20	1.40	0.00
00609	4	9.09	5.73	2.46	0.00
12828	4	12.60	6.14	2.44	0.00
17901	4	0.00	0.00	5.11	1.03*
27605	4	8.93	4.54	1.45	0.00
01506	5	17.00	10.30	3.27	0.00
06515	5	63.90	27.10	8.11	0.00
27201	5	14.00	6.42	2.88	0.00
29802	5	43.40	28.30	2.38	0.00
00607	6	8.42	4.33	1.15	0.00
17020	6	12.60	6.93	1.83	0.00
27902	6	21.10	10.00	3.91	0.00
30412	6	13.30	5.31	1.87	0.00
04802	7	13.90	8.74	2.32	0.00

* Trace amount

Zero values were assigned to levels that were below the limit of detection.

Table A-9 (continued)

WID	Batch	123478-HxCDF	123678-HxCDF	234678-HxCDF	123789-HxCDF
23606	7	28.40	13.80	4.42	0.00
27203	7	21.60	9.87	3.22	0.00
29810	7	25.70	13.60	2.85	0.00
15303	8	26.30	11.50	3.17	0.00
19303	8	26.60	12.70	5.04	0.00
27611	8	10.60	5.06	1.82	0.00
31202	8	9.32	5.25	2.55	0.00
01507	9	18.00	10.50	3.31	0.00
06511	9	10.50	5.92	1.83	0.00
12822	9	8.13	4.21	2.39	0.00
12823	10	16.50	6.16	2.24	0.00
15302	10	20.50	8.04	2.99	0.00
28908	10	17.70	6.76	2.90	0.00
29811	10	12.70	6.15	2.01	0.00
00602	11	14.80	6.39	1.29	0.00
01516	11	9.87	5.18	2.19	0.00
23604	11	11.10	7.28	2.81	0.00
27222	11	14.10	8.60	0.87*	0.00
30413	11	7.42	4.11	1.66	0.00
02302	12	24.40	11.20	3.86	0.00
11201	12	33.60	17.10	3.93	0.00
27302	12	22.80	12.10	3.14	0.00
28002	12	14.20	9.24	2.37	0.00
03501	13	36.00	18.00	6.62	0.00
12818	13	18.60	9.42	1.74	0.00
27220	13	102.00	65.00	26.10	0.00
27602	13	17.30	9.20	1.65	0.00

* Trace amount

Zero values were assigned to levels that were below the limit of detection.

Table A-9 (continued)

WID	Batch	123478-HxCDF	123678-HxCDF	234678-HxCDF	123789-HxCDF
04801	14	23.80	13.30	4.02	0.00
16704	14	16.80	9.58	1.97*	0.00
17303	14	65.20	30.00	8.32	0.00
26703	14	18.10	9.77	4.72	0.00
03503	15	18.60	8.06	3.31	0.00
06512	15	23.30	12.90	2.72	0.00
17005	15	12.30	6.46	2.48	0.36*
27218	15	42.90	22.50	3.60	0.00
15506	16	12.50	6.73	1.57	0.00
17004	16	20.50	8.78	2.78	0.00
17008	16	24.30	10.60	2.23	0.00
17007	17	13.60	7.57	0.00	0.00
27221	17	59.10	40.90	13.50	1.02*
28814	17	14.00	8.13	2.92	0.00
31203	17	13.90	8.49	1.76	0.00
06516	18	25.00	14.10	3.11	0.00
17002	18	20.00	10.20	1.77*	0.00
28903	18	31.30	14.00	6.33	0.00
31204	18	22.00	12.00	5.11	0.00
07505	19	54.60	23.70	13.10	0.00
08803	19	31.00	17.60	3.16	1.19*
18801	19	22.20	9.83	1.94*	0.00
28804	19	13.60	7.00	2.67	0.00
04803	20	12.90	7.49	2.29	0.00
17907	20	47.70	28.40	7.25	0.00
23602	20	30.10	12.60	3.20	0.00

* Trace amount

Zero values were assigned to levels that were below the limit of detection.

APPENDIX B

This section contains the results of the Quality Assurance Program. Data for all compounds are reported in this appendix.

LIST OF TABLES AND FIGURES

I. INTERNALLY SPIKED LIPID SAMPLES

Table B-1. Percent Recovery of Measurements for Compounds from the Twenty Internal Spiked Lipid Samples.

Table B-2. Average Percent Recovery of Measurements of PCDDs and PCDFs From the Spiked Lipid Samples.

Figures B-1 to B-5. Percent Recovery of Spiked Internal QC Samples.

II. SPLIT SAMPLES

Tables B-3 to B-9. Results of Split Sample Analyses.

III. UNSPIKED CONTROL LIPID SAMPLES

Figures B-6 to B-21. Concentration in Unspiked Control Lipid Samples.

Table B-10. Mean Measurements in the Unspiked Control Lipid Samples.

IV. CALIBRATION DATA

Figures B-22 to B-47. RRF Control Charts.

V. INTERNAL QUANTITATION STANDARDS

Figures B-48 to B-56. Internal Quantitation Standards Recovery Plots.

I. INTERNAL SPIKED LIPID SAMPLES

Table B-1 and Figures B-1 through B-5 present the accuracy and precision data for all compounds from the internal spiked lipid samples. Table B-2 shows the % recovery for each measurement broken down by spike level. The recovery data in Figures B-1 through B-5 are plotted in the order of analysis. Three hundred seven of the 320 measurements were within the data quality objectives of 50 - 130% accuracy. The 13 data points that were outside the data quality objectives occurred in five compounds:

Compound	Number of data points below 50% recovery	Number of data points above 130% recovery
2,3,7,8-TCDD	0	1
2,3,4,7,8-PeCDF	1	2
1,2,3,4,7,8-/ 1,2,3,6,7,8-HxCDD	0	6
1,2,3,4,6,7,8-HpCDD	0	1
OCDD	2	0

Eleven of the 13 points outside of the data quality objectives occurred in samples which were spiked at the low spike level. A high background level in the lipid matrix relative to the low spike level resulted in, percentage-wise, more variability in the results. In 10 of these 11 cases the amount of the spike added was less than 14% of the background level. The amount of the low level spike for OCDD was 6% of the background level.

The two data points that were not low-level-spiked samples were for 2,3,7,8-TCDD, which was spiked at the high level and had a recovery of 135%, and for 1,2,3,4,7,8-HxCDD which was spiked at the medium spike level and had a recovery of 143%.

The 1,2,3,4,7,8-HxCDD and 1,2,3,6,7,8-HxCDD isomer concentration levels were reported throughout this study as a combined response. This was necessary because these isomer pairs were not completely resolved on the 60-meter DB5 column. The 1,2,3,4,7,8-HxCDD is typically less than 20% of the 1,2,3,6,7,8-HxCDD concentration.

II. SPLIT SAMPLES

Tables B-3 through B-9 present the data on the split samples. The precision of the measurements are generally very good, with the relative percent differences (RPD) usually less than 20%. Fifty-eight of the 81 RPD values were less than 10%. Seventeen RPD values were between 10 and 20%. Only 6 values were greater than 20% RPD. These measurements are discussed below.

The four highest RPD values (90.8% for OCDF; 80.1% for OCDF; 36.6% for OCDF; 23.6% for 2,3,4,6,7,8-HxCDF) were from samples in which the measurements were close to the detection limits. In each of these four occurrences one of the measurements was a trace value and the other was a positive quantifiable value. The comparison of a trace value to a positive quantifiable value resulted in a high RPD. The remaining two occurrences above 20% RPD were only slightly above (20.2% 1,2,3,6,7,8-HxCDF; 23.6% 2,3,4,6,7,8-HxCDF). In both of these cases, both pairs of measurements were positive quantifiable values.

There were 21 data pairs in which both measurements were "not detected" (ND). Data on the level of detection on these measurements can provide some information on the variability of the detection limit from analysis to analysis.

There were 5 data pairs in which one measurement was a trace value and the other was not detected (ND). In two of those cases the level of detection (LOD) for the ND value was higher than the trace value. In two other cases the LOD for the ND value was lower than the trace value. And in the fifth case the LOD in the ND sample was the same as the trace value.

There were 5 data pairs in which one measurement was a positive quantifiable (PQ) value and the other was a ND value. In 4 of the cases the LOD for the ND value was greater than the PQ value. In one case the LOD was lower.

III. UNSPIKED CONTROL LIPID SAMPLES

Plots of the measurements of the compounds in the unspiked control lipid samples are given in Figures B-6 through B-21. The mean and 95% confidence interval established in the Method Evaluation Study[25] are indicated on each plot.
Three hundred of the 320 data points were within the 95% confidence intervals (CI). The following compounds had data values that were outside the 95% CI:

2,3,4,7,8-PeCDF	1 point higher than 95% CI
1,2,3,4,7,8-HxCDF	5 points lower than 95% CI
1,2,3,4,7,8-/ 1,2,3,6,7,8-HxCDD	8 points lower than 95% CI
1,2,3,4,6,7,8-HpCDD	5 points higher than 95% CI
OCDD	1 point higher than 95% CI

The mean values from the 20 samples run during the study from January 1987 through November 1987, were compared to the mean estimated from the Method Evaluation Study[25] which was run about a year earlier in April 1986. The summary data are presented in Table B-10. The same source of homogenized lipid material was used in both studies.

IV. CALIBRATION DATA

The daily analyses of calibration standards bracketing the sample analysis were conducted. The relative response factors (RRF) for the native compounds and the internal quantitation standards were calculated according to the protocol[25] and are plotted in Figures B-22 through B-47. The data quality objectives for the RRF values stated that the variability for TCDD and TCDF should be within ± 20%, and the variability for the remaining compounds should be within ± 30%. The calibration standard analysis was repeated if any of the 26 measured events were outside the limits.

In some instances data points were noted outside the RRF control limits. When this occurred the analysis for the calibration standard was repeated. Since a small percentage of the data points in any one calibration were expectedly outside the control criteria, the analyst proceeded with the analysis of samples. Typically the compounds for which data points were outside the control limits were the carbon-13 labeled compounds. A comparison of the native PCDD and PCDF RRF values versus the corresponding carbon-13 labeled internal standards demonstrated greater consistency for the native compounds. No exceptions were made for TCDD. If the RRF data for TCDD were outside the control limits, no sample analyses were run until calibration criteria were achieved.

V. INTERNAL QUANTITATION STANDARDS

Figures B-48 through B-56 present the data on the % recovery of the internal quantitation standards from each sample. The data are plotted in the order of analysis. The data quality objective was that the recovery of the internal quantitation standards should be 50 - 115%.

Some of the recoveries were outside the data quality objectives. Even so, the sample analyses were not repeated since the recoveries of the other internal quantitation standards were within the data quality objectives and the observed signal-to-noise ratio was greater than 10.

TABLE B-1
Percent Recovery of Measurements for Compounds from the Twenty Internal Spiked Lipid Samples (%)

Spike Level	% Recovery		
2,3,7,8-TCDF			
Low (10 pg/g):	94.4, 98.0, 91.8, 90.1, 86.1, 91.9, 97.1, 92.3, 96.2	Mean=93.1	CV=4.0%
Medium (25 pg/g):	105, 103, 106, 121, 99.8	Mean=107	CV=7.7%
High (50 pg/g):	99.2, 92.6, 102, 104, 109, 94.8	Mean=100	CV=6.0%
2,3,7,8-TCDD			
Low (10 pg/g):	102, 112, 93.1, 106, 111, 127, 109, 117, 113	Mean=110	CV=8.6%
Medium (25 pg/g):	110, 117, 122, 125, 120	Mean=119	CV=4.8%
High (50 pg/g):	104, 107, 111, 123, 135, 102	Mean=114	CV=11%

TABLE B-1 (continued)

Spike Level	% Recovery		
<u>1,2,3,7,8-PeCDF</u>			
Low (10 pg/g):	106, 104, 105, 104, 117, 104, 108, 101, 115	Mean=107	CV=5.0%
Medium (25 pg/g):	104, 112, 112, 99.6, 109	Mean=107	CV=5.0%
High (50 pg/g):	99.6, 102, 108, 97.8, 103, 101	Mean=102	CV=3.4%
<u>2,3,4,7,8-PeCDF</u>			
Low (10 pg/g):	145, 52, 34.6, 180, 129, 102, 98.3, 95.3, 127	Mean=107	CV=42%
Medium (25 pg/g):	99.6, 113, 125, 95.2, 109	Mean=108	CV=11%
High (50 pg/g):	109, 106, 112, 112, 66.7, 107	Mean=102	CV=17%
<u>1,2,3,7,8-PeCDD</u>			
Low (10 pg/g):	95.0, 101, 86.1, 88.3, 96.7, 103, 112, 105, 101	Mean=98.7	CV=8.3%
Medium (25 pg/g):	86.9, 108, 102, 94.0, 114	Mean=101	CV=11%
High (50 pg/g):	98.2, 102, 90.6, 98.8, 100, 100	Mean=98.3	CV=4.0%

TABLE B-1 (continued)

Spike Level	% Recovery	
1,2,3,4,7,8-HxCDF		
Low (25 pg/g):	76.1, 106, 97.2, 80.3, 85.9, 93.6, 107, 110, 124	Mean=97.8 CV=16%
Medium (62.5 pg/g):	104, 97.1, 84.6, 106, 110	Mean=100 CV=9.9%
High (125 pg/g):	106, 105, 98.7, 99.4, 102, 104	Mean=103 CV=2.9%
1,2,3,6,7,8-HxCDF		
Low (25 pg/g):	118, 109, 96.0, 88.8, 89.9, 95.2, 103, 107, 118	Mean=103 CV=11%
Medium (62.5 pg/g):	100, 97.0, 88.7, 100, 107	Mean=98.5 CV=6.7%
High (125 pg/g):	108, 110, 97.4, 103, 101, 104	Mean=104 CV=4.4%
2,3,4,6,7,8-HxCDF		
Low (25 pg/g):	77.9, 95.2, 86.7, 102, 98.5, 95.2, 97.4, 100, 106	Mean=95.4 CV=8.9%
Medium (62.5 pg/g):	101, 102, 103, 96.2, 97.4	Mean=99.9 CV=3.0%
High (125 pg/g):	96.7, 93.2, 91.7, 98.3, 96.9, 96.2	Mean=95.5 CV=2.6%

TABLE B-1 (continued)

Spike Level	% Recovery	
1,2,3,7,8,9-HxCDF		
Low (25 pg/g):	82.9, 92.0, 79.0, 89.6, 98.0, 91.6, 98.0, 90.8, 102	Mean=91.5 CV=8.0%
Medium (62.5 pg/g):	89.5, 89.3, 88.5, 98.2, 92.7	Mean=91.6 CV=4.4%
High (125 pg/g):	95.2, 92.1, 89.5, 96.8, 100, 89.5	Mean=93.9 CV=4.5%
1,2,3,4,7,8-/1,2,3,6,7,8-HxCDD		
Low (50 pg/g):	79.7, 150, 159, 148, 107, 86.3, 102, 171, 161	Mean=129 CV=27%
Medium (125 pg/g):	103, 109, 143, 95.2, 113	Mean=113 CV=16%
High (250 pg/g):	91.9, 110, 101, 111, 102, 98.0	Mean=102 CV=7.1%
1,2,3,7,8,9-HxCDD		
Low (25 pg/g):	99.2, 93.2, 93.2, 83.1, 105, 84.7, 98.0, 85.1, 94.4	Mean=92.9 CV=8.0%
Medium (62.5 pg/g):	87.6, 98.1, 104, 95.2, 93.3	Mean=95.6 CV=6.3%
High (125 pg/g):	86.0, 91.7, 89.6, 93.4, 91.1, 90.2	Mean=90.3 CV=2.8%

TABLE B-1 (continued)

Spike Level	% Recovery	
1,2,3,4,6,7,8-HpCDF		
Low (25 pg/g):	97.6, 94.2, 98.4, 90.8, 98.0, 75.1, 95.6, 98.8, 97.2	Mean=94.0 CV=8.0%
Medium (62.5 pg/g):	90.1, 95.7, 99.2, 101, 102	Mean=97.6 CV=4.9%
High (125 pg/g):	92.2, 90.6, 92.5, 99.8, 92.4, 93.0	Mean=93.4 CV=3.5%
1,2,3,4,7,8,9-HpCDF		
Low (25 pg/g):	62.2, 97.2, 77.5, 87.6, 119, 97.2, 98.8, 98.4, 109	Mean=94.1 CV=18%
Medium (62.5 pg/g):	82.0, 85.3, 100, 105, 107	Mean=95.9 CV=12%
High (125 pg/g):	90.3, 92.1, 95.0, 106, 110, 102	Mean=99.2 CV=8.0%
1,2,3,4,6,7,8-HpCDD		
Low (25 pg/g):	112, 104, 59.5, 52.2, 68.5, 60.2, 104, 72.3, 137	Mean=85.5 CV=34%
Medium (62.5 pg/g):	85.8, 93.9, 88.1, 90.6, 95.4	Mean=90.8 CV=4.4%
High (125 pg/g):	104, 88.1, 96.0, 104, 96.8, 91.9	Mean=96.8 CV=6.6%

TABLE B-1 (continued)

Spike Level	% Recovery	
OCDF		
Low (50 pg/g):	90.4, 83.4, 74.9, 92.9, 107, 80.3, 86.7, 83.2, 89.9	Mean=87.6 CV=10%
Medium (125 pg/g):	77.7, 105, 94.6, 101, 86.4	Mean=92.9 CV=12%
High (250 pg/g):	86.7, 90.2, 81.6, 108, 95.5, 85.1	Mean=91.2 CV=10%
OCDD		
Low (50 pg/g):	67.7, 130, 27.8, 82.3, 80.6, 112, 70.3, 104, 26.1	Mean=77.9 CV=45%
Medium (125 pg/g):	107, 103, 92.0, 88.1, 105	Mean=99.0 CV=8.5%
High (250 pg/g):	80.6, 100, 88.0, 108, 94.8, 81.1	Mean=92.1 CV=12%

Data quality objectives for the % recovery for the internal spiked lipid samples was 50% - 130%.

$$\% \text{ Recovery} = 100\% \times \frac{\text{conc. spiked sample} - \text{conc. control sample}}{\text{spike level}}$$

FIGURE B-1
Percent Recovery of Spiked Internal QC Samples
Tetrachlorinated Congeners
Batches 1 to 20

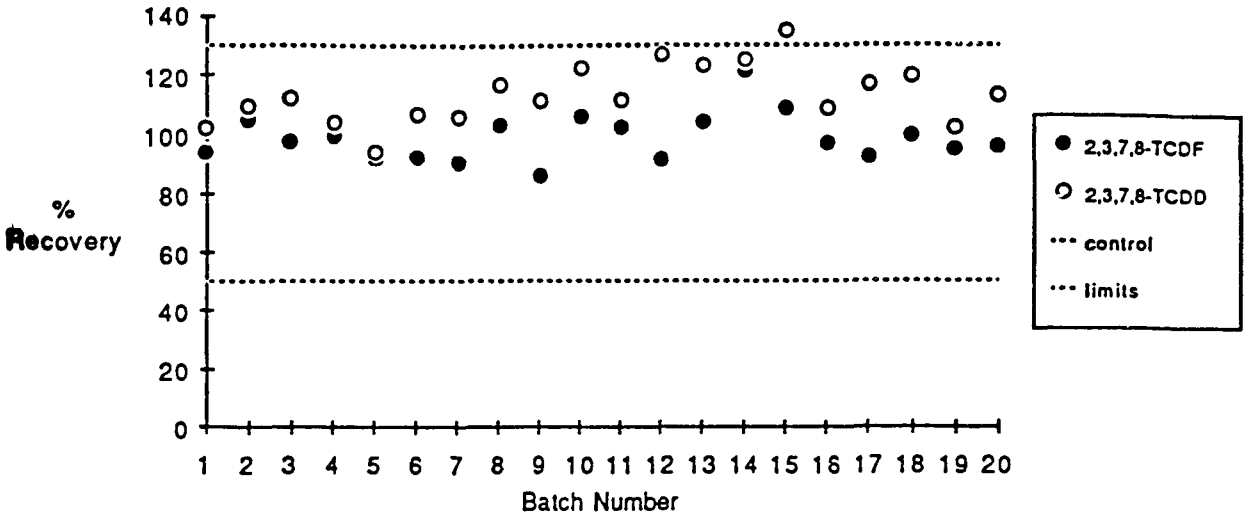


FIGURE B-2
Percent Recovery of Spiked Internal QC Samples
Pentachlorinated Congeners
Batches 1 to 20

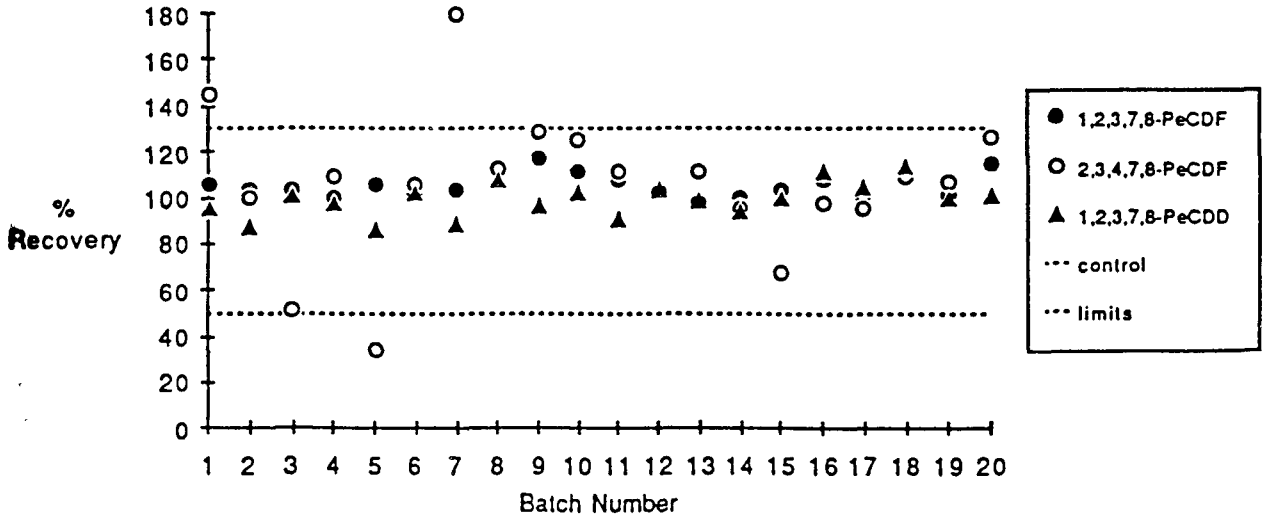


FIGURE B-3
Percent Recovery of Spiked Internal QC Samples
Hexachlorinated Congeners
Batches 1 to20

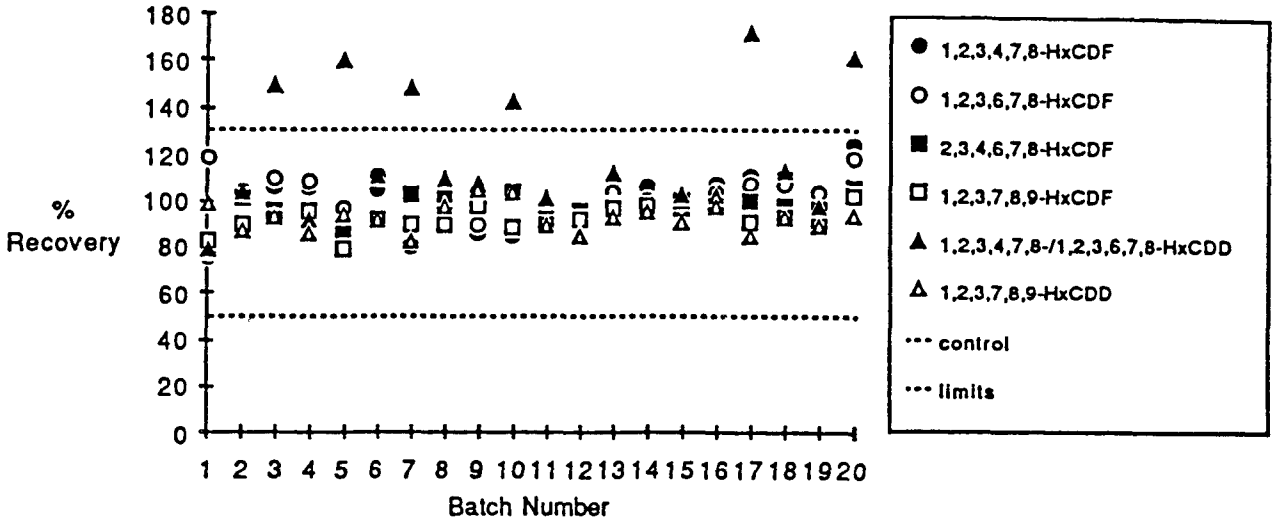


FIGURE B-4
Percent Recovery of Spiked Internal QC Samples
Heptachlorinated Congeners
Batches 1 to20

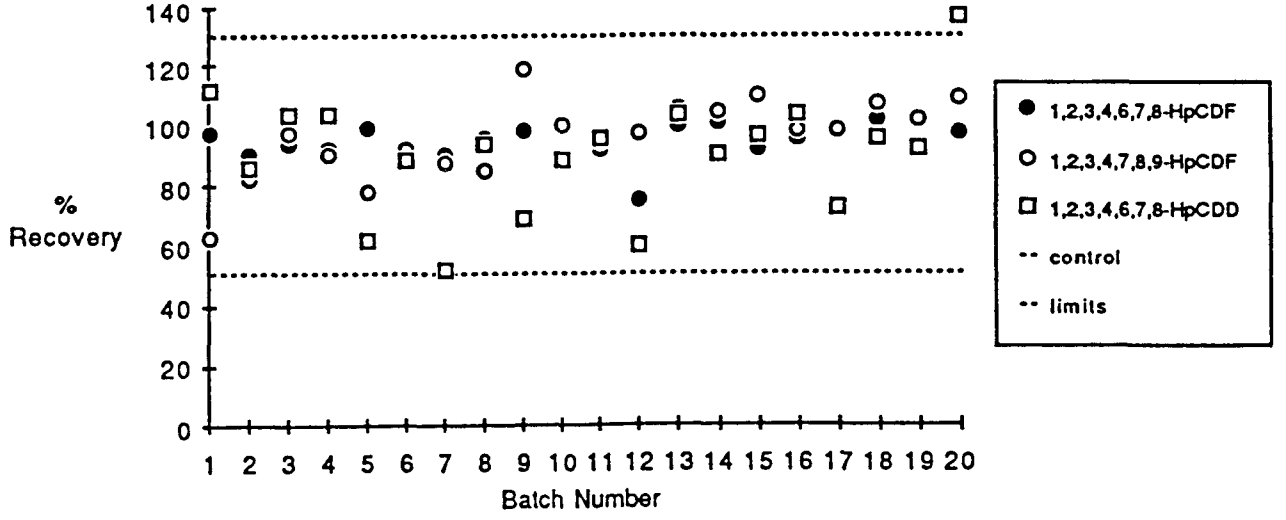


FIGURE B-5
Percent Recovery of Spiked Internal QC Samples
Octachlorinated Congeners
Batches 1 to 20

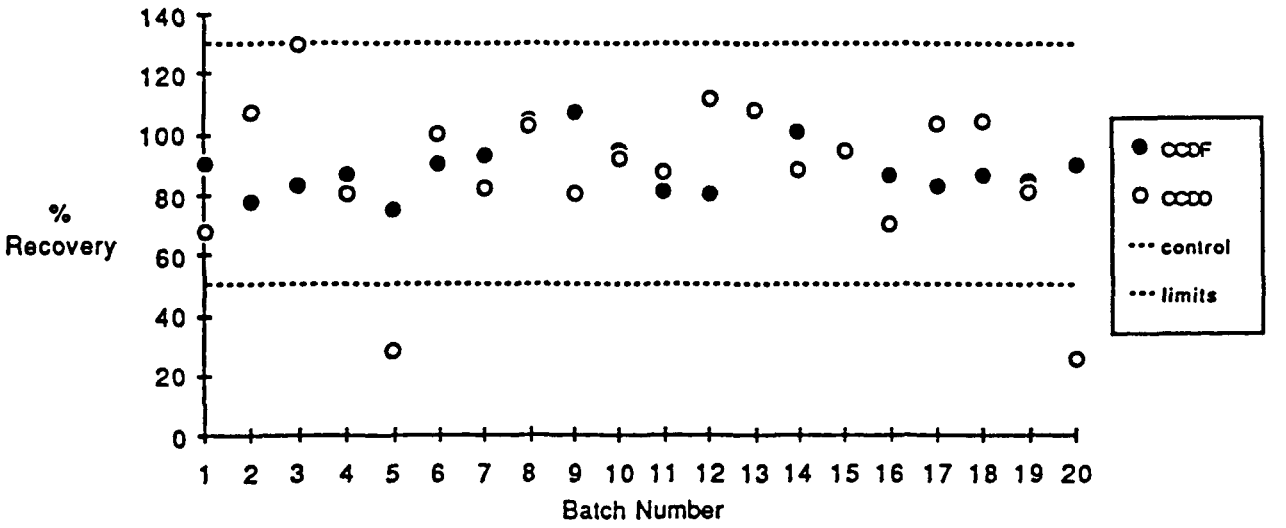


TABLE B-2
Average Percent Recovery of Measurements of
PCDDs and PCDFs From the Spiked Lipid Samples

	Spike Level*		
Compound	Low	Medium	High
2,3,7,8-TCDF	93.1	107	100
2,3,7,8-TCDD	110	119	114
1,2,3,7,8-PeCDF	107	107	102
2,3,4,7,8-PeCDF	107	108	102
1,2,3,7,8-PeCDD	98.7	101	98.3
1,2,3,4,7,8-HxCDF	97.8	100	103
1,2,3,6,7,8-HxCDF	103	98.5	104
2,3,4,6,7,8-HxCDF	95.4	99.9	95.5
1,2,3,7,8,9-HxCDF	91.5	91.6	93.9
1,2,3,4,7,8/ 1,2,3,6,7,8-HxCDD	129	113	102
1,2,3,7,8,9-HxCDD	92.9	95.6	90.3
1,2,3,4,6,7,8-HpCDF	94.0	97.6	93.4
1,2,3,4,7,8,9-HpCDF	94.1	95.9	99.2
1,2,3,4,6,7,8-HpCDD	85.5	90.8	96.8
OCDF	87.6	92.9	91.2
OCDD	77.9	99.0	92.1

* Spike levels based on a 10-gram lipid sample.

Tetra and penta compounds: Low, Medium, High = 10, 25, 50 pg/g.
Hexa and Hepta compounds: Low, Medium, High = 25, 62.5, 125 pg/g.
Octa compounds: Low, Medium, High = 50, 125, 250 pg/g.

TABLE B-3
Results of Split Sample Analysis for #00609

Compound	First Analysis (pg/g)	Second Analysis (pg/g)	Relative Percent Difference (%)
2,3,7,8-TCDF	2.93	2.83	3.47
2,3,7,8-TCDD	8.93	9.09	1.78
1,2,3,7,8-PeCDF	ND[1] (0.7)	ND[1] (0.8)	----
2,3,4,7,8-PeCDF	11.0	13.3	18.9
1,2,3,7,8-PeCDD	17.5	20.6	16.3
1,2,3,4,7,8-HxCDF	9.76	9.09	7.11
1,2,3,6,7,8-HxCDF	5.30	5.73	7.80
2,3,4,6,7,8-HxCDF	1.94	2.46	23.6
1,2,3,7,8,9-HxCDF	ND[1] (0.6)	ND[1] (0.2)	----
1,2,3,4,7,8/ 1,2,3,6,7,8-HxCDD	107	117	8.93
1,2,3,7,8,9-HxCDD	14.5	16.2	11.1
1,2,3,4,6,7,8-HpCDF	10.4	10.6	1.90
1,2,3,4,7,8,9-HpCDF	ND[1] (0.7)	0.69	----
1,2,3,4,6,7,8-HpCDD	164	177	7.62
OCDF	TR[2] (1.34)	3.57	90.8
OCDD	491	594	19.0

[1] Not detected above Limit of Detection (LOD)
[2] Trace

$$\text{Relative Percent Difference (\%)} = \frac{\text{high value} - \text{low value}}{\text{average value}} \times 100$$

TABLE B-4
Results of Split Sample Analysis for #29810

Compound	First Analysis (pg/g)	Second Analysis (pg/g)	Relative Percent Difference (%)
2,3,7,8-TCDF	2.20	2.29	4.0
2,3,7,8-TCDD	11.3	11.8	4.3
1,2,3,7,8-PeCDF	ND[1] (0.3)	ND[1] (0.5)	----
2,3,4,7,8-PeCDF	25.3	24.7	1.6
1,2,3,7,8-PeCDD	14.8	15.0	1.3
1,2,3,4,7,8-HxCDF	21.3	25.7	18.7
1,2,3,6,7,8-HxCDF	11.1	13.6	20.2
2,3,4,6,7,8-HxCDF	2.52	2.85	12.3
1,2,3,7,8,9-HxCDF	ND[1] (0.3)	ND[1] (0.2)	----
1,2,3,4,7,8/ 1,2,3,6,7,8-HxCDD	109	127	15.2
1,2,3,7,8,9-HxCDD	15.9	15.7	1.3
1,2,3,4,6,7,8-HpCDF	33.5	34.1	1.8
1,2,3,4,7,8,9-HpCDF	TR[2] (1.56)	ND[1] (1.9)	----
1,2,3,4,6,7,8-HpCDD	201	210	4.4
OCDF	ND[1] (0.8)	TR[2] (1.6)	----
OCDD	936	868	7.5

[1] Not detected above Limit of Detection (LOD)
[2] Trace

$$\text{Relative Percent Difference (\%)} = \frac{\text{high value} - \text{low value}}{\text{average value}} \times 100$$

TABLE B-5
Results of Split Sample Analysis for #12823

Compound	First Analysis (pg/g)	Second Analysis (pg/g)	Relative Percent Difference (%)
2,3,7,8-TCDF	TR[2] (0.72)	ND[1] (0.6)	----
2,3,7,8-TCDD	10.9	11.2	2.71
1,2,3,7,8-PeCDF	ND[1] (0.2)	ND[1] (0.2)	----
2,3,4,7,8-PeCDF	25.4	24.2	4.84
1,2,3,7,8-PeCDD	18.1	17.0	6.27
1,2,3,4,7,8-HxCDF	16.9	16.5	2.40
1,2,3,6,7,8-HxCDF	6.90	6.16	11.3
2,3,4,6,7,8-HxCDF	2.45	2.24	8.96
1,2,3,7,8,9-HxCDF	ND[1] (0.9)	ND[1] (0.3)	----
1,2,3,4,7,8/ 1,2,3,6,7,8-HxCDD	176	156	12.0
1,2,3,7,8,9-HxCDD	17.8	ND[1] (18.3)	----
1,2,3,4,6,7,8-HpCDF	22.0	22.4	1.80
1,2,3,4,7,8,9-HpCDF	ND[1] (0.5)	ND[1] (0.6)	----
1,2,3,4,6,7,8-HpCDD	138	134	2.94
OCDF	2.52	TR[2] (1.74)	36.6
OCDD	843	822	2.52

[1] Not detected above Limit of Detection (LOD)
[2] Trace

$$\text{Relative Percent Difference (\%)} = \frac{\text{high value} - \text{low value}}{\text{average value}} \times 100$$

A response was noted in the second analysis for 1,2,3,7,8,9-HxCDD but the ratio of the characteristic ions was outside the qualitative criteria.

TABLE B-6
Results of Split Sample Analysis for #29805

Compound	First Analysis (pg/g)	Second Analysis (pg/g)	Relative Percent Difference (%)
2,3,7,8-TCDF	ND[1] (0.9)	ND[1] (0.5)	----
2,3,7,8-TCDD	6.99	7.73	10.0
1,2,3,7,8-PeCDF	ND[1] (0.5)	ND[1] (0.5)	----
2,3,4,7,8-PeCDF	15.5	14.6	6.0
1,2,3,7,8-PeCDD	10.7	9.33	13.7
1,2,3,4,7,8-HxCDF	12.5	12.8	2.40
1,2,3,6,7,8-HxCDF	6.91	7.67	10.4
2,3,4,6,7,8-HxCDF	2.95	ND[1] (2.6)	----
1,2,3,7,8,9-HxCDF	ND[1] (0.6)	ND[1] (0.9)	----
1,2,3,4,7,8/ 1,2,3,6,7,8-HxCDD	129	116	10.6
1,2,3,7,8,9-HxCDD	10.5	9.47	10.3
1,2,3,4,6,7,8-HpCDF	25.6	26.7	4.2
1,2,3,4,7,8,9-HpCDF	ND[1] (2.0)	ND[1] (1.9)	----
1,2,3,4,6,7,8-HpCDD	145	132	9.4
OCDF	2.13	ND[1] (2.3)	----
OCDD	986	997	1.1

[1] Not detected above Limit of Detection (LOD)
[2] Trace

$$\text{Relative Percent Difference (\%)} = \frac{\text{high value} - \text{low value}}{\text{average value}} \times 100$$

A response was noted in the second analysis for 2,3,4,7,8,9-HxCDF but the ratio of the characteristic ions was outside the qualitative criteria.

TABLE B-7
Results of Split Sample Analysis for #29806

Compound	First Analysis (pg/g)	Second Analysis (pg/g)	Relative Percent Difference (%)
2,3,7,8-TCDF	ND[1] (0.7)	ND[1] (1.0)	----
2,3,7,8-TCDD	6.48	6.41	1.1
1,2,3,7,8-PeCDF	ND[1] (0.2)	ND[1] (0.3)	----
2,3,4,7,8-PeCDF	9.06	9.75	7.3
1,2,3,7,8-PeCDD	7.53	7.02	7.0
1,2,3,4,7,8-HxCDF	8.55	8.40	1.8
1,2,3,6,7,8-HxCDF	4.43	4.81	8.3
2,3,4,6,7,8-HxCDF	1.26	TR[2] (1.47)	15.4
1,2,3,7,8,9-HxCDF	ND[1] (0.4)	ND[1] (0.4)	----
1,2,3,4,7,8/ 1,2,3,6,7,8-HxCDD	81.6	86.8	0.3
1,2,3,7,8,9-HxCDD	7.84	7.17	8.9
1,2,3,4,6,7,8-HpCDF	15.0	15.7	4.6
1,2,3,4,7,8,9-HpCDF	ND[1] (0.6)	ND[1] (1.3)	----
1,2,3,4,6,7,8-HpCDD	101	98.9	2.1
OCDF	ND[1] (0.8)	ND[1] (1.0)	----
OCDD	507	562	10.3

[1] Not detected above Limit of Detection (LOD)
[2] Trace

$$\text{Relative Percent Difference (\%)} = \frac{\text{high value} - \text{low value}}{\text{average value}} \times 100$$

A response was noted in the second analysis for 2,3,7,8-TCDF but the ratio of the characteristic ions was outside the qualitative criteria.

TABLE B-8
Results of Split Sample Analysis for #18801

Compound	First Analysis (pg/g)	Second Analysis (pg/g)	Relative Percent Difference (%)
2,3,7,8-TCDF	2.80	2.77	1.1
2,3,7,8-TCDD	7.32	6.74	8.3
1,2,3,7,8-PeCDF	ND[1] (0.3)	ND[1] (0.4)	----
2,3,4,7,8-PeCDF	21.0	20.3	3.4
1,2,3,7,8-PeCDD	12.8	13.0	1.6
1,2,3,4,7,8-HxCDF	21.0	22.2	5.6
1,2,3,6,7,8-HxCDF	9.14	9.83	7.3
2,3,4,6,7,8-HxCDF	1.53	TR[2] (1.94)	23.6
1,2,3,7,8,9-HxCDF	ND[1] (0.4)	ND[1] (0.3)	----
1,2,3,4,7,8/ 1,2,3,6,7,8-HxCDD	120	113	6.0
1,2,3,7,8,9-HxCDD	10.3	9.93	3.7
1,2,3,4,6,7,8-HpCDF	23.6	25.1	6.2
1,2,3,4,7,8,9-HpCDF	ND[1] (1.4)	TR[2] (1.4)	----
1,2,3,4,6,7,8-HpCDD	184	177	3.9
OCDF	TR[2] (2.73)	6.38	80.1
OCDD	1340	1400	4.4

[1] Not detected above Limit of Detection (LOD)
[2] Trace

$$\text{Relative Percent Difference (\%)} = \frac{\text{high value} - \text{low value}}{\text{average value}} \times 100$$

TABLE B-9
Results of Split Sample Analysis for #06509

Compound	First Analysis (pg/g)	Second Analysis (pg/g)	Relative Percent Difference (%)
2,3,7,8-TCDF	ND[1] (0.6)	ND[1] (0.7)	----
2,3,7,8-TCDD	106	113	6.3
1,2,3,7,8-PeCDF	ND[1] (0.7)	ND[1] (0.7)	----
2,3,4,7,8-PeCDF	25.3	24.7	2.4
1,2,3,7,8-PeCDD	19.8	19.6	1.0
1,2,3,4,7,8-HxCDF	28.8	26.6	7.9
1,2,3,6,7,8-HxCDF	11.7	11.0	6.2
2,3,4,6,7,8-HxCDF	TR[2] (2.6)	TR[2] (2.39)	8.4
1,2,3,7,8,9-HxCDF	ND[1] (0.7)	ND[1] (0.5)	----
1,2,3,4,7,8/ 1,2,3,6,7,8-HxCDD	151	164	8.3
1,2,3,7,8,9-HxCDD	20.6	19.9	3.5
1,2,3,4,6,7,8-HpCDF	42.8	47.6	10.6
1,2,3,4,7,8,9-HpCDF	TR[2] (1.8)	ND[1] (2.5)	----
1,2,3,4,6,7,8-HpCDD	235	223	5.2
OCDF	3.1	ND[1] (3.2)	----
OCDD	2500	2530	1.2

[1] Not detected above Limit of Detection (LOD)
[2] Trace

$$\text{Relative Percent Difference (\%)} = \frac{\text{high value} - \text{low value}}{\text{average value}} \times 100$$

The first analysis was in batch 3, the second analysis was in batch 6.

FIGURE B-6

2,3,7,8-TCDD
Concentration in Unspiked Control QC Sample (pg/g)

Control Limits: 95% Confidence Interval for Individual Analyses from Method Evaluation Data

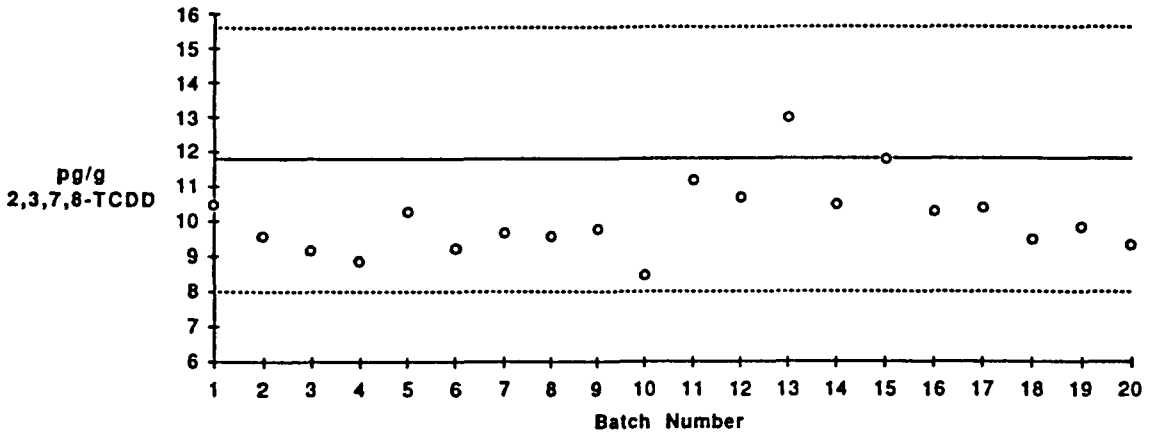


FIGURE B-7

2,3,7,8-TCDF
Concentration in Unspiked Control QC Sample (pg/g)

Control Limits: 95% Confidence Interval for Individual Analyses from Method Evaluation Data

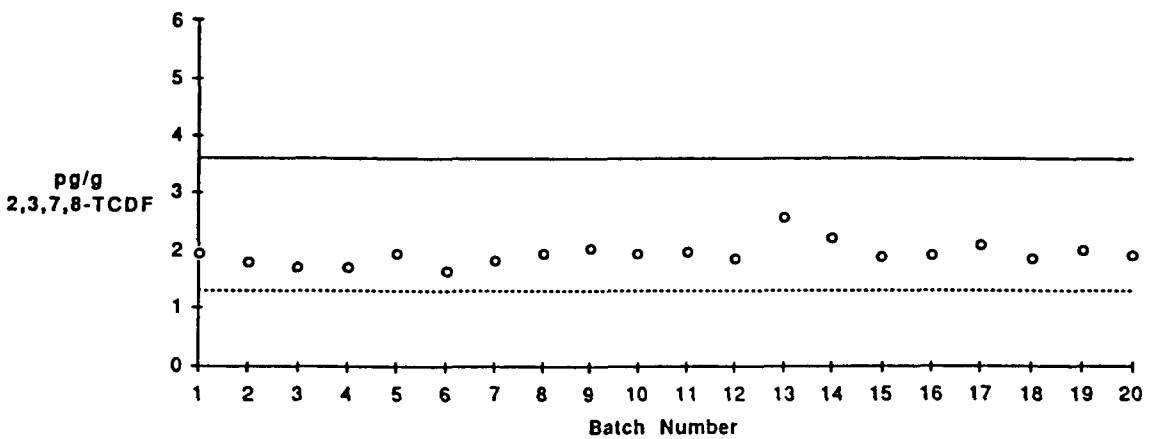


FIGURE B-8

1,2,3,7,8-PeCDF
Concentration in Unspiked Control QC Sample (pg/g)

Control Limits: 95% Confidence Interval for Individual Analyses from Method Evaluation Data

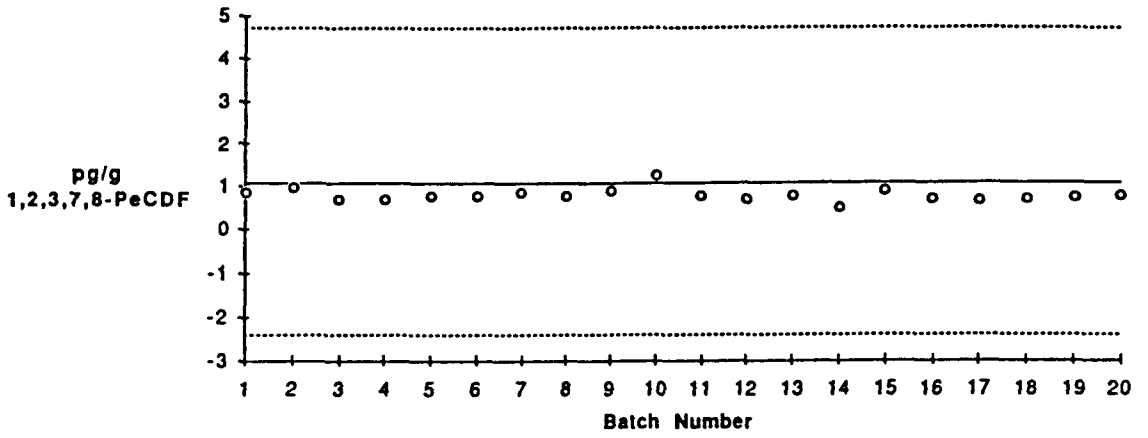


FIGURE B-9

2,3,4,7,8-PeCDF
Concentration in Unspiked Control QC Sample (pg/g)

Control Limits: 95% Confidence Interval for Individual Analyses from Method Evaluation Data

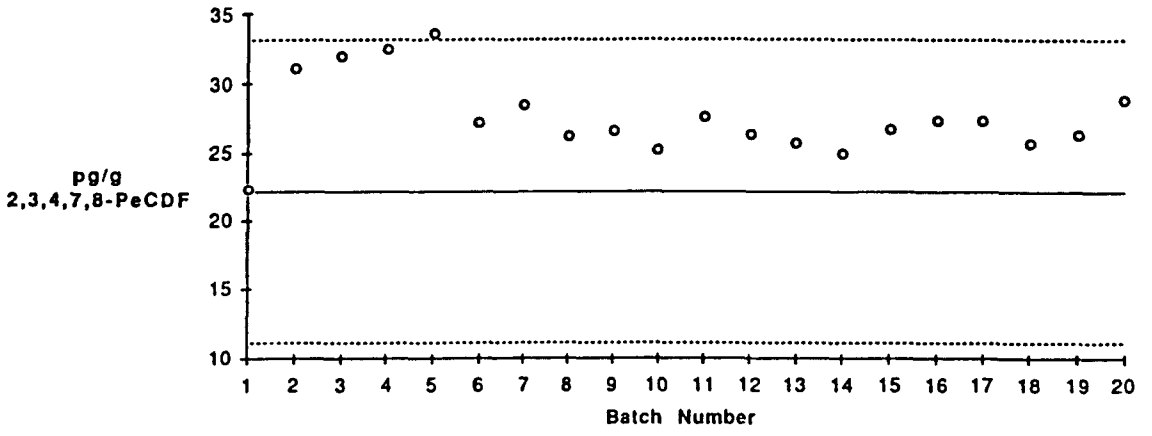


FIGURE B-10

1,2,3,7,8-PeCDD
Concentration in Unspiked Control QC Sample (pg/g)

Control Limits: 95% Confidence Interval for Individual Analyses from Method Evaluation Data

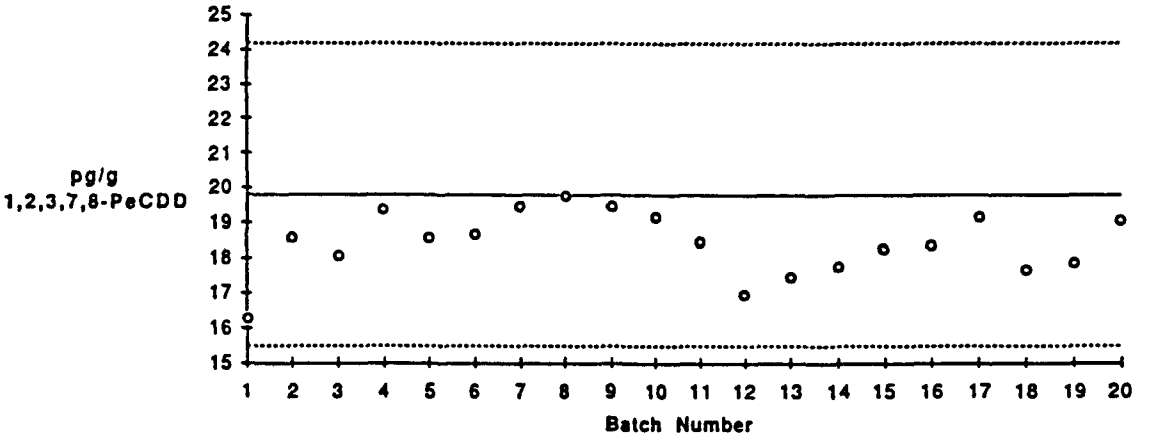


FIGURE B-11

1,2,3,6,7,8-HxCDF
Concentration in Unspiked Control QC Sample (pg/g)

Control Limits: 95% Confidence Interval for Individual Analyses from Method Evaluation Data

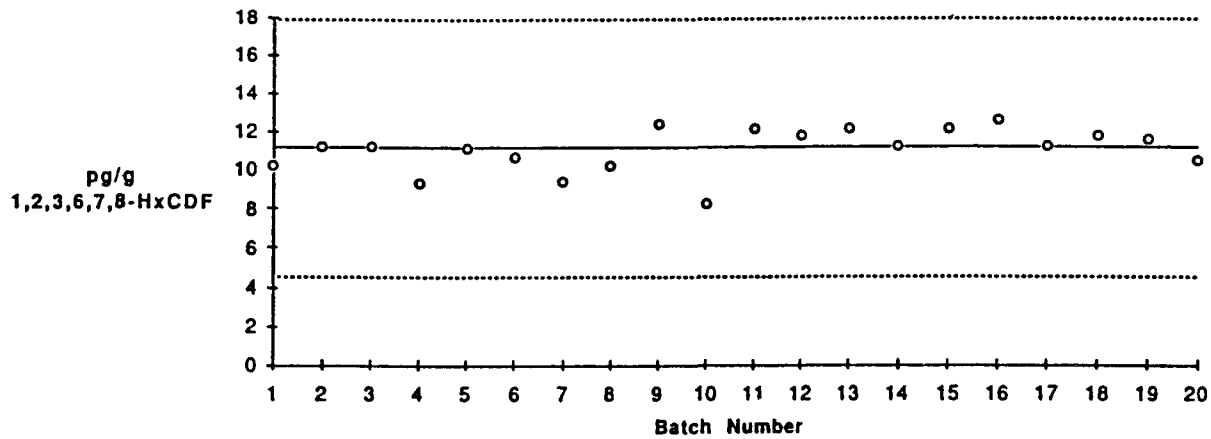


FIGURE B-12

1,2,3,4,7,8-HxCDF
Concentration in Unspiked Control QC Sample (pg/g)

Control Limits: 95% Confidence Interval for Individual Analyses from Method Evaluation Data

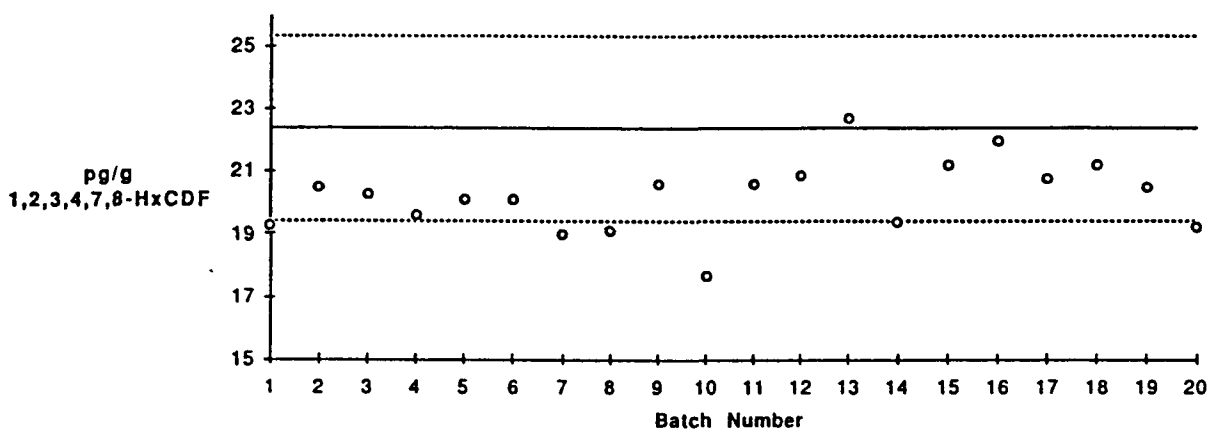


FIGURE B-13

2,3,4,6,7,8-HxCDF
Concentration in Unspiked Control QC Sample (pg/g)

Control Limits: 95% Confidence Interval for Individual Analyses from Method Evaluation Data

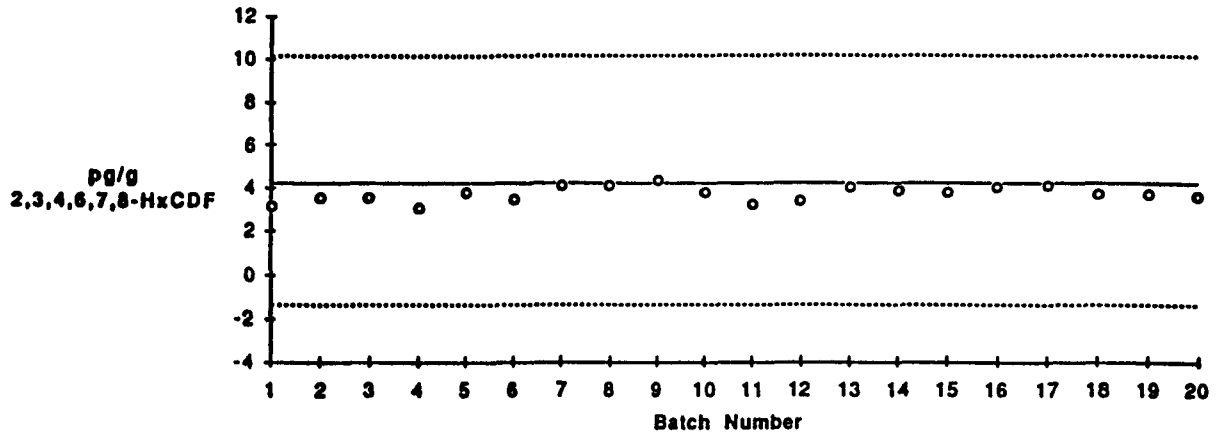


FIGURE B-14

1,2,3,7,8,9-HxCDF
Concentration in Unspiked Control QC Sample (pg/g)

Control Limits: 95% Confidence Interval for Individual Analyses from Method Evaluation Data

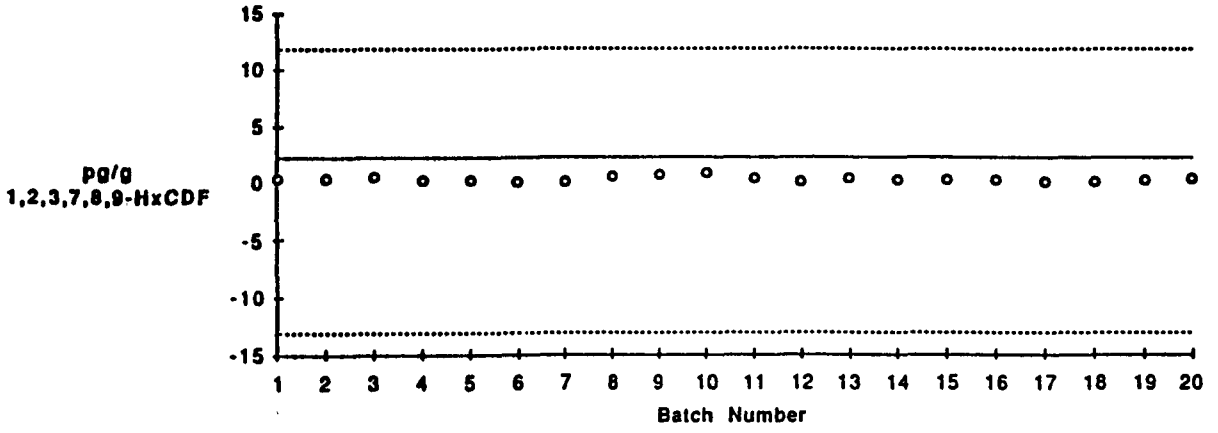


FIGURE B-15

1,2,3,7,8,9-HxCDD
Concentration in Unspiked Control QC Sample (pg/g)

Control Limits: 95% Confidence Interval for Individual Analyses from Method Evaluation Data

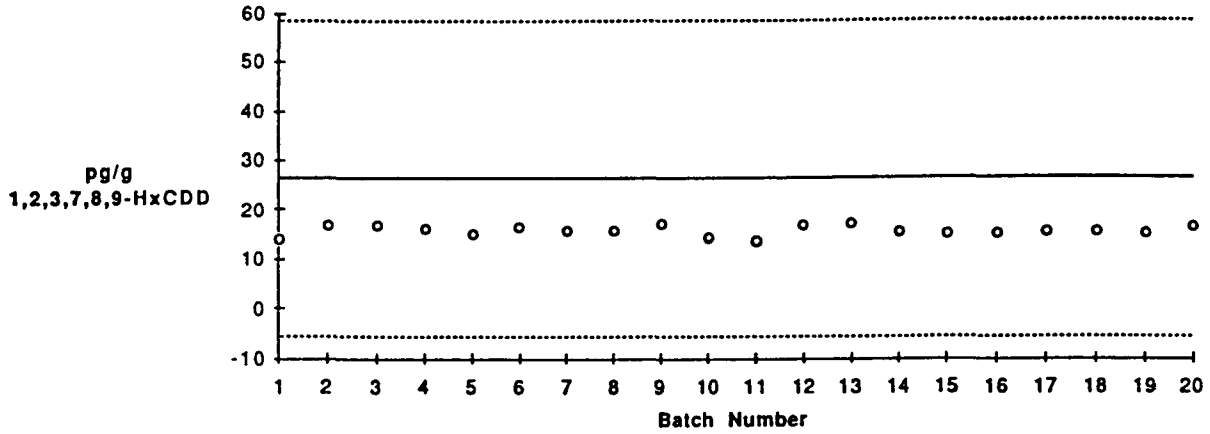


FIGURE B-16

1,2,3,4,7,8/1,2,3,6,7,8-HxCDD
Concentration in Unspiked Control QC Sample (pg/g)

Control Limits: 95% Confidence Interval for Individual Analyses from Method Evaluation Data

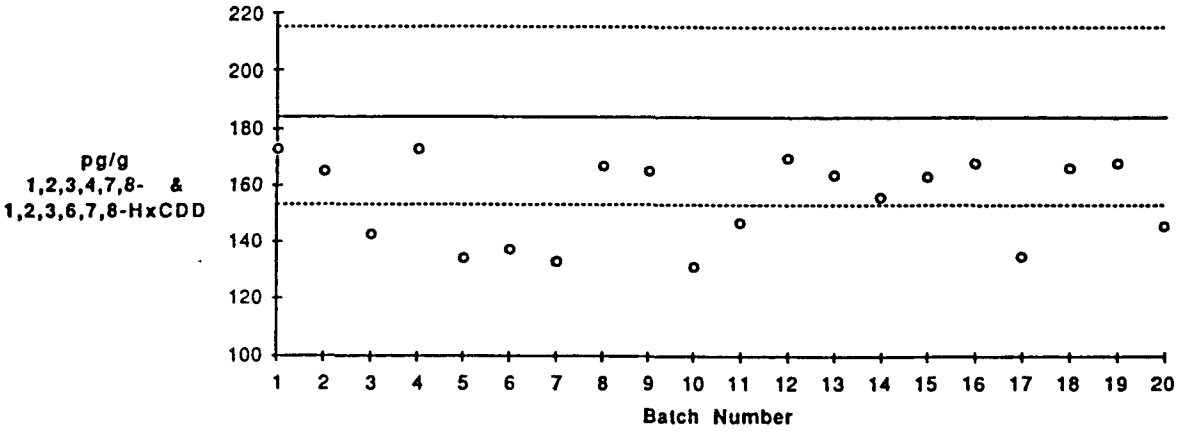


FIGURE B-17

1,2,3,4,7,8,9-HpCDF
Concentration in Unspiked Control QC Sample (pg/g)

Control Limits: 95% Confidence Interval for Individual Analyses from Method Evaluation Data

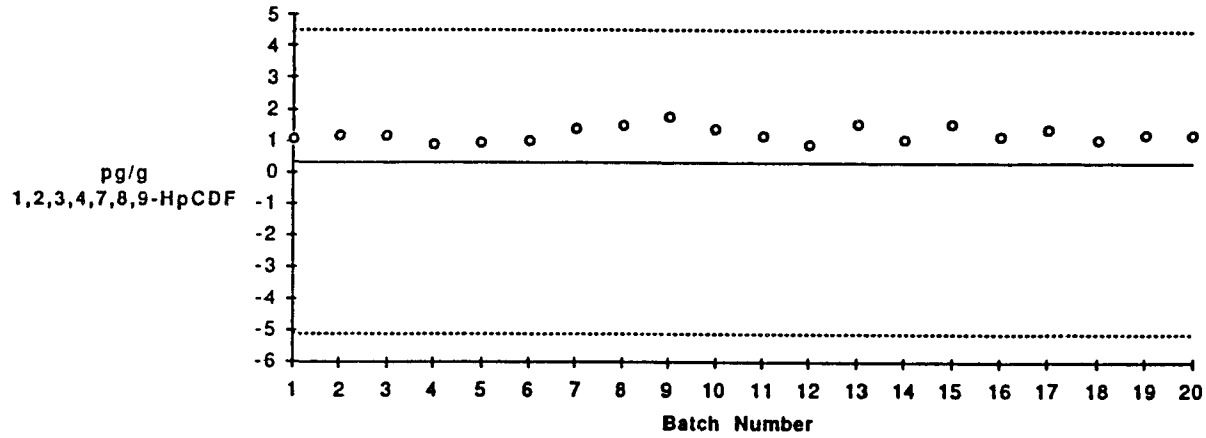


FIGURE B-18

1,2,3,4,6,7,8-HpCDF
Concentration in Unspiked Control QC Sample (pg/g)

Control Limits: 95% Confidence Interval for Individual Analyses from Method Evaluation Data

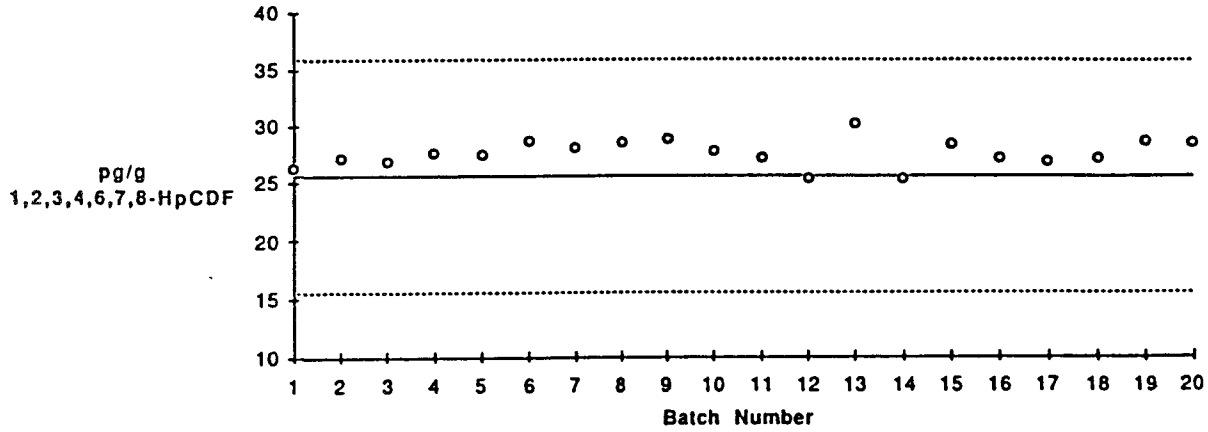


FIGURE B-19

1,2,3,4,6,7,8-HpCDD
Concentration in Unspiked Control QC Sample (pg/g)

Control Limits: 95% Confidence Interval for Individual Analyses from Method Evaluation Data

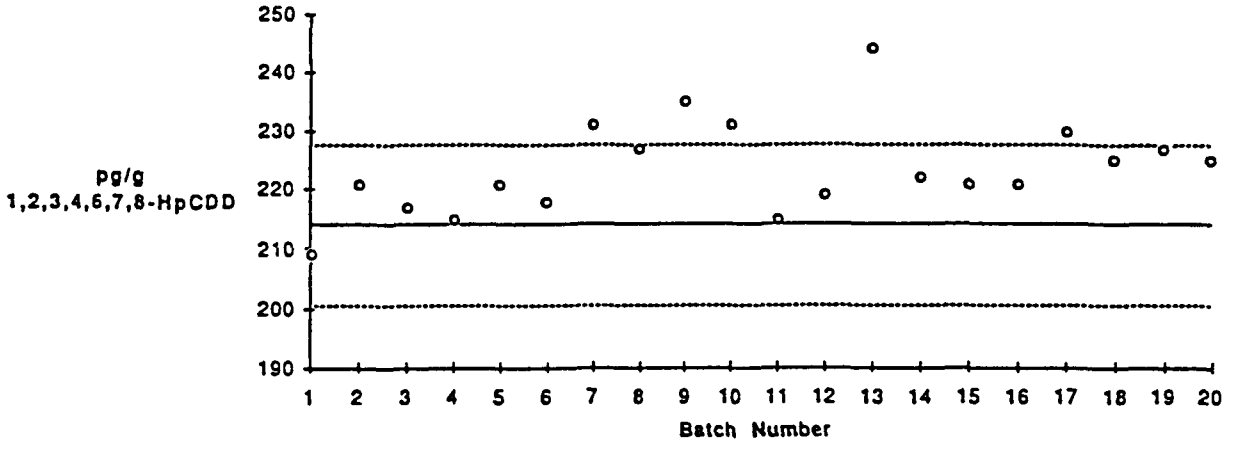


FIGURE B-20

OCDD

Concentration in Unspiked Control QC Sample (pg/g)

Control Limits: 95% Confidence Interval for Individual Analyses from Method Evaluation Data

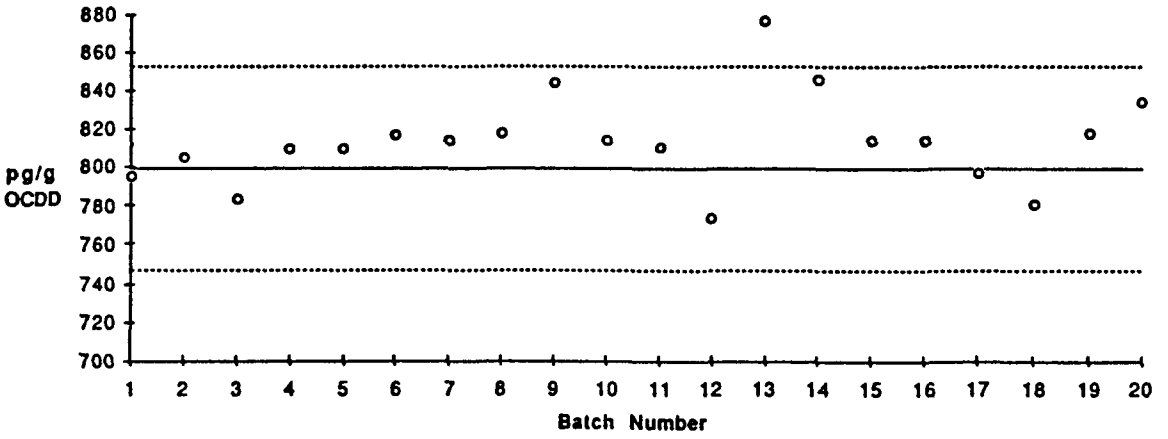


FIGURE B-21

OCDF

Concentration in Unspiked Control QC Sample (pg/g)

Control Limits: 95% Confidence Interval for Individual Analyses from Method Evaluation Data

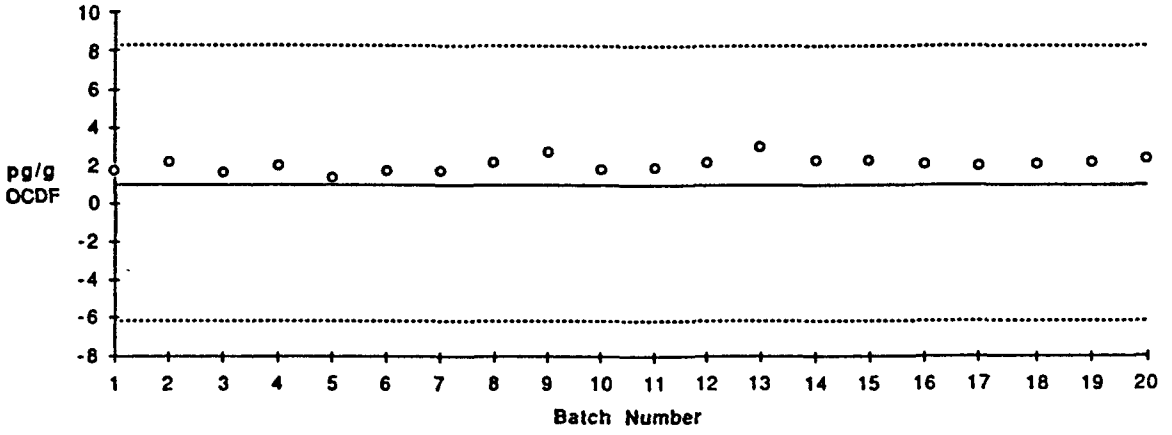


TABLE B-10
Mean Measurements in the Unspiked Control Lipid Samples

Congener	Mean measured in this study (pg/g)	Mean estimated from the Method Evaluation Study (pg/g)
2,3,7,8-TCDF	1.94	ND[1] (3.6)
2,3,7,8-TCDD	10.06	11.8
1,2,3,7,8-PeCDF	0.81	ND[1] (1.1)
2,3,4,7,8-PeCDF	27.65	22.2
1,2,3,7,8-PeCDD	18.46	19.8
1,2,3,4,7,8-HxCDF	20.24	22.4
1,2,3,6,7,8-HxCDF	11.11	11.2
2,3,4,6,7,8-HxCDF	3.76	4.3
1,2,3,7,8,9-HxCDF	0.39	ND[1] (2.3)
1,2,3,4,7,8/ 1,2,3,6,7,8-HxCDD	155.20	184.3
1,2,3,7,8,9-HxCDD	15.70	26.5
1,2,3,4,6,7,8-HpCDF	27.69	25.7
1,2,3,4,7,8,9-HpCDF	1.25	ND[1] (0.3)
1,2,3,4,6,7,8-HpCDD	223.70	214
OCDF	2.11	ND[1] (1.0)
OCDD	813.85	800

[1] Not Detected. The number in parenthesis is the estimated level of detection.

Calculations included all values. Not detected values were set equal to the level of detection and trace values were used as the level reported.

FIGURE B-22

CONTROL CHART 2,3,7,8-TCDD

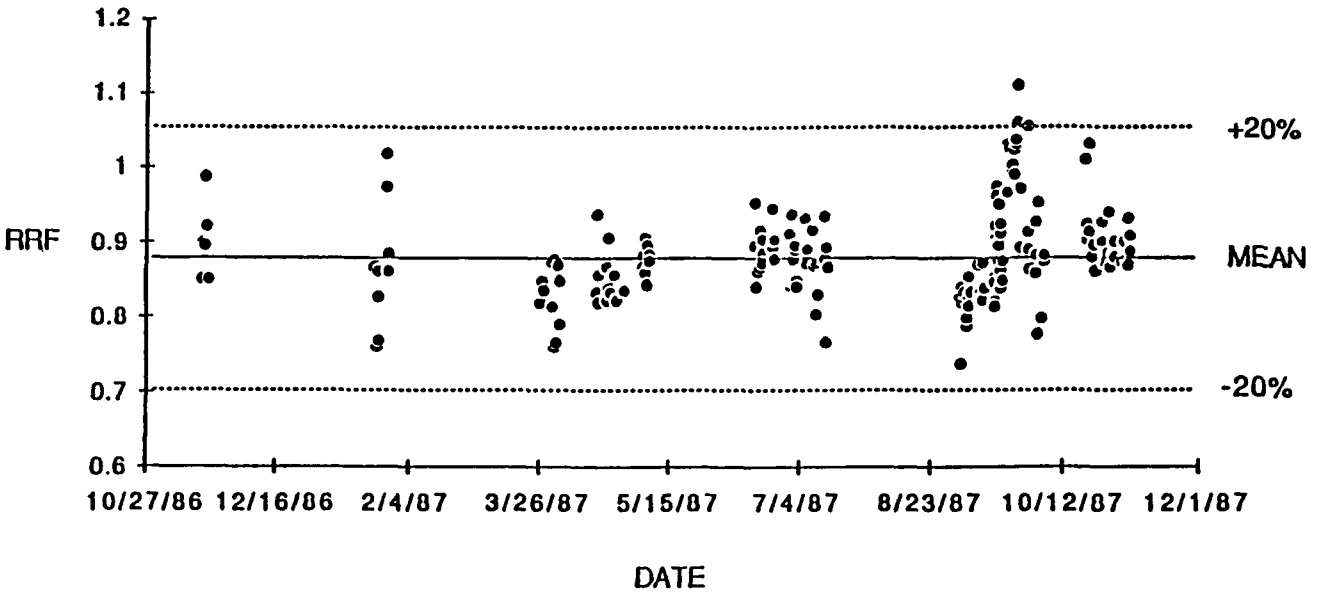


FIGURE B-23

CONTROL CHART 2,3,7,8-TCDF

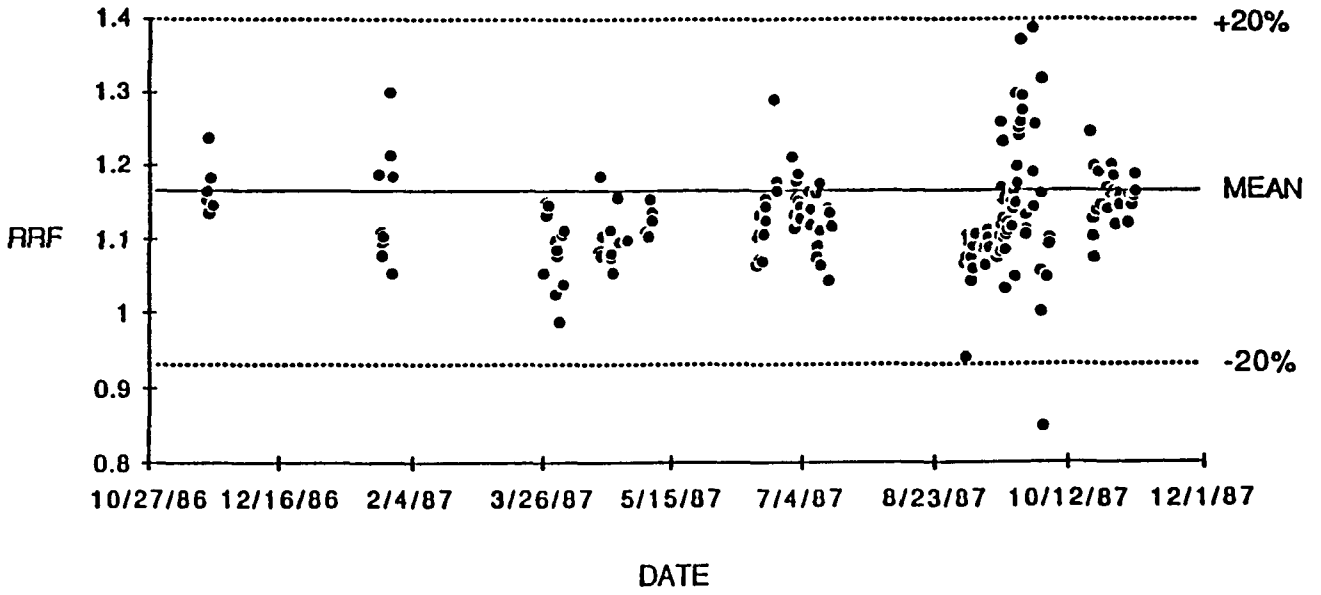


FIGURE B-24

CONTROL CHART 1,2,3,7,8-PeCDD

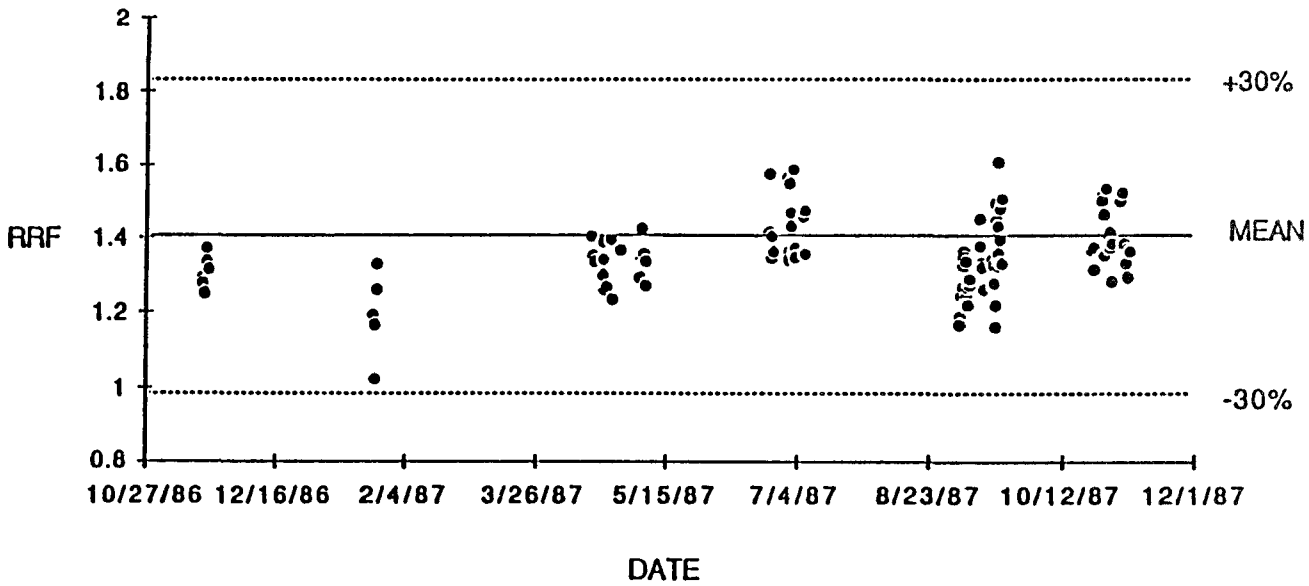


FIGURE B-25

CONTROL CHART 1,2,3,7,8-PeCDF

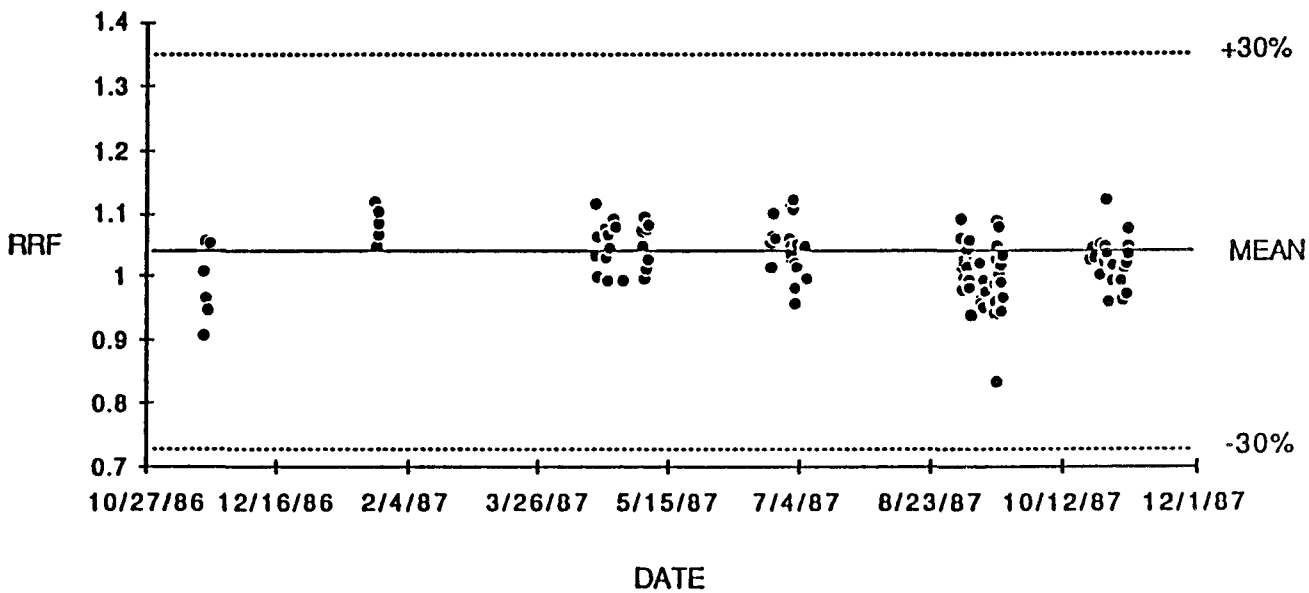


FIGURE B-26

CONTROL CHART 2,3,4,7,8-PeCDF

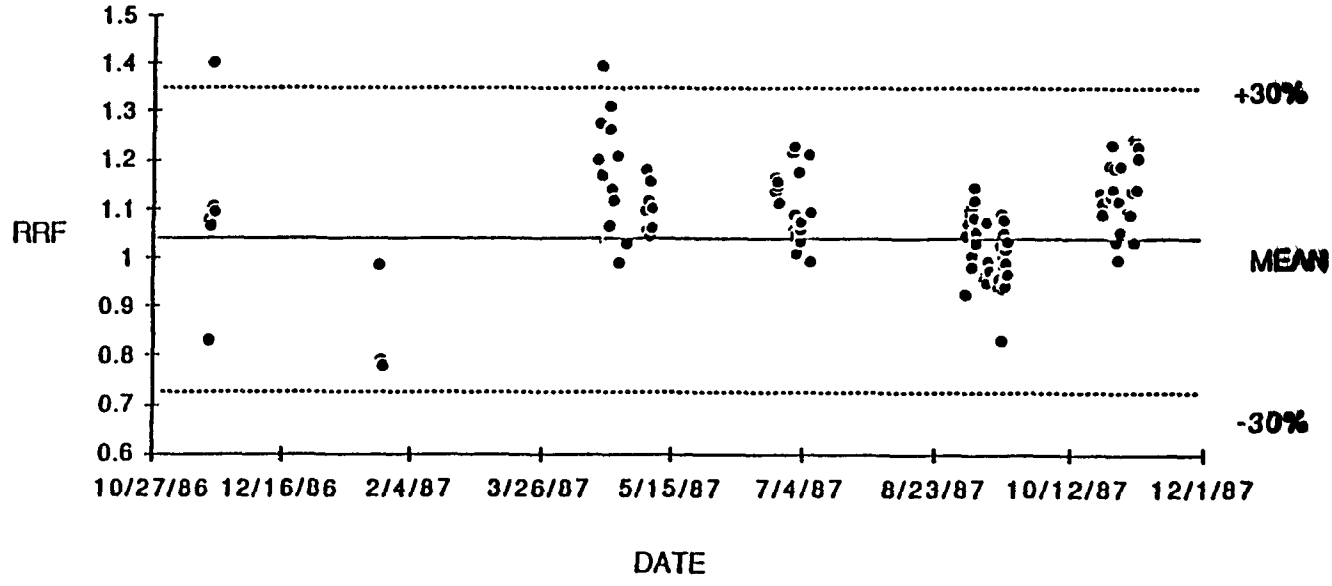


FIGURE B-27

CONTROL CHART 1,2,3,4,7,8-HxCDD

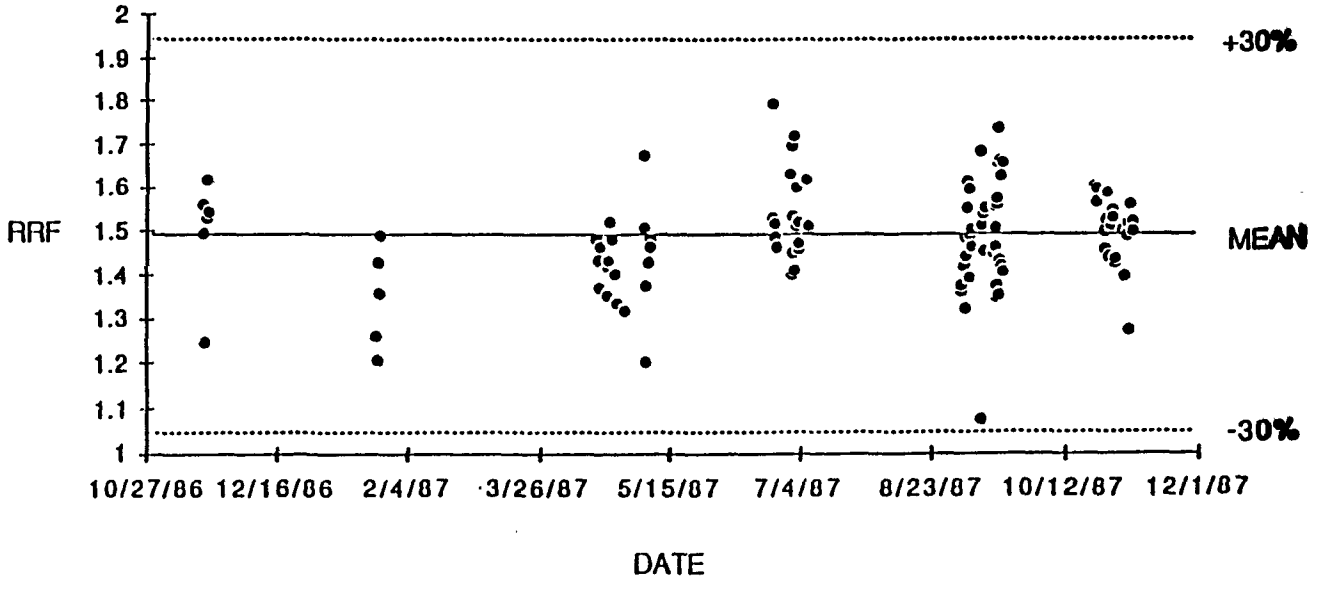


FIGURE B-28

CONTROL CHART 1,2,3,7,8,9-HxCDD

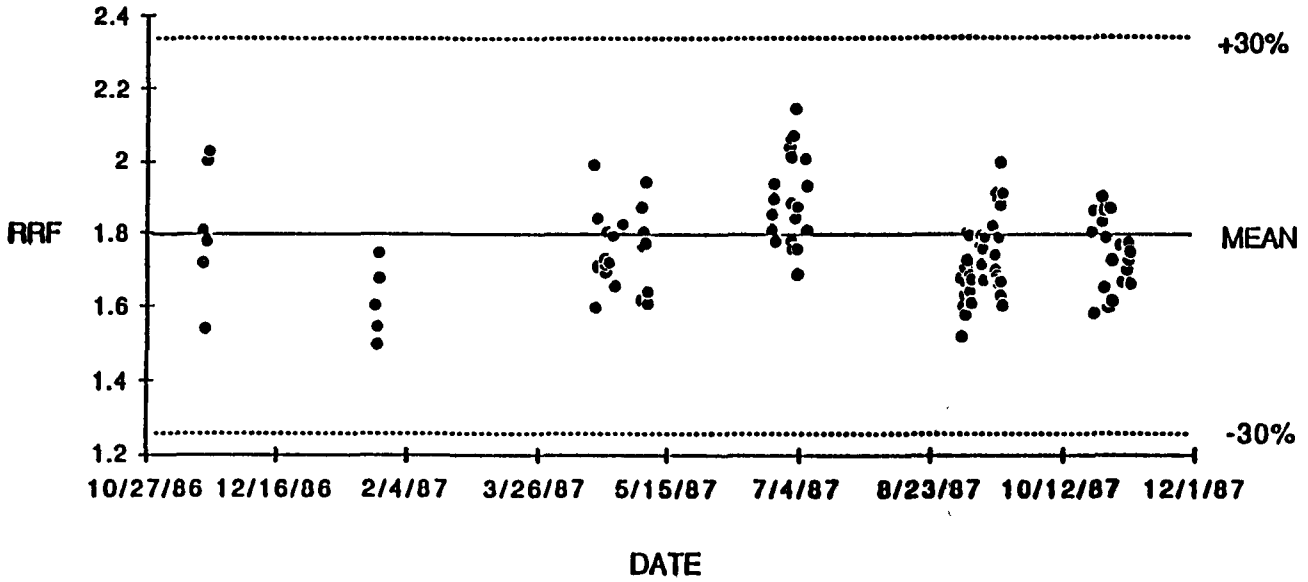


FIGURE B-29

CONTROL CHART 1,2,3,6,7,8-HxCDD

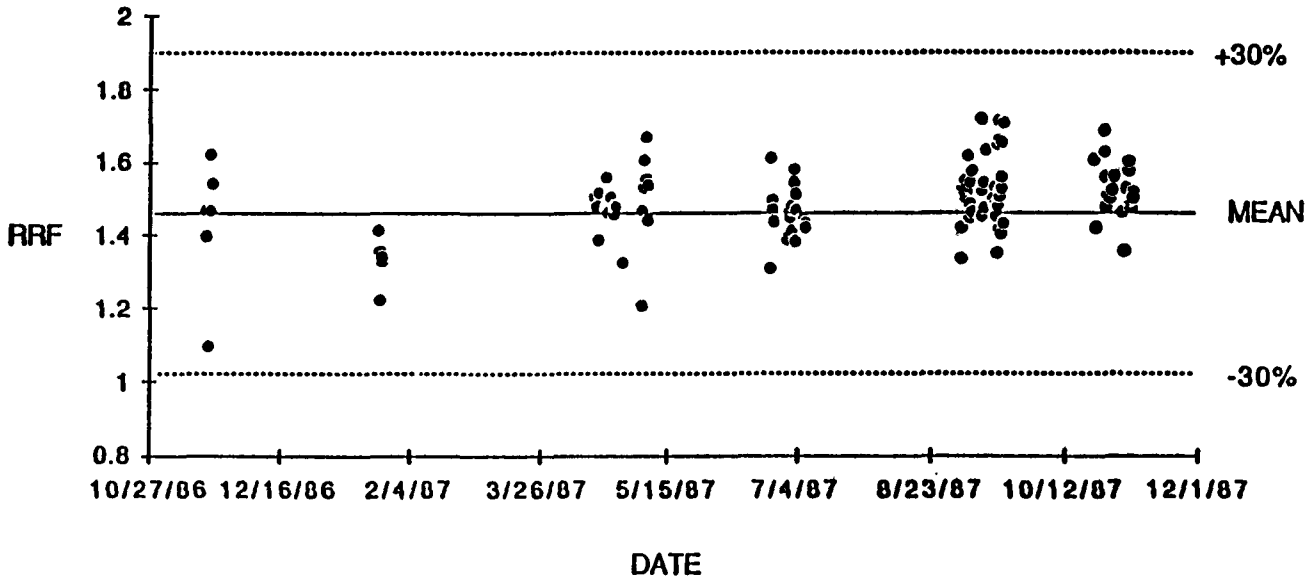


FIGURE B-30

CONTROL CHART 1,2,3,4,7,8-HxCDF

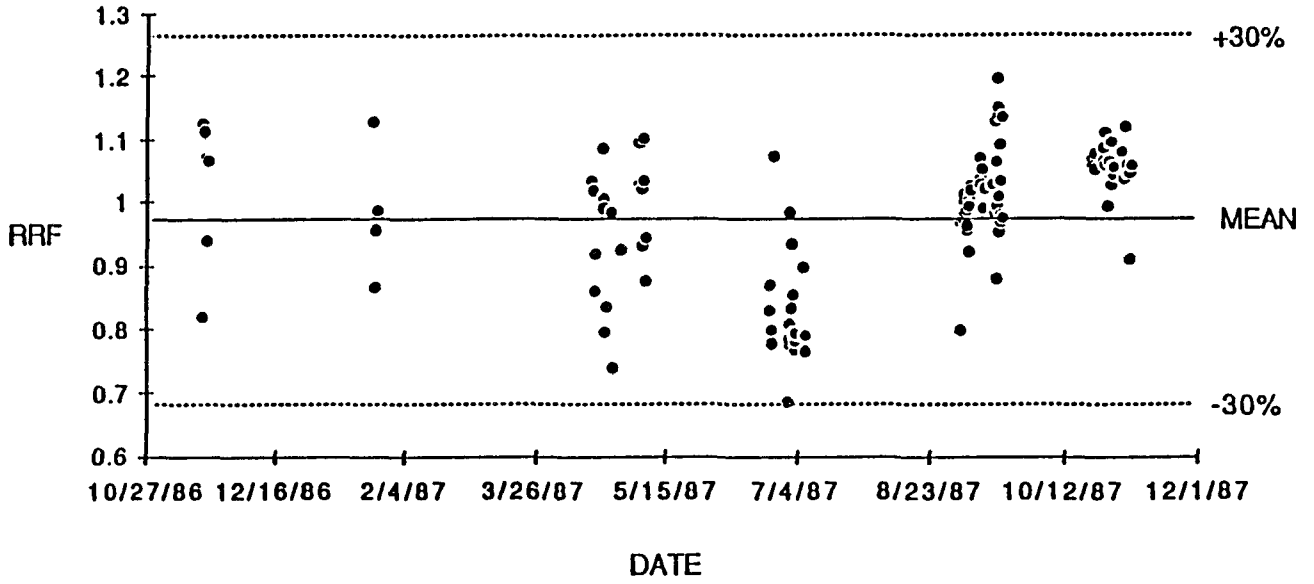


FIGURE B-31

CONTROL CHART 1,2,3,6,7,8-HxCDF

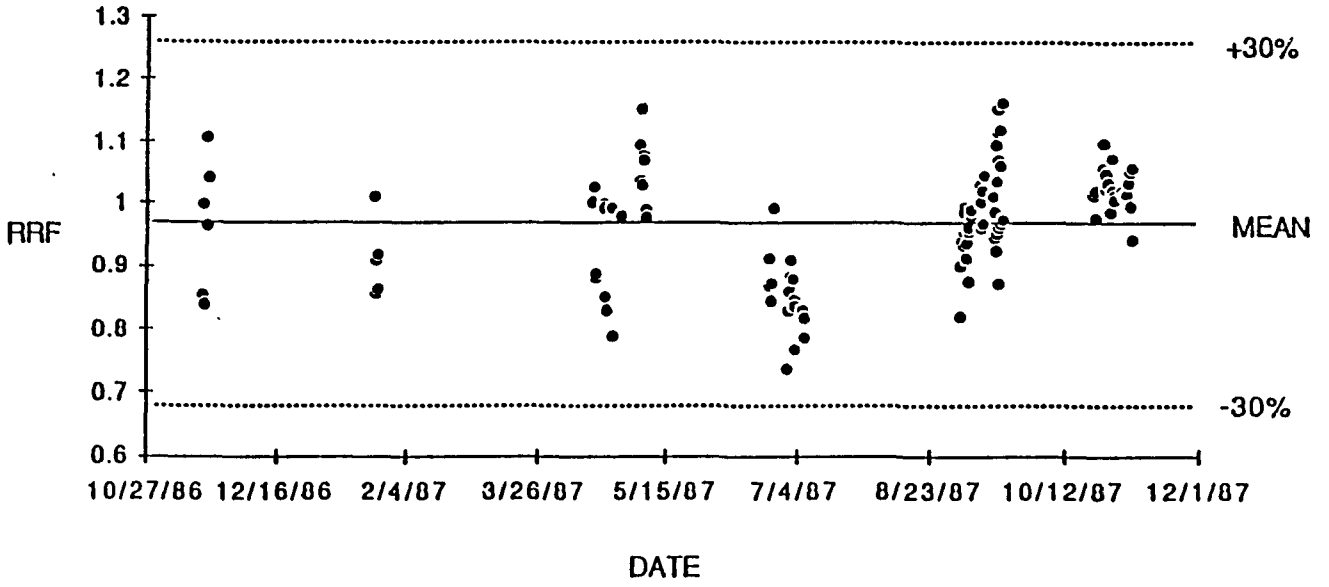


FIGURE B-32

CONTROL CHART 2,3,4,6,7,8-HxCDF

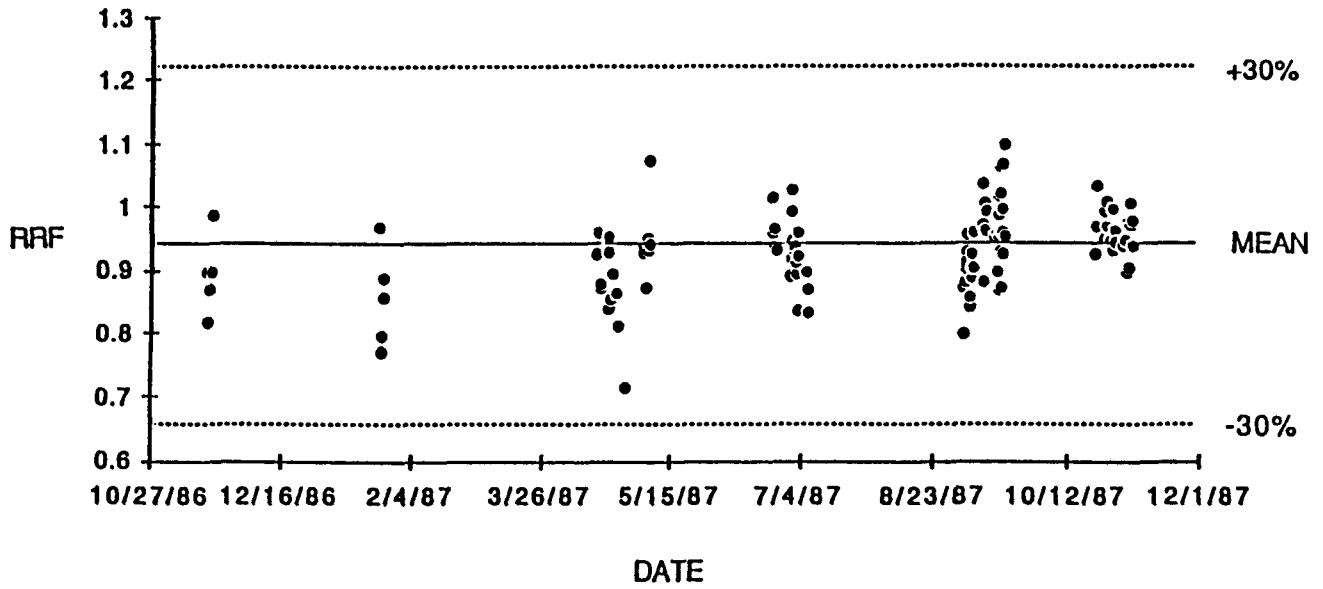


FIGURE B-33

CONTROL CHART 1,2,3,7,8,9-HxCDF

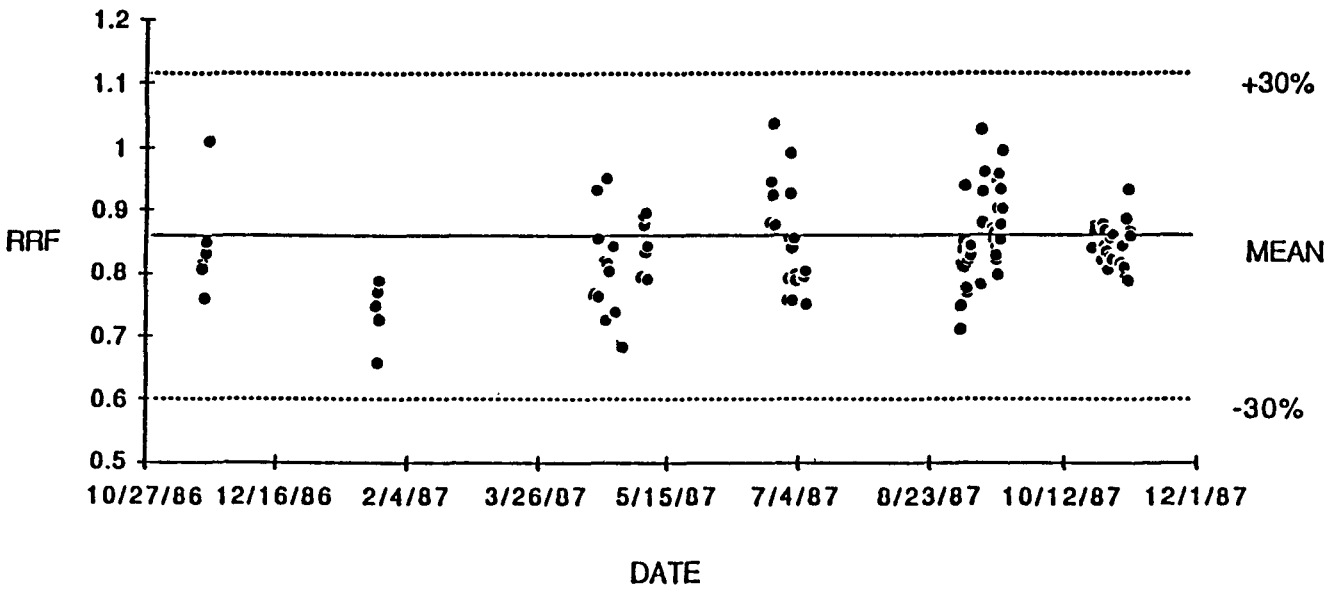


FIGURE B-34

CONTROL CHART 1,2,3,4,6,7,8-HpCDD

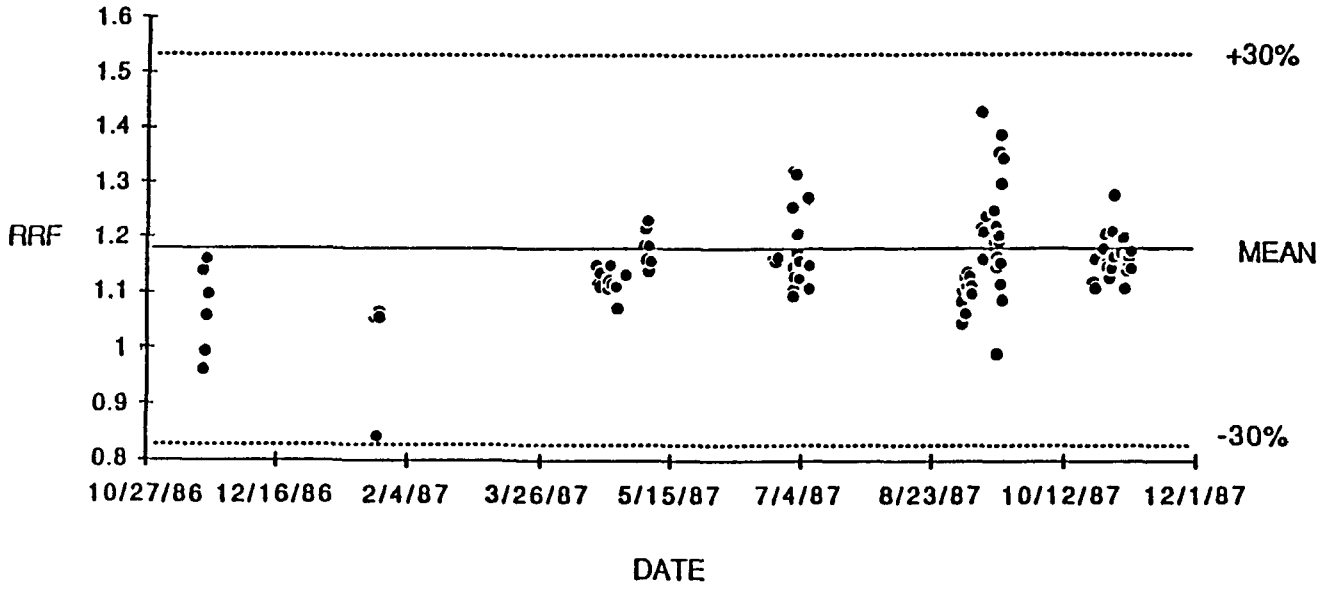


FIGURE B-35

CONTROL CHART 1,2,3,4,6,7,8-HpCDF

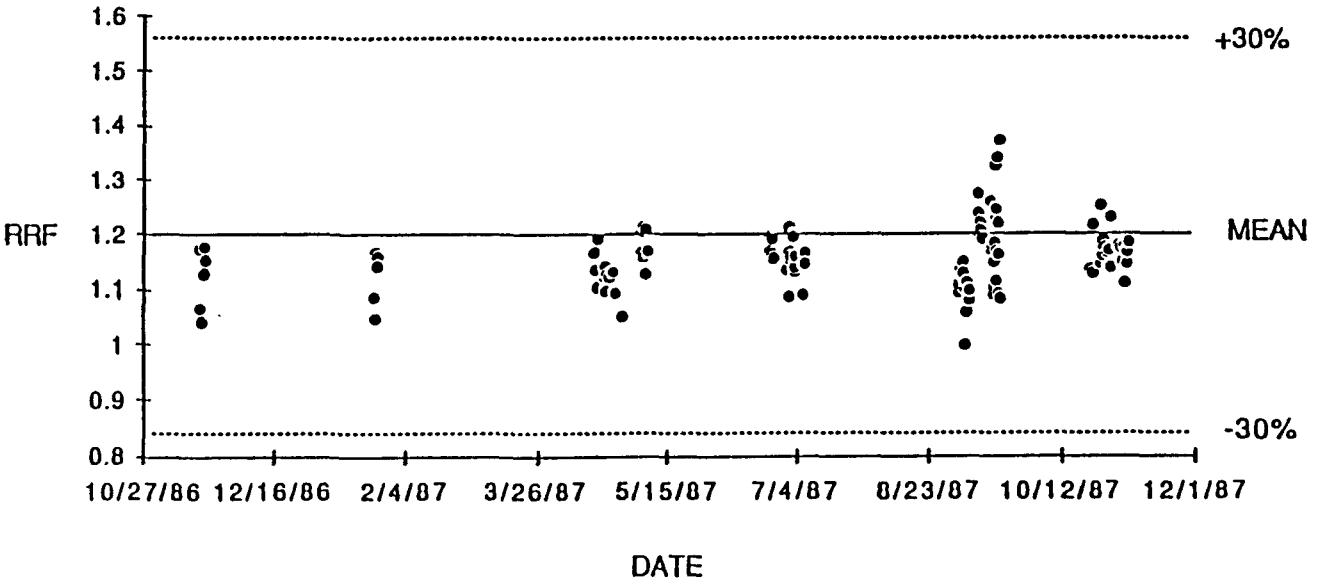


FIGURE B-36

CONTROL CHART 1,2,3,4,7,8,9-HpCDF

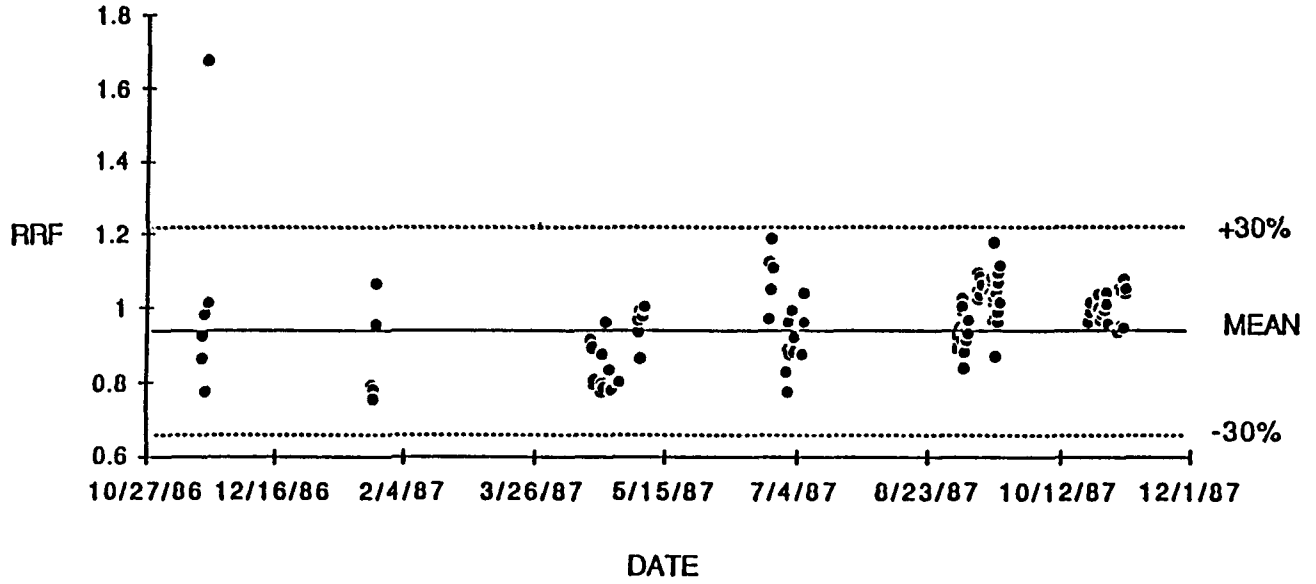


FIGURE B-37

CONTROL CHART OCDF

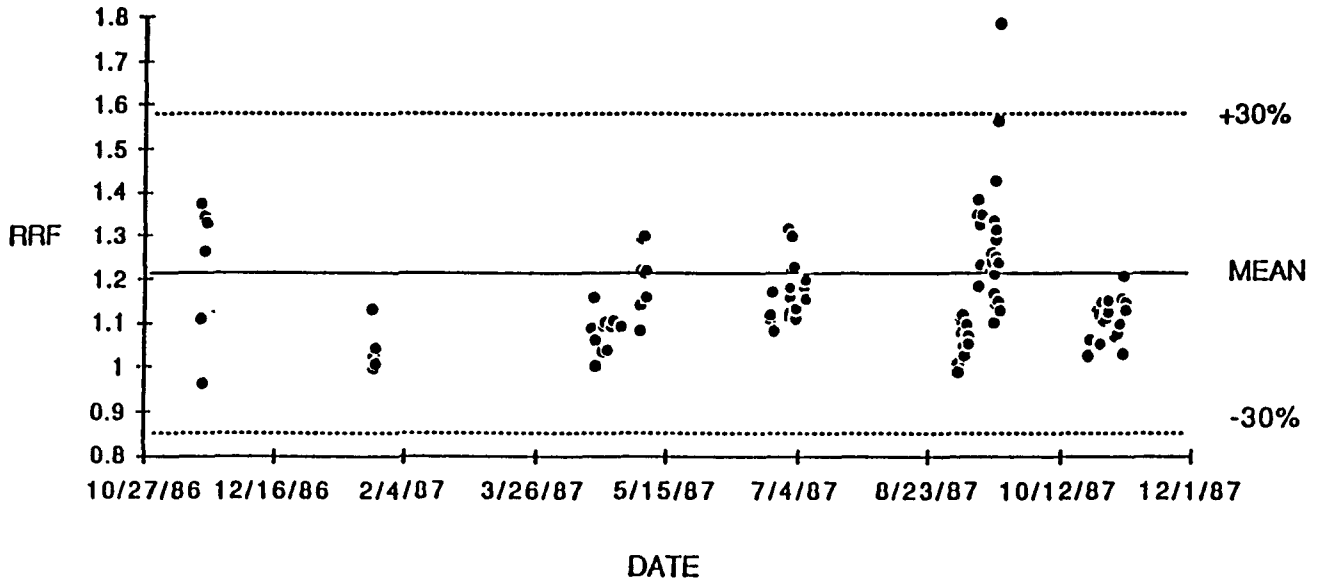


FIGURE B-38

CONTROL CHART OCDD

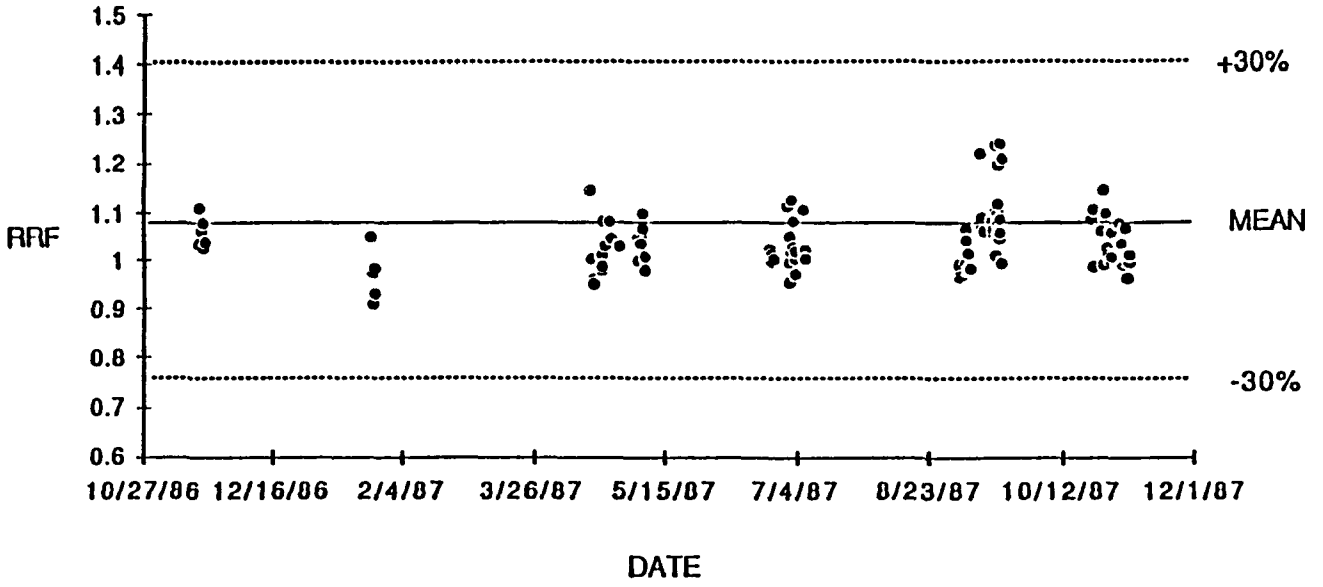


FIGURE B-39

CONTROL CHART 13C12-2,3,7,8-TCDD

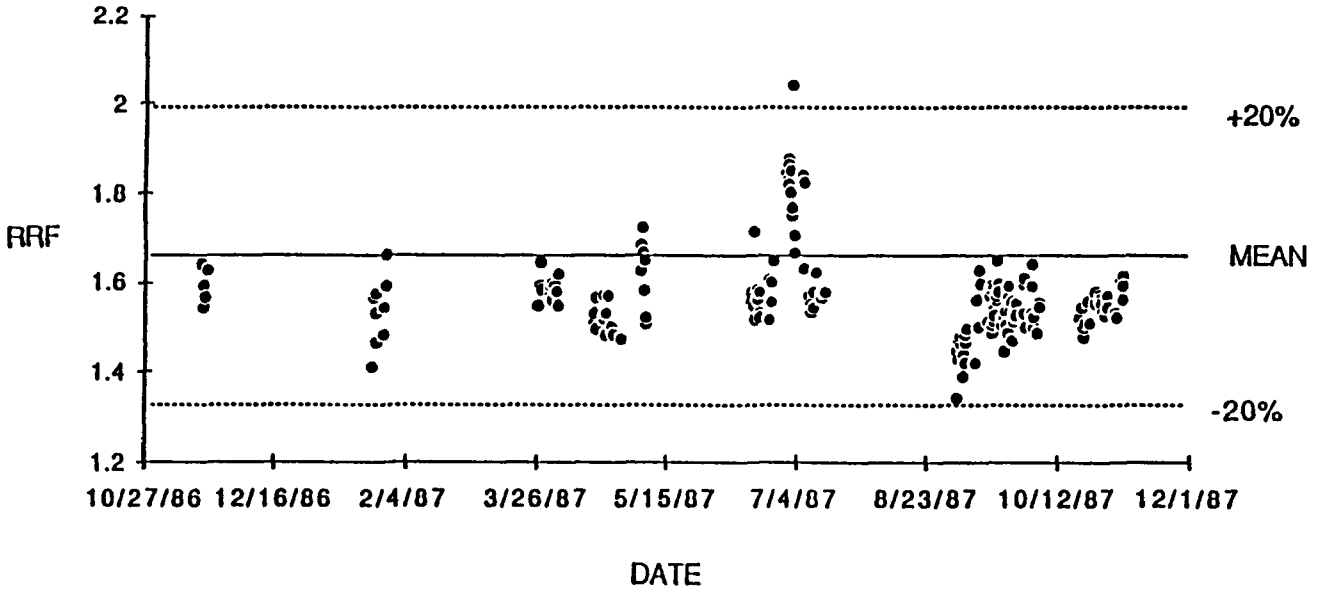


FIGURE B-40

CONTROL CHART 13C12-2,3,7,8-TCDF

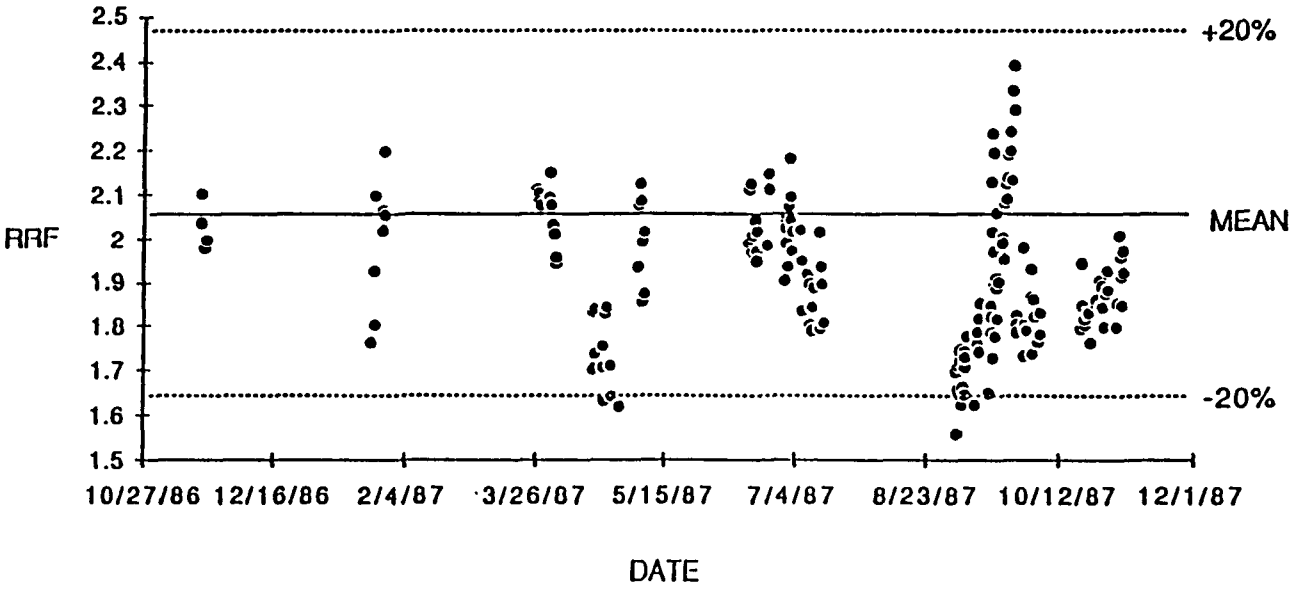


FIGURE B-41

CONTROL CHART 13C12-1,2,3,7,8-PeCDD

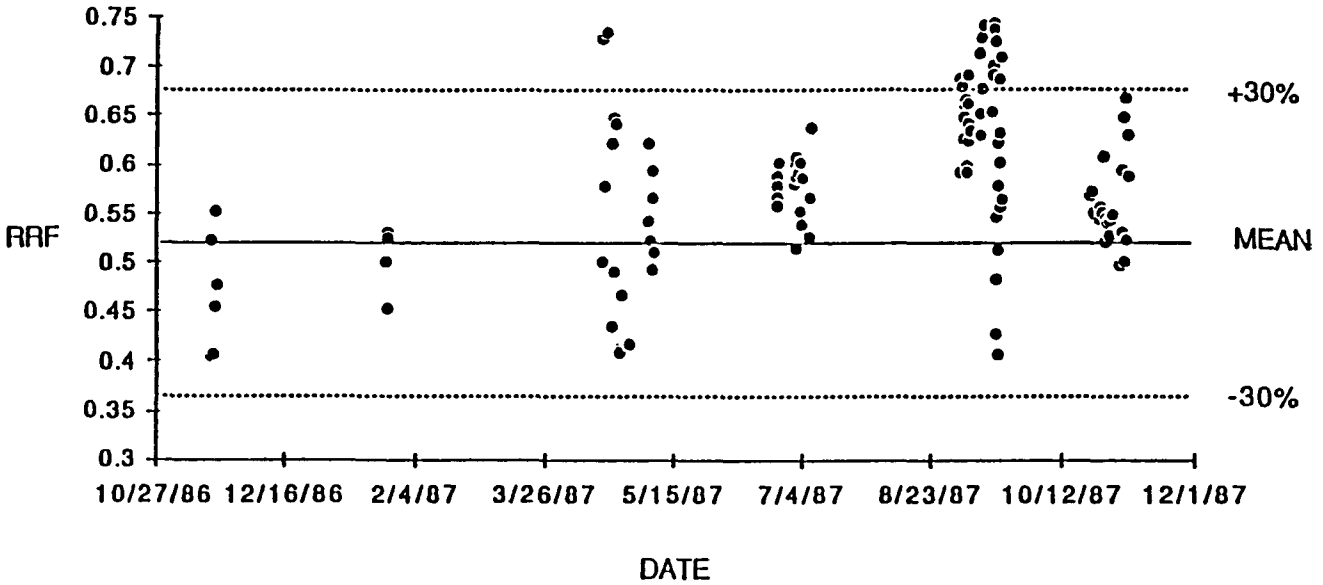


FIGURE B-42

CONTROL CHART 13C12-1,2,3,7,8-PeCDF

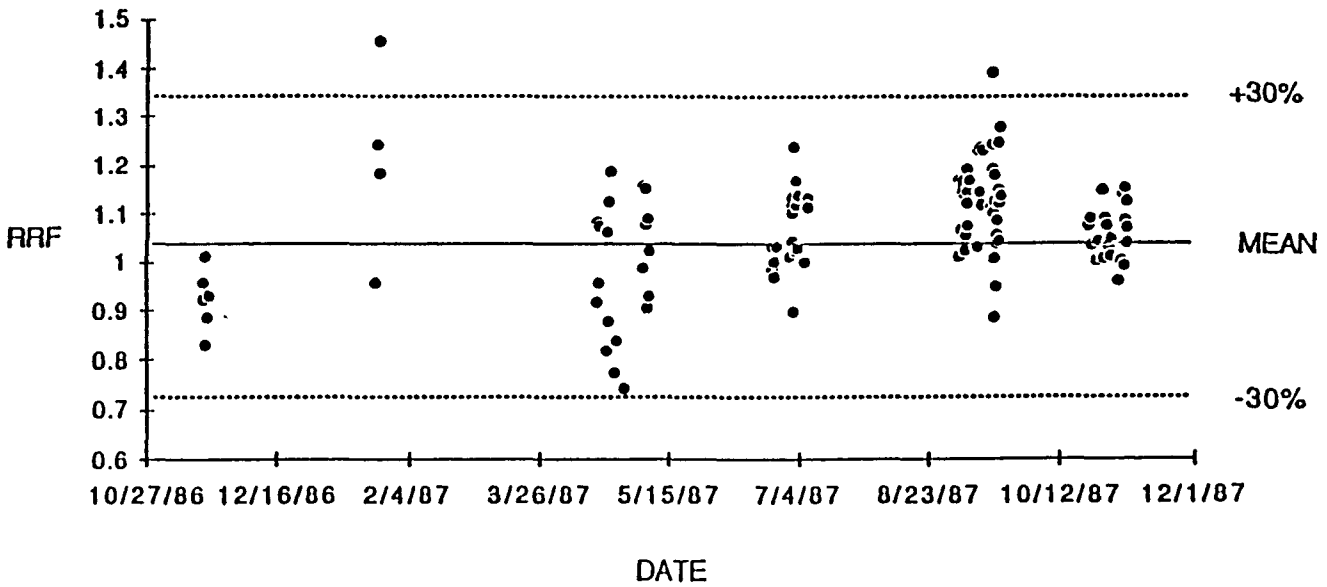


FIGURE B-43

CONTROL CHART 13C12-1,2,3,6,7,8-HxCDD

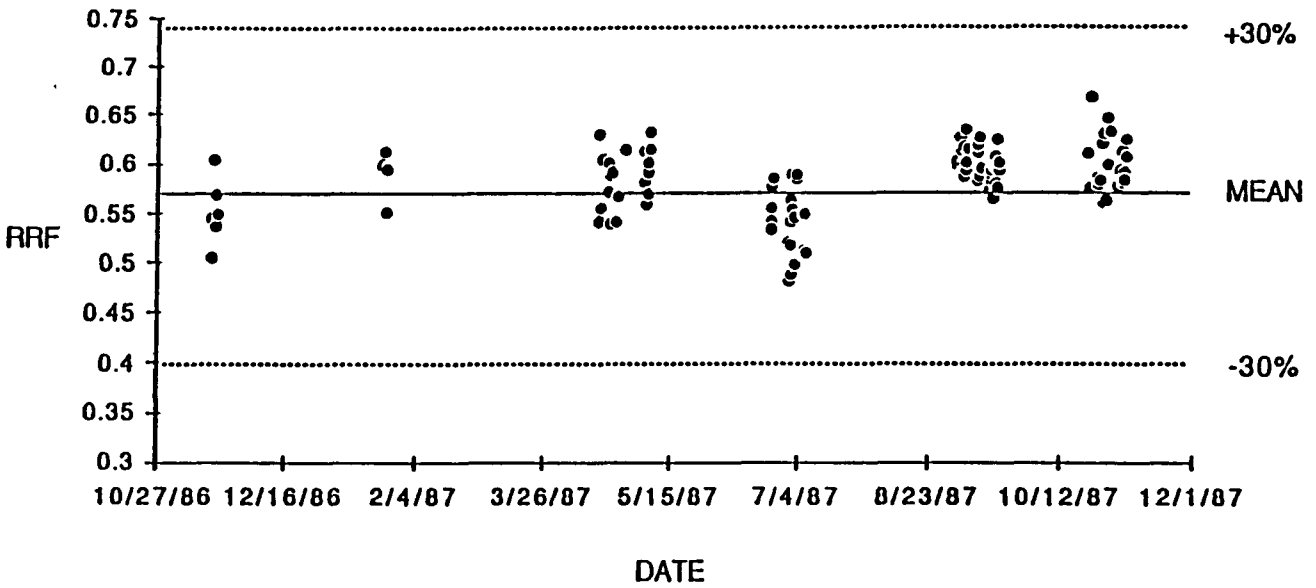


FIGURE B-44

CONTROL CHART 13C12-1,2,3,4,7,8-HxCDF

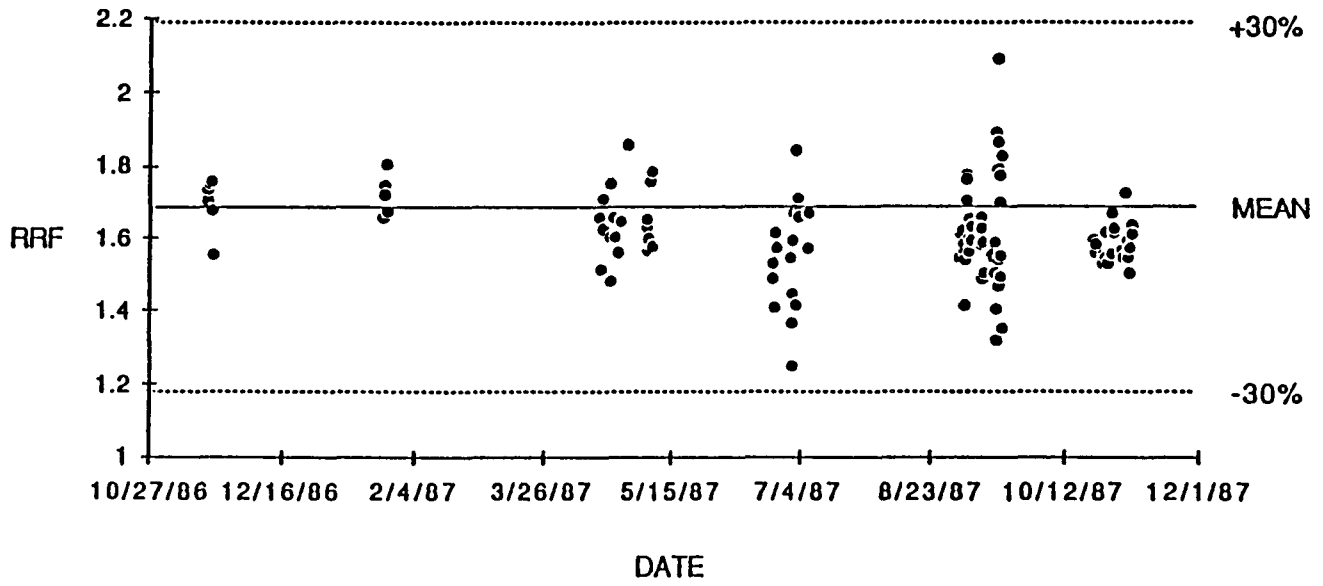


FIGURE B-45

CONTROL CHART 13C12-1,2,3,4,6,7,8-HpCDD

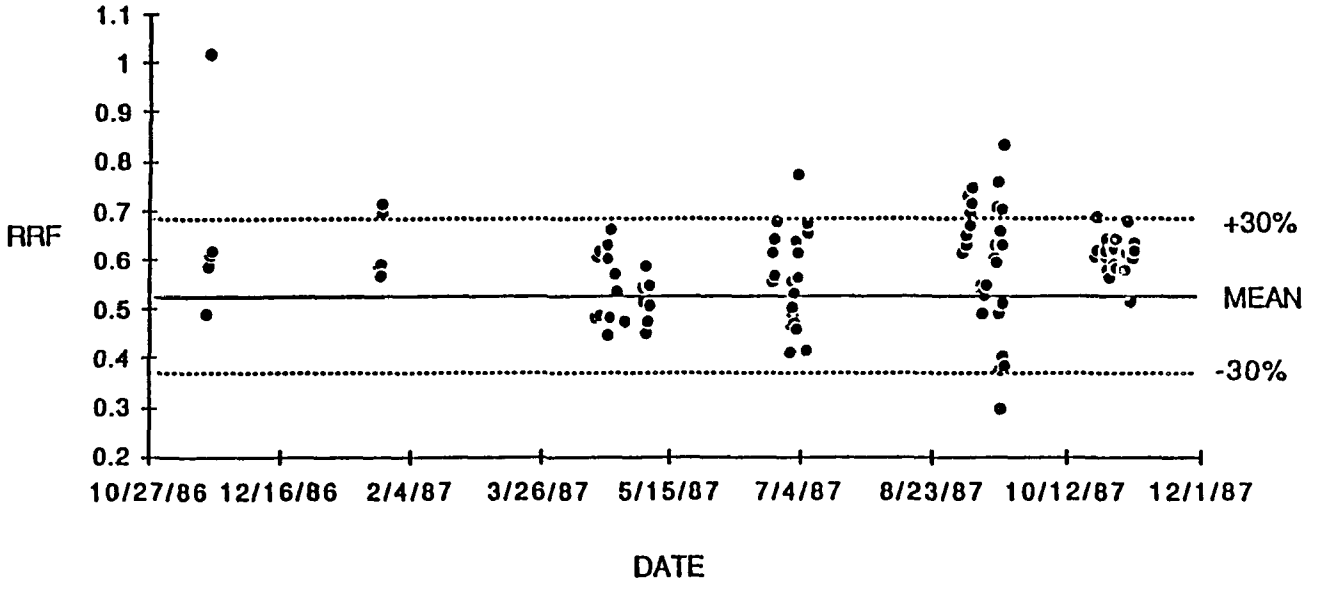


FIGURE B-46

CONTROL CHART 13C12-1,2,3,4,6,7,8-HpCDF

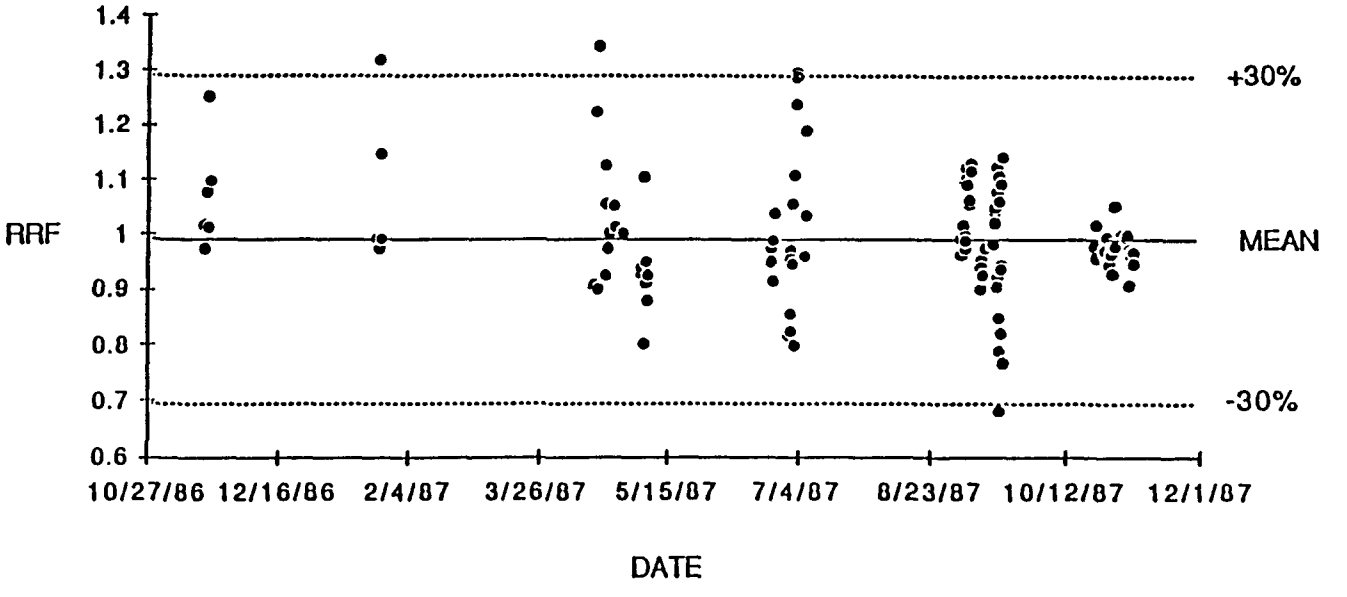


FIGURE B-47

CONTROL CHART 13C12-OCDD

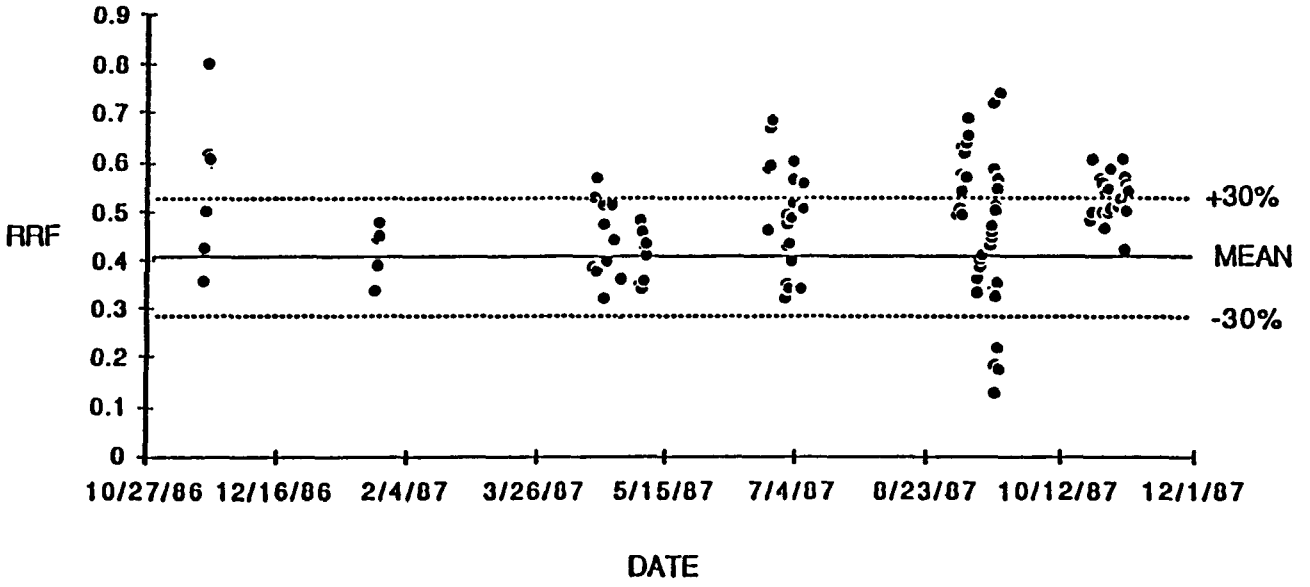


FIGURE B-48

13C12-TCDF Recoveries for Batches 1-20

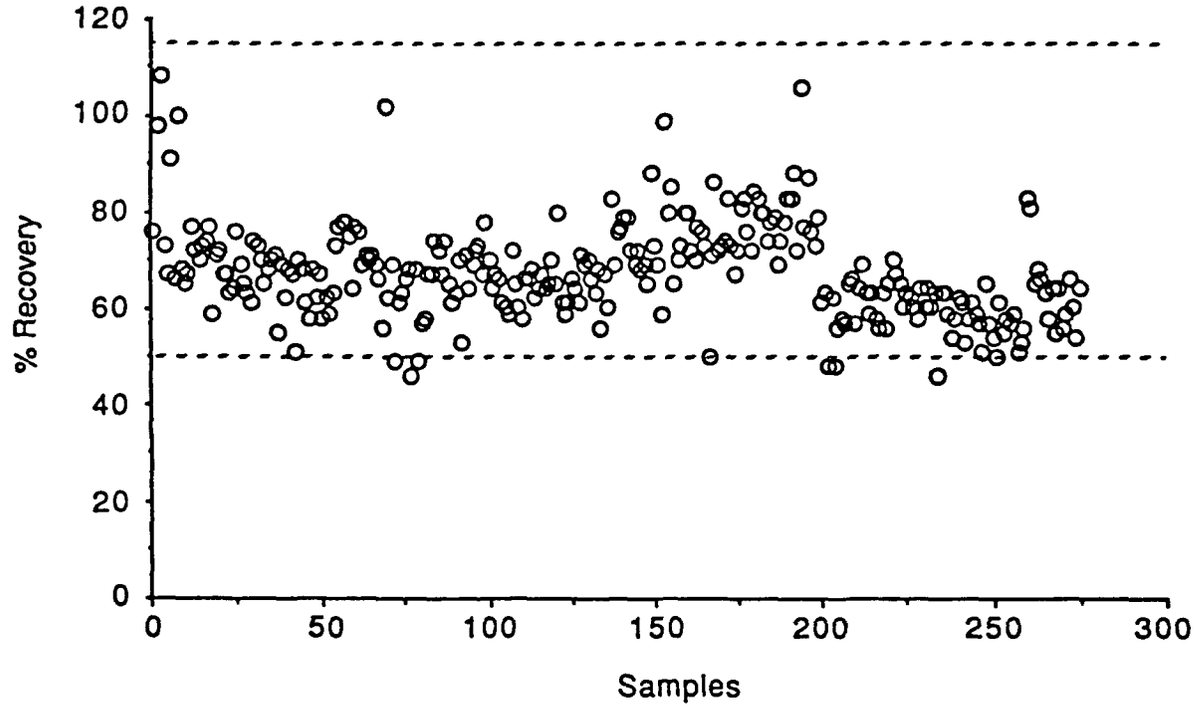


FIGURE B-49

13C12-TCDD Recoveries for Batches 1-20

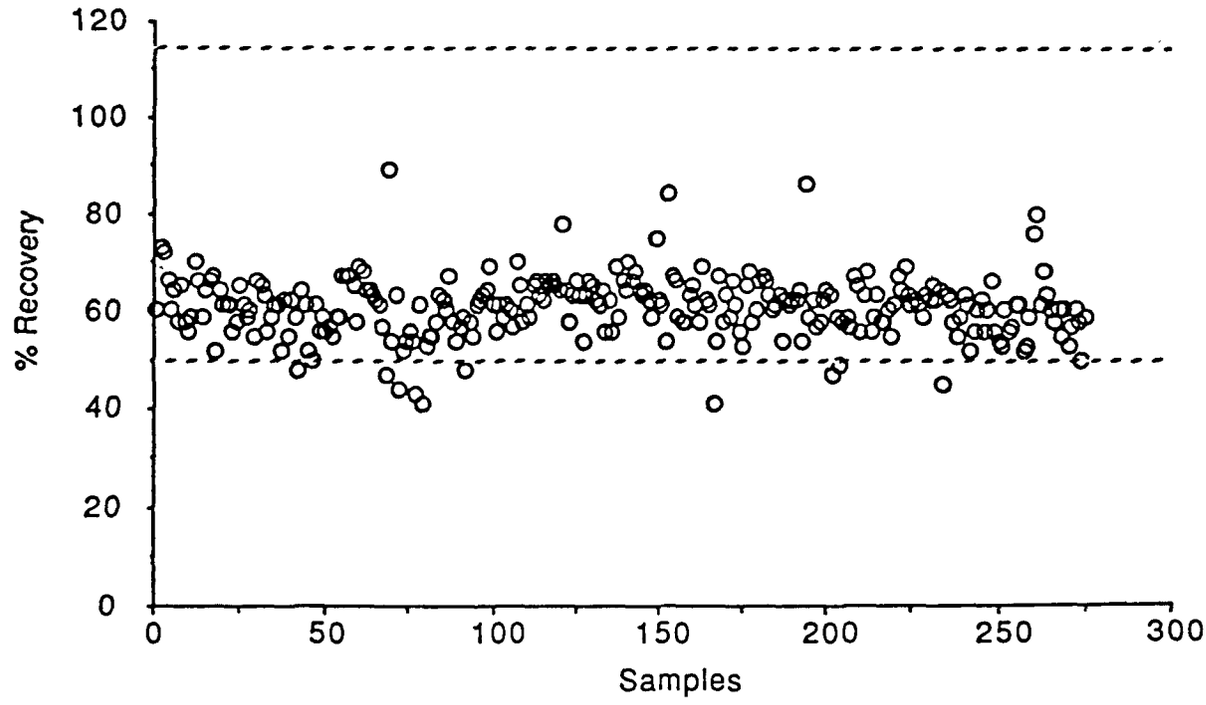


FIGURE B-50

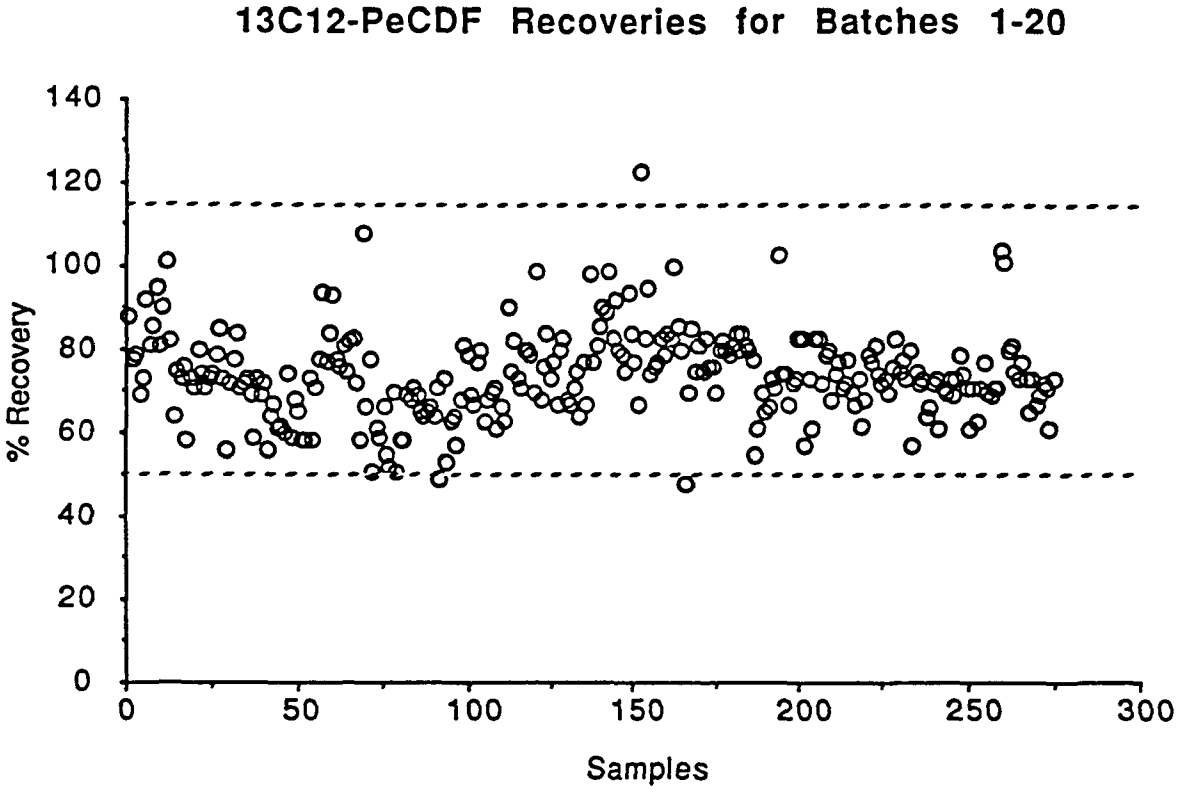


FIGURE B-51

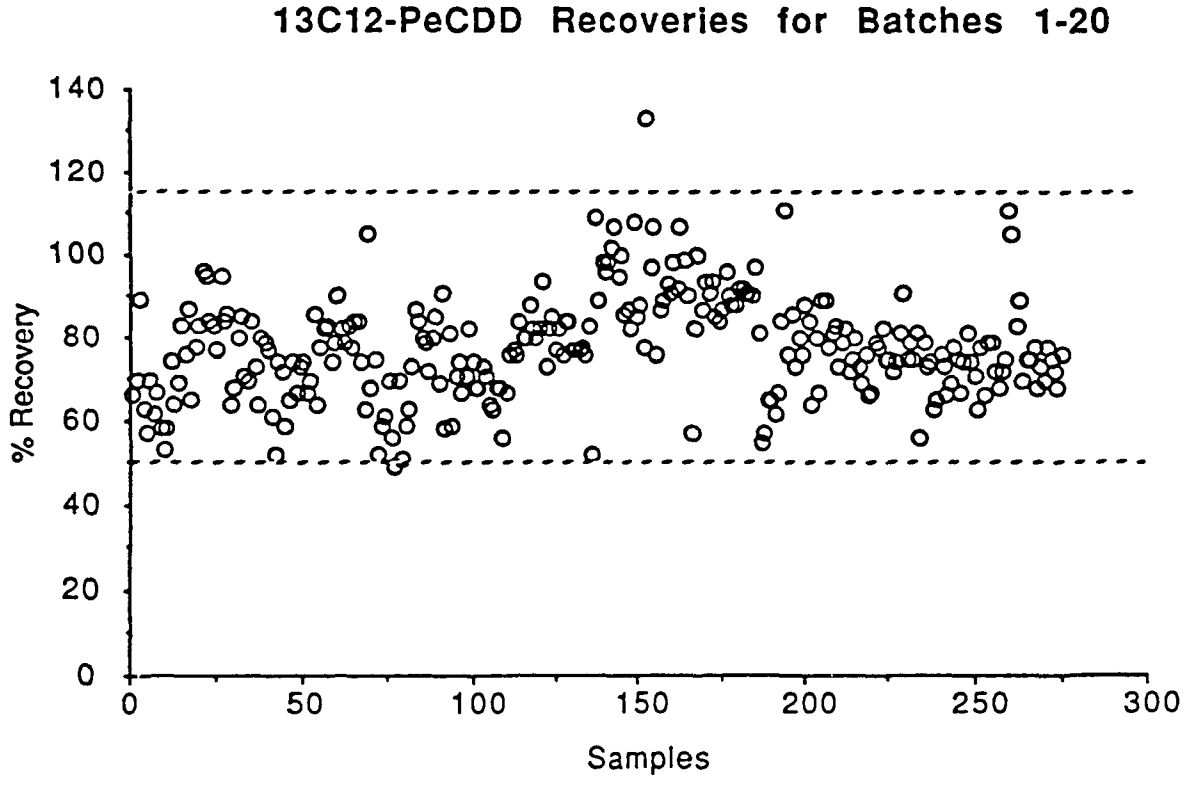


FIGURE B-52

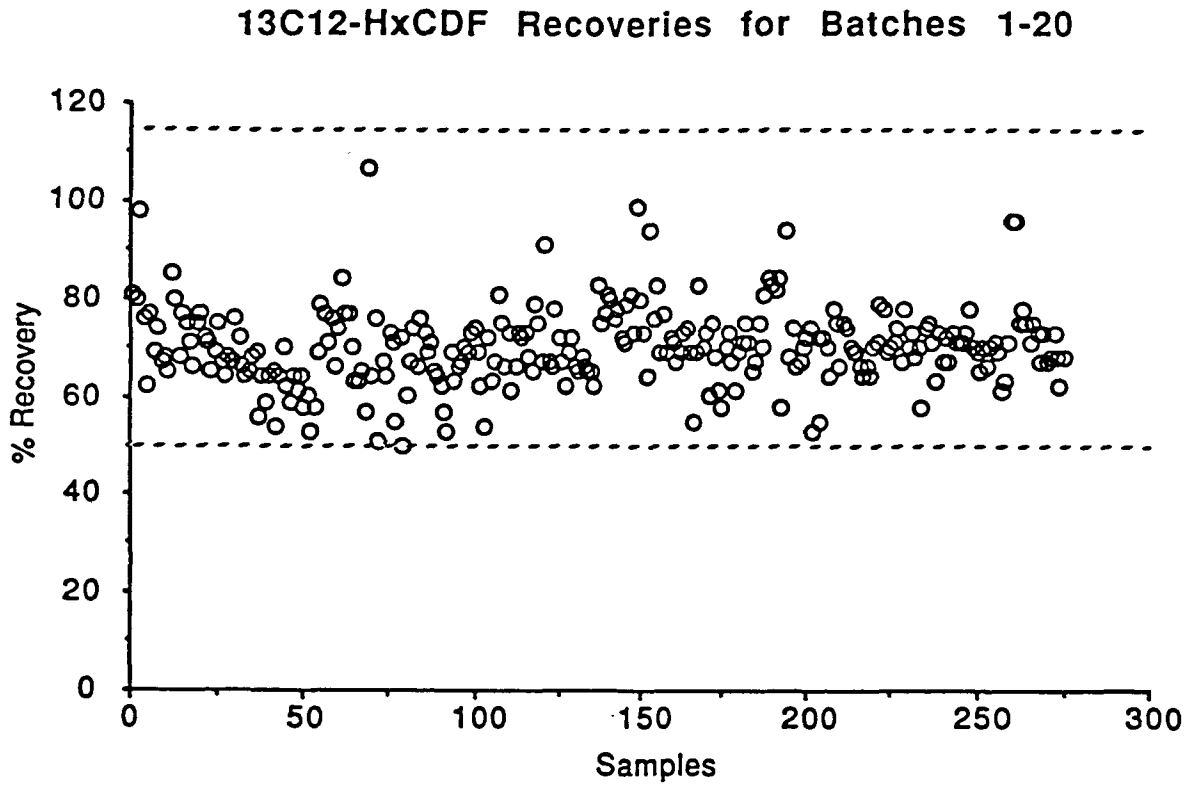


FIGURE B-53

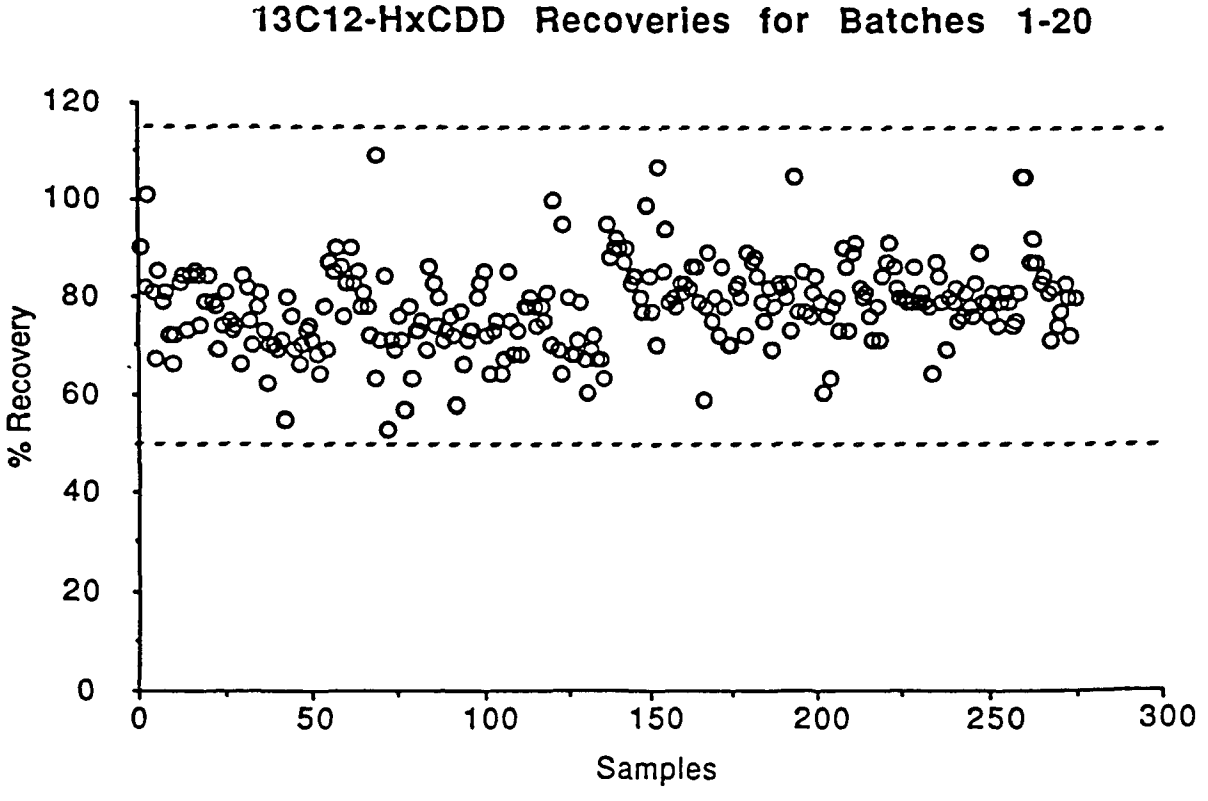


FIGURE B-54

13C12-HpCDF Recoveries for Batches 1-20

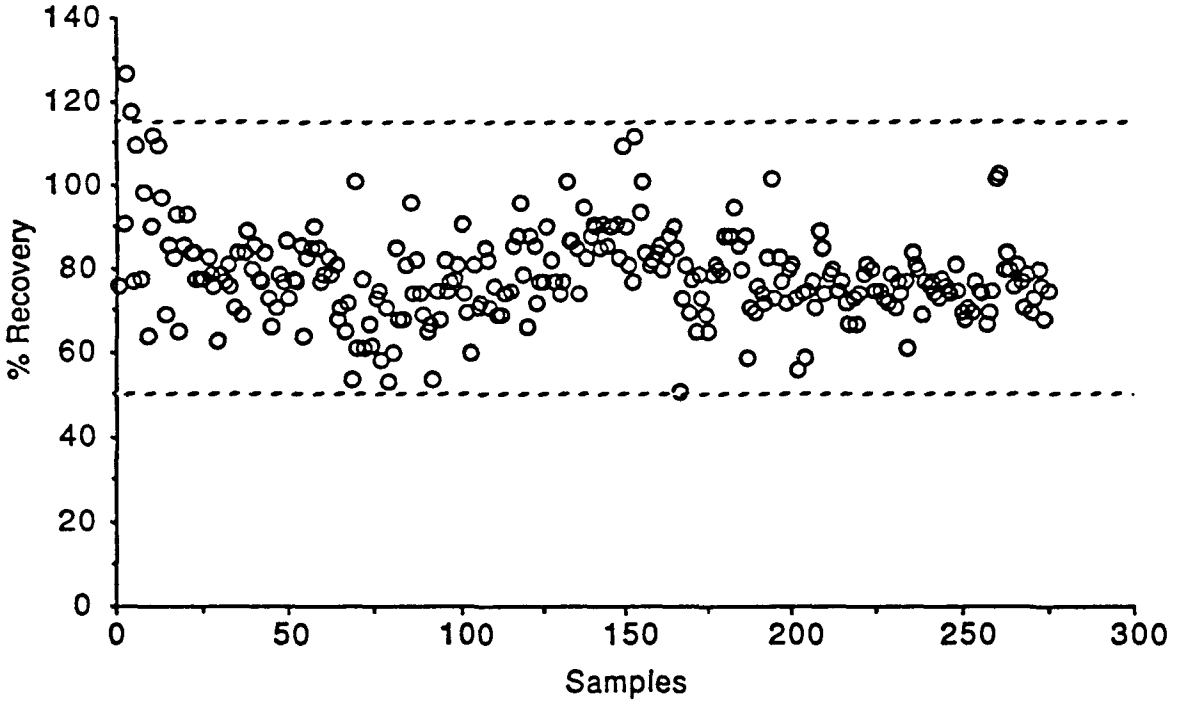


FIGURE B-55

13C12-HpCDD Recoveries for Batches 1-20

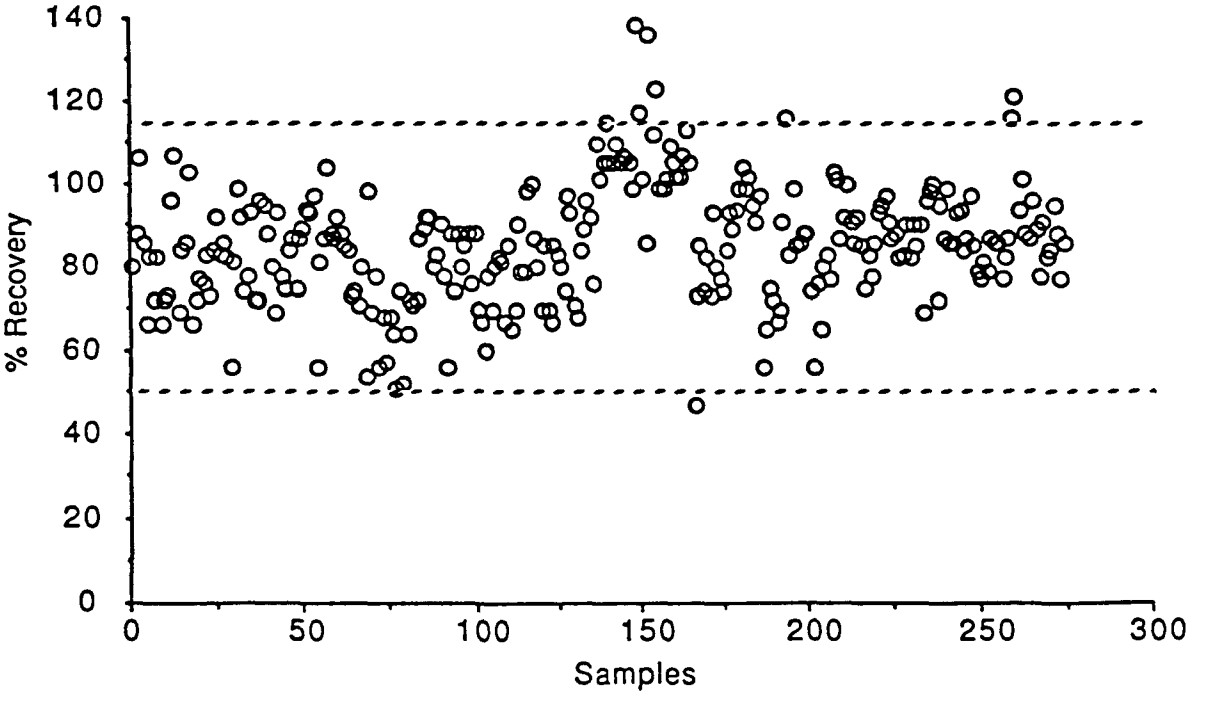
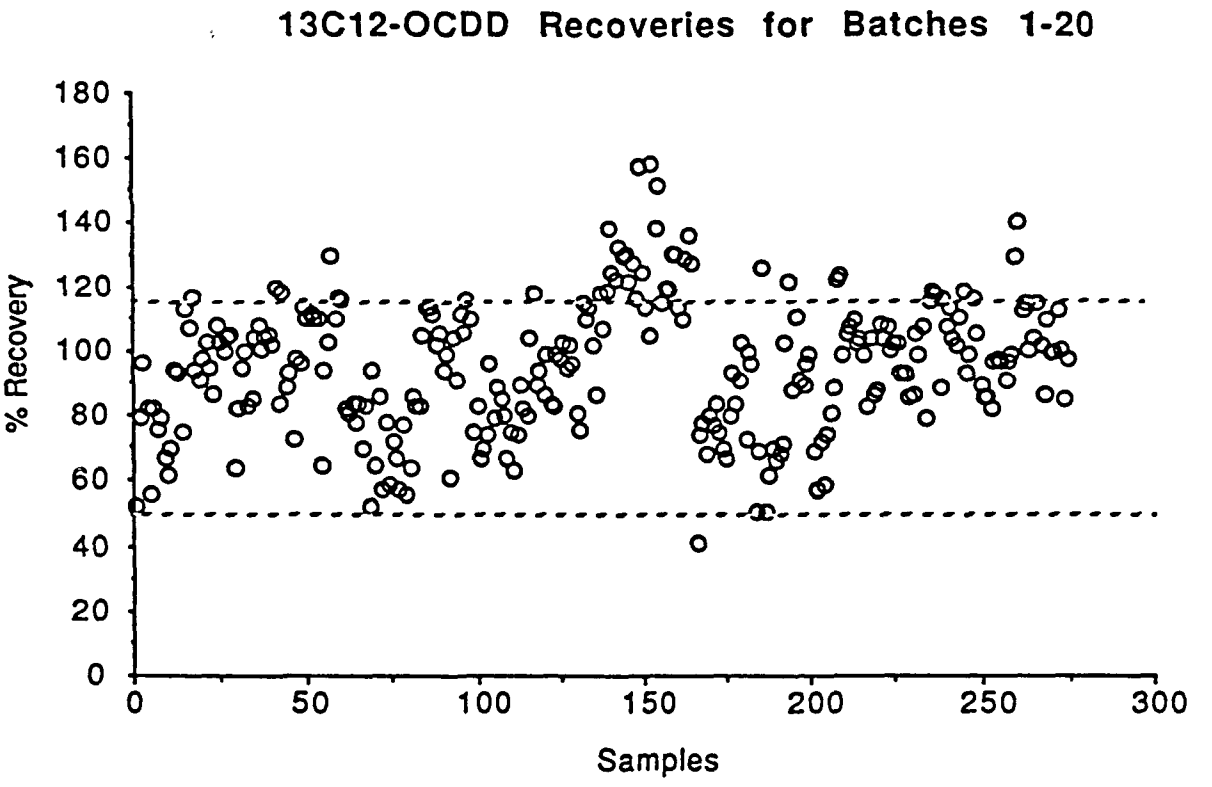


FIGURE B-56


13C12-OCDD Recoveries for Batches 1-20
% Recovery
180
160
140
120
100
80
60
40
20
0
0
50
100
150
200
250
300
Samples

APPENDIX C

This section contains the results of the externally spiked lipid samples. The measurements were precise to three significant digits. This work was conducted under the direction of Jay Glatz, OTS QA Officer. This section was prepared by Jay Glatz.

I. EXTERNAL QUALITY CONTROL AUDIT SAMPLES

The external QC laboratory, Battelle Columbus Division, prepared 3 identical 7-sample sets. Triplicate aliquots were spiked with various PCDD/PCDF congeners at seven concentrations. Spiking was conducted by adding known volumes of 4 stock solutions containing known concentrations of the isomers of interest. The stock solutions were prepared from crystalline material obtained from several suppliers, see Table C-1.

One set of samples was analyzed by Battelle, (reference: Determination of Polychlorinated Dibenzo-p-dioxins and Dibenzofurans in Adipose Tissue; D.G. Aichele, et al.; Battelle Columbus Division, January 8, 1987). The second set was archived at Battelle and the third was sent to the MRI QA manager for incorporation as blind samples into the various batches which were to be analyzed. A solution prepared from the stock spiking solutions was sent to MRI so that comparability of the results would be known. This solution contained the isomers of interest at a nominal concentration of 250 pg/ul. The results of MRI's analysis of this solution are shown in Table C-2. The correction factors obtained from this table were used to adjust the spiking levels reported by Battelle into MRI "measured" spiking levels for recovery calculations.

The results of the external audit samples are listed in Table C-3 through C-10, and summarized in Table C-11. The percent recovery was calculated using the following formula:

$$\% \text{ Recovery} = \frac{[\ ]\text{ Found} - [\ ]\text{ Background}}{[\ ]\text{Spiked level X Conversion factor}} \text{ X } 100\%.$$

Except for sample no. 29, the resolution between 123478-HxCDD and 123678-HxCDD was not sufficient to allow individual quantitation. Therefore, recovery was calculated for the summed isomers. The results are shown graphically in Figure C-1. All data points fell between the stated objectives of 50-130% recovery. The vast majority of data points fell between 80 and 110% recovery indicating highly acceptable performance.

TABLE C-1
Spiking Standards for External Audit Samples

Compound	Commercial Source	Lot Number
2,3,7,8-TCDF	KOR Isotope	55y-7-22
2,3,7,8-TCDD	EPA Repository	CR82-2-2
1,2,3,7,8-PeCDF	Wellington Science	N/A
2,3,4,7,8-PeCDF	Wellington Science	N/A
1,2,3,7,8-PeCDD	KOR Isotope	AA-VIII-185
1,2,3,4,7,8-HxCDF	Wellington Science	N/A
1,2,3,6,7,8-HxCDF	Wellington Science	N/A
2,3,4,6,7,8-HxCDF	Wellington Science	N/A
1,2,3,7,8,9-HxCDF	Cambridge Isotope	MB-13106-47
1,2,3,4,7,8-HxCDD	Cambridge Isotope	830-244
1,2,3,6,7,8-HxCDD	EPA Repository	C25042
1,2,3,7,8,9-HxCDD	EPA Repository	C14829-7
1,2,3,4,6,7,8-HpCDF	Cambridge Isotope	AWN-729-22
1,2,3,4,7,8,9-HpCDF	Cambridge Isotope	13106-7
1,2,3,4,6,7,8-HpCDD	Cambridge Isotope	MLB-706-21
OCDD	Cambridge Isotope	F-2831
OCDF	Cambridge Isotope	F-2832

TABLE C-2
MRI Analysis of External Audit Sample Spiking Solution

PCDD/PDCF	BCL Reported Values (pg/ul)	MRI Analysis Mean (pg/ul)	Conversion Factor
2,3,7,8-TCDD	250	379	1.52
2,3,7,8-TCDF	250	169	0.68
1,2,3,7,8-PCDD	250	163	0.65
1,2,3,7,8-PCDF	250	320	1.28
2,3,4,7,8-PCDF	250	323	1.29
1,2,3,4,7,8-HxCDD	250	266	1.06
1,2,3,6,7,8-HxCDD	250	283	1.13
1,2,3,7,8,9-HxCDD	250	263	1.05
1,2,3,4,7,8-HxCDF	250	281	1.12
1,2,3,6,7,8-HxCDF	250	113	0.45
1,2,3,7,8,9-HxCDF	250	215	0.86
2,3,4,6,7,8-HxCDF	250	232	0.93
1,2,3,4,6,7,8-HpCDD	250	233	0.93
1,2,3,4,6,7,8-HpCDF	250	216	0.86
1,2,3,4,7,8,9-HpCDF	250	223	0.89
OCDD	250	239	0.96
OCDF	250	215	1.26

TABLE C-3
Background Concentrations

PCDD/PCDF	Concentration Mean (ppt)	Standard Deviation	Standard Error	N[1]
2,3,7,8-TCDD	10.064	1.062	0.23739	20
2,3,7,8-TCDF	1.943	0.209	0.04801	19
1,2,3,7,8-PCDD	18.455	0.908	0.20294	20
1,2,3,7,8-PCDF	0.845	0.049	0.03500	2
2,3,4,7,8-PCDF	27.650	2.790	0.62380	20
1,2,3,4,7,8-HxCDD/ 1,2,3,6,7,8-HxCDD	155.200	15.105	3.37764	20
1,2,3,7,8,9-HxCDD	15.695	0.974	0.21770	20
1,2,3,4,7,8-HxCDF	20.240	1.137	0.25417	20
1,2,3,6,7,8-HxCDF	11.147	1.182	0.27849	18
1,2,3,7,8,9-HxCDF	0.880			1
2,3,4,6,7,8-HxCDF	3.788	0.328	0.07517	19
1,2,3,4,6,7,8-HpCDD	223.700	7.981	1.78458	20
1,2,3,4,6,7,8-HpCDF	27.690	1.177	0.26317	20
1,2,3,4,7,8,9-HpCDF	1.380	0.181	0.09065	4
OCDD	813.850	23.725	5.30505	20
OCDF	2.110	0.328	0.08778	14

[1] Number of positive quantifiable results

TABLE C-4
External Audit Sample #29

PCDD/PCDF	Background Level (ppt)	Spiking Level[1]	Conversion Factor	Concentration Found (ppt)	%Recovery[2,3]
2,3,7,8-TCDD	10.1	45.0	1.52	70.5	88
2,3,7,8-TCDF	1.9	35.0	0.68	23.3	90
1,2,3,7,8-PCDD	18.5	72.0	0.65	62.1	93
1,2,3,7,8-PCDF	0.8	35.0	1.28	36.7	80
2,3,4,7,8-PCDF	27.7	72.0	1.29	131.0	111
1,2,3,4,7,8-HxCDD		72.0	1.06	12.1	
1,2,3,6,7,8-HxCDD	155.2	630.0	1.13	761.0	78
1,2,3,7,8,9-HxCDD	15.7	72.0	1.05	85.1	92
1,2,3,4,7,8-HxCDF	20.2	72.0	1.12	86.2	82
1,2,3,6,7,8-HxCDF	11.1	45.0	0.45	26.6	77
1,2,3,7,8,9-HxCDF	0.9	35.0	0.86	26.2	84
2,3,4,6,7,8-HxCDF	3.8	45.0	0.93	37.2	80
1,2,3,4,6,7,8-HpCDD	223.7	630.0	0.93	757.0	91
1,2,3,4,6,7,8-HpCDF	27.7	72.0	0.86	86.2	94
1,2,3,4,7,8,9-HpCDF	1.4	35.0	0.89	23.9	72
OCDD	813.9	630.0	0.96	1170.0	59
OCDF	2.1	45.0	1.26	38.3	64

1 Concentration (pg/g,ppt) based on a weighed amount of crystalline material and on a 10g adipose extract.
2 Control limits are 50-130%.
3 Not corrected for differences in sample weight.

$$\% \text{ Recovery} = \frac{[\ ] \text{ Found} - [\ ] \text{ Background}}{[\ ] \text{ Spiked level X Conversion factor}} \text{ X } 100\%.$$

TABLE C-5
External Audit Sample #10

PCDD/PCDF	Background Level (ppt)	Spiking Level[1]	Conversion Factor	Concentration Found (ppt)	%Recovery[2,3]
2,3,7,8-TCDD	10.1	54.0	1.52	94.1	102
2,3,7,8-TCDF	1.9	49.0	0.68	33.2	94
1,2,3,7,8-PCDD	18.5	96.0	0.65	87.1	110
1,2,3,7,8-PCDF	0.8	49.0	1.28	39.7	62
2,3,4,7,8-PCDF	27.7	96.0	1.29	104.0	62
1,2,3,4,7,8-HxCDD		96.0	1.06		
1,2,3,6,7,8-HxCDD	155.2	810.0	1.13	1040.0	87
1,2,3,7,8,9-HxCDD	15.7	96.0	1.05	111.0	95
1,2,3,4,7,8-HxCDF	20.2	96.0	1.12	116.0	89
1,2,3,6,7,8-HxCDF	11.1	54.0	0.45	31.2	83
1,2,3,7,8,9-HxCDF	0.9	49.0	0.86	36.8	85
2,3,4,6,7,8-HxCDF	3.8	54.0	0.93	53.0	98
1,2,3,4,6,7,8-HpCDD	223.7	810.0	0.93	919.0	92
1,2,3,4,6,7,8-HpCDF	27.7	96.0	0.86	111.0	101
1,2,3,4,7,8,9-HpCDF	1.4	49.0	0.89	38.6	85
OCDD	813.9	810.0	0.96	1350.0	69
OCDF	2.1	54.0	1.26	56.2	80

1 Concentration (pg/g,ppt) based on a weighed amount of crystalline material and on a 10g adipose extract.
2 Control limits are 50-130%.
3 Not corrected for differences in sample weight.

$$\% \text{ Recovery} = \frac{[\ ] \text{ Found} - [\ ] \text{ Background}}{[\ ] \text{ Spiked level X Conversion factor}} \text{ X } 100\%.$$

TABLE C-6
External Audit Sample #8

PCDD/PCDF	Background Level (ppt)	Spiking Level[1]	Conversion Factor	Concentration Found (ppt)	%Recovery[2,3]
2,3,7,8-TCDD	10.1	9.0	1.52	24.3	104
2,3,7,8-TCDF	1.9	7.0	0.68	6.29	92
1,2,3,7,8-PCDD	18.5	12.0	0.65	27.1	110
1,2,3,7,8-PCDF	0.8	7.0	1.28	8.96	91
2,3,4,7,8-PCDF	27.7	12.0	1.29	38.8	72
1,2,3,4,7,8-HxCDD		12.0	1.06		
1,2,3,6,7,8-HxCDD	155.2	45.0	1.13	222.0	105
1,2,3,7,8,9-HxCDD	15.7	12.0	1.05	26.4	85
1,2,3,4,7,8-HxCDF	20.2	12.0	1.12	34.1	103
1,2,3,6,7,8-HxCDF	11.1	9.0	0.45	15.3	104
1,2,3,7,8,9-HxCDF	0.9	7.0	0.86	5.80	81
2,3,4,6,7,8-HxCDF	3.8	9.0	0.93	12.7	106
1,2,3,4,6,7,8-HpCDD	223.7	45.0	0.93	263.0	94
1,2,3,4,6,7,8-HpCDF	27.7	12.0	0.86	37.9	99
1,2,3,4,7,8,9-HpCDF	1.4	7.0	0.89	6.29	78
OCDD	813.9	45.0	0.96	858.0	102
OCDF	2.1	9.0	1.26	13.9	104

1 Concentration (pg/g,ppt) based on a weighed amount of crystalline material and on a 10g adipose extract.
2 Control limits are 50-130%.
3 Not corrected for differences in sample weight.

$$\% \text{ Recovery} = \frac{[\ ] \text{ Found} - [\ ] \text{ Background}}{[\ ] \text{ Spiked level X Conversion factor}} \text{ X } 100\%.$$

TABLE C-7
External Audit Sample #20

PCDD/PCDF	Background Level (ppt)	Spiking Level[1]	Conversion Factor	Concentration Found (ppt)	%Recovery[2,3]
2,3,7,8-TCDD	10.1	49.5	1.52	101.0	121
2,3,7,8-TCDF	1.9	42.0	0.68	31.5	104
1,2,3,7,8-PCDD	18.5	84.0	0.65	75.0	103
1,2,3,7,8-PCDF	0.8	42.0	1.28	48.0	88
2,3,4,7,8-PCDF	27.7	84.0	1.29	137.0	101
1,2,3,4,7,8-HxCDD		84.0	1.06		
1,2,3,6,7,8-HxCDD	155.2	720.0	1.13	1100.0	105
1,2,3,7,8,9-HxCDD	15.7	84.0	1.05	101.0	97
1,2,3,4,7,8-HxCDF	20.2	84.0	1.12	108.0	93
1,2,3,6,7,8-HxCDF	11.1	49.5	0.45	30.6	88
1,2,3,7,8,9-HxCDF	0.9	42.0	0.86	31.9	86
2,3,4,6,7,8-HxCDF	3.8	49.5	0.93	47.2	94
1,2,3,4,6,7,8-HpCDD	223.7	720.0	0.93	920.0	104
1,2,3,4,6,7,8-HpCDF	27.7	84.0	0.86	102.0	103
1,2,3,4,7,8,9-HpCDF	1.4	42.0	0.89	41.5	107
OCDD	813.9	720.0	0.96	1410.0	86
OCDF	2.1	49.5	1.26	58.9	91

1 Concentration (pg/g,ppt) based on a weighed amount of crystalline material and on a 10g adipose extract.
2 Control limits are 50-130%.
3 Not corrected for differences in sample weight.

$$\% \text{ Recovery} = \frac{[\ ] \text{ Found} - [\ ] \text{ Background}}{[\ ] \text{ Spiked level X Conversion factor}} \text{ X } 100\%.$$

TABLE C-8
External Audit Sample #18

PCDD/PCDF	Background Level (ppt)	Spiking Level[1]	Conversion Factor	Concentration Found (ppt)	%Recovery[2,3]
2,3,7,8-TCDD	10.1	36.0	1.52	73.3	115
2,3,7,8-TCDF	1.9	21.0	0.68	16.8	104
1,2,3,7,8-PCDD	18.5	24.0	0.65	32.7	91
1,2,3,7,8-PCDF	0.8	21.0	1.28	21.5	77
2,3,4,7,8-PCDF	27.7	24.0	1.29	51.2	76
1,2,3,4,7,8-HxCDD		24.0	1.06		
1,2,3,6,7,8-HxCDD	155.2	360.0	1.13	585.0	99
1,2,3,7,8,9-HxCDD	15.7	24.0	1.05	40.8	100
1,2,3,4,7,8-HxCDF	20.2	24.0	1.12	45.3	93
1,2,3,6,7,8-HxCDF	11.1	36.0	0.45	29.4	113
1,2,3,7,8,9-HxCDF	0.9	21.0	0.86	16.1	84
2,3,4,6,7,8-HxCDF	3.8	36.0	0.93	34.6	92
1,2,3,4,6,7,8-HpCDD	223.7	360.0	0.93	554.0	99
1,2,3,4,6,7,8-HpCDF	27.7	24.0	0.86	49.3	105
1,2,3,4,7,8,9-HpCDF	1.4	21.0	0.89	21.1	105
OCDD	813.9	360.0	0.96	1170.0	103
OCDF	2.1	36.0	1.26	44.2	93

1 Concentration (pg/g,ppt) based on a weighed amount of crystalline material and on a 10g adipose extract.
2 Control limits are 50-130%.
3 Not corrected for differences in sample weight.

$$\% \text{ Recovery} = \frac{[\ ] \text{ Found} - [\ ] \text{ Background}}{[\ ] \text{ Spiked level X Conversion factor}} \text{ X } 100\%.$$

TABLE C-9
External Audit Sample #26

PCDD/PCDF	Background Level (ppt)	Spiking Level[1]	Conversion Factor	Concentration Found (ppt)	%Recovery[2,3]
2,3,7,8-TCDD	10.1	18.0	1.52	36.6	97
2,3,7,8-TCDF	1.9	14.0	0.68	11.7	102
1,2,3,7,8-PCDD	18.5	18.0	0.65	32.9	123
1,2,3,7,8-PCDF	0.8	14.0	1.28	17.6	94
2,3,4,7,8-PCDF	27.7	18.0	1.29	51.6	103
1,2,3,4,7,8-HxCDD		18.0	1.06		
1,2,3,6,7,8-HxCDD	155.2	90.0	1.13	264.0	90
1,2,3,7,8,9-HxCDD	15.7	18.0	1.05	33.4	94
1,2,3,4,7,8-HxCDF	20.2	18.0	1.12	39.2	94
1,2,3,6,7,8-HxCDF	11.1	18.0	0.45	20.1	111
1,2,3,7,8,9-HxCDF	0.9	14.0	0.86	11.8	91
2,3,4,6,7,8-HxCDF	3.8	18.0	0.93	20.4	99
1,2,3,4,6,7,8-HpCDD	223.7	90.0	0.93	312.0	105
1,2,3,4,6,7,8-HpCDF	27.7	18.0	0.86	44.3	107
1,2,3,4,7,8,9-HpCDF	1.4	14.0	0.89	14.6	106
OCDD	813.9	90.0	0.96	903.0	103
OCDF	2.1	18.0	1.26	22.7	91

1 Concentration (pg/g,ppt) based on a weighed amount of crystalline material and on a 10g adipose extract.
2 Control limits are 50-130%.
3 Not corrected for differences in sample weight.

$$\% \text{ Recovery} = \frac{[\ ] \text{ Found} - [\ ] \text{ Background}}{[\ ] \text{ Spiked level X Conversion factor}} \text{ X } 100\%.$$

TABLE C-10
External Audit Sample #30

PCDD/PCDF	Background Level (ppt)	Spiking Level[1]	Conversion Factor	Concentration Found (ppt)	%Recovery[2,3]
2,3,7,8-TCDD	10.1	40.5	1.52	71.7	100
2,3,7,8-TCDF	1.9	28.0	0.68	20.4	95
1,2,3,7,8-PCDD	18.5	48.0	0.65	52.1	108
1,2,3,7,8-PCDF	0.8	28.0	1.28	34.5	94
2,3,4,7,8-PCDF	27.7	48.0	1.29	95.3	109
1,2,3,4,7,8-HxCDD		48.0	1.06		
1,2,3,6,7,8-HxCDD	155.2	450.0	1.13	655.0	89
1,2,3,7,8,9-HxCDD	15.7	48.0	1.05	65.7	99
1,2,3,4,7,8-HxCDF	20.2	48.0	1.12	75.9	104
1,2,3,6,7,8-HxCDF	11.1	40.5	0.45	31.6	112
1,2,3,7,8,9-HxCDF	0.9	28.0	0.86	26.2	105
2,3,4,6,7,8-HxCDF	3.8	40.5	0.93	44.9	109
1,2,3,4,6,7,8-HpCDD	223.7	450.0	0.93	659.0	104
1,2,3,4,6,7,8-HpCDF	27.7	48.0	0.86	72.5	109
1,2,3,4,7,8,9-HpCDF	1.4	28.0	0.89	28.8	110
OCDD	813.9	450.0	0.96	1260.0	103
OCDF	2.1	40.5	1.26	50.4	95

1 Concentration (pg/g,ppt) based on a weighed amount of crystalline material and on a 10g adipose extract.
2 Control limits are 50-130%.
3 Not corrected for differences in sample weight.

$$\% \text{ Recovery} = \frac{[\ ] \text{ Found} - [\ ] \text{ Background}}{[\ ] \text{ Spiked level X Conversion factor}} \text{ X } 100\%.$$

TABLE C-11
Summary Recovery Data for External Audit Samples

Compounds	ID	% Recovery (7 trials)							X	% RSD
2,3,7,8-TCDD	A	88	102	104	121	115	96	100	104	9.9
2,3,7,8-TCDF	B	90	94	92	104	104	103	95	97	5.8
1,2,3,7,8-PCDD	C	93	110	110	103	91	123	108	105	9.7
1,2,3,7,8-PCDF	D	80	62	91	88	77	94	94	84	12.8
2,3,4,7,8-PCDF	E	111	62	72	101	76	103	109	91	20.3
1,2,3,4,7,8-HxCDD										
1,2,3,6,7,8-HxCDD	F/G	78	87	105	105	99	90	89	93	10.0
1,2,3,7,8,9-HxCDD	H	92	95	85	97	100	94	99	95	4.9
1,2,3,4,7,8-HxCDF	I	82	89	103	93	93	94	104	94	7.5
1,2,3,6,7,8-HxCDF	J	77	83	104	88	113	111	112	98	14.4
1,2,3,7,8,9-HxCDF	K	84	85	81	86	84	91	105	88	8.5
2,3,4,6,7,8-HxCDF	L	80	98	106	94	92	99	109	97	9.2
1,2,3,4,6,7,8-HpCDD	M	91	92	94	104	99	105	104	98	5.7
1,2,3,4,6,7,8-HpCDF	N	94	101	99	103	105	107	109	103	4.6
1,2,3,4,7,8,9-HpCDF	O	72	85	78	107	105	106	110	95	15.4
OCDD	P	59	69	192	86	103	103	103	89	19.3
OCDF	Q	64	80	104	91	93	91	95	88	13.5

FIGURE C-1

VA EXTERNAL AUDIT SAMPLE RECOVERIES

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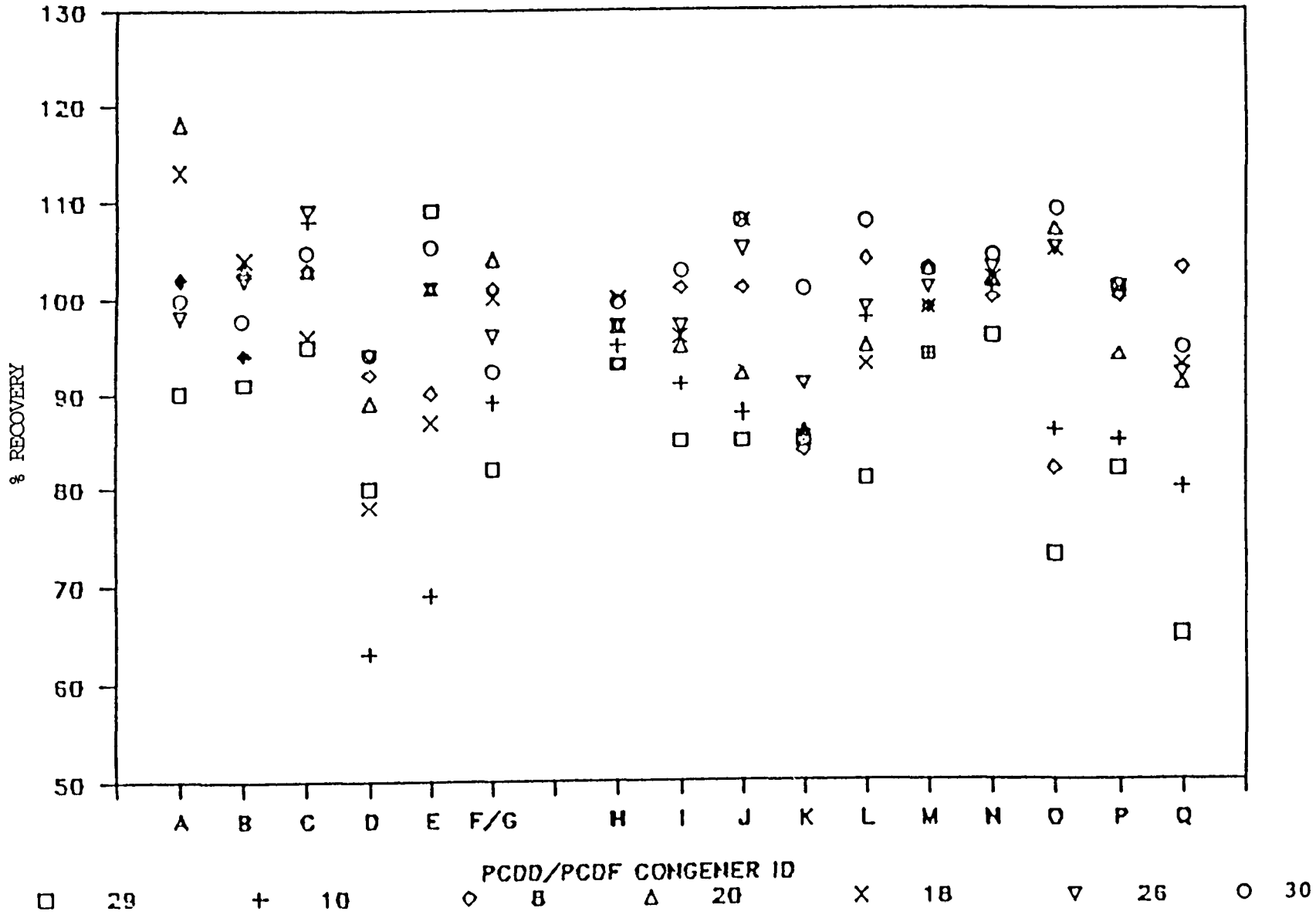

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15. Supplementary Notes

16. Abstract (Limit: 200 words)

Concern about the adverse effects of exposure to Agent Orange is for the most part attributable to its toxic contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). TCDD accumulates preferentially in body fat and has a long half life in humans. Therefore, TCDD levels in adipose tissue can serve as a biological marker of exposure to Agent Orange. The adipose tissue collected for the EPA's National Human Adipose Tissue Survey (NHATS) was made available to the study as a source of tissue specimens. A total of 40 Vietnam veterans, 80 non-Vietnam veterans and 80 civilian men were selected from males born between 1936 and 1954 and their adipose tissues were analyzed for 17 2,3,7,8-substituted dioxins and dibenzofurans. TCDD levels were log normally distributed and the mean level of 2,3,7,8-TCDD in adipose tissue of the Vietnam veterans (13.4 ppt) was not significantly different from that of the non-Vietnam veterans (12.5 ppt) or civilian men (15.8 ppt). Adjusting for demographic variables did not change the conclusions. The study results suggest that heavy exposure to Agent Orange for most Vietnam veterans was very unlikely and that there is no readily available and reliable indirect method of assessing exposure to Agent Orange for Vietnam veterans.

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