ANALYSIS OF TECHNICAL GRADE PESTICIDES FOR TCDD AT PARTS-PER-BILLION LEVEL

by

Joseph G. Montalvo, Jr., James F. Ryan, III, and Roy Flagg
Physical and Engineering Sciences Division
Gulf South Research Institute
New Orleans, Louisiana 70186

Number
68-01-3981

Project Officer

Patricia Ott
Environmental Fate Branch of the Hazard Evaluation Division
Office of Pesticide Programs
Washington, D.C. 20460

OFFICE OF PESTICIDE PROGRAMS
OFFICE OF TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

DISCLAIMER

This report has been reviewed by the Environmental Fate Branch of the Hazard Evaluation Division, U.S. Environmental Protection Agency, and not approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

ABSTRACT

Gulf South Research Institute (GSRI) analyzed 69 technical grade pesticide batch samples (Silvex; 2,4,5-trichlorophenoxyacetic acid; an organophosphate; 2,4,5-trichlorophenol; and a chlorinated phenolic antiseptic), 31 replicate samples of these batch samples, and 41 quality control samples for total tetrachlorodibenzo-p-dioxin (TCDD). The samples were extracted with hexane and the extracts were dried, concentrated, and chromatographed on alumina. Samples were qualitatively and quantitatively analyzed by gas chromatography/mass spectrometry (GS/MS) in the selective ion monitoring (SIM) mode. Certainty of TCDD detection was assessed via summation of six qualitative criteria. Based on this assessment, the data were rated positive, doubtful, or nondetectable. For those authentic samples in which detection was judged positive, levels of total TCDD ranged from nondetectable to 30 ppb; quality control samples contained up to 3984 ppb. The use of high efficiency capillary column GC/MS is recommended for future TCDD investigations.

CONTENTS

FIGURES

TABLES

ACKNOWLEDGMENT

The cooperation of Mr. Ronald F. Thomas, Supervisory Chemist, Benefits and Field Studies Division, Environmental Protection Agency, in sampling design and shipment of the samples to Gulf South Research Institute is appreciated. The assistance of Ms. Patricia Ott, Project Officer, is gratefully acknowledged. The assistance of Dr. Mary M. McKown, GSRI senior analytical chemist, throughout the project performance is appreciated.

SECTION 1

INTRODUCTION

Polychlorinated dibenzo-p-dioxins (PCDDs) may be formed as undesirable trace impurities in the production of technical grade chlorophenols. The latter are employed extensively as pesticides, as wood preservatives and as starting materials for a series of other products. Through careful control over conditions in the pesticide manufacturing process, only minimal quantities of PCDDs are formed, but at temperatures above 200°C a condensation of chlorophenols may occur with formation of increasing quantities of PCDDs (1).

Several PCDDs have been found to be highly toxic and possibly carcinogenic and are stable in biological systems (2). Theoretically, a total of 75 different PCDDs exist, including 22 tetrachlorodibenzo-p-dioxins. The toxicological properties of positional isomers of the same dioxin may vary significantly. Since the 2,3,7,8-tetrachlorodibenzo-p-dioxin (Figure 1) is extremely toxic (3), TCDDs deserve special consideration. Although the synthetic route for TCDD formation during chlorophenol manufacture favors 2,3,7,8-TCDD, other tetrachlorodibenzo-p-dioxin isomers may also be formed (4).

Under the aegis of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the U.S. Environmental Protection Agency (EPA) has set maximum TCDD contamination limits in technical grade hexachlorophene at 100 ppb (0.1 ppm) in order to reduce human and environmental exposure. The purpose of the GSRI research effort was to determine levels of TCDD present in technical grade pesticide samples submitted by EPA.

In view of the inherent toxicity of TCDD, special precautions in handling are required during analysis. Concentration by several orders of magnitude is sometimes necessary, and an ultrasensitive detection method is preferred. A combination of gas chromatography and mass spectrometry has been shown to be an excellent method for detecting TCDDs at very low levels (5). Identification of individual TCDD isomers requires an initial, high efficiency separation such as glass capillary gas chromatography (GC) prior to mass spectrometric (MS) analysis. Low resolution GC columns, i.e., packed columns, provide a convenient means for TCDD isomer summation (total TCDD) by GC/MS.

The present study has focused on determination of total TCDD in technical grade samples by low resolution gas chromatography/mass spectrometry. Sample preparation included addition of ^{37}Cl 2,3,7,8-TCDD to assess

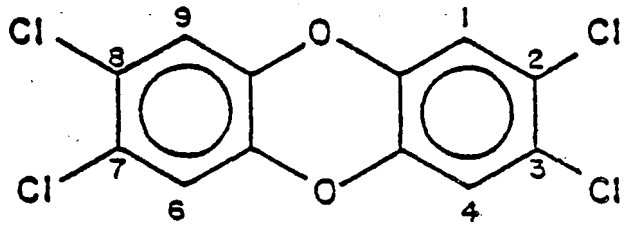


Figure 1. Structure of 2,3,7,8-TCDD.

dioxin recovery, solvent extraction, and cleanup on alumina. GC/MS analyses were carried out using a packed column; the standard addition injection technique was used. Selective ion monitoring (SIM) and complete mass spectral data were obtained. Six qualitative criteria for TCDD detection were developed incorporating the precision of the analytical measurement system. The project quality assurance protocol included internal quality control samples (precision and accuracy) and quality control charting.

SECTION 2

CONCLUSIONS

The results of low resolution gas chromatographic/mass spectrometric analysis of 141 samples (including quality controls and authentic pesticide samples containing unknown quantities of TCDD) for total tetrachlorodibenzo-p-dioxin have indicated levels ranging from nondetectable to 30 ppb for 69 authentic pesticide batch and 31 replicate samples and nondetectable to 3984 ppb for 41 quality control samples. TCDD was not detected (detection limit 10 ppb) in 66 percent of the authentic samples, detection was doubtful in 9 percent and positive in 25 percent. Whereas TCDD could not be detected in a majority of authentic samples, positive samples contained 10-30 ppb total TCDD.

SECTION 3

RECOMMENDATIONS

The investigation of total tetrachlorodibenzo-p-dioxin levels in compounds for whose synthesis 2,4,5-trichlorophenol is used should be continued. In view of the known toxicity of the 2,3,7,8-TCDD isomer and incomplete separation of the isomers using packed columns, future investigations should be performed using high efficiency gas chromatography (capillary column)/mass spectrometry. These data are of major importance from the point of view of environmental protection.

In spite of the eminent need for standardized methods for TCDD analysis in technical grade pesticide formulations and environmental samples, such procedures are still lacking. The establishment of standardized methods would permit substantial progress in baseline and on-going studies. Such methods should be directed toward improved sample preparation (to reduce interferences) and improved resolution/detection of the 2,3,7,8-TCDD isomer, with the consideration in mind that such methods should be suitable for use by moderately well equipped laboratories.

SECTION 4

EXPERIMENTAL PROCEDURES

SAFE HANDLING IN THE LABORATORY

Disposable rubber gloves were worn while handling samples and solutions, and all evaporations were performed in a hood. Waste materials were stored in a capped steel drum labeled TCDD WASTE. Personnel associated with the project had periodic physical examinations.

GENERAL PROCEDURES

After taking custody of samples furnished by the EPA, TCDD residues were identified and quantified using a three-stage analytical protocol including sample preparation, mass spectral data aquisition, and data analysis.

TCDD Standards

TCDD standards in benzene were supplied by Dr. Aubry Dupuy, Pesticide Monitoring Laboratory, EPA. The 2,3,7,8-TCDD standard concentration was 500 picograms (pg)/microliter (µl). Concentration of the ^{37}Cl labeled 2,3,7,8-TCDD standard was 480 pg/µl. Labeled TCDD was used to determine dioxin recovery through the entire extraction/cleanup procedure. Nonlabeled TCDD was used to quantify dioxin levels in the samples.

Sample Preparation

Ethanol, hexane, benzene, carbon tetrachloride, and methylene chloride were nanograde (pesticide) quality; inorganic salts, acids and bases were ACS grade. Distilled water was prepared by filtering through a mixed bed ion exchanger, activated carbon, and distilling in an all glass still. Aluminum oxide Woelm neutral, activity grade I, was used for column chromatography.

The procedure used in these analyses is a modification of that provided by EPA in the contract. In general, it involved caustic KOH saponification of the pesticide followed by extraction of the neutral TCDD into hexane. The hexane solution was cleaned up by extracting several times with sodium hydroxide solution and sulfuric acid, followed by chromatography on alumina. The eluate was concentrated for GC/MS analysis. Details of the procedures follow.

A 1 g quantity of sample was transferred quantitatively to a 500 ml separatory funnel containing 60 ml of pesticide grade ethanol. Exactly 48 ng of ^{37}Cl labeled 2,3,7,8-TCDD was added with a microsyringe and the mixture swirled. Four milliliters of 40 percent KOH was added and the mixture immediately shaken. The room temperature digestion was allowed to proceed for 30 minutes, with occasional swirling. Two-hundred milliliters of distilled water was added to dissolve the precipitate formed upon base addition, and the resulting solution was extracted with three 100 ml portions of nanograde hexane. The combined hexane extracts were washed sequentially with three 100-ml volumes of 1N NaOH, one 80-ml volume of distilled water, and 95 percent H_2SO_4 until no further color appeared in H_2SO_4. A final washing with 80 ml distilled water completed the extraction.

The hexane extract was filtered through a 15 mm x 20 cm column of granular anhydrous Na_2CO_3 followed by concentration in a Kuderna-Danish evaporator to a volume of about 4 ml. The anhydrous Na_2CO_3 had been purified by pre-extraction with nanograde CH_2Cl_2 in a Soxhlet extractor and drying at 100°C in a vacuum oven. The concentrate was reduced to 0.5 ml under a stream of UHP or ultra high purity nitrogen (Matheson, Rutherford, N.J.). The residue was reconstituted in 2 ml nanograde hexane and chromatographed through a 4.5 cm column. The column consisted of activated alumina over which a 1/4 inch layer of purified granular Na_2SO_4 was placed. The column was preconditioned by washing with 5 ml of CH_2Cl_2, followed by removal of residual CH_2Cl_2 by blowing dry N_2 through the column, and heating for 48 hours at 240°C. The sample was eluted from the column with 6 ml CCl_4 followed by 4 ml of CH_2Cl_2. The carbon tetrachloride layer was discarded and the CH_2Cl_2 fraction evaporated under UHP N_2 to 20 µl. The concentrate was reconstituted to 2 ml with hexane, evaporated to 20 µl, and rediluted with hexane to 2 ml. The final extract volume was concentrated to 200 µl. The three evaporations were necessary to remove residual CH_2Cl_2.

The extract was divided equally into two glass ampules. Ampules were flame sealed, and the extract volume noted by marking the liquid level. After coding the ampules were stored in a freezer at -10°C.

Samples were processed in "sets". Each set of samples consisted of internal and EPA supplied quality control (QC) and pesticide samples. Generally each set of samples contained two quality control samples and four pesticide samples processed from left to right as indicated below:

$$\cdots \overrightarrow{A_i\ X_j\ X_{j+1}\ X_{j+2}\ X_{j+3}\ B_k} \cdots$$

where A_i = ith observation of the accuracy QC sample (for example, pesticide sample #0093, furnished by EPA, accepted value for TCDD = 2.3 ppm).

X_j = the jth EPA pesticide sample (unknown TCDD level),

and B_k = kth observation of the zero level TCDD QC sample (solvent blank spiked only with ^{37}Cl TCDD, 48 ng).

QC and EPA sample numbers for each paired extract were coded with a numerical suffix: one ampule, -2(for immediate GC/MS assay) and the other, -1 (for backup analysis). EPA sample numbers ranged from 2 to 9 digits. Replicate samples in this report are denoted by an "I" (initial), "D" (duplicate), "T" (triplicate), or "Q" (quadruplicate) after the sample batch number. The series sample number (e.g., 148920) represents one source of technical grade material; the -1, -2, -3, etc., following the series sample numbers each represents a different batch sampled from that source.

TCDD and ^{37}Cl TCDD standards were transferred to Mininert (Supelco) valve vials. A back-up seal in the Teflon valve had been removed and the assembly prewashed. Standards were coded and frozen until utilized.

Mass Spectral Data Aquisition

The low resolution gas chromatograph/mass spectrometer used was a Hewlett-Packard (HP) 5985 GC/MS in the electron ionization (EI) source configuration. The control unit displayed the ion current (peak area) on a Tektronix 4012 CRT computer terminal, and hard copies were provided by the Tektronix 4631 hard copy unit.

Generally spectrometer operating conditions were as follows: column 3 ft x 2 mm ID glass; packing 3 percent OV-11 on 100/120 mesh Supelcoport; column temperature 200°C for 1 minute to 240°C at 6°C per minute; injection heater temperature 250°C; jet separator interface temperature 260°C; ion source temperature 150°C; carrier gas, helium 32 ml/min; electron impact source 70 eV; emission 0.3 mA; electron multiplier 2.6 KV; mass range 150-330 amu; scan time 3 ms/amu. The mass spectrometer was tuned to monitor three molecular ions at m/e values of 320, 322, and 328. Retention time of TCDD was approximately 3.6 minutes under the above conditions.

The GC/MS protocol for TCDD measurement was designed to permit simultaneous data acquisition for: ^{37}Cl TCDD recovery, TCDD detection limit, TCDD quantification, and confirmation via mass spectra (molecular ion abundances). In this procedure, sample extracts and TCDD standards were thawed to room temperature. Ampules containing sample extract were broken above the liquid level and firmly positioned in a plastic block. TCDD solutions were always handled under a hood, and liquid and solid wastes were transferred daily to the TCDD waste drum.

A 10 µl Hamilton 701N microsyringe was used to inject sample and standard aliquots into the GC/MS. GC column performance and MS linearity were checked by co-injecting the following mixtures of standards, one injection per mixture in the sequence tabulated:

1 µl native TCDD + 1 µl ^{37}Cl TCDD

2 µl native TCDD + 2 µl ^{37}Cl TCDD

1 µl native TCDD + 1 µl ^{37}Cl TCDD

2 µl native TCDD + 2 µl ^{37}Cl TCDD

Data for each injection were displayed on the CRT computer terminal. Peaks (m/e = 320, 322 and 328) corresponding to the TCDD retention time region were integrated using a HP-21MXE data system and results displayed. Collected data for each injection were recorded on a Tektronix 4610 hard copy unit.

Quality control and pesticide samples were run in the same order in which the extracts were prepared. Co-injection of mixtures, one injection per mixture, was as follows:

COMPOSITION OF ANALYSIS SET
(µl co-injected)

Injection Number	Sample Volume (µl)		TCDD Native Standard		^{37}Cl TCDD Standard
1	2	+	0	+	0
2	2	+	1	+	2
3	2	+	0	+	0
4	2	+	2	+	1

During the TCDD retention time-span the m/e 320, 322 and 328 peak areas were integrated, and a photocopy prepared for each injection (see sample hard copies in Appendices). Background noise level was integrated from the baseline adjacent to the m/e 322 peak. The ion current chromatogram from injection 1 was used for noise determination if the 322 peak was present; the chromatogram from injection 2 was used if the 322 peak was not found. A photocopy of the noise level was prepared.

Hard copies from injections 1 and 3 were inspected to determine if both m/e 320 and 322 peaks were found for both injections. A sample meeting these criteria was subjected to GC/MS mass fragmentation confirmation by evaporating the extract under N_2 to about 10 µl. Two microliters of the concentrated extract was injected. Both mass spectrum and absolute abundances for m/e values were obtained and hard copies prepared (see sample copies in Appendices).

Data Analysis

Integrated peak areas for TCDD standard solutions were subjected to linear regression analysis (y = peak area; X = µl injected). Correlation coefficient (r), slope (s), intercept (i), and the ratio i/s were computed for m/e = 320, 322, and 328. Instrumental sensitivity, expressed as area/pg, was computed for m/e 320 and 328 by dividing "s" by 500 (TCDD standard concentration in pg/µl) and 480 (^{37}Cl TCDD standard concentration in pg/µl). An average 320/322 ratio was computed from the (2 + 0 + 0) injections. Pertinent data were plotted on a QC chart to monitor instrument performance. Expressed as percent, i/s x 100, average values were < ± 5 percent. Large negative values, i.e., 20 percent, could be indicative of TCDD adsorption or degradation in the system. In any case, this problem was usually rectified by changing the GC column.

Peak areas for the method blank (QC), accuracy (QC), and EPA samples were tabulated on data sheets, designed exclusively for this project, along with other pertinent information. A sample data sheet is shown in Appendix A; the method for computing percent ^{37}Cl TCDD recovery, TCDD sample concentration, detection limit, and results of the confirmation analysis are also illustrated. Note that reported TCDD levels are corrected for recovery.

The contract specification for ^{37}Cl TCDD recovery in the pesticide samples was 50 to 120 percent, inclusive. If the initial GC/MS recovery assay was outside the specified range, the backup aliquot of the original extract was assayed. If the average of the two recovery values was within the specification range, contract requirements were satisified. If the average recovery was outside the specification range, the sample was re-extracted and assayed one additional time. Confirmation of TCDD detection by recording complete mass spectra was optional at TCDD contamination levels <10 ppb and a contract requirement at ≥10 ppb.

The standard addition technique was not successful with samples containing large quantities (parts-per-million) of TCDD because the amount of TCDD standard co-injected was insignificant compared to the TCDD level in the pesticide extract. Determination of regression slope and intercept for TCDD quantifications with such samples was variable due to poor correlations. An alternative GC/MS protocol was used for these samples. An external calibration curve was constructed by injecting varying amounts of the TCDD standard into the GC/MS. The resultant linear regression equation for the standard, in terms of picograms rather than microliters injected was:

$$y = X s + i \qquad (1)$$

where y = peak area (m/e = 322)
s = slope (area/pg TCDD)
i = intercept
X = pg TCDD injected

Rearranging Eq. (1):

$$X_p = (y_p - i)/s \qquad (2)$$

where X_p = pg TCDD in the pesticide sample extract and y_p = average m/e 322 area for a 2 µl sample extract. TCDD concentration (ppm) in the sample is equal to:

$$ppm = X_p \frac{(200\ \mu l\ \text{sample extract volume})}{(2\ \mu l\ \text{injected into GC/MS})} / (R \cdot \text{sample weight} \cdot 10^4)$$

where R is the percentage recovery of ^{37}Cl TCDD.

SECTION 5

RESULTS AND DISCUSSION

QUALITY ASSURANCE

Several aspects of this project were monitored for quality assurance. These included (1) taking custody of standards and samples, (2) materials and reagents used in the analysis, (3) standard procedures, (4) calibrations, (5) record keeping, and (6) quality control charts. Chain of custody records were transmitted to GSRI along with samples and standards. Sample receipt was acknowledged by dating, signing, and returning a copy of the chain of transfer record to EPA. Discrepancies in labeling or replacement requests were noted on the transfer record.

Materials and reagents used in the analysis in general were ACS or pesticide grade. New lots of material were tested for TCDD interference by subjecting them to the analytical protocol. The sample extraction and clean-up procedure described in Section 4 was used throughout the project, without modification, and therefore constituted a standard operating procedure. Generally, the GC/MS protocol followed the standard procedure outlined in the previous section; certain modifications, i.e., changes in column temperature programming, were necessary to overcome interferences and refine the method.

The GC/MS instrument was tuned daily prior to analyses. GC/MS performance and linearity standards bracketed 5-6 successive analyses. Record keeping included an instrumental preventive maintenance log. GC/MS data and computer file reference number, date of extraction, and the sample weight were recorded in this log for each sample assayed.

Sample processing accountability was an important part of record keeping. Results were compiled on a master sample status form (see Appendix B) in the order samples were received. Results were also compiled on a cumulative data form (Appendix C) in the order samples were assayed. Cumulative quality control charts were useful indicators of whether or not the measurement process was in control. The charts showed trends or runs, sudden shifts in the mean, increased variability and often indicated the nature of a problem.

Figures 2 through 4 show quality control data for GC/MS performance, method blank, and accuracy of TCDD determinations. Control chart analysis of graphs A, B, C, and D (Figure 2) shows systematic variations with probable causes. A series of increasing and decreasing values (graphs B and D, observation numbers 1-6 and 29-34) was obtained after the instrument

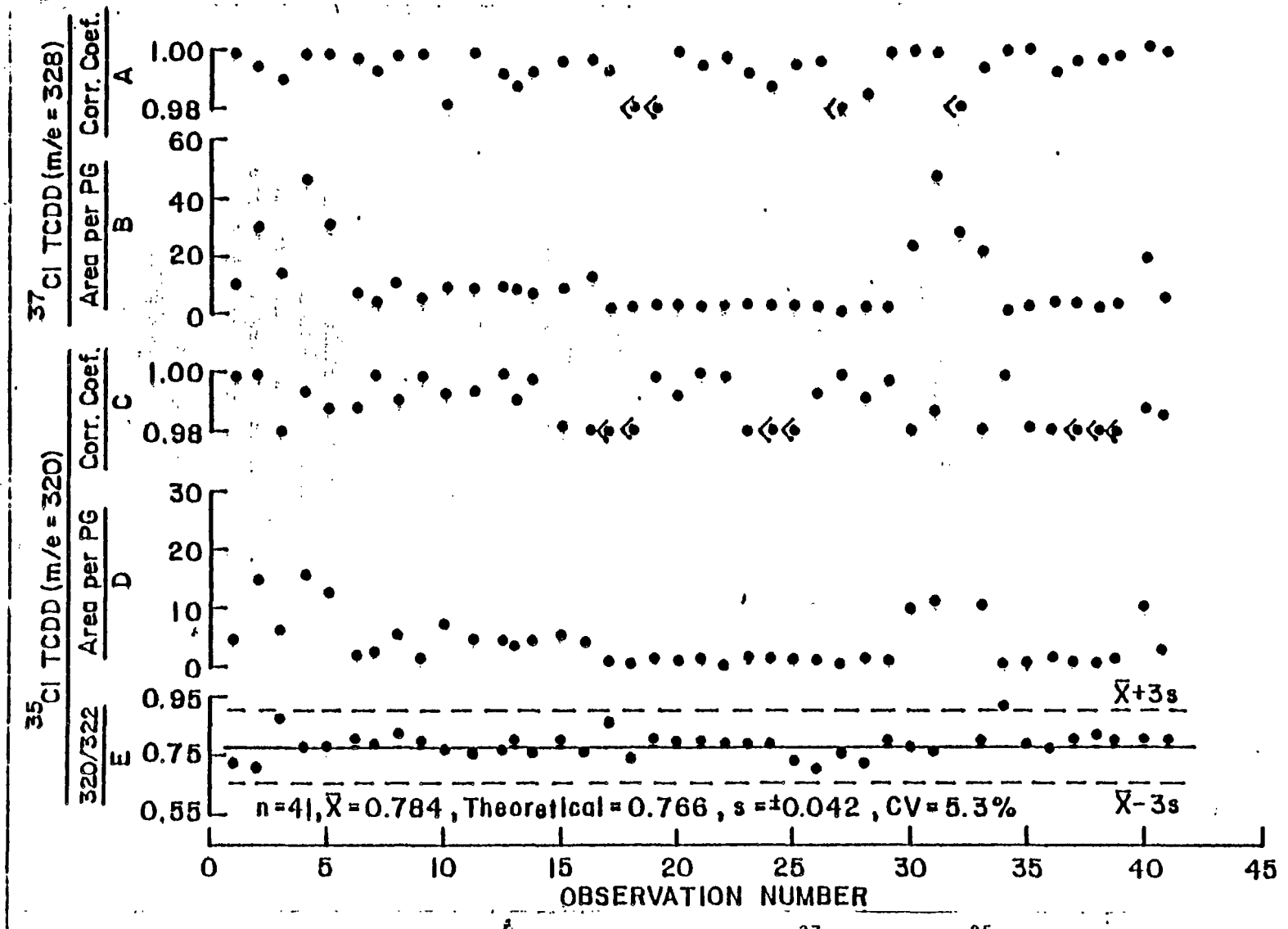


Figure 2. Quality control chart for GC/MS performance (Sample: ^{37}Cl TCDD and ^{35}Cl TCDD standards).

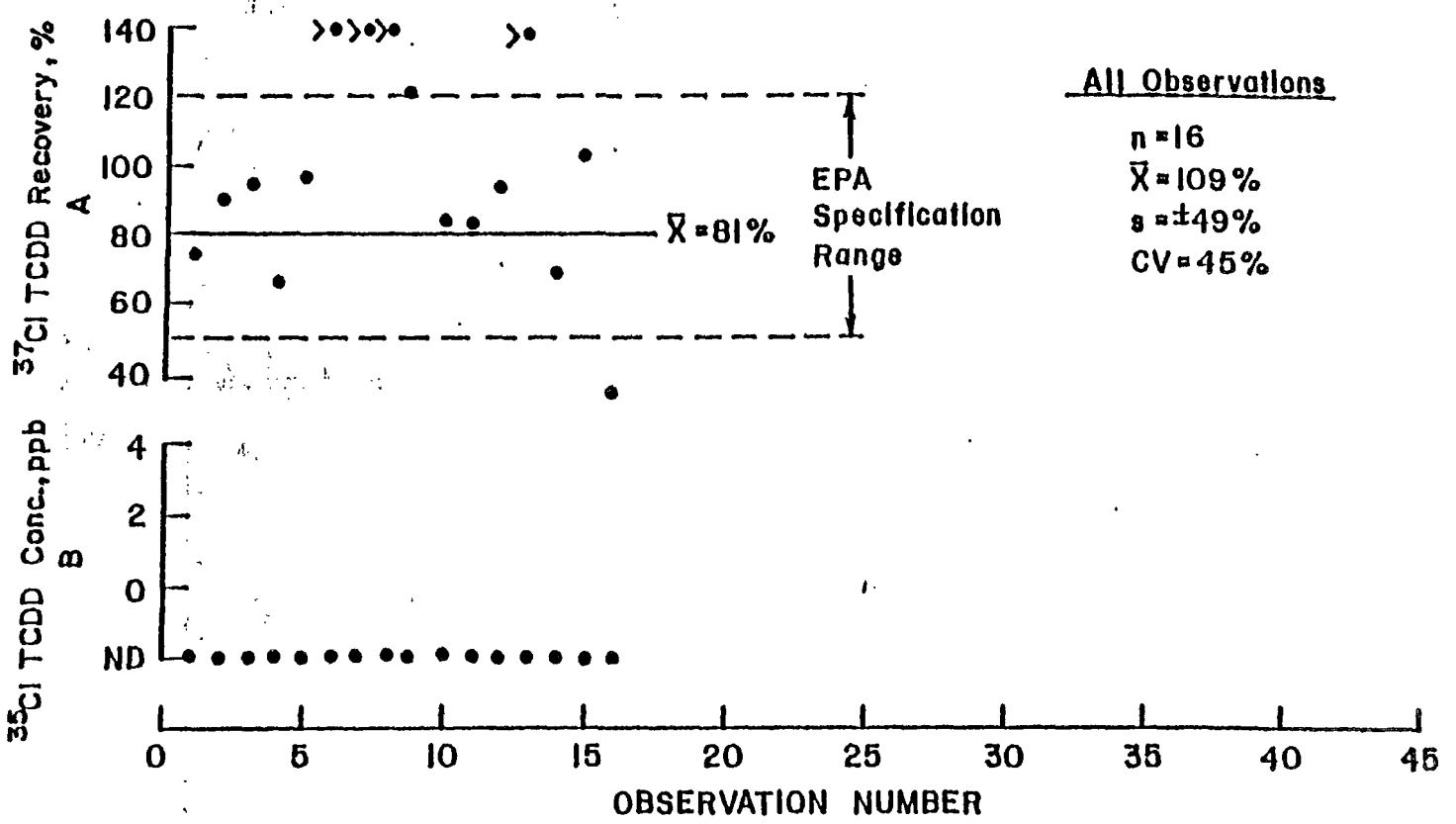


Figure 3. Method blank quality control chart (Sample: solvent blank with added ^{37}Cl TCDD).

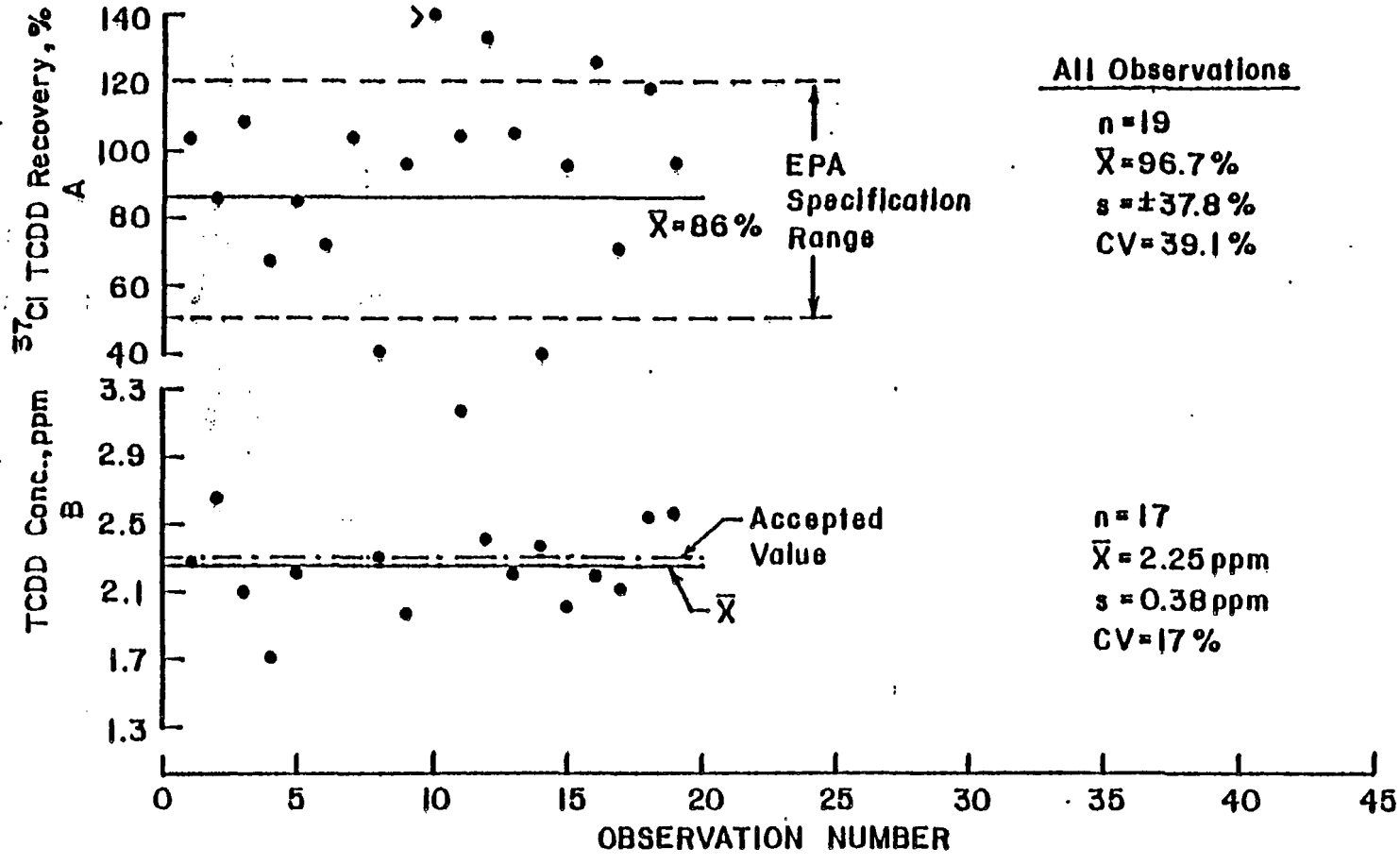


Figure 4. Accuracy quality control chart (Sample: EPA #0093).

was shut down and the ion source cleaned. Observation numbers 18-29 and 35-39 show decreased sensitivity prior to source maintenance. Ion current chromatograms for observation 21 are shown in Appendix D. Concomitant with lower sensitivity, runs of 2 to 4 observations (graphs A and C) were observed with regression correlation coefficients <0.980. Nonetheless, graph E shows that only 1 observation out of 41 was outside the arbitrarily chosen ±3 standard deviation limits for 320/322 ion current peak areas. The theoretical ratio of 0.766 (6) compares favorably with the observed mean value ($\bar{X}$ = 0.784, percent difference = + 2.3 percent). The conclusion was that the measurement process (320/322 ratio) was in control for 97.6 percent of the observations is based on Duncan's (7) criteria that define statistical control to be any point within 3 standard deviation limits. No limits were placed on A-D graphs.

Figures 3 and 4 show the method blank (solvent spiked only with ^{37}Cl labeled TCDD) and accuracy (EPA reference pesticide sample #0093) quality control charts with EPA recovery specification range. Mean recovery observations falling within EPA specifications were 81 and 86 percent, respectively. The EPA accepted value for total TCDD concentration in the pesticide reference sample is 2.3 ppm (8). A mean of 2.25 ± 0.38 ppm was obtained, with a coefficient of variation of 17 percent. No TCDD was observed in any method blank.

Several recovery observations were outside the specification range. A run of out-of-specification values (Figure 3, curve A, observations 6-8) was due in part to a partially clogged jet separator. Cleaning the jet separator rectified the problem. Random observations outside specification limits probably reflect overall variance of the total procedure.

A majority of out-of-specification recovery values were on the high side, i.e. >120 percent, probably because the upper one-sided tolerance of the analytical process is only 20 percent above the theoretical recovery value of 100 percent; the corresponding lower one-sided tolerance is 2.5 times as great, i.e., 50 percent.

Criteria for a Positive Sample

Qualitative criteria developed for the detection of TCDD in the pesticide extract matrix were: (1) a recovery of ^{37}Cl TCDD between 50 and 120 percent, inclusive; (2) both m/e 320 and 322 peaks existing at the TCDD retention time region; (3) well defined m/e 320 and 322 peaks whose retention time exactly equals that of TCDD standards; (4) signal-to-noise ratio of at least 2.5/1 at m/e 322; (5) ratio of m/e 320/322 peak areas in the proper isotopic proportion; and (6) TCDD confirmation by molecular ion spectra. Data for the first five requirements were obtained by selective ion monitoring at m/e 328, 322, and 320; data for TCDD confirmation were generated by recording spectra in the m/e range 150-350 and computer tabulation of absolute abundances of 119-131 m/e. The range of interest was m/e 320-328; the wide range facilitated background measurement. Pertinent information explaining these binary (positive/negative) decision making processes is shown in Table 1.

Criterion #1 was based solely on recovery specifications in the contract. Criteria 5 and 6 incorporate variance associated with the measurement system. Statistical limits imposed are shown in Table 1. The theoretical isotopic abundance ratio of m/e 320/322/324/326 relative to 322 is 0.766/1.000/0.489/0.001 (6). Criterion #6 was divided into three subcriteria (Table 1); eight binary permutations of the subcriteria are possible as shown in Table 2. Overall criterion #6 test characteristic was arbitrarily chosen from the three subcriteria. The rational for subcriteria decision making correlates with isotopic abundance ratios. Greater emphasis was given to 6a (m/e 320/322) since these two m/e values are the largest. More precise ratio measurements are anticipated with 6a rather than the smaller 324/322 ratio for 6b or 6c. Table 3 shows the evaluation of the certainty of TCDD detection based on summation of qualitative criteria characteristics. Criteria 1 through 4 were absolute or necessary for determining if TCDD was detected except for the D (duplicate), T (triplicate) and Q (quadruplicate) reruns of original samples. Detection limits and mass confirmation spectra were not required for the latter.

Appendices D-I include representative hard copy data for GC/MS instrumental performance and TCDD detection.

Sample Data

A wide variety of pesticide samples were analyzed for total TCDD residues. Submitted EPA samples (unknowns and quality control samples) were analyzed on a blind basis. The two sample types were visually and experimentally indistinguishable. EPA provided decoding information only after completion of the assays and reporting of the TCDD data. Table 4 summarizes the decoding information. Generally, a 6-digit sample code represents a series of pesticide samples of the same generic class; individual samples were denoted by a hyphenated (in some cases) suffix made up of numbers and/or letters. For example, 137832-5D relates to the duplicate determination of the fifth subsample in the chlorinated phenolic antiseptic 137832 series. Each subsample represents a different batch of the manufactured technical grade pesticides.

Based on EPA decoding information, replicate sample data, if available, were collated according to sample batch number. After first combining replicate information with the appropriate first-run results, a TCDD detection judgement was made for each batch sample, regardless of the number of replicates assayed. These judgements were either positive, negative, or doubtful. For example, if the first run (I) sample of batch number 1234 was positive (e.g., 1234I is +), the duplicate (D) run negative (1234D is -), and the triplicate (T) run questionable (1234T is doubtful), then the judgement was that TCDD detection was doubtful in sample 1234. On the other hand, if a sample batch were judged TCDD positive, regardless of how many replicates were run, total TCDD level found was reported, including replicate data and the average value, if appropriate.

A total of 69 authentic pesticide batch samples were assayed. TCDD was detected in 17 batch samples (25 percent); TCDD detection was doubtful in 6 samples (9 percent); and TCDD was not detected in 46 batch samples (66

percent). Tables 5-12 summarize data for TCDD in various types of authentic pesticide samples. In those instances where all or most batch samples were positive for a given series, tabulation of mean value, standard deviation, and coefficient of variation is based on positive sample data. NA (not applicable) refers to information not available or not requested. For example, qualitative detection criteria #4 and #6 were not required for -D, -T, or -Q samples. Data for EPA quality controls are shown in Table 13.

Variation of TCDD detection judgements versus EPA sample type is shown in Table 14. For the authentic batch samples TCDD was nondetectable (detection limit 10 ppb) in 100 percent of the 2,4,5-trichlorophenol samples analyzed (n, number of batch samples assayed = 16). For comparative purposes, batch samples of 2,4,5-T, the butoxyethyl ester of 2,4,5-T, and the isooctyl ester of 2,4,5-T are grouped under 2,4,5-T as shown below.

2,4,5-T SAMPLE TYPE (16 BATCHES)

Type	Number of Batches	Number of Batches/(percent) Positive	Doubtful	Nondetectable
2,4,5-T	3	1/(33.3)	1/(33.3)	1/(33.3)
butoxyethyl ester of 2,4,5-T	10	9/(90)	1/(10)	0
isooctyl ester of 2,4,5-T	3	0	3/(100)	0
	16	10/(63)	5/(31)	1(6)

Overall, detection of TCDD was positive in 63 percent (n=10) of the batch samples, doubtful in 31 percent (n=5), and nondetectable in 6 percent (n=1). Specifically, detection of total TCDD was positive in 90 percent (n=9) of the butoxyethyl ester of 2,4,5-T batch samples. In contrast, detection of TCDD was judged equally positive, doubtful and nondetectable in all (n=3) of the 2,4,5-T batch samples, and TCDD detection was questionable in all (n=3) of the isooctyl ester of 2,4,5-T batch samples.

Eight Silvex type batch samples were assayed. TCDD detection was positive in 86 percent (n=6) of the butoxypropyl ester of Silvex and questionable in the remaining sample (n=1) of this type. Only one batch of isooctyl ester of Silvex was assayed; the TCDD detection judgement was positive. One-hundred percent of both the chlorinated phenolic antiseptic (n=22) and the organophosphate (n=7) batch samples gave negative TCDD detection characteristics.

Summarizing the TCDD detection judgements for authentic pesticide batch samples in terms of decreasing percentages, 66 percent were nondetectable, 25 percent positive, and 9 percent doubtful. Likewise, judgements for the 20 different EPA QC samples analyzed were 40 percent nondetectable, 30 percent doubtful, and 30 percent positive.

Variation of total TCDD level found versus sample type for positive samples is shown in Table 15. All authentic pesticide batch samples for which TCDD detection was judged positive contained total TCDD in the range 10-30 ppb. By contrast, total TCDD content of EPA quality controls varied from a low range of 31-99 ppb to a high of 3000-3499 ppb.

Precision of TCDD determinations in the EPA quality controls is shown in Table 16. Relative standard deviation ranged from 2.6 percent to 83 percent. Standard deviation range for authentic unknown replications was 3.1 percent to 52 percent, as shown in Table 17.

The prevalence of doubtful TCDD detection judgements in authentic pesticide samples and EPA quality controls suggests the need for improvements in the measurement system. A significant number of doubtful detection instances are related to apparent TCDD levels <10 ppb; in addition, TCDD confirmation was not a contract requirement at these low levels. Mean detection limit was 4.7 ppb $\pm$ 3.5 ppb (standard deviation). Mean value plus two standard deviations = 11.7 ppb; thus questionable TCDD detection at the 10 ppb level can be expected. Therefore, any result found below 10 ppb in authentic samples was reported as nondetectable (ND). Actual values below 10 ppb in the quality controls are tabulated in Table 13 to demonstrate the inaccuracy in reporting such data. The qualitative criteria were used to define the judgement below 10 ppb (i.e., positive, doubtful, or negative).

Problems encountered in the project - low and high recoveries, unreliability of standards, inability to distinguish matrix problems from instrumental ones, variability in machine sensitivity, changes in chromatography, and interferences - contributed to observable variations in relative standard deviation and detection limits. Extracts yielded interferences of varying severity from other substances depending upon the efficiency and reproducibility of the cleanup procedure and sample type. Varying interferences also affected ^{37}Cl TCDD recovery. For example, excellent recoveries were obtained for samples 148921-3, 148921-3D, and 148921-4D. Zero percent recoveries were obtained with 148921-3T and 148921-4T. Internal quality control samples in the same sample sets (pertaining to extraction) gave excellent recoveries.

Low recoveries resulting from interferences might be prevented by employing a second Al_2O_3 cleanup column or other modification in the extraction procedure. Recoveries higher than the theoretical value were anticipated when m/e 328 peaks co-eluted with ^{37}Cl-TCDD.

Interfering m/e 320 and 322 peaks co-eluting with TCDD contributed to variability in calculated TCDD levels. In some instances peaks eluting prior to or immediately after TCDD prevented accurate peak interpretation.

Application of high resolution gas chromatography should help to overcome problems with interferences.

Problems with standard reliability included irreproducibility of lots and concentration upon standing. TCDD standards were provided by EPA and lots were checked against each other to identify suspect solutions. TCDD standards were provided in benzene solvent. Aliquots of the standards were transferred to small vials sealed with Teflon-lined rubber septums. Standards were withdrawn with a 10 µl microsyringe. Repeated insertions of the microsyringe needle (four insertions were required per sample assay) resulted in formation of holes in the septum. Benzene evaporated through the porous septum and concentration of the TCDD standard increased. An attempt was made to solve the problem by adding a small amount of mercury to the standard vials to seal punctures in the septum. Between injections, vials were stored in an inverted position which prevented benzene evaporation. However, after a few days the solution turned cloudly, indicating instability of the standard solutions. The problem was rectified by using Mininert vials as outlined in the experimental section.

Variability in machine sensitivity contributed to variable TCDD detection limits and regression correlation coefficients. Approximately one hour was required to complete the four GC/MS runs needed to construct the regression line. Drift in GC/MS sensitivity and specificity over this period contributed to a poorer correlation coefficient with increased variability in reported TCDD recovery and quantification.

Distinguishing matrix problems from instrumental ones was difficult. Replicate recovery assays of the same extract provided data to elucidate problems. Poor precision was indicative of instrumental problems. High precision combined with recoveries outside the specification range suggested matrix or extraction problems.

Total TCDD was judged nondetectable in over 50 percent of the authentic pesticide batch samples assayed. Extending the lower working range of the measurement system seems desirable. This task might be accomplished by further cleanup of the extract, evaporation of the extract to a smaller volume, and employing high efficiency gas chromatography/mass spectrometry.

Application of the more tedious standard addition technique to these analyses appears justified over external calibration. Variations in the linear regression slope were observed for some samples, which may be indicative of matrix effects. The standard addition technique also tends to compensate for instrument drift. Therefore, more accurate data would be anticipated with the standard addition approach than with external working curve techniques.

REFERENCES

1. Blair, E.H. Chlorodoxins—Origin and Fate. Preface, Advances in Chemistry Series #120, American Chemical Society, Washington, D.C., 1973. p. 141.

2. Schwetz, B.A., J.M. Norris, G.L. Sparschu, V.K. Rowe, P.J. Gehring, J.L. Emerson, and C.G. Gerbig. Toxicology of Chlorinated Dibenzo-Para-Dioxins. Environ. Health Perspect. 5:87-99, 1973.

3. IARC (WHO). Some Fumigants, the Herbicides 2,4-D and 2,4,5-T, Chlorinated Dibenzodioxins and Miscellaneous Industrial Chemicals. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man, 15, 1977. 354 pp.

4. Rappe, C., H.R. Buser, and H.P. Bosshardt. Identification and Quantification of Poly-Chlorinated Dibenzo-p-Dioxins (PCDDs) and Dibenzo-Ferans (PCDFs) in 2,4,5-T-Ester Formulations and Herbicide Orange. Chemosphere, 7(5): 431-438, 1978.

5. Firestone, D. The 2,3,7,8-Tetrachlorodibenzo-p-Dioxin Problem: A Review. In: Ecological Bulletins (Stockholm), 27:39-52, 1978.

6. Beynon, J.H. Mass Spectrometry and its Applications to Organic Chemistry. D. Van Nostrand Co., Princeton, N.J. 1960. 640 pp.

7. Duncan, A.J. Quality Control and Industrial Statistics, 4th ed., Richard D. Irwin, Inc., Homewood, Ill., 1974. p. 207.

8. Personal Communication, Mr. Ronald Thomas, Environmental Protection Agency, Beltsville, Md., 1978.

TABLE 1. QUALITATIVE CRITERIA EXPLANATION

No.	Criteria	Explanation
1	$50\% \leq R \leq 120\%$	If initial R value exceeds specifications backup extract is assayed. If R still out-of-specification, sample is extracted and assayed one additional time.
2	existence of both m/e 320 and m/e 322	If only one of two peaks exists, sample listed as ND, not detected.
3	well defined m/e 320 and 322 peaks at TCDD retention time	A change in slope from negative to positive if the TCDD peak is a shoulder on a contaminating peak.
4	m/e 322 integrated peak area at least 2.5 times greater than baseline integrated noise	If less than 2.5, sample listed as ND, not detected
5	m/e 320/322 isotopic ratio, selected ion monitoring	Control limits set by mean value $\pm$ 3 standard deviations as determined by quality control data obtained with EPA reference pesticide sample #0093.
6	m/e range 320-326, absolute abundances, molecular ion spectra	Divided into three subcriteria: (a) m/e 320/322 control limits set by mean value $\pm$ 3 standard deviations as determined by quality control data obtained with EPA reference pesticide sample #0093. (b) m/e 324/322 control limits set by mean value $\pm$ 3 standard deviations as determined by quality control data obtained with EPA reference pesticide sample #0093. (c) Existence of m/e 320, 322, 324, and 326 in absolute abundance table.

TABLE 2. DEPENDENCE OF CRITERION #6 OVERALL BINARY CHARACTERISTIC ON SUBCRITERIA RESULTS

Overall Criterion #6 Characteristic	Permutation 6a	6b	6c
- (negative)	-	-	-
	-	+	-
	-	-	+
	-	+	+
+ (positive)	+	+	+
	+	+	-
	+	-	+
	+	-	-

TABLE 3. TCDD DETECTION JUDGEMENT BASED ON QUALITATIVE CRITERIA CHARACTERISTICS

Qualitative Criteria No.						Judgement
1	2	3	4	5	6	
For First-Run Samples:						
+	+	+	+	+	+	TCDD detected
+	+	+	+	-	+	
+	+	+	+	-	-	TCDD detection questionable
+	+	+	+	+	-	
Any or all negative[a]				NA[b]	NA	TCDD not detected
For Duplicate (-D), Triplicate (-T), or Quadruplicate (-Q) Samples:						
+	+	+	NA	+	NA	TCDD detected if also detected in first-run sample
						TCDD detection questionable if first-run sample also questionable or not detected.
+	+	+	NA	-	NA	TCDD detection questionable regardless of first-run judgement
Any or all negative (-)			NA	±	NA	TCDD not detected regardless of first-run judgement

[a]Qualitative criteria 1 through 4 all absolute or necessary for TCDD detected (positive) judgement.

[b]NA = not applicable.

TABLE 4. VARIOUS EPA SAMPLES ANALYZED FOR TOTAL TCDD

Sample Series	Number of Batch Samples	Pesticide Sample Type (Generic Name)
		AUTHENTIC PESTICIDES
137401	1	2,4,5-T[a]
137402	1	
137403	1	
137832	14	chlorinated phenolic
137834	8	antiseptic
137833	2	2,4,5-trichlorophenol
137835	4	
148917	5	
148918	5	
141996	1	isooctyl ester of 2,4,5-T
141998	2	
141997	1	isooctyl ester of Silvex[b]
148919	10	butoxyethyl ester of 2,4,5-T
148920	7	butoxypropyl ester of Silvex
148921	7	organophosphate insecticide
		QUALITY CONTROLS
229617		
226617		
225511		
222841		
222455		
220777		
93-01		
14-01		
0093		
1-01		
305		
304		
303		
90		
11		
4		
3 W		
3 X		
3 Y		
3 Z		

[a] 2,4,5-T is 2,4,5-trichlorophenoxyacetic acid.

[b] Silvex is 2,4,5-TP

TABLE 5. TCDD DATA FOR AUTHENTIC 2,4,5-TRICHLOROPHENOL (16 Batch Samples)

EPA Series Number	EPA Batch Sample Number	Percent Recovery C137TCDD	Limit of Detection (ppb)	Qualitative Criteria 1	2	3	4	5	6	Judgement Pos.	Dtfl.[a]	ND	Total TCDD (ppb)
137833	-1	95	4.6	+	-	NA	NA	NA	NA			✓	ND
	-2	79	3.5	+	-	NA	NA	NA	NA			✓	ND
137835	-1	102	3.7	+	-	NA	NA	NA	NA			✓	ND
	-2	104	5.0	+	-	NA	NA	NA	NA			✓	ND
	-3	82	9.3	+	-	NA	NA	NA	NA			✓	ND
	-4	89	2.1	+	-	NA	NA	NA	NA			✓	ND
148917	-1	89	2.6	+	-	NA	NA	NA	NA			✓	ND
	-2	69	1.9	+	+	+	+	+	NA			✓	ND
	-3	100	2.5	+	-	NA	NA	NA	NA			✓	ND
	-4	80	8.8	+	-	NA	NA	NA	NA			✓	ND
	-5	86	2.5	+	-	NA	NA	NA	NA			✓	ND
148918	-1	51	1.8	+	+	+	+	+	NA			✓	ND
	-2	93	5.6	+	-	NA	NA	NA	NA			✓	ND
	-3	96	2.6	+	-	NA	NA	NA	NA			✓	ND
	-4	95	5.0	+	-	NA	NA	NA	NA			✓	ND
	-5	61	1.7	+	-	NA	NA	NA	NA			✓	ND

[a] Dtfl. = Doubtful

TABLE 6. TCDD DATA FOR AUTHENTIC 2,4,5-T (2,4,5-TRICHLOROPHENOXYACETIC ACID)
(3 Batch Samples)

EPA Series Number	EPA Batch Sample Number	Percent Recovery C137TCDD	Limit of Detection (ppb)	Qualitative Criteria 1	2	3	4	5	6	Judgement Pos.	Dtfl.	ND	Total TCDD (ppb)	Average Total TCDD (ppb)
137400	-137401	71	2.4	+	+	+	+	+	NA			✓	ND	
	-137402									✓				18
	-137402I	68	5.2	+	+	+	+	-	+	✓			17	
	-137402D	105	NA	+	+	+	NA	+	NA	✓			18	
	-137403										✓			Dtfl.
	-137403I	71	4.2	+	+	+	+	+	+	✓[a]			14	
	-137403D	58	NA	+	-	NA	NA	NA	NA			✓	ND	

[a]Probably false positive since the duplicate sample (137403D) was ND.

TABLE 7. TCDD DATA FOR AUTHENTIC BUTOXYETHYL ESTER OF 2,4,5-T
(10 Batch Samples)

EPA Series Number	EPA Batch Sample Number	Percent Recovery C137TCDD	Limit of Detection (ppb)	Qualitative Criteria 1	2	3	4	5	6	Judgement Pos.	Dtfl.	ND	Total TCDD (ppb)	Average Total TCDD (ppb)
148919	-1													21
	-1I	80	6.8	+	+	+	+	+	+	✓			23	
	-1D	114	NA	+	+	+	NA	+	NA	✓			19	
	-2									✓				23
	-2I	67	2.5	+	+	+	+	+	+	✓			25	
	-2D	99	NA	+	+	+	NA	+	NA	✓			21	
	-3	104	8.0	+	+	+	+	+	+	✓			24	
	-4	82	6.4	+	+	+	+	+	+	✓			29	
	-5									✓				23
	-5I	89	4.3	+	+	+	+	+	+	✓			22	
	-5D	114	NA	+	+	+	NA	+	NA	✓			23	
	-6									✓				25
	-6I	108	3.0	+	+	+	+	+	+	✓			28	
	-6D	78	NA	+	+	+	NA	+	NA	✓			22	
	-6T	72	1.1	+	+	+	+	+	+	✓			21	
	-7										✓			Dtfl.
	-7I	53	2.0	+	+	+	+	+	-		✓		33	
	-7D	111	NA	+	+	+	NA	+	NA		✓		19	
	-8									✓				22
	-8I	117	1.8	+	+	+	+	+	+	✓			14	
	-8D	116	NA	+	+	+	NA	+	NA	✓			30	
	-9									✓				22
	-9I	97	3.1	+	+	+	+	+	+	✓			23	
	-9D	70	NA	+	+	+	NA	+	NA	✓			23	
	-9T	99	4.5	+	+	+	+	+	+	✓			19	
	-10									✓				23
	-10I	61	3.5	+	+	+	+	+	+	✓			27	
	-10D	104	NA	+	+	+	NA	+	NA	✓			19	

Statistical information for TCDD positive batch samples:		
	n	7
	mean total TCDD (ppb)	22.7
	standard deviation (ppb)	1.3
	coefficient of variation (%)	5.7

TABLE 8. TCDD DATA FOR AUTHENTIC ISOOCTYL ESTER OF 2,4,5-T
(3 Batch Samples)

EPA Series Number	EPA Batch Sample Number	Percent Recovery C137TCDD	Limit of Detection (ppb)	Qualitative Criteria 1	2	3	4	5	6	Judgement Pos.	Dtfl.	ND	Total TCDD (ppb)	Average Total TCDD (ppb)
141996														Dtfl.
	-141996I	103	2.0	+	+	+	+	-	+		✓		15	
	-141996D	61	NA	+	+	+	NA	+	NA		✓		30	
	-141996T	78	5.9	+	+	+	+	+	+	✓[a]			21	
	-141996Q	103	NA	+	+	+	NA	-	NA		✓		27	
141998														
	-1										✓			Dtfl.
	-1I	110	2.5	+	+	+	+	+	+	✓			28	
	-1D	73	NA	+	+	+	NA	-	NA		✓		13	
	-2										✓			Dtfl.
	-2I	108	2.2	+	-	NA	NA	NA	NA			✓	ND	
	-2D	79	1.5	+	+	+	+	+	+	✓[a]			27	

[a]Other replicate(s) doubtful or nondetectable, therefore, probably not dioxin-contaminant or interferences present.

TABLE 9. TCDD DATA FOR AUTHENTIC BUTOXYPROPYL ESTER OF SILVEX
(7 Batch Samples)

EPA Series Number	EPA Batch Sample Number	Percent Recovery C137TCDD	Limit of Detection (ppb)	Qualitative Criteria 1	2	3	4	5	6	Judgement Pos.	Dtfl.	ND	Total TCDD (ppb)	Average Total TCDD (ppb)
148920														
	-1	71	4.4	+	+	+	+	+	+	✓			21	
	-2									✓				19
	-2I	83	2.3	+	+	+	+	+	+	✓			19	
	-2D	115	NA	+	+	+	NA	+	NA	✓			17	
	-2T	83	6.3	+	+	+	+	+	+	✓			22	
	-3									✓				19
	-3I	66	5.5	+	+	+	+	+	+	✓			21	
	-3D	77	NA	+	+	+	NA	+	NA	✓			17	
	-4										✓			Dtfl.
	-4I	67	1.0	+	+	+	+	-	NA		✓		20	
	-4D	113	NA	+	+	+	NA	+	NA		✓		20	
	-5									✓				21
	-5I	97	5.2	+	+	+	+	+	+	✓			24	
	-5D	79	NA	+	+	+	NA	+	NA	✓			17	
	-5T	66	7.2	+	+	+	+	-	+	✓			23	
	-6	103	0.9	+	+	+	+	+	+	✓			12	
	-7									✓				22
	-7I	74	5.6	+	+	+	+	+	+	✓			22	
	-7D	111	NA	+	+	+	NA	+	NA	✓			21	

Statistical information for TCDD positive batch samples:

n	4
Mean total TCDD (ppb)	20.3
Standard deviation (ppb)	1.5
Coefficient of variation (%)	7.4

TABLE 10. TCDD DATA FOR AUTHENTIC ISOOCTYL ESTER OF SILVEX
(1 Batch Sample)

EPA Series Number	EPA Batch Sample Number	Percent Recovery C137TCDD	Limit of Detection (ppb)	Qualitative Criteria 1	2	3	4	5	6	Judgement Pos.	Dtfl.	ND	Total TCDD (ppb)
141997	141997	81	9.7	+	+	+	+	+	+	✓			20

TABLE 11. TCDD DATA FOR AUTHENTIC CHLORINATED PHENOLIC ANTISEPTIC
(22 Batch Samples)

EPA Series Number	EPA Batch Sample Number	Percent Recovery C137TCDD	Limit of Detection (ppb)	Qualitative Criteria 1	2	3	4	5	6	Judgement Pos.	Dtfl.	ND	Total TCDD (ppb)
137832													
	-1	83	16	+	-	NA	NA	NA	NA			✓	ND
	-2	77	12	+	-	NA	NA	NA	NA			✓	ND
	-3											✓	ND
	-3I	102	8.9	+	-	NA	NA	NA	NA			✓	
	-3D	68	NA	+	-	NA	NA	NA	NA			✓	
	-4											✓	ND
	-4I	50	1.8	+	-	-	-	-	NA			✓	
	-4D	61	NA	+	-	NA	NA	NA	NA			✓	
	-5											✓	ND
	-5I	91	4.0	+	-	NA	NA	NA	NA			✓	
	-5D	79	NA	+	-	NA	NA	NA	NA			✓	
	-6	90	3.9	+	-	NA	NA	NA	NA			✓	ND
	-7	84	9.4	+	-	NA	NA	NA	NA			✓	ND
	-8	93	2.2	+	-	NA	NA	NA	NA			✓	ND
	-9	68	8.4	+	-	NA	NA	NA	NA			✓	ND
	-10	64	3.6	+	-	NA	NA	NA	NA			✓	ND
	-11	78	2.3	+	-	NA	NA	NA	NA			✓	ND
	-12	74	4.4	+	-	NA	NA	NA	NA			✓	ND
	-13	96	2.7	+	-	NA	NA	NA	NA			✓	ND
	-14	68	2.6	+	-	NA	NA	NA	NA			✓	ND
137834													
	-1	93	7.1	+	-	NA	NA	NA	NA			✓	ND
	-2	111	2.4	+	-	NA	NA	NA	NA			✓	ND
	-3	91	1.9	+	-	NA	NA	NA	NA			✓	ND
	-4	78	3.0	+	-	NA	NA	NA	NA			✓	ND
	-5	100	1.7	+	-	NA	NA	NA	NA			✓	ND
	-6	93	2.3	+	-	NA	NA	NA	NA			✓	ND
	-7	117	4.3	+	-	NA	NA	NA	NA			✓	ND
	-8	102	1.9	+	-	NA	NA	NA	NA			✓	ND

TABLE 12. TCDD DATA FOR AUTHENTIC ORGANOPHOSPHATE
(7 Batch Samples)

EPA Series Number	EPA Batch Sample Number	Percent Recovery C137TCDD	Limit of Detection (ppb)	Qualitative Criteria 1	2	3	4	5	6	Judgement Pos.	Dtfl.	ND	Total TCDD (ppb)
148921													
	-1	51	9.9	+	-	NA	NA	NA	NA			✓	ND
	-2	110	2.9	+	-	NA	NA	NA	NA			✓	ND
	-3											✓	ND
	-3I	100	5.2	+	-	NA	NA	NA	NA			✓	
	-3D	100	NA	+	-	NA	NA	NA	NA			✓	
	-3T	0	NA	-	-	NA	NA	NA	NA			✓	
	-4											✓	ND
	-4I	111	2.2	+	-	NA	NA	NA	NA			✓	
	-4D[a]	95	NA	+	+	+	NA	+	NA		✓		
	-4T	0	NA	-	-	NA	NA	NA	NA			✓	
	-5	78	1.5	+	-	NA	NA	NA	NA			✓	ND
	-6	69	4.5	+	-	NA	NA	NA	NA			✓	ND
	-7	93	9.3	+	-	NA	NA	NA	NA			✓	ND

[a]Probably false positive due to contamination or interferences present, since replicate samples were ND.

TABLE 13. TCDD DATA FOR QUALITY CONTROLS
(20 Different QC Samples; 41 Assays)

EPA QC Sample Number	Percent Recovery Cl37TCDD	Limit of Detection (ppb)	Qualitative Criteria 1	2	3	4	5	6	Judgement Pos.	Defl[a]	ND	Total TCDD (ppb)	Average Total TCDD (ppb)
222455											✓		ND
222455I	92	3.1	+	+	+	+	+	+	✓			3.6	
222455D	76	NA	+	-	NA	NA	NA	NA			✓	ND	
220777										✓			Defl.
220777I	71	2.5	+	+	+	+	+	+	✓			39	
220777D	119	NA	+	+	+	NA	+	NA	✓			45	
226617										✓			Defl.
226617I	95	4.9	+	+	+	+	+	+		✓		23	
226617D	116	NA	+	+	+	NA	+	NA		✓		6	
225511										✓			Defl.
225511I	76	5.9	+	+	+	+	-	+	✓			74	
225511D	77	NA	+	+	+	NA	+	NA	✓			43	
229617											✓		ND
229617I	58	3.6	+	+	-	+	-	NA		✓		7	
229617D	82	NA	+	-	NA	NA	NA	NA			✓	ND	
1-01									✓				212
1-01I	101	12.7	+	+	+	+	+	+	✓			236	
1-01D	61	NA	+	+	+	NA	+	NA	✓			126	
1-01T	94	NA	+	+	+	NA	-	NA	✓			274	
11									✓				81
11I	80	0.7	+	+	+	+	+	+	✓			80	
11D	84	NA	+	+	+	NA	+	NA	✓			83	
11T	100	NA	+	+	+	NA	+	NA	✓			79	
3W	77	9.9	+	-	NA	NA	NA	NA			✓	ND	
3Y											✓		ND
3YI	59	4.9	+	+	-	+	-	+			✓	ND	
3YD	83	NA	+	-	NA	NA	NA	NA			✓	ND	
3X									✓				87
3XI	83	2.4	+	+	+	+	-	+	✓			56	
3XD	92	NA	+	+	+	NA	+	NA	✓			124	
3XT	89	NA	+	+	+	NA	+	NA	✓			82	
93-01	115	5.5	+	+	+	+	+	+	✓			1620	
4									✓				3627
4I	113	13	+	+	+	+	+	+	✓			3270	
4D	90	NA	+	+	+	NA	+	NA	✓			3984	
14-01											✓		ND
14-01I	83	3.1	+	+	+	+	+	NA		✓		5.7	
14-01D	107	NA	+	-	NA	NA	NA	NA			✓	ND	
14-01T	89	NA	+	+	+	NA	+	NA		✓		9.6	
3Z											✓		ND
3ZI	68	2.5	+	-	NA	NA	NA	NA			✓	ND	
3ZD	106	NA	+	-	NA	NA	NA	NA		✓		ND	
222841											✓		ND
222841I	89	2.0	+	+	+	+	+	NA		✓		2.2	
222841D	76	NA	+	-	NA	NA	NA	NA			✓	ND	
0093	97	82	+	+	+	+	+	+	✓			2250	
90										✓			Defl.
90I	88	2.8	+	+	+	+	+	+	✓			85	
90D	60	NA	+	+	+	NA	-	NA		✓		57	
303										✓			Defl.
303I	79	2.1	+	+	+	+	+	-		✓		16	
303D	81	NA	+	+	+	NA	+	NA		✓		18	
304											✓		ND
304I	98	1.2	+	+	+	+	+	+	✓			9.6	
304D	72	NA	+	+	+	NA	+	NA	✓			7.1	
305										✓			Defl.
305I	116	14	+	+	+	+	+	+	✓			1610	
305D	94	NA	+	+	+	NA	+	NA	✓			1732	

[a] Replicate doubtful or ND or other reruns suggest probably not dioxin-contaminant or interferences present.

TABLE 14. VARIATION OF TCDD DETECTION JUDGEMENT VERSUS EPA SAMPLE TYPE

Sample Type	Number Batch Samples Analyzed	Number/(Percent) of Batch Samples Versus Detection Judgement		
		Positive	Doubtful	Nondetectable
		AUTHENTIC PESTICIDE BATCH SAMPLES		
2,4,5-Trichlorophenol	16			16/100
2,4,5-T	3	1/(33.3)	1/(33.3)	1/(33.3)
Butoxyethyl Ester of 2,4,5-T	10	9/(90)	1/(10)	
Isooctyl Ester of 2,4,5-T	3		3/(100)	
Butoxypropyl Ester of Silvex	7	6/(86)	1/(14)	
Isooctyl Ester of Silvex	1	1/(100)		
Chlorinated Phenolic Antiseptic	22			22/(100)
Organophosphate	7			7/(100)
TOTALS	69	17(25)	6/(9)	46/66
		QUALITY CONTROLS		
Twenty different QC Samples Assayed		6/(30)	6/(30)	8/(40)

TABLE 15. VARIATION OF TOTAL TCDD LEVEL VERSUS SAMPLE TYPE FOR POSITIVE SAMPLES

Sample Type	Number Batch Samples Positive for TCDD	TCDD Range (ppb)							
		10-30	31-99	100-499	500-999	1000-1499	1500-1999	2000-2499	2500-2999	3000-3499	3500-3999
		Number Batch Samples/(Percent)									
AUTHENTIC PESTICIDE BATCH SAMPLES											
2,4,5-T	1	1/(18)									
Butoxyethyl Ester of 2,4,5-T	9	9/(100)									
Butoxypropyl Ester of Silvex	6	6/(100)									
Isooctyl Ester of Silvex	1	1/(100)									
QUALITY CONTROLS											
Six different QC Samples Positive for TCDD			2/(32.8)	1/(16.8)			1/(16.8)		1/(16.8)		1/(16.8)

TABLE 16. COMPARISON OF REPLICATE RESULTS FOR EPA QUALITY CONTROL SAMPLES

Sample	Total TCDD Concentration (ppb)			Statistical Analysis		
	Run No. 1	Run No. 2(-D)	Run No. 3(-T)	Average (ppb)	Standard Deviation (ppb)	Relative Standard Deviation (%)
1-01	236	126	274	212	77	36
11	80	83	79	81	2.1	2.6
3Y	ND	ND	NA	NA	NA	NA
3X	56	124	82	87	34	39
4	3270	3984	NA	3627	504	14
14-01	5.7[a]	ND	9.6[a]			
3Z	ND	ND	NA			
222841	2.2[a]	ND	NA			
222455	3.6	ND	NA			
220777	39	45	NA	42	4.2	9.4
226617	23[a]	6[a]	NA	15	12	83
225511	74	43	NA	59	22	37
229617	7[a]	ND	NA			
90	85	57[a]	NA	71	20	28
303	16[a]	18[a]	NA	17	1.4	8.3
304	9.6	17.1	NA	8.4	1.8	21
305	1610	1732	NA	1671	86	5.2

[a]Qualitative criteria judgement: TCDD detection doubtful.

TABLE 17. COMPARISON OF REPLICATE RESULTS FOR AUTHENTIC PESTICIDE BATCH SAMPLES

Type Sample	Batch Sample	Total TCDD Concentration (ppb) Run No. 1	Run No. 2(-D)	Run No. 3(-T)	Run No. 4(-Q)	Statistical Analysis Average (ppb)	Standard Deviation (ppb)	Relative Standard Deviation (%)
2,4,5-T	137402	17	18			18	0.71	4
	137403	14	ND					
Chlorinated Phenolic Antiseptic	137832-3	ND	ND					
	137832-4	ND	ND					
	137832-5	ND	ND					
Isooctyl Ester of 2,4,5-T	141996[a]	15	30	21	27	23	7	30
	141998-1	28	13[a]			21	11	52
Butoxyethyl Ester of 2,4,5-T	148919-1	23	19			21	2.8	14
	148919-2	25	21			23	2.8	13
	148919-5	22	23			23	0.71	3.1
	148919-6	28	22	21		24	3.8	15.8
	148919-7	33[a]	19[a]			26	9.9	38
	148919-8	14	30			22	11	51
	148919-9	23	23	19		22	2.3	10.3
	138919-10	27	19			23	5.7	25
Butoxypropyl Ester of Silvex	148920-2	19	17	22		19	2.5	13.2
	148920-3	21	17			19	2.8	15
	148920-4	20[a]	20[a]			20	-	-
	148920-5	24	17	23		21	3.8	18.1
	148920-7	22	21			22	0.71	3.3
Organophosphate Formulations[b]	148921-3	ND	ND					
	148921-4	ND	14[a]					

[a]Qualitative criteria judgement: TCDD detection doubtful.

[b]A third sample of 148921-3 and 148921-4 was also run but not reported due to a 0 percent recovery.

Appendix A. Data Sheets for Sample 148919-5-$\overline{2}$

ANALYSIS OF TECHNICAL GRADE PESTICIDES FOR TCDD

Data Sheet

Sample No. 148919-5-2 Date Received 2/8/78 Date of GC/MS Analysis 8/2/78
Date and Type of Extraction / Cleanup 7/18/78 / Hexane - NaOH - H_2SO_4 - Al_2O_3
Sample Weight 1.0084g Sample Volume 2 µl Total Extract 200 µl
GC Conditions: Column 3' OV-11, 3% Injector Temperature 250°C Column Temperature 210(3)@
(6)260t
Carrier Gas and Flow Rate UHP He / 32 ml/min

GC/MS Data

µl Co-Injected			Peak Area			
			m/e			Ratio
Sample	^{35}Cl-TCDD	^{37}Cl-TCDD	320	322	328	320/322
2	0	0	269	338	1146	0.796
2	1	2	828	1060	3547	X
2	0	0	219	289	1023	0.758
2	2	1	1436	1906	2471	X
		Corr.(r)=	0.9993	0.9991	0.9971	
		Slope.(s)=	594.1	791.7	1245	
		Intcp.(I)=	241.8	304.4	1112	
		I/s =	0.406	0.385	0.893	
		Avg. Ratio =			0.777	± 0.027

ANALYSIS OF TECHNICAL GRADE PESTICIDES FOR

148919-521-2 TCDD

Results

RECOVERY

^{37}Cl-TCDD Std. Conc.(pg/µl) = 480

Added to Sample(ng) = 48 (I)

Recovered(ng):

i/s x 48 = 42.8 (II)

% Recovery (R)

$R = \frac{II}{I} \times 100 = 89$ %

QUANTIFICATION

^{35}Cl-TCDD Std. Conc.(pg/µl) = 500

	320	322	
Amount in Extract(ng) = i/s x 50	20.3	19.3	(III)
Amount in Sample(ng) = III/R	22.8	21.7	(IV)
Sample Conc.(ppb) = IV/g Sample	22.6	21.5	

Avg.Conc.(ppb) = 22.1 ± 0.78

LIMIT OF DETECTION

N/A _____ A ✓

m/e = 322

L.D., 2.5(S/N) = 4.3 ppb

CONFIRMATION ANALYSIS (Fragmentation Pattern Recorded)

N/A _____ A ✓

Result Positive ✓

Result Negative _____

Appendix B. Master Sample Status Form

Master Sample Status For 328-884-11

Sample	Recovery, %			TCDD Level	Full Scale Configurations		Calculated LD	Sample Completed
	Screening	Repeat	Average	(ppb)	Needed	Done	(ppt)	

Appendix C. Cumulative Tabulated Analytical Data Form

CUMULATIVE
TABULATED ANALYTICAL DATA FOR 328-884-11

Sample	Recovery, %			TCDD Level (ppb)	Confirmation	Calculated LD (ppb)	Sample Completed	
	Screening	Repeat	Average				GSRI	EPA

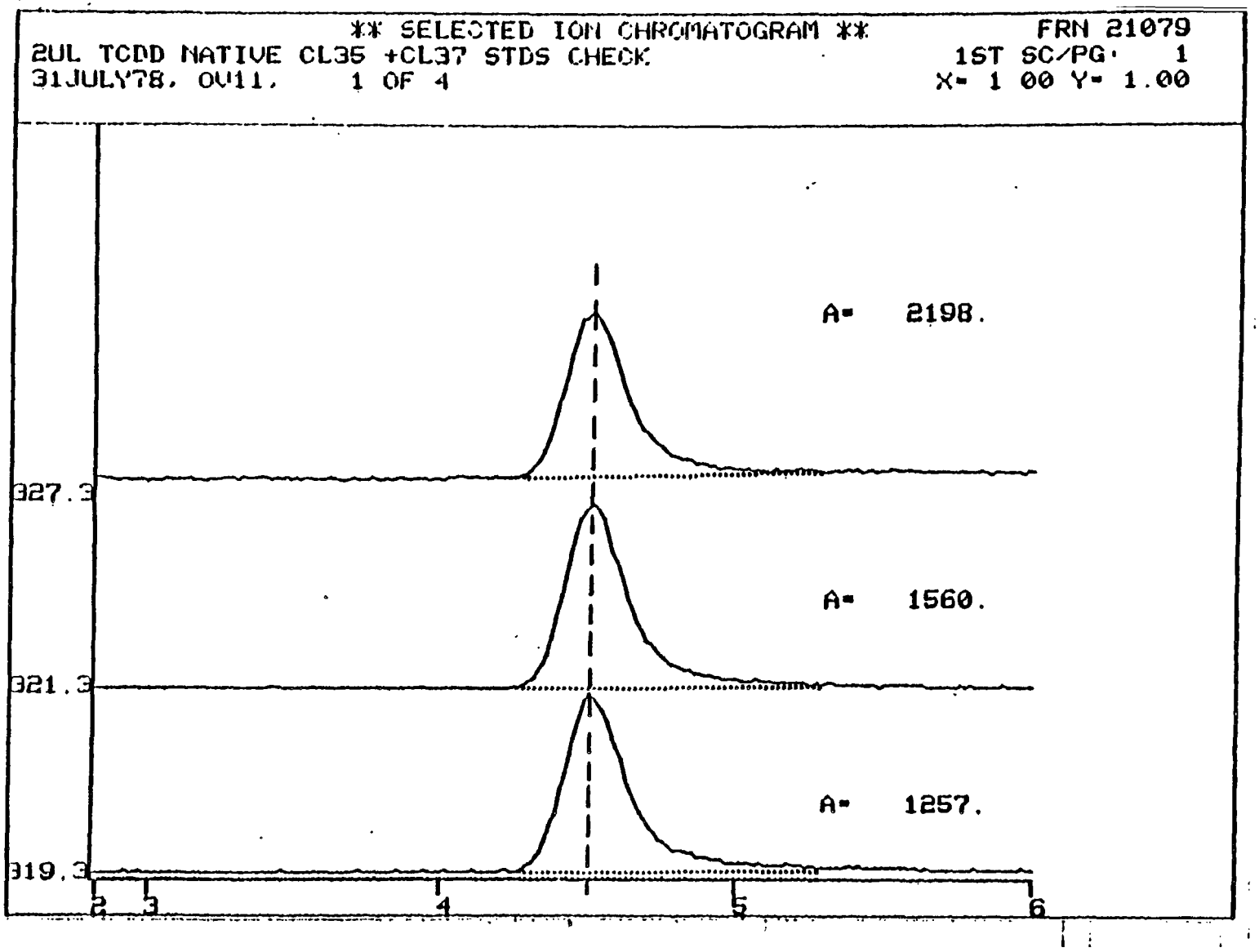
Code: NA – not applicable ND – not detectable * – task performed Page___
√ – sample completed

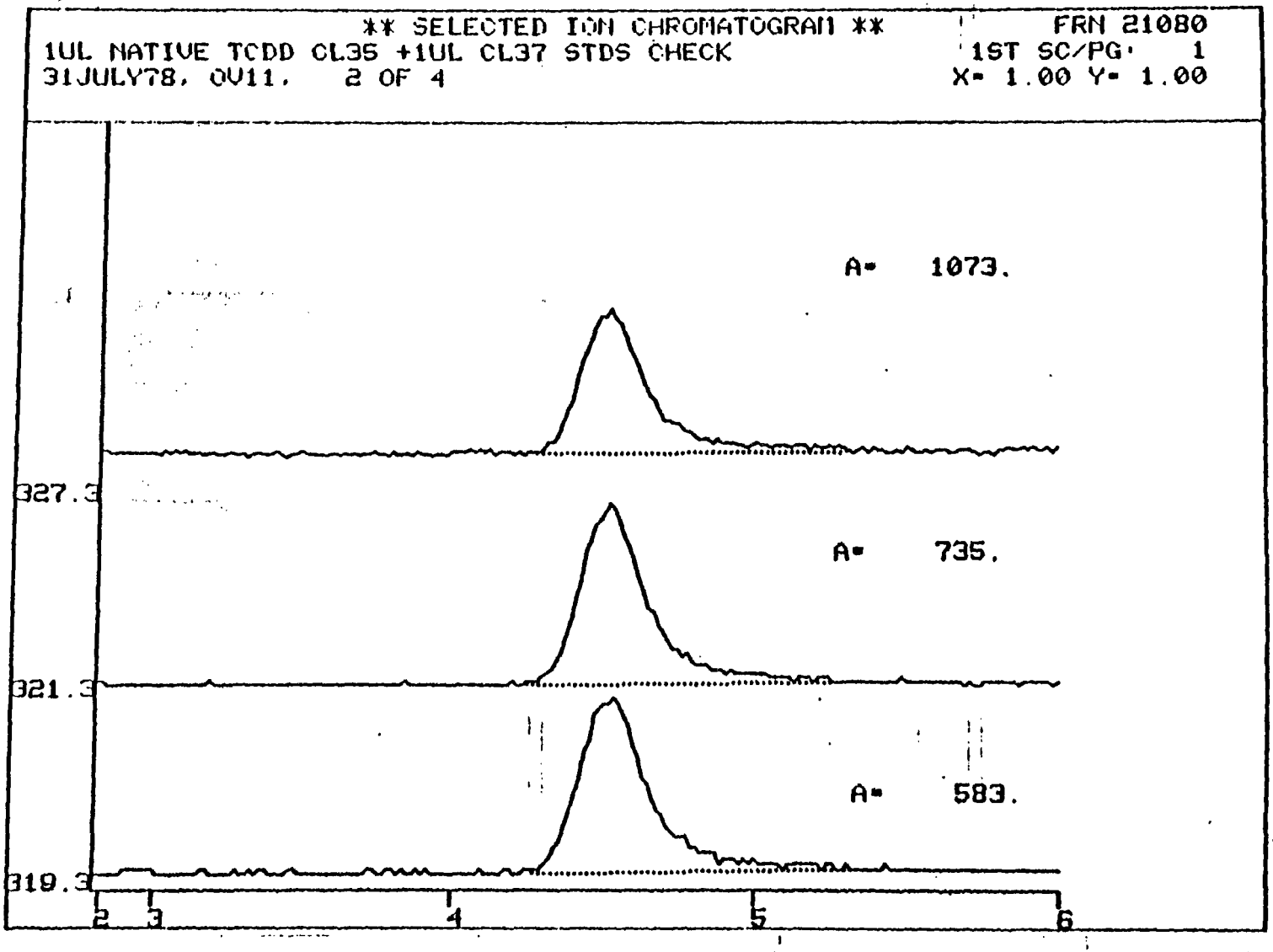
Appendix D. GC/MS Performance Ion Current Chromatograms

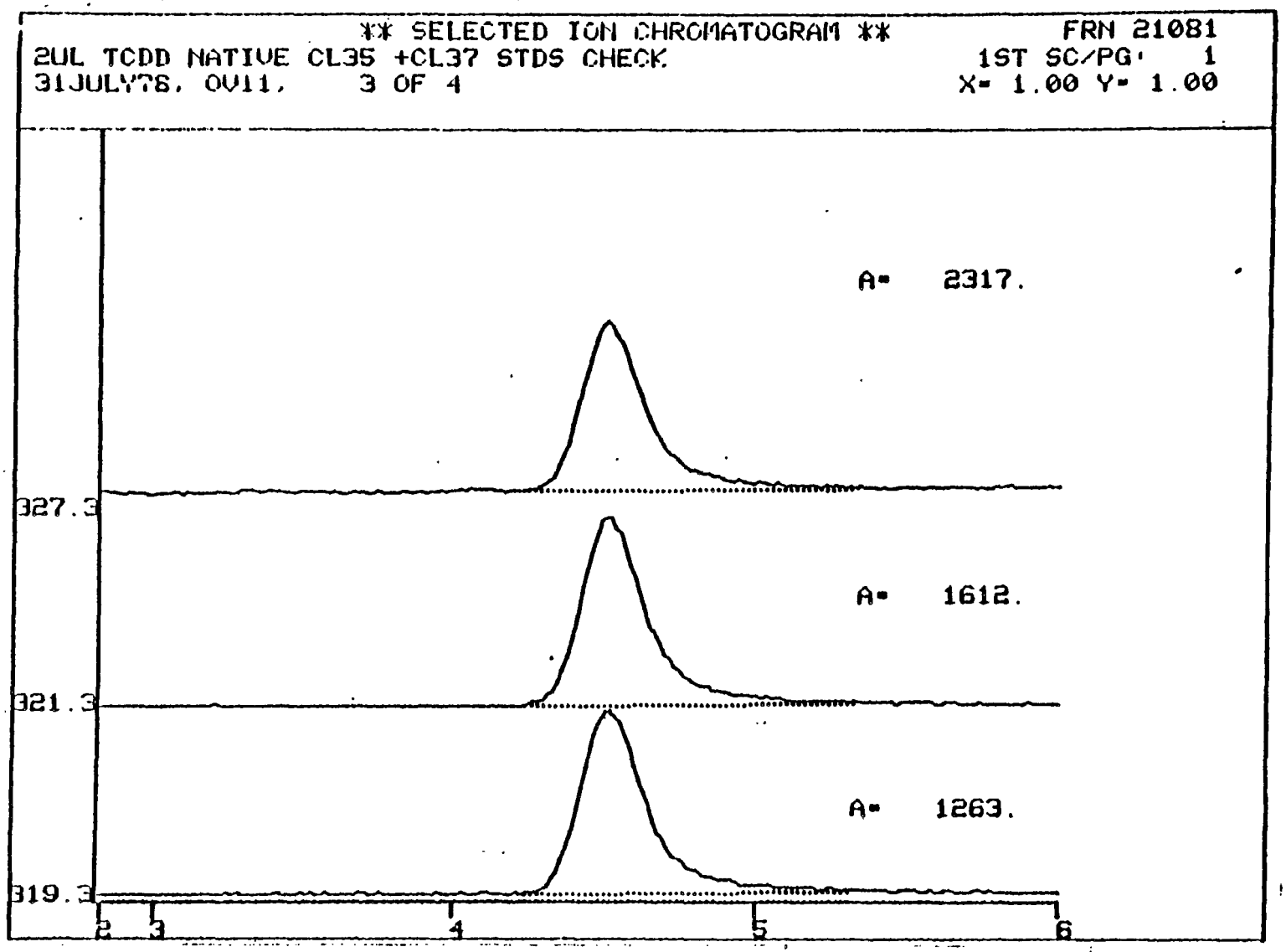
(Observation #21)

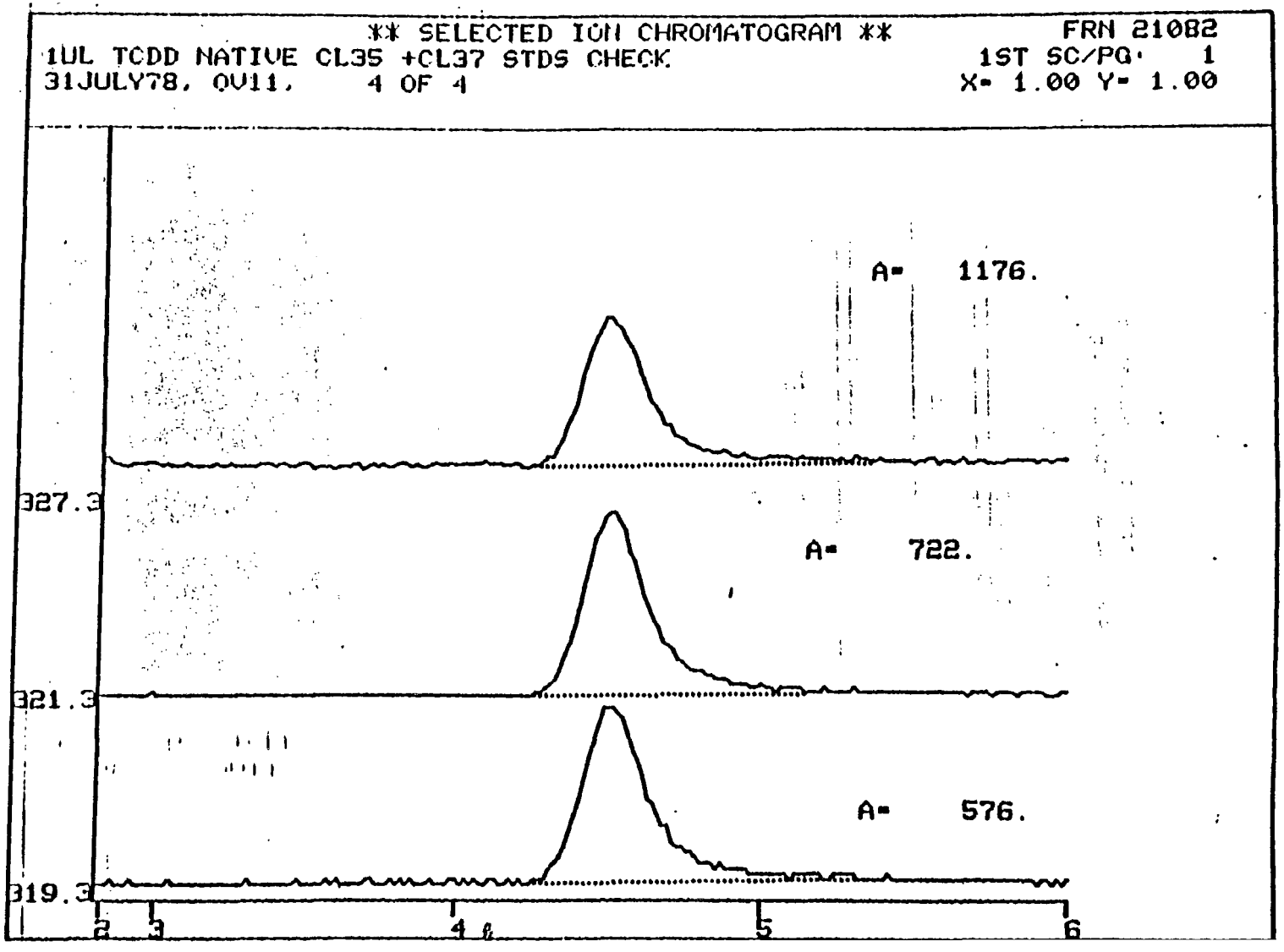
OBSERVATION #21 DATA

ul co-injected			m/e Peak area		
^{35}Cl TCDD		^{37}Cl TCDD	320	322	328
2	+	2	1257	1560	2198
1	+	1	583	735	1073
2	+	2	1203	1612	2317
1	+	1	576	722	1176
	Corr (r)		0.9999	0.9990	0.9952
	Slope (s)		680.5	857.5	1133
	Intercept (i)		-101	-129	-8.5
	s/500 or 480		1.36	1.71	2.36
	Avg. 320/322 = 0.795				


** SELECTED ION CHROMATOGRAM **
FRN 21079
2UL TCDD NATIVE CL35 +CL37 STDS CHECK
1ST SC/PG: 1
31JULY78. 0U11. 1 OF 4
X= 1 00 Y= 1.00
A= 2198.
327.3
A= 1560.
321.3
A= 1257.
319.3
2
3
4
5
6


** SELECTED ION CHROMATOGRAM **
FRN 21080
1UL NATIVE TCDD CL35 +1UL CL37 STDS CHECK
1ST SC/PG: 1
31JULY78, 0V11. 2 OF 4
X= 1.00 Y= 1.00
A= 1073.
327.3
A= 735.
321.3
A= 583.
319.3
2
3
4
5
6


** SELECTED ION CHROMATOGRAM **
FRN 21081
2UL TCDD NATIVE CL35 +CL37 STDS CHECK
1ST SC/PG: 1
31JULY'86. OV11. 3 OF 4
X= 1.00 Y= 1.00
A= 2317.
327.3
A= 1612.
321.3
A= 1263.
319.3
2
3
4
5
6

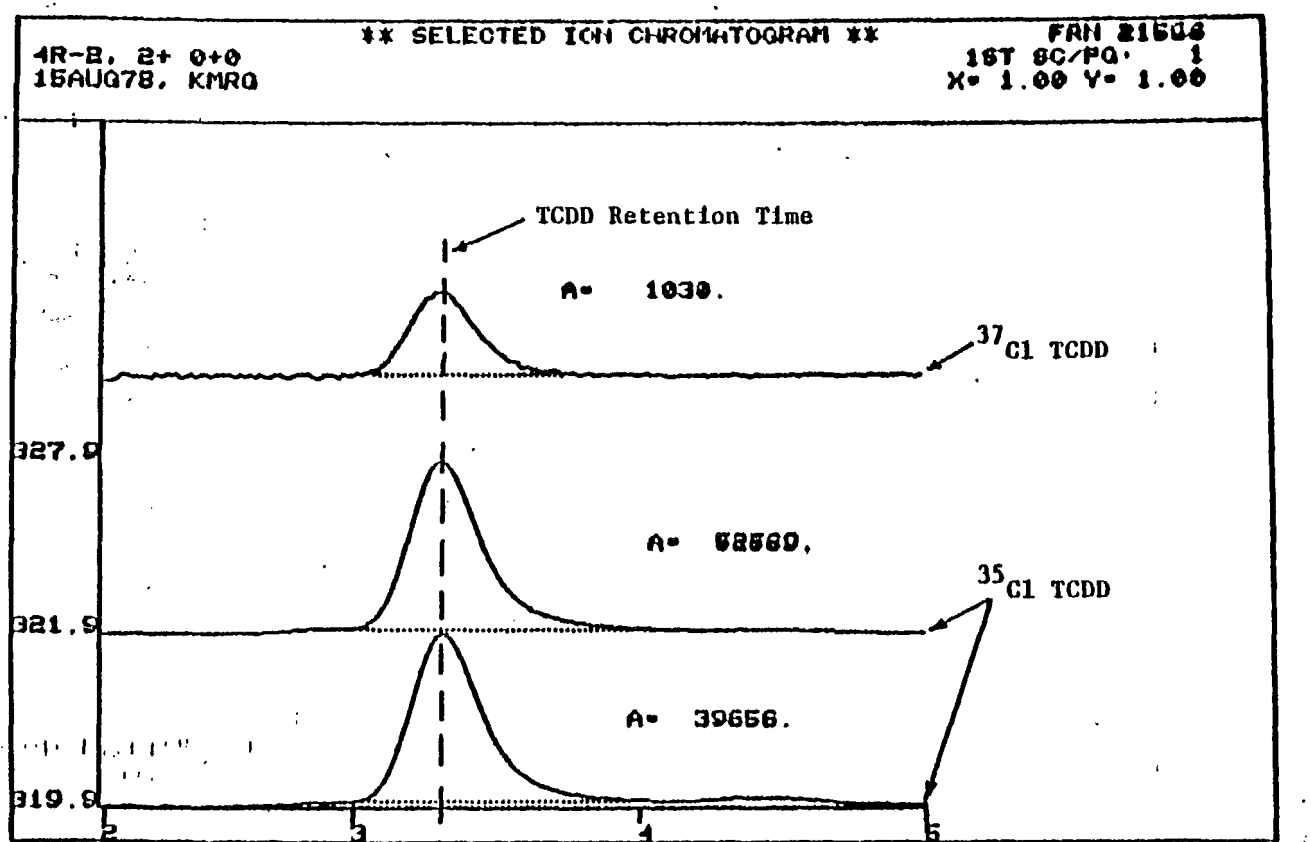

** SELECTED ION CHROMATOGRAM **
FRN 21082
1UL TCDD NATIVE CL35 +CL37 STDS CHECK
1ST SC/PG: 1
31JULY78, QU11. 4 OF 4
X= 1.00 Y= 1.00
A= 1176.
327.3
A= 722.
321.3
A= 576.
319.3
2
3
4
5
6

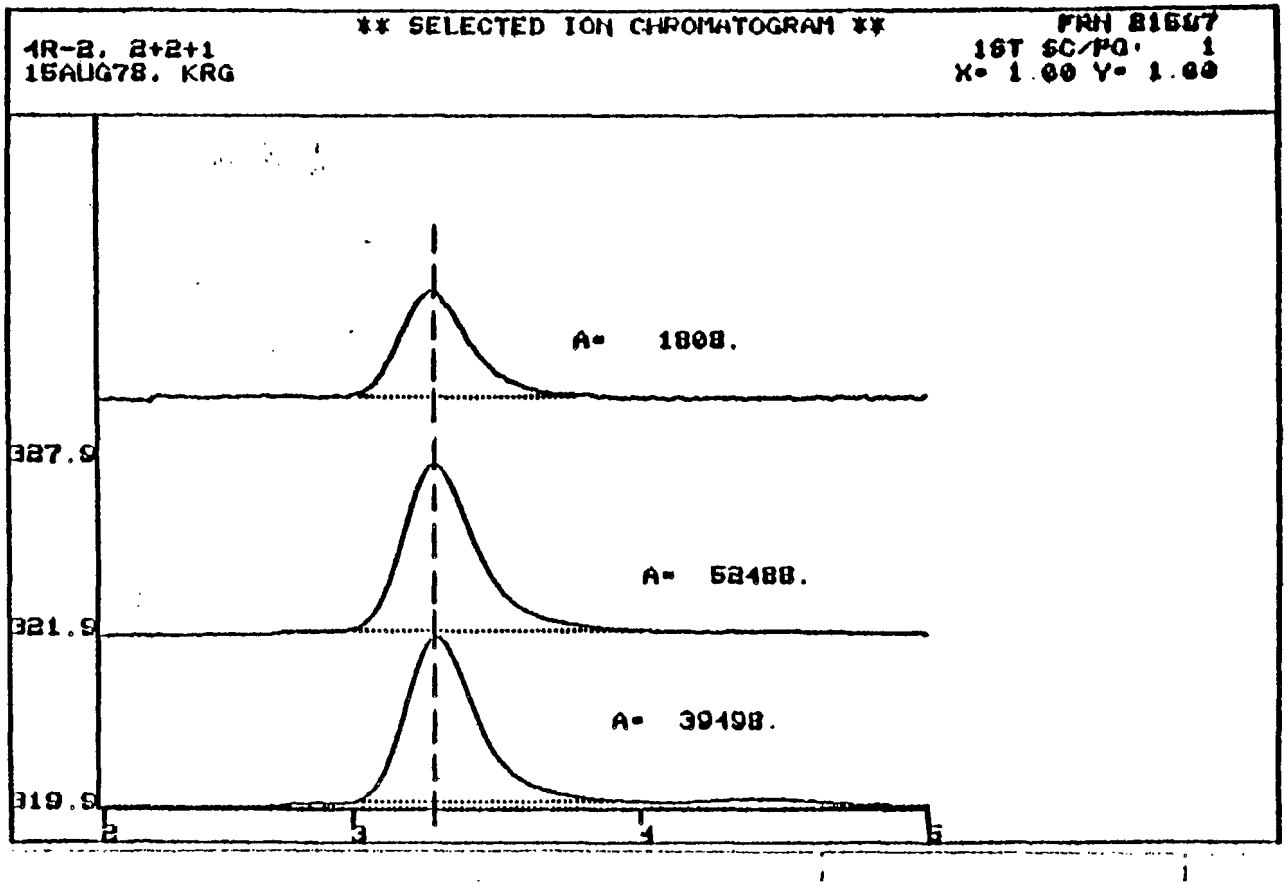
Appendix E. Ion Current Chromatograms for TCDD Detected in a Pesticide Sample

(EPA Sample #4)

QUALITATIVE CRITERIA					
1	2	3	4	5	6
+	+	+	+	+	+

JUDGEMENT: TCDD detected in the sample because all six criteria were positive.

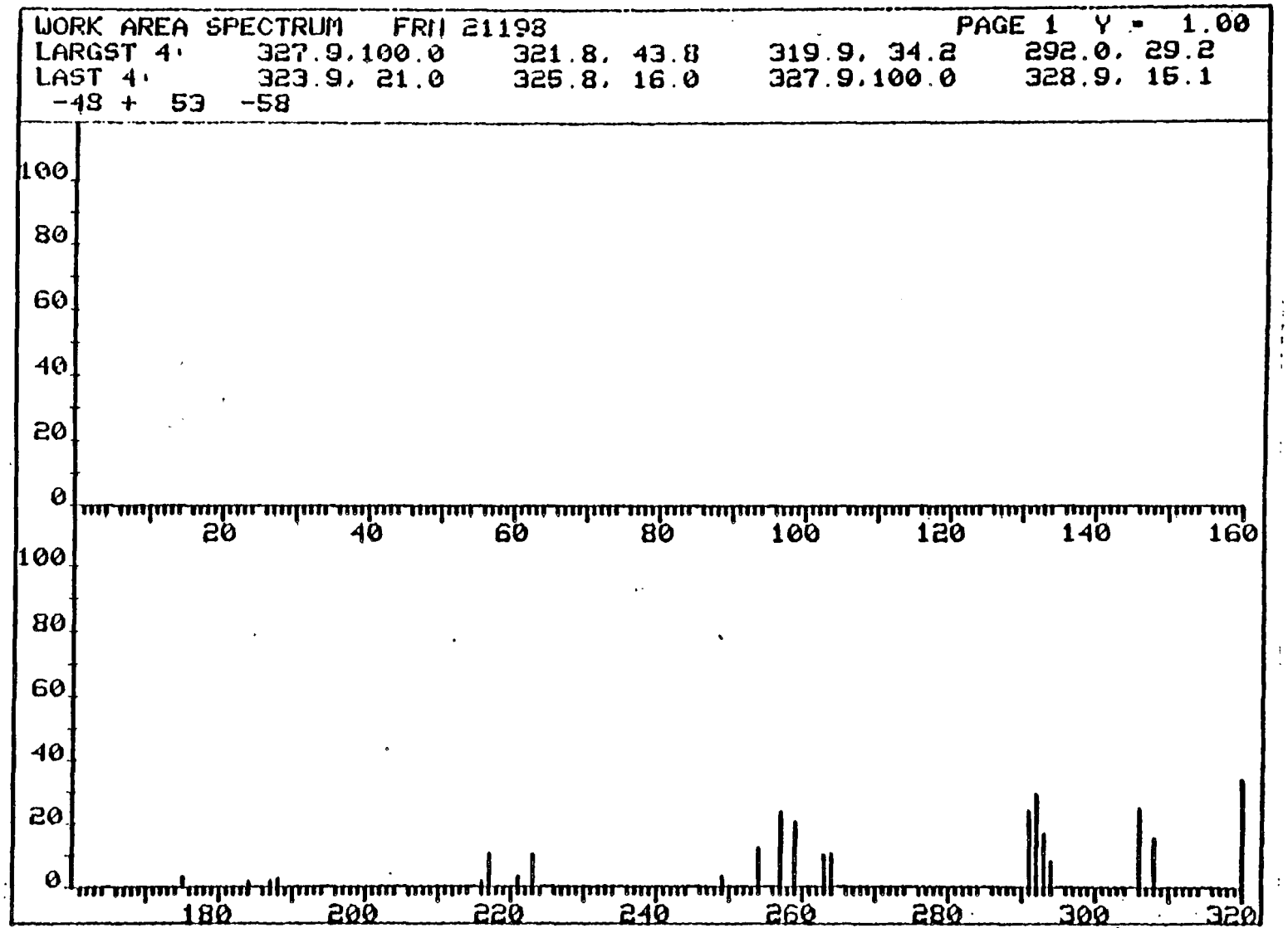

** SELECTED ION CHROMATOGRAM **
4R-B, 2+ 0+0
15AUG78, KMRQ
FRN 21508
1ST SC/PQ: 1
X= 1.00 Y= 1.00
TCDD Retention Time
A= 1030.
37Cl TCDD
327.9
A= 52560.
321.9
35Cl TCDD
A= 39656.
319.9
2
3
4
5

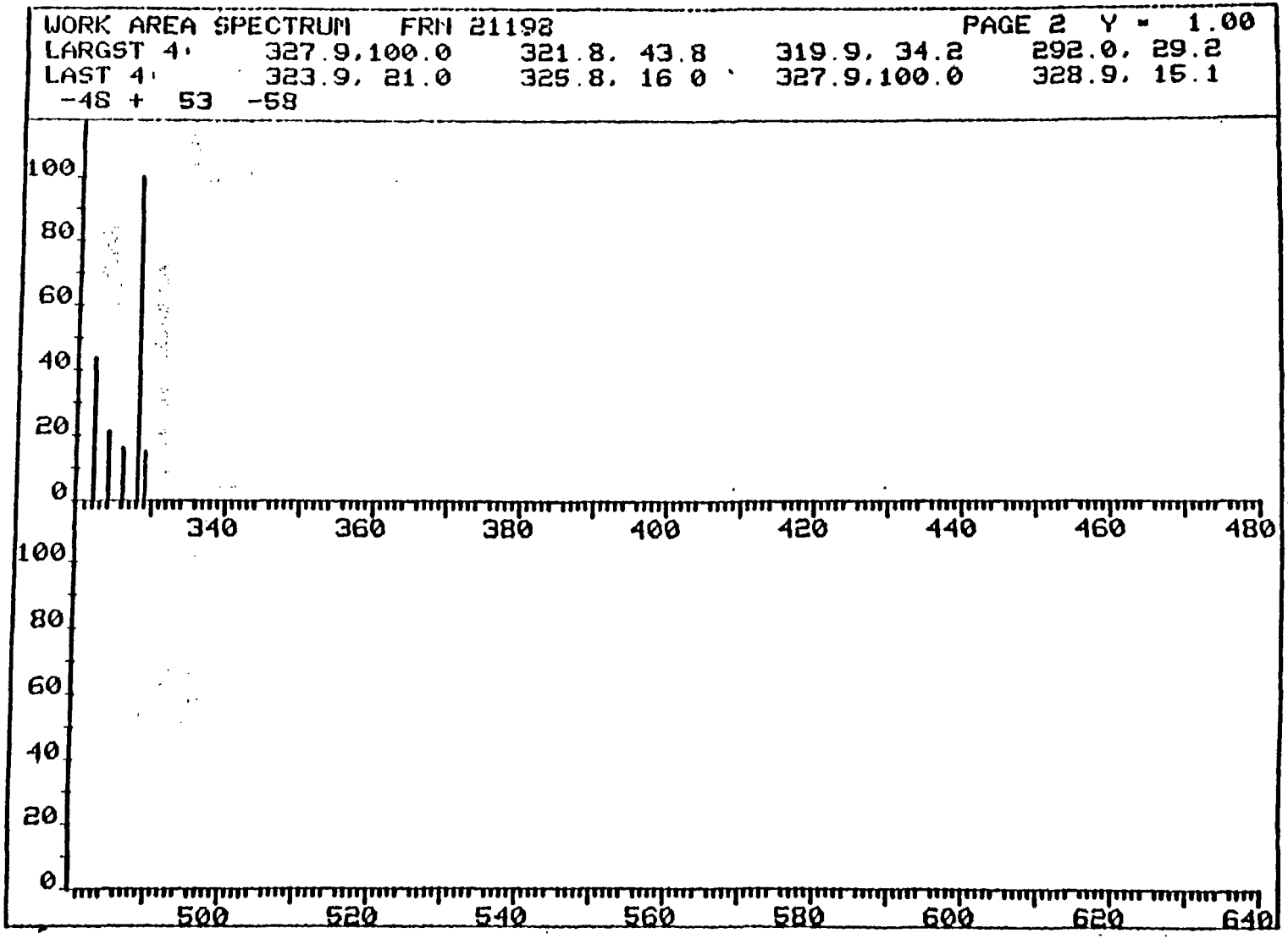

** SELECTED ION CHROMATOGRAM **
FRN 81507
4R-2, 2+2+1
15AUG78, KRG
1ST SC/PO: 1
X= 1.00 Y= 1.00
A= 1808.
327.9
A= 52488.
321.9
A= 39498.
319.9
2
3
4
5

Appendix F. Mass Spectrum for TCDD Detected in a Pesticide Sample

(EPA Sample #148919-5)

NOTE: Existence of m/e lines at 320, 322, 324 and 326 indicative of line at m/e 328 due to ^{37}Cl TCDD.


WORK AREA SPECTRUM FRI 21198
PAGE 1 Y = 1.00
LARGST 4: 327.9,100.0 321.8, 43.8 319.9, 34.2 292.0, 29.2
LAST 4: 323.9, 21.0 325.8, 16.0 327.9,100.0 328.9, 15.1
-48 + 53 -58
100
80
60
40
20
0
20 40 60 80 100 120 140 160
100
80
60
40
20
0
180 200 220 240 260 280 300 320


WORK AREA SPECTRUM FRN 21198
PAGE 2 Y = 1.00
LARGST 4: 327.9,100.0 321.8, 43.8 319.9, 34.2 292.0, 29.2
LAST 4: 323.9, 21.0 325.8, 16 0 327.9,100.0 328.9, 15.1
-48 + 53 -58
100
80
60
40
20
0
340
360
380
400
420
440
460
480
100
80
60
40
20
0
500
520
540
560
580
600
620
640

Appendix G. Mass Abundance Tabulation for TCDD Detected in a Pesticide Sample

(EPA Sample #90)

NOTE: Mass abundances at m/e 320, 322, 324 and 326 indicative of TCDD.

FRN 21643 SPECTRUM 47 RET. TIME = 4.7
>PAUSE

MASS	ABUND	MASS	ABUND	MASS	ABUND	MASS	ABUND
247	15.	271	8.	297	4.	319	7.
248	11.			297	3.	319	7.
249	19.	272	11.	298	4.	320	22.
250	11.	273	14.	299	7.	321	10.
251	15.	274	135.			322	29.
251	15.	275	81.	300	3.	324	14.
252	8.	276	23.	300	3.	325	9.
253	45.	277	11.	301	5.	326	7.
254	22.	278	8.	302	5.		
255	40.	279	8.	303	7.	328	38.
256	15.	280	6.	304	5.	329	10.
257	59.	281	40.	305	4.	330	5.
		282	13.	306	8.	331	15.
259	473.	283	15.	307	11.	332	11.
260	130.	284	6.	307	10.	333	9.
261	41.	285	12.	308	9.	334	5.
262	21.			309	13.	334	4.
263	28.	287	12.	310	5.	336	4.
264	8.	288	26.	311	7.	336	4.
264	7.	289	11.	312	4.	337	6.
264	7.	290	26.	313	9.	337	6.
265	9.	291	15.			338	6.
266	8.	292	11.	314	6.	339	4.
267	19.	293	21.	315	45.	340	2.
268	10.	294	8.	316	19.	341	7.
269	15.	295	7.	317	53.		
270	7.	296	4.	318	18.	342	4.

<CONT>

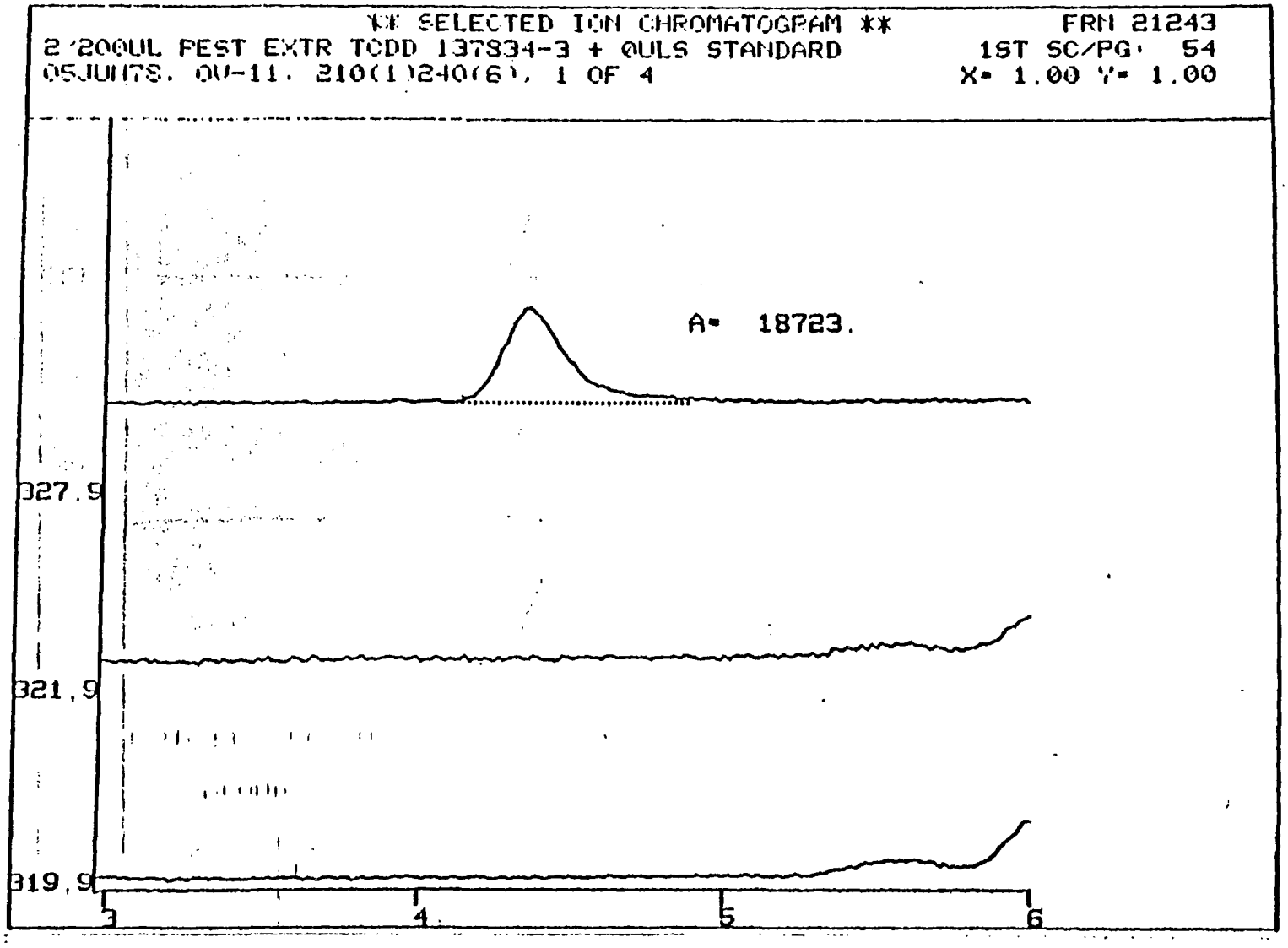
Appendix H. Ion Current Chromatograms for Nondetectable TCDD in a Pesticide Sample

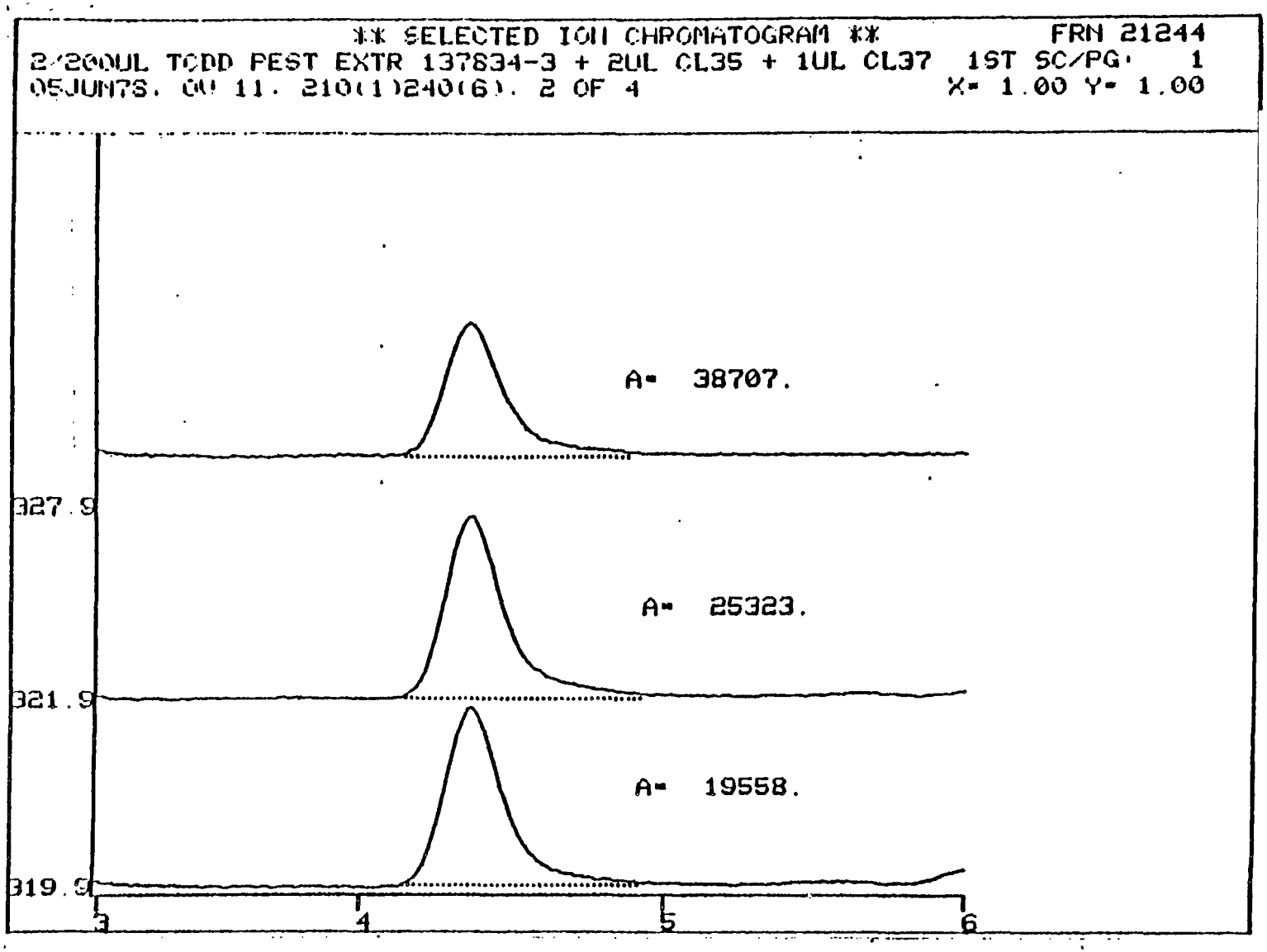
(EPA Samples #137834-3 and 3YD)

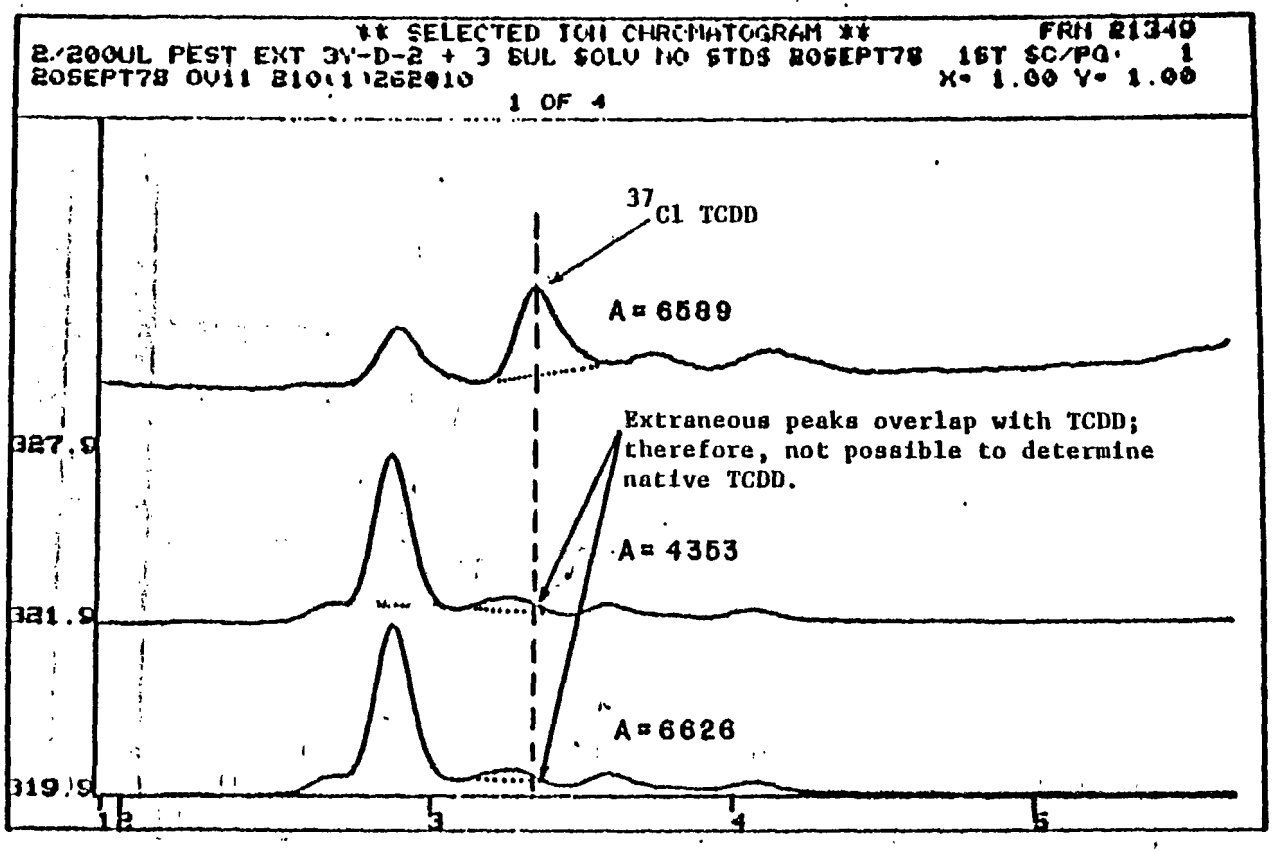
QUALITATIVE CRITERIA

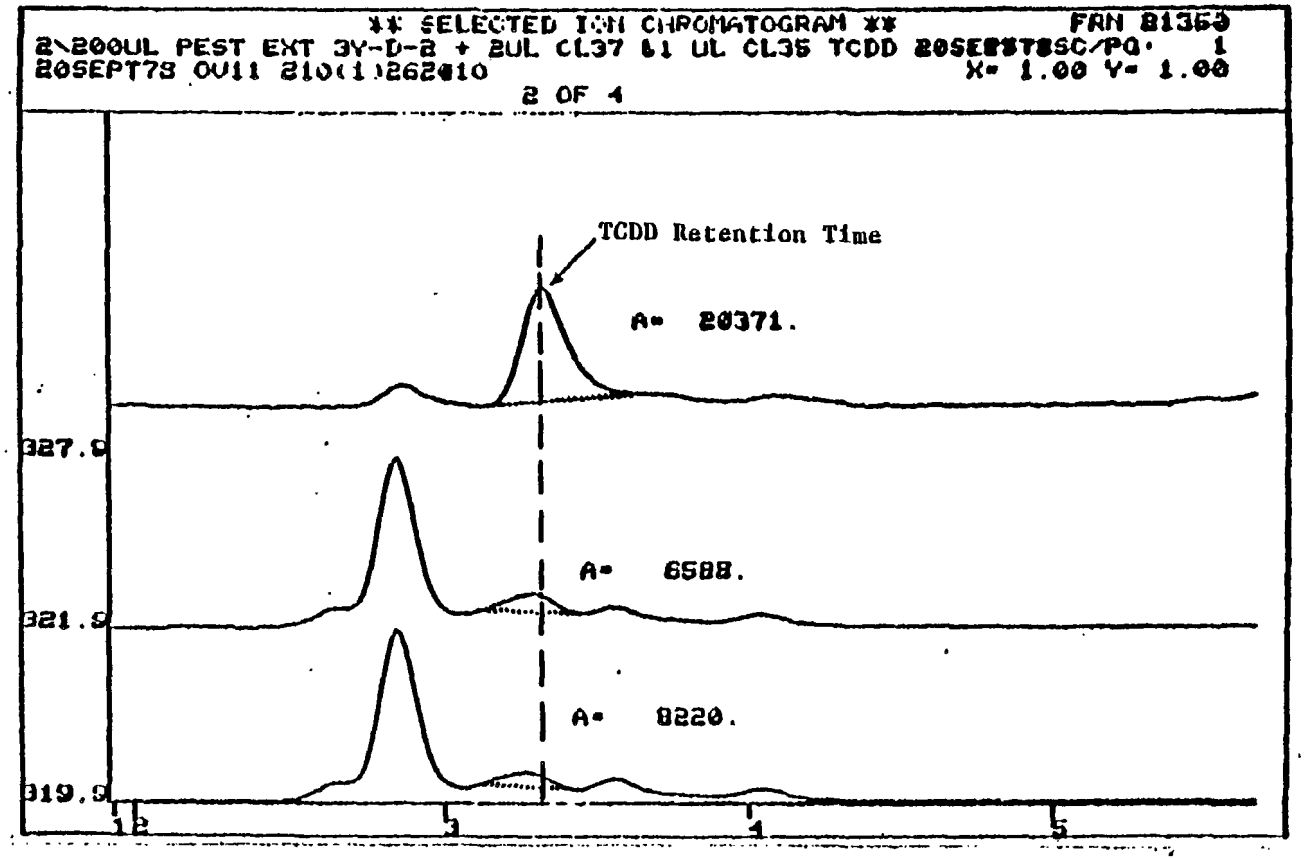
Sample	1	2	3	4	5	6
137834-3	+	-	NA	NA	NA	NA
3YD	+	-	NA	NA	NA	NA

JUDGEMENT: TCDD not detected in the sample because criteria #2 was negative due to absence of both m/e 320 and 322 peaks.


** SELECTED ION CHROMATOGRAM **
FRN 21243
2/20QUL PEST EXTR TCDD 137834-3 + QULS STANDARD
1ST SC/PG: 54
05JUN7S. QV-11. 210(1)240(6), 1 OF 4
X= 1.00 Y= 1.00
A= 18723.
327.9
321.9
319.9
3
4
5
6


** SELECTED ION CHROMATOGRAM **
FRN 21244
2/200UL TODD PEST EXTR 137834-3 + 2UL CL35 + 1UL CL37 1ST SC/PG: 1
05JUN7S. 00 11. 210(1)240(6). 2 OF 4
X= 1.00 Y= 1.00
A= 38707.
327.9
A= 25323.
321.9
A= 19558.
319.9
3
4
5
6


** SELECTED TOT CHROMATOGRAM **
FRI 21340
8./200UL PEST EXT 3V-D-2 + 3 BUL $OLV NO TD 20SEPT78 16T $C/PO 1
20SEPT78 OV11 B10(1)262@10
X= 1.00 Y= 1.00
1 OF 4
37Cl TCDD
A= 6589
Extraneous peaks overlap with TCDD; therefore, not possible to determine native TCDD.
327.9
A= 4353
321.9
A= 6626
319.9
1
2
3
4
5


** SELECTED ION CHROMATOGRAM **
FRN 81353
2\200UL PEST EXT 3Y-D-2 + 2UL CL37 &1 UL CL35 TCDD 20SEPT8SC/PQ 1
20SEPT78 0U11 210(1)262@10
X= 1.00 Y= 1.00
2 OF 4
TCDD Retention Time
A= 20371.
327.9
A= 6588.
321.9
A= 8220.
319.9
1
2
3
4
5

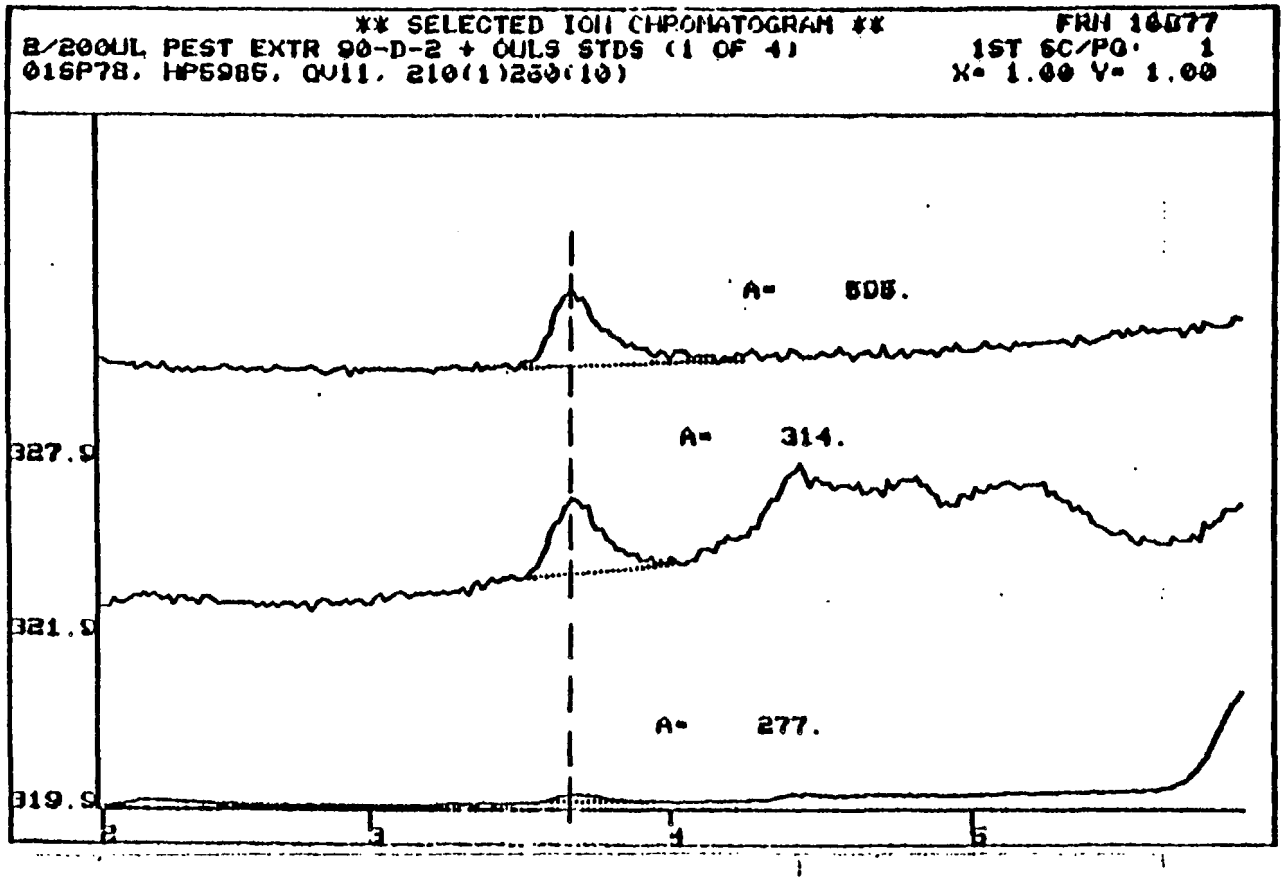
Appendix I. Ion Current Chromatograms for Questionable TCDD Detection in a Pesticide Sample

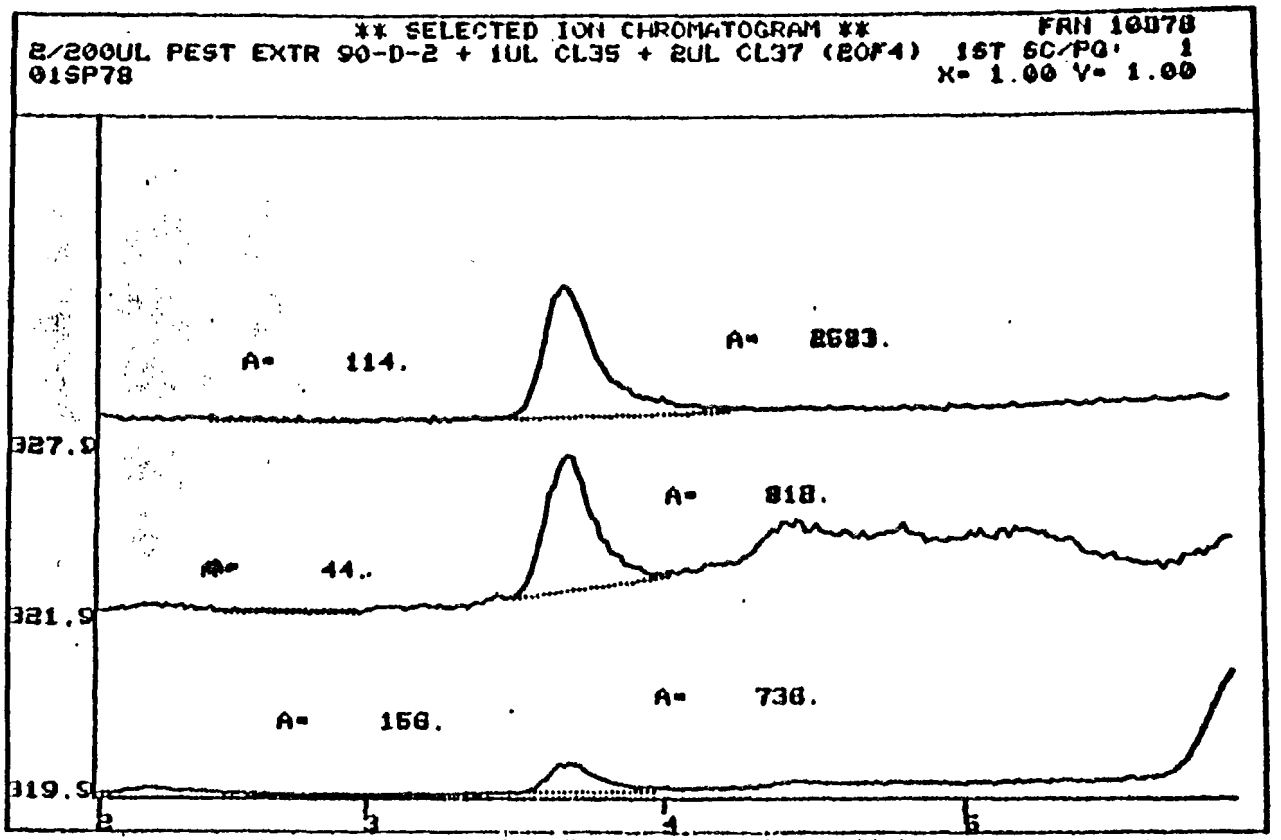
(EPA Sample #90D)

QUALITATIVE CRITERIA

1	2	3	4	5	6
+	+	+	NA	-	NA

JUDGEMENT: TCDD detection questionable because criteria #5 was negative due to m/e ratio out-of-specification.


** SELECTED ION CHROMATOGRAM **
FRN 16077
B/200UL PEST EXTR 90-D-2 + 0ULS STDS (1 OF 4)
1ST SC/PG: 1
016P78. HP5985. QU11. 210(1)250(10)
X= 1.00 Y= 1.00
A= 505.
327.9
A= 314.
321.9
A= 277.
319.9
2
3
4
5


** SELECTED ION CHROMATOGRAM **
FRN 10078
2/200UL PEST EXTR 90-D-2 + 1UL CL35 + 2UL CL37 (20F4) 1ST SC/PG' 1
01SP78
X= 1.00 Y= 1.00
A= 114.
A= 2583.
327.9
A= 44..
A= 818.
321.9
A= 158.
A= 738.
319.9
2
3
4
5