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Carborundum Co.
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DETERMINATION OF TCDD IN INDUSTRIAL AND MUNICIPAL WASTEWATERS

by

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Contract No. 68-03-2635

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16. ABSTRACT

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In conjunction with the development of an analytical method, the stability of TCDD in organic solvents and chlorinated water samples was also studied. It was found that TCDD solutions prepared in benzene, acetone and methanol remained stable during both cold storage (4°C) and at room temperature (25°C). However, degradation of TCDD in water was observed as a result of chlorinated followed by prolonged storage.

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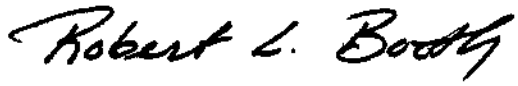
FOREWORD

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The Environmental Monitoring and Support Laboratory - Cincinnati conducts research to:

- Develop and evaluate techniques to measure the presence and concentration of physical, chemical, and radiological pollutants in water, wastewater, bottom sediments, and solid waste.
- Investigate methods for the concentration, recovery, and identification of viruses, bacteria, and other microbiological organisms in water. Conduct studies to determine the responses of aquatic organisms to water quality.
- Conduct an Agency-wide quality assurance program to assure standardization and quality control of systems for monitoring water and wastewater.

Under provisions of the Clean Water Act, the Environmental Protection Agency is required to promulgate guidelines establishing test procedures for the analysis of pollutants. The Clean Water Act Amendments of 1977 emphasize the control of toxic pollutants and declare the 65 priority pollutants and classes of pollutants to be toxic under Section 307(a) of the Act. This report is one of a series that investigates the analytical behavior of selected priority pollutants and suggests a suitable test procedure for their measurement.



Robert L. Booth, Acting Director
Environmental Monitoring and Support
Laboratory - Cincinnati

ABSTRACT

This program was undertaken in an effort to develop an analytical method for the determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in industrial and municipal wastewaters. The method includes extraction of TCDD with methylene chloride clean up, with columns and quantitative determination with gas chromatography (electron capture detector) and gas chromatography/mass spectrometry (GC/MS). The detection limit was found to be 0.003 µg/L.

In conjunction with the development of an analytical method, the stability of TCDD in organic solvents and chlorinated water samples was also studied. It was found that TCDD solutions prepared in benzene, acetone and methanol remained stable during both cold storage (4°C) and at room temperature (25°C). However, degradation of TCDD in water was observed as a result of chlorination followed by prolonged storage.

This report was submitted in fulfillment of Contract No. 68-03-2635 by California Analytical Laboratory working as a subcontractor to The Carborundum Company under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period May 1978 to June 1979.

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SECTION 1

INTRODUCTION

One of the most toxic synthetic compounds is 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD). It is an undesirable by-product in the production of 2,4,5-trichlorophenol, an intermediate for chemicals such as the herbicide 2,4,5-trichlorophenoxy acetic acid and the bacteriacide hexachlorophene. Due to the extreme toxicity of TCDD and its potential presence in wastewater and the effluents of chemical plants manufacturing 2,4,5-trichlorophenol, it should be closely monitored.

The purpose of the present study was to develop and evaluate several techniques for analysis of TCDD by gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS). In conjunction with the development of the analytical method, studies to determine the optimum conditions for water samples storage were also conducted by evaluating the effects of pH and residual chlorine. The stability of TCDD in organic solvents such as benzene, acetone and methanol was also studied to determine the shelf life of both the standard and the extract solutions.

While the focus of this study was to develop an analytical method and to conduct related studies for the 2,3,7,8-TCDD, the technique and results could apply to other isomers and analogs.

SECTION 2

CONCLUSION AND RECOMMENDATIONS

Based on the experimental part of this project, an analytical protocol was developed to determine the concentration of TCDD in wastewater. The recovery percentages and the standard deviations obtained from spiked samples were quite comparable to other published or accepted methods for TCDD analyses in various substrates, with the exception of the low recoveries whenever the charcoal-silica gel column was employed. The consistent performance as indicated by the small value of the standard deviation and clean up capability of the charcoal-silica gel column, made it possible to include it in the recommended protocol. Almost all methods for TCDD analysis call for the use of either Cl^{37}-TCDD or C^{13}-TCDD as internal standards. These labelled compounds, which must be analyzed by GC/MS, not only improve the accuracy in quantitation, but sometimes act as a carrier, especially during column chromatographic clean up, to improve recoveries. The use of labelled internal standards is also recommended for wastewater when charcoal-silica gel clean up is required.

One of the aims of this study was to develop an analytical method employing only single column chromatographic clean up followed by gas chromatographic determination with packed column and electron capture detector. Unfortunately, the common existance of other chlorinated compounds in high concentrations when trying to measure low levels of TCDD in wastewater samples made it necessary to recommend the use of sophisticated techniques and equipment such as the GC/MS and capillary columns. The original intent of this research effort was to develop a routine monitoring method which government and industrial laboratories would have the equipment and technical ability to follow. The protocol developed in this study can be considered a monitoring method, but may require relatively sophisticated laboratories and technique development.

As expected, standard solutions prepared in organic solvents remain very stable during both cold storage (4°C) and at room temperature (25°C). A laboratory should have no trouble in storing extracts and standard solutions for periods up to several months.

In contrast to the stability of TCDD standards in organic solvents, degradation of the compound in water was observed as a result of chlorination followed by storage. If the mechanism of the destruction of TCDD by chlorine is due to the oxidizing power of chlorine, then other oxidation processes such as ozonation in water treatment must be studied. The loss of TCDD in chlorinated water suggests that water samples from chlorinated

effluents should be extracted and analyzed as soon as possible unless a non-reactive preservative can be found. It is recommended that additional studies be conducted to determine the shelf life of TCDD in both natural and wastewaters.

SECTION 3

LITERATURE REVIEW OF CURRENT ANALYTICAL METHODS

ENVIRONMENTAL AND BIOLOGICAL SAMPLES

Environmental samples such as soil and water usually require little clean up prior to GC determination. However, biological samples, especially those with high fat content, must be processed through vigorous digestion and extraction steps.

Woolson and Ensor[1] determined the amount of TCDD in soil by extracting the acidified sample with 1:1 hexane:acetone, and washing the extract with 1N KOH to remove 2,4-D and 2,4,5-T. The hexane phase was extracted repeatedly with concentrated H_2SO_4 until the acid was clear. The H_2SO_4 was removed, the extract was drained through Ha_2CO_3 and anhydrous Ha_2SO_4 after a water wash. The volume was adjusted for GC/EC determination.

For biological samples, Hass[2] homogenized beef liver and fat tissues in a blender with 2:1 $CHCl_3$:MeOH and washed the extract with a small amount of 1.19% KCl. The organic solvent was then evaporated and the residue redissolved in CCl_4 and washed with conc. H_2SO_4. The CCl_4 was then pipetted off and evaporated and the residue dissolved in hexane for further clean up prior to GC /MS determination.

One of the most elaborate methods for biological samples was used by Baughman and Meselson.[3] After the normal extraction and clean up procedures, the sample was purified by preparative GC and the presence of TCDD determined by high resolution mass spectrometry. When beef liver samples were spiked at 0.02 ppb of TCDD, the average recovery was 34 ± 7.2%. Recovery for 1 ppb of Cl^{37}-TCDD was 27 ± 5.0%.

In addition to the normal column chromatography clean up steps, Lamparski[4] and coworkers also used elevated temperature, reverse-phase liquid chromatography to purify extracts of fish tissues. Their technique enabled them to detect 10 to 100 parts per trillion TCDD by multiple ion mode GC/MS.

FORMULATIONS

The method used by Edmunds[5] and associates to determine the amount of TCDD in 2,4,5-T includes most of the basic techniques for analyzing chlorodioxins. It includes the following steps:

a. Digestion of the formulation with methanolic lithium hydroxide.

b. Extraction of the neutral compounds into hexane.

c. Clean up with an alumina column.

d. Determination with gas chromatography (Electron capture and flame ionization detectors).

Woolson[6] and his group examined 129 samples of 17 different pesticides derived from chlorophenols for chlorodioxins. They extracted TCDD from an alcoholic KOH mixture and performed a column chromatographic clean up with an alumina column (450 x 19 mm i.d.), which was eluted with 100 mL of petroleum ether followed by 50 mL of 5% diethyl ether in petroleum ether, which was discarded. 100 mL of 50% diethyl ether in petroleum ether was collected and TCDD determined by GC/EC, using a 5% OV-225 column.

Samples which were difficult to clean up were further treated by first evaporating the eluate from the alumina column just to dryness, cooling to about 0°C on an ice bath and adding 10 mL of 1:1 HNO_3:H_2SO_4 mixture. The mixture was gradually warmed to room temperature and added to 50 mL of ice water in a separatory funnel after 15 minutes. The beaker was rinsed with five 10 mL aliquots of hexane. The combined hexane rinses were shaken for one minute and washed with water, and the hexane was drained through a $NaHCO_3$-Na_2SO_4 column. The volume was adjusted for analysis.

Due to the presence of electron capture interferences, a more specific detector is generally needed for the determination of TCDD. Buser and Bosshardt[7] used essentially the same extraction and clean up method as Edmunds, but they employed the technique of mass fragmentography for the determination step. By monitoring the intensity of a single ion, m/e 320 (M+) in this case, a detection limit of 0.5 ng was routinely obtained. For gas chromatography, a glass column 1.5 m x 2 mm i.d. packed with 3% silicone OV-225 on Chromasorb W., AW-DMCS was used.

Vogel and Weeren[8] dissolved 2,4,5-T in a mixture of dimethyl formamide-acetronitrile-water (1:1:1) and extracted TCDD from the mixture with hexane. The hexane extract wasd purified first with an alumina column. Impurities were then eluted with hexane-ether (95:5). TCDD was eluted with ether and was purified again on a silica gel plate with hexane as the mobile phase. TCDD was then determined by GC/FID. Recovery was 95% and the detection limit was 30 ppb.

To handle a large number of analyses, Ramstad[9] and coworkers developed an automated clean up system, employing a "high-purity" silica gel column (Curtin-Matheson Scientific, No. 531-178) in their clean up step. After TCDD was eluted from the column using 1:4 benzene:hexane eluent, the column was re-generated by removing the ester from the silica surface with THF-benzene (15:85) passing through the column in the reverse direction. They were able to obtain a standard deviation for the same formulation, after 36 analyses, of 2 ppb with a mean TCDD concentration of 26 ppb.

SPECIAL GC AND MS TECHNIQUES

Besides packed GC columns, capillary columns are sometimes employed to achieve better resolution and sensitivities. Passivirta[10] and associates were able to see 0.5 pg TCDD with an EC detector by employing a 35 m, 0.35 mm i.d. glass capillary column coated with Silar 10C. Buser[11] used high-resolution OV-101 and OV-17 glass capillary columns to separate several TCDD isomers.

In addition to electron impact techniques in the MS determination, chemical ionization[12] and the negative ion chemical ionization technique are also being used. Using oxygen as a reagent gas, Hunt et al,[13] obtained a spectrum containing the molecular ion at m/e 320, and isotope clusters at m/e 176, 301, and 335. The sensitivity is at least 25 times better than the high resolution procedure.

CONCLUSION

Most methods call for a rather polar solvent or mixture of solvents such as chloroform, hexane-acetone mixture, followed by column chromatographic clean up with either silica gel or alumina columns. The present study evaluated and adopted the published silica gel and alumina columns, but studied methylene chloride and a mixture of 15% methylene chloride in hexane for extraction. The choice of methylene chloride was based on both its compar- able polarity and its being used as the extraction solvent for most of the chemicals such as the pesticide and the base/neutral compounds that are on the priority pollutants list.

Both the packed and the capillary gas chromatographic columns used in all the methods were common ones suitable for most neutral compounds. Columns such as the 1.5% OV-17 + 1.95% QF-1 on Gas Chrom Q that are already being used for pesticide analysis were also evaluated and eventually adopted.

Based on information from the literature, it was decided an analytical procedure similar to the existing ones for pesticides among the priority pollutants should be evaluated. The results of the current study actually produced a protocol that is interchangeable with the pesticide method, enabling the analyst to use either the pesticide or the base/neutral extract to screen for TCDD on most wastewater samples that do not have major interferences.

SECTION 4

METHOD DEVELOPMENT

DESCRIPTION OF SAMPLES

Wastewater samples used in this study were composites of samples submitted by other sampling teams to the laboratory. They were mainly from sources such as pesticide manufacturers, wood treatment plants, organic chemical manufacturers and leachates from chemical burial sites. Many of the samples were highly contaminated with chlorinated organic compounds especially the ones that frequently associate with the chemical generation of TCDD, such as chlorinated benzenes and phenols.

ANALYTICAL SCHEME

The conventional scheme of solvent extraction, column chromatographic clean up procedures and instrumentation analysis was employed. Figure 1 presents the flowchart of all the steps involved in the analytical protocol. Since wastewater samples range from relatively "clean" to extremely "dirty" with very high concentration of other chlorinated compounds such as tetrachlorobenzenes, chlorinated phenols and chlorinated aliphatic compounds, the analyst may be able to analyze samples at any of the four steps marked for GC/MS analysis.

EXTRACTION

The purpose of this experiment was to assess the effect of pH of the water sample on extraction efficiency. Two extraction solvent systems commonly used in pesticide analysis were used to extract TCDD from water at acidic, neutral and basic pH.

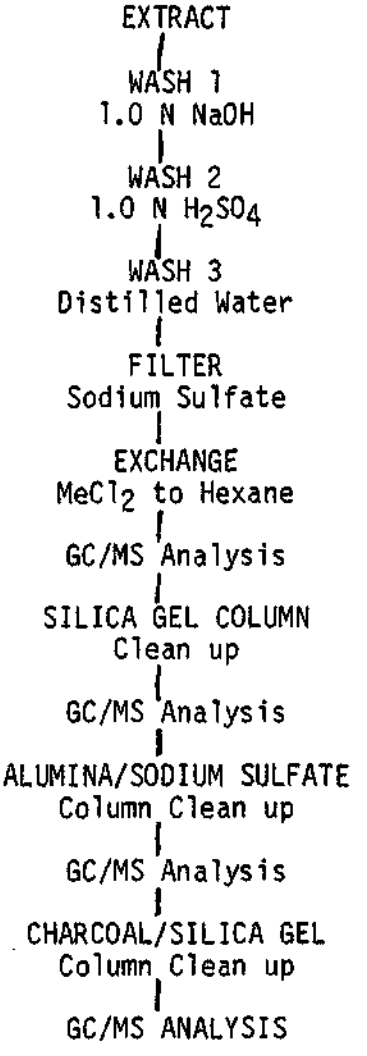
The following variables were used for evaluation of the extraction efficiency:

Solvent Systems	pH Levels of Water Sample		
(1) Methylene chloride	2	7	10
(2) 15% methylene chloride in hexane	2	7	10

The water sample was prepared by placing one liter of steam-distilled water in a 2.0 liter separatory funnel. The desired pH level was obtained by addition of the necessary amount of either 0.1 N HCl or 0.1 N NaOH. The

FIGURE 1.

Laboratory flow chart of protocol for processing wastewater extracts



pH was determined by using multirange pH paper which is sensitive to ± 0.2 pH unit. After pH adjustment, 100 ng of TCDD dissolved in 100 µl of methanol was added to the water. This was then briefly shaken. Then 60 mL of the respective solvent was added, and the sample shaken vigorously for approximately one minute.

This was repeated twice using 60 mL of solvent. These extracts were then combined and passed through 50 gm hexane-washed Na_2SO_4 into a Kuderna-Danish (K-D) apparatus and placed on a water bath. The sample is concentrated to approximately 10 mL, at which time, 1.0 mL of toluene was introduced. This was further concentrated to about 5 mL. The concentrate was transferred to a 10 mL graduated test tube and concentrated to about 1 mL with a gentle stream of nitrogen at room temperature. Two portions of 5 mL of hexane were added to exchange for the methylene chloride by taking the solvent down to 1 mL after each addition with nitrogen. The final volume of the extract was adjusted to 1.0 mL. Each data point represents results from duplicate injections of the analyses as compared to the response of a standard of TCDD in hexane prepared on the day of analysis. Comparative results are given in Table 1.

TABLE 1. EXTRACTION OF TCDD FROM WATER BY ORGANIC SOLVENTS*

	pH 2		pH 7		pH 10	
Solvent	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
$MeCl_2$	92%	6.2%	92%	5.5%	91%	5.5%
15% $MeCl_2$ in hexane	93%	3.7%	100%	6.8%	95%	7.7%

*Values given are from triplicate samples.

As indicated in Table 1, both solvent systems were proven effective in extracting TCDD from all pH levels, and recoveries in the 90% + range were consistently obtained. Pure methylene chloride is recommended in the protocol because the chance of emulsion formation is less than when a mixture of methylene chloride in hexane is used. It is also the solvent used in extracting neutral compounds and pesticides in a number of other EPA procedures; therefore, these extracts can be used for TCDD determination.

Since there was no significant difference in recovery of TCDD at different pH levels of the water, it is not necessary to adjust the pH of the sample before extraction.

Since TCDD is a trace impurity in 2,4,5-trichlorophenol or in final products manufactured from the phenol such as 2,4,5-trichlorophenoxy acetic acid (2,4,5-T), it often occurs as a neutral compound in minute quantity together with much higher concentrations of acidic chlorinated compounds, so it is desirable to remove the acidic compounds from the extract by washing

the organic extract with a dilute solution (1 N) of sodium hydroxide or potassium hydroxide. Such a technique was well documented to be successfully in many methods especially those dealing with formulations of 2,4,5-T, and it was adopted in this study.

RECOVERY OF TCDD FROM EVAPORATION

TCDD is somewhat volatile and a significant loss can occur during evaporation of the extraction of solvents. By following the evaporation procedures in the recommended protocol CAREFULLY, the recoveries shown in Table 2 were measured:

TABLE 2. PERCENTAGE RECOVERY OF TCDD FROM EVAPORATION

Run	1st	2nd	3rd	Mean	Std. Dev.
Hexane	100	90	100	97	5.8
15% $MeCl_2$ in hexane	105	100	103	103	2.5
MeCl2	110	106	95	104	7.8

COLUMN CHROMATOGRAPHIC CLEAN UP

Silica Gel Column

The silica gel column method was adapted from the procedure developed by Dow Chemical Co. in analyzing TCDD in 2,4,5-trichlorophenoxyacetic acid formulations. A glass column (30 cm x 1 cm i.d.) was packed with 14 gm of silica gel (Curtin Matheson Scientific Inc., Davidson 923 brand, 100-200 mesh, high purity grade) and equilibrated with 20% benzene in hexane. After the addition of either sample extract or TCDD standard, 20% benzene in hexane mixture was added to the column as necessary to maintain liquid above the silica bed, until 40 mL were collected. The eluate was condensed and analyzed for TCDD by GC/EC and/or GC/MS. This column is particularly useful in cleaning water samples obtained from effluents of manufacturing plants which formulate herbicides such as 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). The acidic compounds are retained by this column. TCDD can be eluted using a mixture of 20% benzene in hexane. Triplicate studies in our laboratory have shown that adding 100 ng of TCDD to this column enables a mean recovery of 90%, with a standard deviation of 8%.

Alumina Column

A 15 cm x 1 cm i.d. column was packed with neutral alumina (Fisher Scientific Co., Fisher A540, neutral, activated at 130°C for 24 hours). The sample was introduced after the column was developed with 50 mL of hexane, and TCDD was eluted with the following solvent systems:

1) 50 mL 3% methylene chloride in hexane

2) 50 mL 20% methylene chloride in hexane

The 20% methylene chloride in hexane portion was analyzed for TCDD by GC/EC and/or GC/MS.

Tests which include eluting 100 ng of TCDD through this column show that TCDD is recovered from the 20% methylene chloride in hexane with a mean recovery of 86% (standard deviation 9%) from three runs. No TCDD was detected in the 3% methylene chloride mixture or in the pure methylene chloride eluate. This column is routinely used in our laboratory to clean up hexachlorophene samples and to separate PCBs from TCDD. These procedures have proved to be effective with wastewater samples originating from manufacturers of 2,4,5-trichlorophenol, hexachlorophene and related products.

Charcoal-silica Gel Column

Many evaluations of columns packed with activated cocoanut charcoal were performed. However, due to the strong absorption of TCDD in charcoal, recoveries of TCDD were poor. This was true even with solvents such as methanol, acetone, ethyl acetate, methylene chloride and benzene. In an attempt to reduce the amount of charcoal in this column, silica gel was added as a dilutent.

The column consisted of a disposable Pipette (Fisher Scientific Co. Cat., No. 13-678-6A, 5 mm i.d.) packed to a length of 5 cm with a mixture of one part activated cocoanut charcoal (Fisher Scientific Co. 5-690-A. 50-200 mesh) to 140 parts of silica gel (Curtin Matheson 100-200 mesh, Davidson 923, high purity grade). It was eluted in the following manner:

1) Equilibrate the column with 5 mL of hexane.

2) Transfer extract into the column with hexane (any benzene or methylene chloride in the extract must be exchanged into hexane prior to addition to the column).

3) Elute with 10 mL of hexane, discard.

4) Elute with 10 mL of benzene.

5) Concentrate the benzene fraction carefully for analysis.

Results were obtained by adding to the charcoal silica gel column 10 ng and 100 ng of TCDD and eluting as mentioned above. Recoveries from six replicate runs are given in the following table:

TABLE 3. RECOVERIES OF TCDD FROM CHARCOAL/SILICA GEL COLUMN

TCDD Added	Mean Recovery	Std. Dev.
10 ng	46	3.6
100 ng	43	5.4

The above data indicated no significant difference in the percentage of recovery at two spiking levels, and despite the somewhat poor recoveries the standard deviations were excellent.

Florisil Column

The florisil column was prepared by packing a column (10 cm x 2.5 cm i.d.) with florisil which was activated overnight at 110°C. The sample was introduced after the column was washed with 25 mL of hexane, and then eluted first with 200 mL of 6% ethyl ether in hexane followed by 200 mL of 15% ethyl ether in hexane. Each fraction was collected and analyzed separately.

The results indicated that when 100ng of TCDD were eluted through a florisil column, the mean recoveries of five runs was 62% (standard deviation 5.5%) in the 15% ether-hexane fraction and 30% (standard deviation 3.0%) in the 6% ether-hexane fraction.

It is recommended that internal standards such as Cl^{37}-TCDD or 1,2,3,4,-TCDD be incorporated whenever a column clean up procedure is required. It should be emphasized, however, that Cl^{37}TCDD cannot be used when an electron capture detector is employed. Experience shows that if a sample requires charcoal-silica gel column clean up, it is usually so contaminated that it requires analysis by mass fragmentography techniques.

GAS CHROMATOGRAPHY

This laboratory has experienced both clean samples that could be analyzed with an electron capture detector and packed columns and very contaminated samples that could only be GC/MS coupled with capillary columns. Although it is more convenient to use the computerized multiple-ion-detection mode of the GC/MS system, the Programmable Multiple Ion Monitor (PROMIN) was also found to be very satisfactory. The following is a discussion on the various gas chromatographic columns used on this project.

Packed Columns

1) 1.5% OV-17 + 1.9% QF-1 on Gas Chrom Q (or 1.5% SP-2250 + 1.95% SP-2401 on Supelcoport), 100/120 mesh, 108 cm, 4 mm i.d., glass.

2) 3% OV-17 on Gas Chrom Q, 100/120 mesh, 180 cm, 2 mm i.d., glass.

3) 1% SP-2250 on Supelcoport, 120 cm, 2 mm i.d. glass.

While all three packed columns performed equally well, column (1) is recommended because it is the same column frequently used by the Environmental Protection Agency in the analysis for pesticides and has been successfully used routinely in our laboratory.

Capillary Columns

1) SP-2250, 30 meters, 0.25 mm i.d., 0.80 mm o.d. glass.

2) SP-2100, 30 meters, 0.25 mm i.d., 0.80 mm o.d. glass.

Again, both columns performed well, but the SP-2250 is recommended because it separated 1,2,3,4-TCDD and 2,3,7,8-TCDD iosmers better than SP-2100 column. When 1,2,3,4-TCDD is used as the internal standard, SP-2250 capillary column must be used.

Detection Limit

The instrumental detection limits were determined by measuring five times the signal to noise ratio for both ECD and GC/MS. The following table summarizes the instruments and column used, the operational conditions and instrumental sensitivities.

TABLE 4. INSTRUMENTAL DETECTION LIMITS

Instrument	Column	Splitting Ratios	Sensitivity
GC/ECD[1]	Packed[3]	---	10-20 pg
	Capillary[4]	1/50 to 1/75	10-20-pg
GC/MS[2]	Packed[3]	---	10-20 pg
	Capillary[4]	Splitless	10-20 pg

1. Varian Model 3700 equipped with Nickel 63 detector.
2. Finnigan Model 4000 GC/MS system coupled with INCOS computer, operated on the Multiple Ion Detection (MID)/Mass Fragmentography mode for ions 320, 322 and 324.
3. Supelcoport 100/120 mesh coated with 1.5% SP-2250 + 1.95% SP-2401 packed in a 180 cm x 2 mm i.d. glass column. Column temperature was 220°C. Carrier gas for GC/ECD was 5% methane + 95% Argon at 25 mL/min, and helium at 25 mL/min for GC/MS.
4. SP-2250 coated on a 3 m x 0.25 mm i.d. glass column (Supelco No. 2-3714 or equivalent) with helium as carrier gas at 30 cm/sec linear velocity. Column temperature was 210°C.

The sensitivity of both the GC/EC and GC/MS were found to be quite comparable. Figure 2 shows the response of an EC detector when 20 pg of TCDD is injected into the SP-2100 capillary column, Figures 3, 4 and 5 are mass chromatograms of 10, 20 and 40 pg, respectively, of TCDD analyzed using mass fragmentographic techniques of the mass spectroscopy system Figure 6 shows the mass chromatograms of ions 320 (M^+), 322 $(M+2)^+$, 324 $(M+4)^+$ and the total ion current chromatograms obtained when the GC/MS was operated on the computerized Multiple Ion Detection (MID) mode.

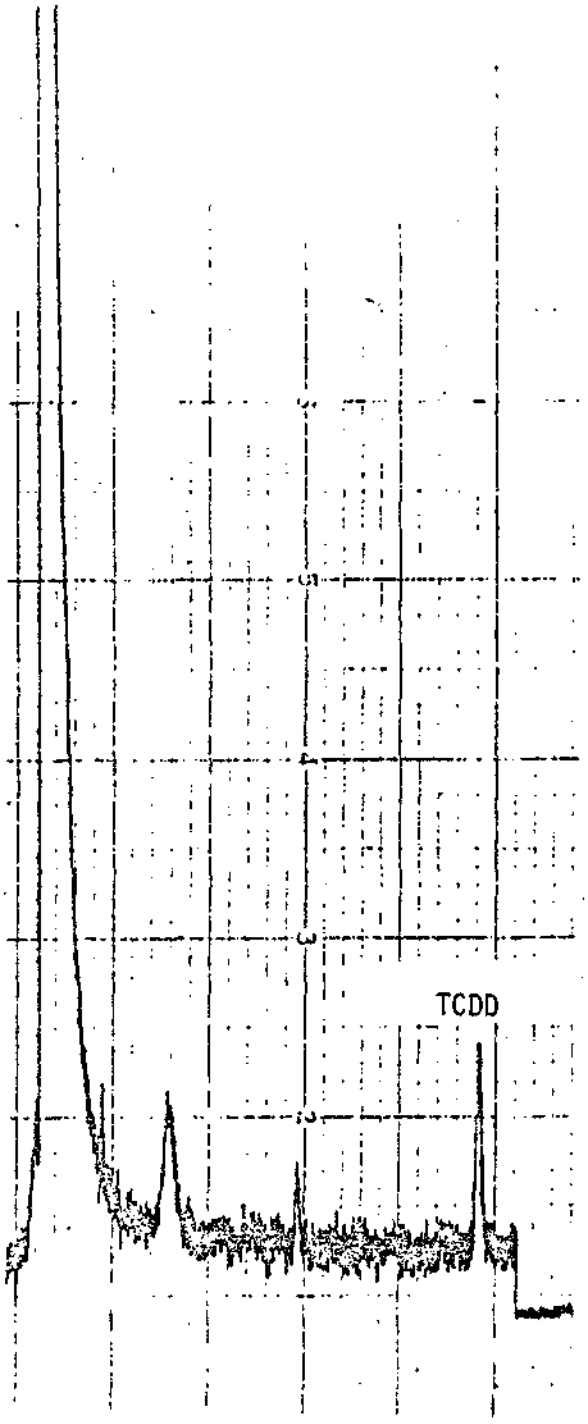


Figure 2. GC/EC chromatogram of 20 pg of TCDD. SP-2100 capillary column.

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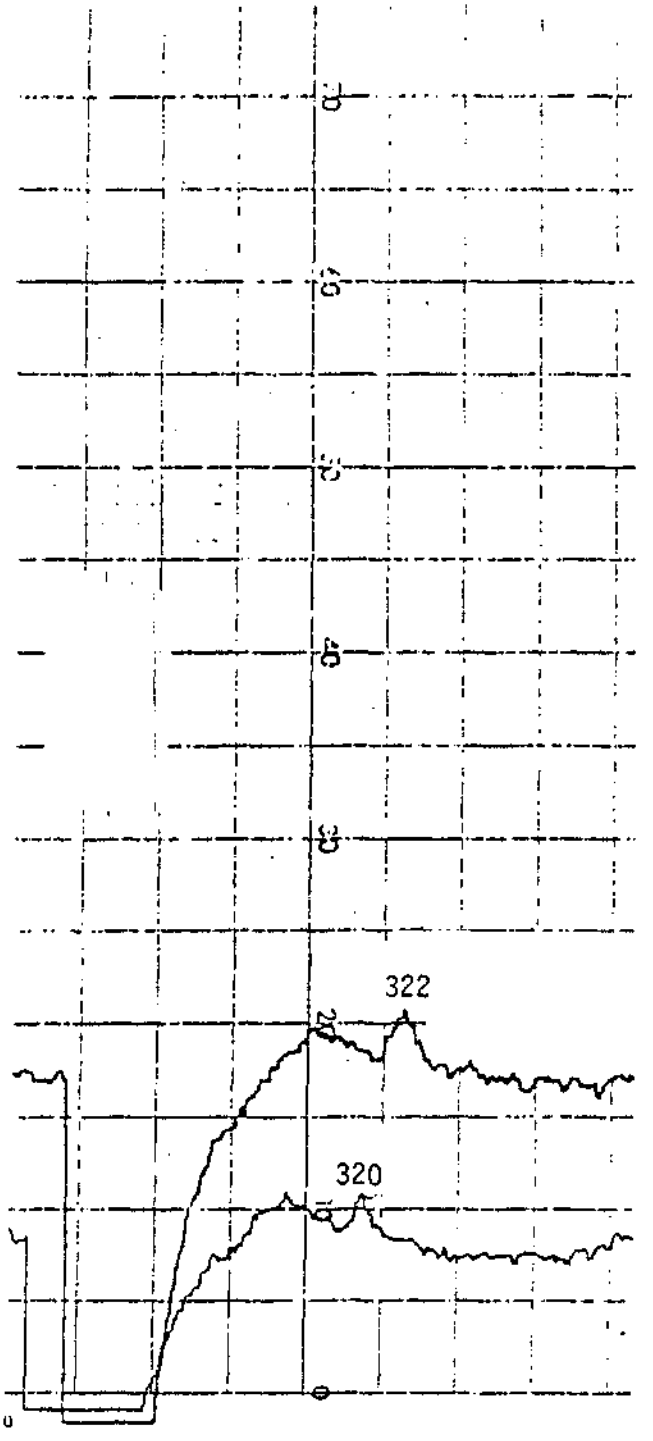

Figure 3. Mass chromatograms of ions 320 $(M)^+$ and 322 $(M+2)^+$ of 10 pg of TCDD. PROMIN. 1% SP-2250 on Supelcoport column.

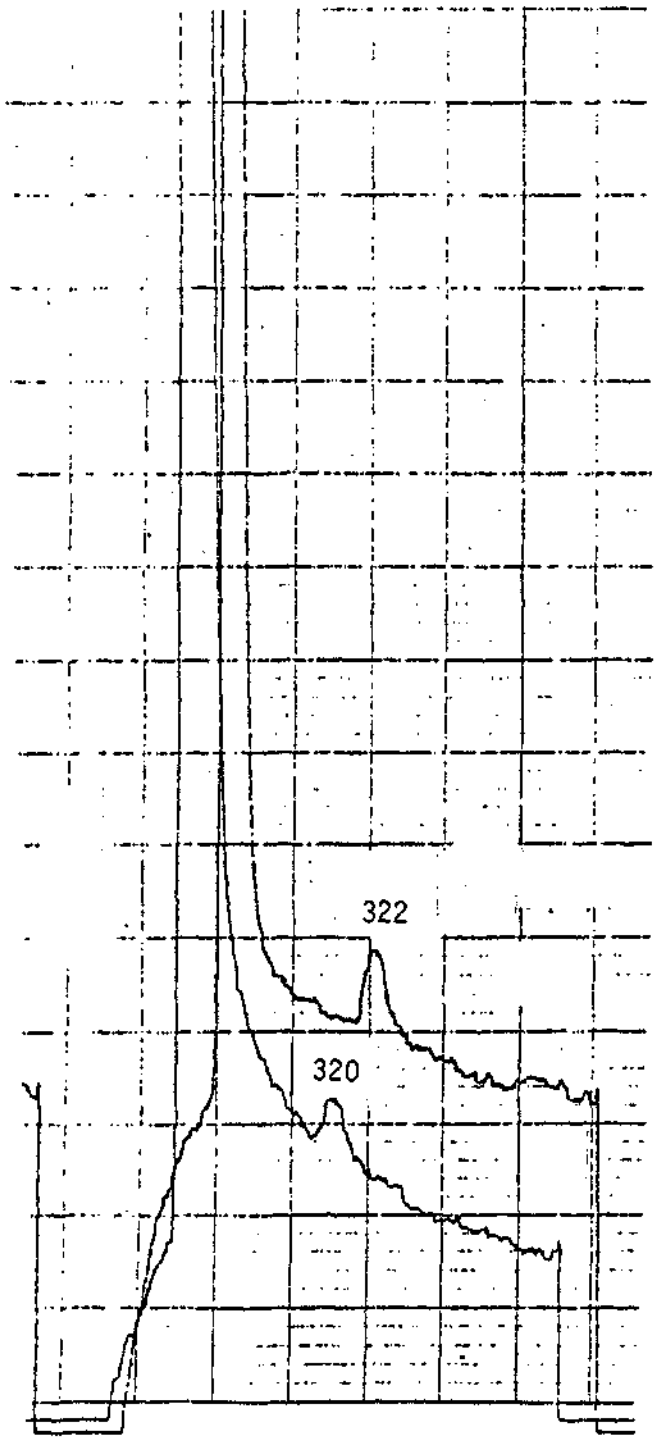

Figure 4. Mass chromatograms of ions 320 $(M)^+$ and 322 $(M+2)^+$ of 40 pg of TCDD. PROMIN. 1% SP-2250 on Supelcoport column.

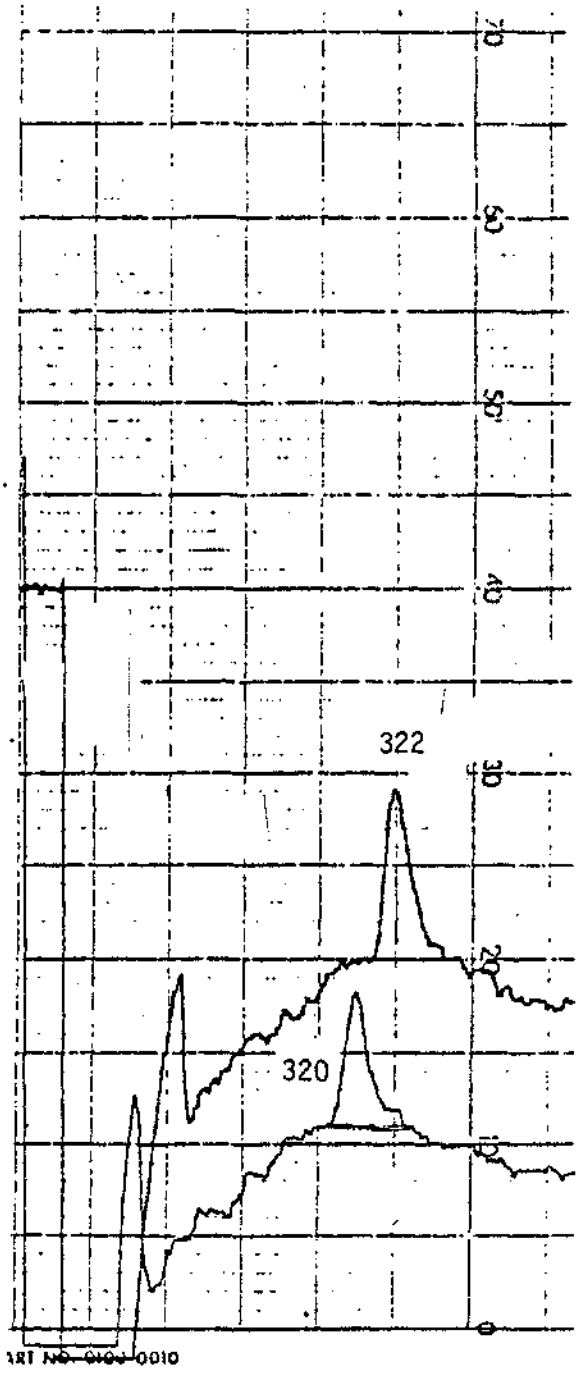

Figure 5. Mass chromatograms of ions 320 $(M)^+$ and 322 $(M+2)^+$ of 40 pg of TCDD. PROMIN. 1% SP-2250 on Supelcoport column

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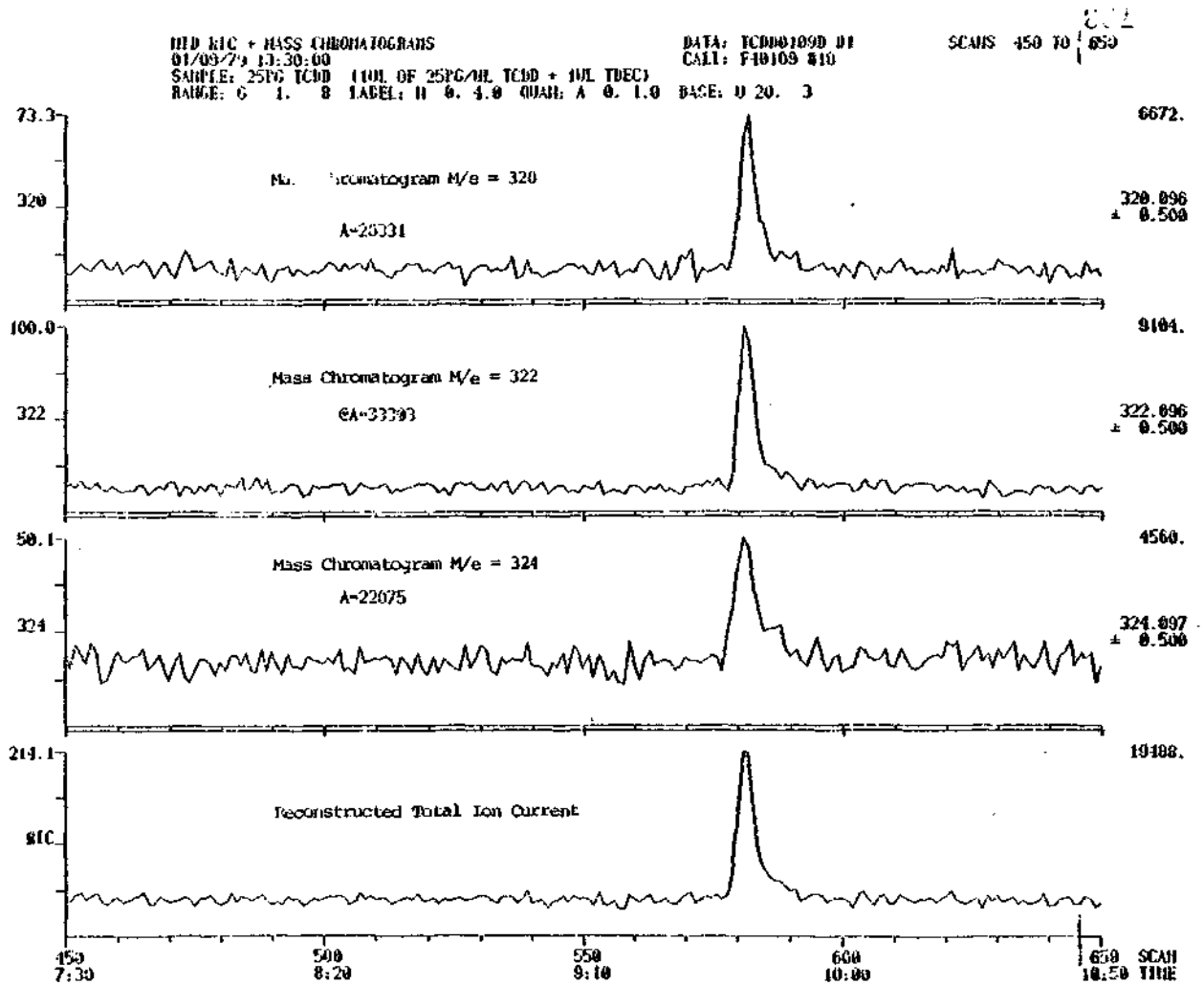

Figure 6. Mass chromatograms of ions 320 $(M)^+$, 322 $(M+2)^+$, 324 $(M+4)^+$ and reconstructed total ion current of 25 pg of TCDD. Computerized Multiple Ions Detection, SP-2250 capillary column.

While the electron capture detector is sensitive enough for most samples, it has the drawback of not being compound specific, and it cannot handle samples that contain large concentrations of electron capturing compounds. Since TCDD is often associated with other chlorinated compounds at concentrations several magnitudes higher than that of TCDD, the analyst often encounters wastewater samples that, even after rigorous clean up, still require measurement by mass fragmentography.

Both GC/EC and GC/MS MID have about the same sensitivity. The following Table lists the projected detection limits for TCDD in wastewater samples based upon typical extraction, concentration and instrumentation; relative retention time of TCDD as well as some potential surrogates and aldrin are also given.

TABLE 5. GAS CHROMATOGRAPHY AND DETECTION LIMIT FOR TCDD IN WASTEWATER

Column	Retention Time (Min) TCDD	Cl^{37}TCDD	1,2,3,4-TCDD	Aldrin	Det.[1] Limit (µg/L)
Packed[2]	7.3	7.3	7.4	2.3	0.003
Glass Capillary[3]	9.5	9.5	9.7	3.3	0.003

1. Detection limit is calculated from the minimum detectable GC response being equal to five times the GC background noise, assuming a 1 mL effective volume of the 1 liter sample extract, and assuming a GC injection of 5 microliters. Detection levels apply to both electron capture and GC/MS detection.
2. Same packed column and conditions as in Table 4.
3. Same capillary column and conditions as in Table 4.

Internal Standards

In addition to the normal usages of internal standards for quantitative computation, a surrogate compound to be added to the water, which is less toxic than the hazardous 2,3,7,8-TCDD, is useful to varify the efficiency of the entire analytical method. For GC/MS determination, Cl^{37}-TCDD and C^{13}-TCDD are the most common internal standards that are added to the extract. The former has the advantage of yielding a single peak (m/e=328) with good intensity for the MID mode while the C^{13}-TCDD gives a cluster of isotopic peaks for better confirmation. Figure 7 is a MID chromatogram when a mixture of Cl-2,3,7,8-TCDD and Cl^{37}-2,3,7,8-TCDD was analyzed.

When an electron capture detector is used, compounds other than labeled ones must be used as internal standards. When 1,2,3,4-TCDD was tried, it was found that it behaved very similarly to the 2,3,7,8-TCDD. The only drawback with the 1,2,3,4-TCDD is that it elutes very close to 2,3,7,8-TCDD

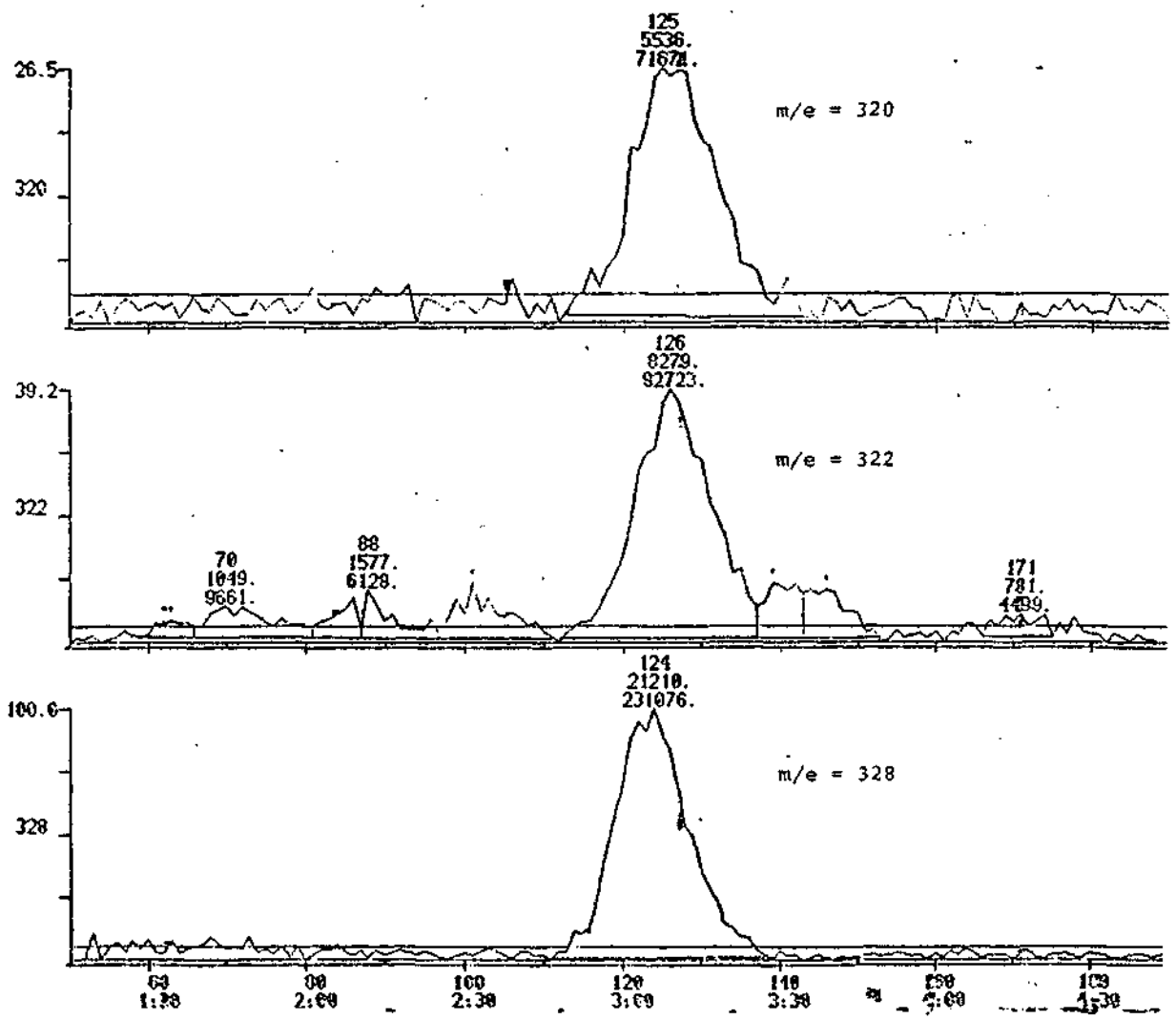

Figure 7. MID Chromatogram of TCDD and Cl^{37}-TCDD.

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from packed gas chromatographic columns, and capillary columns must be employed to separate the two isomers. Some other TCDD isomers can be better separated than 1,2,3,4-TCDD from 2,3,7,8-TCDD, but they are generally not available commercially and typically not pure enough to be used.

For clean water samples which do not require column chromatography clean up, 3,3'-4,4'-tetrachlorodiphenyl ether which can be fully extracted by methylene chloride (mean recovery was found to be 95% from three runs, with a standard deviation of 4.8%) is a very suitable surrogate compound. The elution patterns of this compound from the clean up columns were so different from the 2,3,7,8-TCDD that it cannot be recommended as an internal standard without further and extensive studies.

The Recommended Protocol

Based on the findings mentioned in the above sections, a protocol was formulated to analyze TCDD in industrial wastewaters. Figure 1 is a flow chart of these procedures, and a detailed protocol is attached with this report. The protocol includes three clean up columns investigated during the course of this study, the order of employing each of the columns as indicated in the flow chart is the same order which our laboratory follows in analyzing our wastewater samples; the analyst should experiment with the particular type of wastewater before choosing the number and type of columns. Since some loss of TCDD is almost unavoidable during sample manipulation, it is recommended that the analyst reduce to a minimum the number of clean up steps.

In order to determine the precision and accuracy of the protocol submitted with this report, five water samples were spiked with 100 ppt of 2,3,7,8-TCDD and then carried through the entire protocol. Analyses were performed after extraction as well as after passage through each of three columns. The results obtained were as follows:

TABLE 6. % OF RECOVERIES OF SPIKED WATER SAMPLES

Runs	After Extraction	1 Column[a] Clean up	2 Columns[b] Clean up	3 Columns[c] Clean up
1	90.	83	89	25
2	94.	87	62	37
3	96.	88	66	41
4	90.	83	81	46
5	93.	85	85	43
Mean	93.	85	77	38
Standard Deviation	2.6	2.3	12	8.2

a. Silica gel
b. Silica gel + alumina
c. Silica gel + alumina + mixture of charcoal and silica gel.

In addition to recoveries performed on distilled water, seven industrial wastewater samples were also spiked with various amounts of 2,3,7,8-TCDD and then analyzed. The spiking levels and results are as follows:

TABLE 7. RECOVERIES OF TCDD FROM WASTEWATER

Sample Identification[1]	Spiking Level, ppb	# of Clean Up Columns	Instrument	% Recovery
1A	20	One	GC/MS	63
1B	0	One	GC/MS	*
2A	40	One	GC/MS	70
2B	0	One	GC/MS	*
3A	80	One	GC/MS	75
3B	0	One	GC/MS	*
4A	20	One	GC/MS	71
4B	0	One	GC/MS	*
5A	100	Three	GC/EC	38
5B	0	Three	GC/EC	*
6A	100	Three	GC/EC	51
6B	0	Three	GC/EC	*
7A	100	Three	GC/EC	41
7B	0	Three	GC/EC	*

1. Industrial wastewater from other EPA projects, original source unknown.

* No TCDD was detected on any of the unspiked samples.

The above data indicated the four samples which were cleaned up with the silica gel column only yielded slightly lower percentage of recovery than the spiked distilled water while the recoveries of the last three samples were very close to that of spiked distilled water after going through all three clean up columns. Taking the experimental errors, the differences between levels of spiking as well as the qualities of the wastewater into consideration, the recoveries between clean and industrial waters are actually rather close.

Two sets of seven municipal wastewater samples, each obtained from the Sacramento Wastewater Treatment plant, were spiked with TCDD at levels of 0.015 µg/L, or 5 x detection limit and 0.15 µg/L, 50 x detection limit and analyzed for precision and accuracy studies according to the recommended protocol. Preliminary studies indicated that the alumina column clean up was required before the sample can be analyzed by GC/MS. The results obtained were given in the following table.

TABLE 8. PRECISION AND ACCURACY STUDIES OF TCDD ANALYSIS FOR WASTEWATER (FROM SEVEN REPLICATES)

Spiking Level	% Recovery of 7 Replicates	Mean Recovery	Standard Deviation
0.015 µg/L	85.0; 81.2; 74.7, 90.6 87.1; 85.3; 81.7	83.7%	5.1%
0.150 µg/L	84.6; 82.2; 79.0; 81.5	82.2	4.2%

NOTE: No TCDD was found in two unspiked samples. Detection limit was 0.003 µg/L.

SECTION 5

STABILITY STUDIES

SHELF LIFE OF TCDD STANDARDS IN ORGANIC SOLVENTS

Since standard solutions of priority pollutants are generally prepared and sent to other laboratories by EPA, it is important to know optimum storage conditions to prevent undesirable degradation of the chemical. The purpose of this study was to measure the shelf life of TCDD standards prepared in benzene, acetone and methanol at both the refrigerated temperature of 4°C and the shipping temperature of 25°C.

Procedure

2,3,7,8-tetrachlorodibenzo-1,4-dioxin (TCDD) stock standard of 0.1 mg/mL in benzene was diluted to a final concentration of 100 µg/mL in the following solvents: benzene, acetone, and methanol. After each of these standards were prepared, 1.0 mL was pipetted into a 2.0 mL vial and sealed with a Teflon lined screw cap. These vials, 18 for each solvent used, were placed in a refrigerator at 4°C for the duration of the study. Immediately prior to each sampling period at 30, 60 and 90 days, six vials of each solvent were removed from the refrigerator and left at room temperature -- 25°C -- for a period of five to seven days. During this period, the vials remained in the dark.

Using a 10.0 µL syringe, 4 µL injections of each sample were injected for GC/EC analysis. This was done in triplicate for each vial, resulting in 18 injections for each solvent. TCDD in hexane standard was made fresh daily from the stock standard (0.1 mg/mL). Injections of 2 through 6 µg/L were used to establish standard curves. Finally, using the curves, the average recovery for each solvent group was determined. Also a linear regression program was used to determine the average recovery. In most cases these values are close to those obtained from the standard curve.

Results and Discussion

30 Days Storage.

TABLE 9. % OF RECOVERY OF TCDD AFTER 30 DAYS OF STORAGE

Vials	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	Mean	Std. Dev.
Benzene	99%	99%	100%	101%	102%	104%	101%	1.9%
Acetone	95%	94%	92%	92%	90%	90%	92%	2.0%
Methanol	99%	96%	99%	96%	96%	88%	96%	4.0%

60 Days Storage

TABLE 10. % OF RECOVERY OF TCDD AFTER 60 DAYS OF STORAGE

Vials	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	Mean	Std. Dev.
Benzene	100%	100%	108%	113%	86%	95%	100%	9.8%
Acetone	105%	105%	94%	93%	101%	98%	99%	5.2%
Methanol	84%	94%	103%	96%	97%	100%	96%	6.5%

90 Days Storage

TABLE 11. % OF RECOVERY OF TCDD AFTER 90 DAYS OF STORAGE

Vials	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	Mean	Std. Dev.
Benzene	101%	105%	105%	103%	110%	100%	104%	3.6%
Acetone	106%	106%	105%	106%	105%	104%	105%	0.8%
Methanol	94%	95%	98%	97%	102%	107%	99%	4.9%

The above data clearly indicate that TCDD is very stable in benzene, acetone, and methanol when stored at 4°C and during shipping at 25°C for five to seven days. The mean values of recovery are generally within or very close to ± 1 standard deviation except in one case in which the mean value is 105% and the standard deviation has an exceptionally low value of 0.8%. It can be concluded that the changes in TCDD concentration is not significant and within experimental error.

THE EFFECT OF CHLORINATION ON STABILITY OF TCDD IN WATER, A PRESERVATION STUDY

Since chlorine, a very powerful oxidizing agent, is the most common disinfectant used in water treatment, its effect on the level of TCDD was investigated. A preservation study was conducted to determine the changes, if any, of TCDD in water with and without chlorine treatment at three pH levels. The results of this study can serve the purpose in determining the optimum sampling, storage of water samples as well as how soon such samples must be extracted after sampling.

Procedure

Water samples of 1 liter in a gallon bottle at pH levels of 2, 7, and 10 were spiked with 1 ppb of TCDD together with 10 ppm chlorine (from sodium

hypochlorite) and stored at temperatures of 4°C and 25°C for 14 days. A similar set of samples without chlorine spiked with 1 ppb of TCDD served as controls. The water was extracted and analyzed by GC/MS, because sodium hypochlorite causes interference with GC/EC determinations.

Results and Discussion

The following results were obtained. Each value represents the mean of triplicate runs.

TABLE 12. % RECOVERIES OF 1 µg/L OF TCDD IN WATER AFTER CHLORINE TREATMENT

	0 Day		14 Days 4°C		14 Days 25°C	
pH Level	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
2	91.3	10	43.6	6.2	50.5	6.0
7	81.7	8.2	49.7	5.5	37.6	10.3
10	84.0	5.7	42.5	8.2	32.1	8.6

TABLE 13. % RECOVERIES OF 1 ppb OF TCDD IN WATER WITHOUT CHLORINE TREATMENT

	0 Day		14 Days 4°C		14 Days 25°C	
pH Level	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
2	81.1	9.0	65.5	8.0	77.5	10.9
7	90.9	9.1	67.5	7.6	77.0	5.9
10	97.7	8.1	74.4	4.6	79.1	7.2

When the mean recoveries of the above two tables were combined and the loss of TCDD due to the net effect of chlorination after 14 days of storage computed, the following results were obtained:

TABLE 14. LOSS OF TCDD DUE TO CHLORINATION IN 14 DAYS

	% of TCDD Loss	
pH	4°C	25°C
2	33	35
7	26	51
10	43	59

Since the study was conducted only at a single storage period of 14 days, two temperatures and three pH levels, it is difficult to establish any trend and generalization from the above data. One conclusion that can be drawn is that the loss of 30% to 60% of TCDD due to chlorination followed by 14 days of storage even at a low temperature of 4°C, can cause significant discrepancies between the actual level of TCDD in the wastewater in the environment and the analytical results. In order to study further the rate of the disappearance of TCDD, a second experiment which was designed to eliminate potential loss due to transferring, extraction and evaporation in the normal analytical procedure was conducted. In this experiment, 90 ng of TCDD in 9.0 µL of acetone was spiked into 90 mL water contained in a 100 mL volumetric flask. It was then chlorinated with 10 ppm of chlorine (from sodium hypochlorite), and 1.0 mL of purified hexane was added to the solution to extract the TCDD at the end of the storage period. The hexane layer was then analyzed by GC/MS. Storage periods of 0, 3, 6, 9, 12, 15 and 21 days were studied. The results obtained are given in Table 15 as well as graphically plotted in Figure 8. No degradation was observed in a control set which was held at identical experimental conditions except it was not chlorinated.

TABLE 15. THE EFFECT OF CHLORINATION ON TCDD CONCENTRATION IN WATER

Storage Period	pH 7 at 25°C % of TCDD Recovered	% of TCDD Loss
0	100	0
3	94.9	5.1
6	89.6	10.4
9	82.9	17.1
12	81.2	18.8
15	77.9	22.1
21	71.6	28.4

The degradation rate observed in this experiment is somewhat slower than those reported in the first, this is probably due to the modified procedure used in the second experiments to eliminate losses due to non-oxidative causes such as evaporation. The results reported in the above table should be considered much more reliable since seven data points are used to construct the degradation while only two data points, although each computed by triplicate runs, were obtained in the first experiment (0 and 14 days).

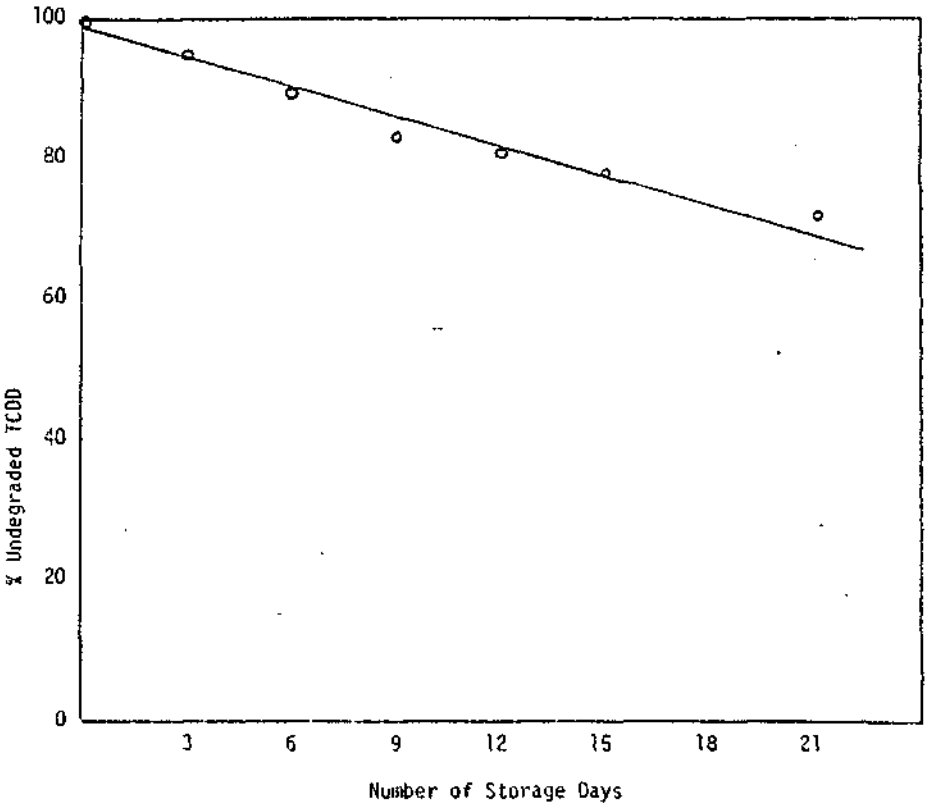

Figure 8. Degradation of TCDD as a result of chlorination.

SECTION 6

RECOMMENDED PROTOCOL: TCDD METHOD 613

The recommended protocol given here, with the exception of the section on precision and accuracy, was written by the U.S. EPA project officer. The basis of the protocol were experimental results obtained from this study.

SCOPE AND APPLICATION

This method covers the determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The following parameter may be determined by this method:

Parameter	Storet No.
TCDD	34675

This method is applicable to the determination of TCDD in municipal and industrial discharges. It is designed to be used to meet the monitoring requirements of the National Pollutant Discharge Elimination System (NPDES). As such, it presupposes the potential for finding trace levels of TCDD in the sample. The method incorporates techniques that can also be used to screen samples for TCDD using the highly sensitive electron capture detector.

The sensitivity of this method is usually dependent upon the level of interferences rather than instrumental limitations. The limit of detection listed in Table 16 represents the sensitivity that can be achieved in wastewaters under optimum conditions.

This method is recommended for use only by experienced residue analysts or under the close supervision of such qualified persons.

Because of the extreme toxicity of this compound, the analyst must take elaborate precautions to prevent exposure to himself, or to others, of materials known or believed to contain TCDD.

TABLE 16. GAS CHROMATOGRPAHY OF TCDD

Column	Retention Time (min.)	Det. Limit (µg/L)[a]
Packed[b]	7.3	0.003
Glass Capillary[c]	9.5	0.003

[a]Detection Limit is calculated from the minimum detectable GC response being equal to five times the GC background noise, assuming a 1 mL effective final volume of the 1 liter sample extract, and assuming a GC injection of 5 microliters. Detection levels apply to both electron capture and GC/MS detection.

[b]Packed column conditions: Supelcoport 100/120 mesh coated with 1.5% SP-2250/1.95% SP-2401 packed in a 180 cm long x 2 mm I.D. glass column with 5% Methane/95% Argon carrier gas at 25 mL/min flow rate. Column temperature is 220°C.

[c]Glass capillary column conditions: SP-2250 coated on a 30 m long, x 0.25 mm I.D. glass column (Supelco No. 2-3714 or equivalent) with helium carrier gas at 30 cm/sec linear velocity run splitless. Column temperature is 210°C.

Section 7 contains guidelines and protocols that should serve as minimum safe handling standards for the laboratory.

SUMMARY OF METHOD

1. A 1 liter sample of wastewater is extracted with methylene chloride using separatory funnel techniques. The extract is dried and exchanged to hexane while being concentrated to a volume of 1.0 mL or lower. Capillary column GC/MS conditions and internal standards techniques are described which allow for the accurate measurement of TCDD in the extract. Electron capture gas chromatographic conditions are also provided to permit the analyst to use this equipment to prescreen samples before GC/MS analysis.

2. If interferences are encountered, the method provides selected general purpose clean up procedures to aid the analyst in their elimination.

INTERFERENCES

1. Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and or elevated baselines causing misinterpretation of gas chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by running method blanks. Specific selection of reagents and purification of solvents by distillation in all glass systems may be required.

2. Interferences coextracted from the samples will vary considerably from source to source, depending upon the diversity of the industrial complex or municipality being sampled. TCDD is often associated with other interfering chlorinated compounds which are at concentrations several magnitudes higher than that of TCDD. While general clean up techniques are provided as part of this method, unique samples may require additional clean up approaches to achieve the sensitivity stated in Table 16.

3. The other isomers of tetrachlorodibenzo-p-dioxin may interfere with the measurement of 2,3,7,8-TCDD. Capillary column gas chromatography is required to resolve those nine isomers that yield virtually identical mass fragmentation patterns.

APPARATUS AND MATERIALS

1. Sampling equipment, for discrete or composite sampling.

   a. Grab sample bottle -- amber glass, liter or quart volume. French or Boston Round design is recommended. The container must be washed and solvent rinsed before use to minimize interferences.

   b. Bottle caps -- Threaded to screw on sample bottles. Caps must be lined with Teflon. Foil may be substituted if sample is not corrosive.

   c. Compositing equipment -- Automatic or manual compositing system. Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated during sampling. No tygon or rubber tubing or fittings may be used in the system.

2. Separatory funnels -- 2000 mL and 500 mL, with Teflon stopcock.

3. Drying column -- A 20 mm I.D. pyrex chromatographic column with coarse frit.

4. Kuderna-Danish (K-D) Apparatus

   a. Concentrator tube -- 10 mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked. Ground glass stopper (size 19/22 joint) is used to prevent evaporation of extracts.

   b. Evaporative flask -- 500 mL (Kontes K-57001-0500 or equivalent). Attach to concentrator tube with springs. (Kontes K-672750-0012).

   c. Snyder column -- three ball macro (Kontes K-503000-0121 or equivalent).

   d. Snyder column -- two ball micro (Kontes K-569001-0219 or equivalent).

   e. Boiling chips -- extracted, approximately 10/40 mesh.

5. Water bath -- heated, with concentric ring cover, capable of temperature control (± 2°C). The bath should be used in a hood.

6. Gas chromatograph -- Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including electron capture, packed and capillary column supplies, recorder, gases, syringes. A data system for measuring peak areas is recommended.

7. GC/Mass Spectrometer system -- electron impact source, capable of selected ion monitoring in groups of two or more (Finnigan 1500 or equivalent).

8. Chromatography column -- 30 mm long x 10 mm I.D. with coarse fritted disc at bottom and Teflon stopcock.

9. Chromatography column -- 400 mm long x 11 mm I.D. with coarse fritted disc at bottom and Teflon stopcock.

10. Pipets -- Disposable, Pasteur, 150 mm long x 5 mm I.D. (Fisher Scientific Co., No. 13-678-6A or equivalent).

REAGENTS

1. Sodium hydroxide -- (ACS) 10 N and 1 N in distilled water. Wash the solutions with methylene chloride and with hexane.

2. Sulfuric acid -- (ACS) Mix equal volumes of conc. H_2SO_4 with distilled water (1+1) and 1 N. Wash the solutions with methylene chloride and with hexane.

3. Methylene chloride, hexane, benzene, tetradecane -- Pesticide quality or equivalent.

4. Sodium sulfate -- (ACS) Granular, anhydrous (purified by heating at 400°C for 4 hours.

5. Stock standards -- In a glovebox, prepare stock standard solutions of TCDD and ^{37}Cl-TCDD. The stock solutions are stored in the glovebox, and checked frequently for signs of degradation or evaporation, especially just prior to preparing working standards from them.

6. Silica gel -- high purity grade, 100/120 mesh, (Fisher Scientific Co., No. S-679 or equivale).

7. Alumina -- neutral, 80/200 mesh (Fisher Scientific Co., No. A-540 or equivalent). Before use activate for 24 hours at 130°C in a foil covered glass container.

8. Activated Coconut Charcoal -- 50/200 mesh (Fisher Scientific Co., No. 5-690A or equivalent).

CALIBRATION

1. Prepare calibration standards for the internal standard technique that will allow for measurement of relative response fractors of at least three TCDD/^{37}Cl-TCDD ratios. The ^{37}Cl-TCDD concentration in the standards should be fixed and selected to yield a reproducible response at the most sensitive setting of the mass specrometer.

2. Assemble the necessary GC or GC/MS appparatus and establish operating parameters equivalent to those indicated in Table 16. Calibrate the GC/MS system according to Eichelberger, et al (1975). By injecting calibration standards, establish the response factors for TCDD vs ^{37}Cl-TCDD.

3. Before using any clean up procedure, the analyst must process a series of calibration standards through the system to validate elution patterns and the absence of interferences from the reagents.

QUALITY CONTROL

1. Before processing any samples, the analyst should demonstrate through the analysis of a distilled water method blank, that all glassware and reagents are interference free. Each time a set of samples is extracted or there is a change in reagents, a method blank should be processed as a safeguard against chronic laboratory contamination.

2. Standard quality assurance practices should be used with this method. Field replicates should be collected to validate the precision of the sampling technique. Laboratory replicates should be analyzed to validate the precision of the analysis. Fortified samples should be analyzed to validate the accuracy of the analysis, although surrogate spikes are recommended because of the toxicity of TCDD. Where doubt exists over the identification of a peak on the electron capture chromatogram, mass spectroscopy must be used for clarification or confirmation.

SAMPLE COLLECTION, PRESERVATION AND HANDLING

1. Grab samples must be collected in glass containers. Conventional sampling practices should be followed, except that the bottle must not be prewashed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be free of tygon and other potential sources of contamination.

2. The samples must be iced or refrigerated from the time of collection until extraction. Chemical preservatives should not be used in the field unless more than 24 hours will elapse before delivery to the laboratory. If the samples will not be extracted within 48 hours of collection, the sample should be adjusted to a pH range of 6.0-8.0 with sodium hydroxide or sulfuric acid.

3. All samples must be extracted within seven days and completely analyzed within 30 days of collection.

SAMPLE EXTRACTION

CAUTION: If there is a remote possibility that the sample contains TCDD at measureable levels, all of the following operations must be performed in a limited access laboratory with the analyst wearing full protective covering for all exposed skin surfaces.

1. Mark the water meniscus of the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2 liter separatory funnel. Check the pH with wide-range paper and adjust to within the range of 5 to 9 with sodium hydroxide or sulfuric acid.

2. Add 60 mL methylene chloride to the sample bottle and shake 30 seconds to rinse the walls. Transfer the solvent into the separatory funnel, and extract the sample by shaking the funnel for two minutes with periodic venting to release vapor pressure. Allow the organic layer to separate from the water phase for a minimum of ten minutes. If the emulsion interface between layers is more than one third the size of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, or centrifugation. Collect the methylene chloride extract in a 250 mL separatory funnel.

3. Add a second 60 mL volume of methylene chloride to the sample bottle and complete the extraction procedure a second time, combining the extracts in the 500 mL separatory funnel.

4. Perform a third extraction in the same manner. To the combined extracts in the separatory funnel add 100 mL 1 N NaOH. Shake the funnel for 30 to 60 seconds. Allow the layers to separate and draw the organic layer into a 250 mL Erlenmeyer flask. Discard the aqueous layer and return the organic layer to the separatory funnel. Perform a second wash of the organic layer with 1 N NaOH and discard the aqueous layer.

5. In the same manner wash the organic layer twice with 100 mL 1 N N_2SO_4, discarding the aqueous layers.

6. Wash the organic layer three times with 100 mL H_2O, discarding the aqueous layers.

7. Pour the organic layer extract through a drying column containing 3 to 4 inches of anhydrous sodium sulfate, and collect it in a 500 mL K-D flask equipped with a 10 mL concentrator tube. Rinse the Ehrlenmeyer flask and column with 20 to 30 mL methylene chloride to complete the quantitative transfer.

8. Add one to two clean boiling chips to the flask and attach a three ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a steaming hot (60-65^{o}C) water bath so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed in steam. Adjust the vertical position of the apparatus and the water temperatures required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain for at least 10 minutes while cooling.

9. Momentarily remove the Snyder column, add 50 mL hexane and a new boiling chip and replace the Snyder column. Increase the temperature of the water bath to 80°C. Prewet the Snyder column by adding about 1 mL hexane to the top. Evaporate the solvent as in Step 8. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane. A 5 mL syringe is recommended for this operation.

10. Add a clean boiling chip and attach a micro Snyder column. Prewet the column by adding about 1 mL hexane to the top. Place the K-D apparatus on the 80°C water bath so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 5 to 10 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches about 0.5 mL, remove the K-D apparatus and allow it to drain for at least 10 minutes while cooling. Remove the micro Snyder column and rinse its lower joint into the concentrator tube with 0.2 mL hexane. Adjust the extract volume to 1.0 mL with hexane. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately.

11. Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1000 mL graduated cylinder. Record the sample volume to the nearest 5 mL.

12. The analyst has several options available to him depending upon the nature of the sample and the availability of resources:

    a. If the appearance of the extract or previous experience with the matrix indicates clean up will be required, the analyst should proceed with one or more techniques as described in the clean up and separation steps.

    b. If the analyst wishes to screen the sample for the possible presence of TCDD before GC/MS analysis, he can analyze the extract by packed column or capillary column electron capture detection.

    c. The analyst may proceed directly to GC/MS analysis.

CLEAN UP AND SEPARATION

1. Several clean up column choices are offered to the analyst in this section. Although any of them may be used independently, the silica gel column followed immediately by the alumina column has been used frequently to overcome background problems encountered by the GC/MS.

2. Silica Gel Column Clean up for TCDD.

    a. Fill a 400 mm long x 11 mm I.D. chromatography column with silica gel to the 300 mm level, tapping the column gently to settle the silica gel. Add 10 mm anhydrous sodium sulfate to the top of the silica gel.

b. Preelute the column with 50 mL 20% benzene/80% hexane (V/V). Adjust the elution rate to 1 mL/min. Discard the eluate and just prior to exposure of the sodium sulfate layer to the air, transfer the entire 1.0 mL sample extract onto the column, using two 2 mL portions of 20% benzene/80% hexane to complete the transfer.

c. Just prior to exposure of the sodium sulfate layer to the air, add 40 mL 20% benzene/80% hexane to the column. Collect the eluate in a 500 mL K-D flask equipped with a 10 mL concentrator tube.

d. Evaporate the fraction to 1.0 mL by standard K-D techniques. Analyze by ECGC, GC/MS or continue clean up as described below.

3. Alumina Column Clean up for TCDD.

a. If the extract is not in hexane, add 0.1 to 0.2 mL tetradecane keeper and concentrate it at room temperature down to this volume using a stream of dry nitrogen gas. Dilute to 1.0 mL with hexane.

b. Fill a 300 mm long x 10 mm I.D. chromatography column with activated alumina to the 150 mm level, tapping the column gently to settle the alumina. Add 10 mm anhydrous sodium sulfate to the top of the alumina.

c. Preelute the column with 50 mL hexane. Adjust the elution rate to 1 mL/min. Discard the eluate and just prior to exposure of the sodium sulfate layer to the air, transfer the entire 1 mL sample extract onto the column, using two additional 2 mL portions of hexane to complete the transfer.

d. Just prior to exposure of the sodium sulfate layer to the air, add 50 mL 3% methylene chloride/97% hexane (V/V) and continue the elution of the column. Discard the eluate.

e. Next elute the column with 50 mL 20% methylene chloride/80% hexane (V/V) into a 50 mL K-D flask equipped with a 10 mL concentrator tube. Concentrate the collected fraction to 1.0 mL by standard K-D technique. Analyze by ECGC, GC/MS or continue clean up as described below.

4. Charcoal and Silica-gel Column Clean up for TCDD.

a. Prepare a homogeneous mixture of one part activated charcoal to 140 parts silica-gel. Fill a 5 mm I.D. disposable pipet to a length of 50 mm, tapping the column to settle the mixture.

b. Preelute the column with 5 mL hexane. Discard the eluate and just prior to exposure of the top of the column to the air, transfer an 0.5 mL aliquot of sample extract onto the column, using an additional 0.5 mL hexane to complete the transfer.

c. Just prior to exposure of the top of the column to the air, add 10 mL hexane and continue the elution of the column. Discard the eluate.

d. Next, elute the column with 10 mL benzene into a 10 mL K-D concentrator tube. Concentrate the eluate to 1.0 mL with micro K-D concentration on a boiling water bath. Analyze by ECGC or GC /MS.

ELECTRON CAPTURE SCREENING

1. The sample extracts cam be screened by electron capture gas chromatography at the option of the analyst in an effort to reduce the workloal on the GC/MS system. Either packed or capillary column techniques may be used for this purpose. The only acceptable conclusions that can be reached with this technique are: (a) TCDD is not detectable at the detection limit of the procedure, (b) TCDD is not present above a stated concentration or control level, and (c) the presence or absence of TCDD is unresolved.

2. Table 16 summarizes some recommended gas chromatographic column materials and operating conditions for the instrument. Included in this table are estimated retention times and sensitivities that should be achieved by this method. Examples of the Chromatography achieved by these columns are shown in Figures 2 and 6. Calibrate the system daily with a minimum of three injections of calibration standards.

3. For packed column GC, inject a 2 to 5 µL of the sample extract using the solvent-slush technique. A splitless injector is recommended for the capillary system, but solvent exchange to tetradecane is required. Record the volume injected to the nearest 0.05 µL, and the resulting peak size, in area units.

4. If there is no measureable baseline deflection at the retention time of TCDD, report the result as less than the detection limit of the electron capture system.

5. If a measureable peak appears within the tolerances of the TCDD retention time of the system, the analyst should proceed to GC/MS.

6. If the complexity of the chromatogram defies interpretation, the analyst may want to pursue clean up followed by reanalysis by ECGC, or proceed directly to GC/MS.

GC/MS ANALYSIS

1. Table 16 summarizes the recommended capillary column gas chromatographic materials and operating conditons for the instrument. Included in this table is the estimated retention time and sensitivity that should be achieved by this method. An example of the chromatography achieved by this column is shown in Figure 6. Calibrate the system daily, with a minimum at three injections of standard mixtures.

2. Add a known amount of ^{37}Cl-TCDD to the sample extract.

3. Analyze samples with selected ion monitoring of at least two ions characteristic of TCDD (m/e 320 and m/e 322). Proof of the presence of TCDD exists if the following conditions are met:

   a. The retention time of the peak in the sample must match that in the standard, within the performance specifications of the analytical system.

   b. The ratio of ions (320:322) must agree within 10% of that in the standard.

   c. The retention time of the peak maximum for the m/e 320 peak must exactly match that of the 322 peak.

4. Quantitate the TCDD peak from the response relative to the ^{37}Cl-TCDD internal standards.

5. If a response is obtained for both ions but is outside the expected ratio, then a co-eluting impurity may be suspected. In this case, another set of ions, in the m-COCl (257-259) group should be analyzed. These ions are useful in characterizing the molecular structure of TCDD. Other ions as arising from suspected impurities such as DDE, DDD or PCB residues may also be determined. The choice of these ions would be based upon the discretion of the analyst with a knowledge of the particular matrix under study. In particular, analysis of the EC chromatogram will provide insight into the complexity of the problem and will determine the manner in which the mass spectrometer will be used.

6. If broad background interference restricts the sensitivity of the GC/MS analysis, the anlyst should employ clean up procedures and reanalyze by GC/MS.

7. In those circumstances where these procedures do not yield a definitive conclusion, then the use of high resolution mass spectrometry is suggested.

CALCULATIONS

1. Determine the concentration of individual compounds according to the formula:

$$\text{Concentration, } \mu g/L = \frac{(A)\quad(V_t)}{(V)_i\quad(V_s)}$$

where A = Nanograms TCDD injected into the GC/MS.

V_i = Volume of extract injected (µL)

V_t = Volume of total extract (µL)

V_s = Volume of water extracted (mL)

2. Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, all data obtained should be reported.

PRECISION AND ACCURACY

The following precision and accuracy information were obtained from five water samples spiked with 100 ppt of TCDD.

	No. Column Clean up	One Column Clean up[a]	Two Column Clean up[b]	Three Column Clean up[c]
Mean, %	93	85	77	38
Std. Dev. %	2.6	2.3	12	8.2

[a] Silica gel column
[b] Silic gel column + alumina column
[c] Silica gel column + Alumina column + charcoal/silica gel mixture column

SECTION 7

SAFE HANDLING PRACTICES FOR TCDD

Dow Chemical Co. has issued the following description of safe handling practices for TCDD in the laboratory. In addition to these practices, the following points are also helpful.

1. Contamination of the laboratory will be minimized by conducting all manipulations in the hood.

2. Effluent of the gas chromatography (from the Nickel-63 detector or as a result of splitting when capillary columns are used) should pass through either a column of activated charcoal or bubbled through a trap containing oil or high-boiling alcohols.

3. Liquid waste can be dissolved in methanol or ethanol and irradiated with ultraviolet light with wavelength greater than 290 nm for several days.

SUMMARY OF SAFE HANDLING OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) IN THE LABORATORY

Biochemical Research Laboratory
The Dow Chemical Company
Midland, Michigan
February 10, 1970

The followings statements on safe handling are as complete as possible on the basis of available toxicological information. The precautions for safe handling and use are necessarily general in nature since detailed, specific recommendations can be made only for the particular exposure and circumstances of each individual use. Inquiries about specific operations or uses may be addressed to the Dow Chemical Co. Assistance in evaluating the health hazards of particular plant conditions may be obtained from certin consulting laboratories and from State Departments of Health or of Labor, many of which have an industrial health service.

* * * *

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is extremely toxic. However, it has been handled for years without injury in analytical and biological laboratories. Techniques used in handling radioactive and infectious materials are applicable to TCDD.

Protective Equipment: Throw away plastic gloves, apron or lab coat, safety glasses and lab hood adequate for radioactive work. Workers must be trained in the proper method of getting out of contaminated gloves and clothing WITHOUT CONTACTING THE EXTERIOR SURFACES.

Personal Hygiene: Thorough washing of hands, and forearms after each manipulation and before breaks, coffee, lunch and a change of shift.

Confinement: Isolated work area, posted with signs, segregated glassware and tools, plastic backed absorbent paper on bench tops.

Waste: Good technique includes minimizing contaminated waste. Plastic bag liners should be used in waste cans. Janitors must be trained in safe handling of waste (one accidental case of chloracne resulted from handling laboratory waste in a routine manner).

Disposal of Wastes: TCDD decomposes above 800°C. Low level waste such as the absorbent paper, tissues, animal remains and plastic gloves may be burned in a good incinerator. Gross quantities (milligrams) should be packaged securely and disposed of through commercial or governmental channels which are capable of handling high level radioactive wastes or extremely toxic wastes. Liquids should be allowed to evaporate in a good hood and in a disposable container. Residues may then be handled as above.

Decomtamination: Personal -- any mild soap with plenty of scrubbing action. Glassware, Tools and Surfaces -- Chlorothene NU is the least toxic solvent shown to be effective. Satisfactory cleaning may be accomplished by rinsing with chlorothene, then washing with any detergent and water. Dish water may be disposed of to the sewer. It is prudent to minimize solvent wastes because they may require special disposal through commercial sources which are expensive.

Laundry: Clothing known to be contaminated should be disposed of with the precautions described under "Disposal of Wastes." Lab coats or other clothing worn in TCDD work may be laundered. Clothing should be collected in plastic bags. Persons who convey the bags and launder the clothing should be advised of the hazard and trained in proper handling. The clothing may be put into a washer without contact if the laundryman knows the problem. The washer should be run through a cycle before being used again on other clothing.

Wipe Tests: A useful method of determining cleanliness of work surfaces and tools is to wipe the surface with a piece of filter paper which is extracted for 30 minutes, with agitation, in 5 mL of chloroform or benzene at room temperature. For prompt analysis gas chromatograhy may be used. Less than 1 microgram TCDD per sample indicates acceptable cleanliness; anything higher warrents further cleaning. More than 10 micrograms on a wipe sample indicates an acute hazard and requires prompt cleaning before further use of the equipment or work space. It indicates further that unacceptably sloppy habits have ben employed in the past.

Wipe test extracts may be applied to the rabbit ear according to the technique of Adams, et al. This method is more sensitive but does not give answers for three weeks. Any positive response indicates the need for further cleaning; any response greater than slight indicates the need for prompt cleaning and improvement in work habits.

Inhalation: Any procedure that may produce airborne contamination must be done with good ventilation. Gross losses to a ventilation system must not be allowed. Handling of the dilute solutions normally used in analytical and animal work presents no inhalation hazards except in case of an accident.

Accidents: Remove contaminated clothing immediately, taking precautions not to contaminate skin or other articles. Carefully place such clothing in a plastic bag for burning. Wash exposed skin vigorously and repeatedly until medical attention is obtained.

For clinical advice, contact B. B. Holder, M.D., Midland Division Medical Director, The Dow Chemical Company, Midland, Michigan, 48640, telephone (Area Code 517) 636-2243. For detailed safe handling precautions for specific procedures, contact L. G. Silverstein, Biochemical Research Laboratory, The Dow Chemical Company, Midland, Michigan, 48640, telephone (Area Code 517) 636-4676.

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