QUANTITATIVE CAPILLARY COLUMN GAS CHROMATOGRAPHY-MASS SPECTROMETRY METHODS OF ANALYSIS FOR TOXIC ORGANIC COMPOUNDS

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ABSTRACT

High resolution glass capillary column gas chromatography (GC) coupled with low or high resolution mass spectrometry (HRMS) is one of the most powerful tools in analytical chemistry. Low concentrations, nanogram- and picogram-per-gram (ng/g and pg/g) levels, of toxic organic compounds in complex sample media can be unambiguously identified and quantified utilizing this technique.

A capillary column GC-HRMS multiple ion selection method of analysis was developed and utilized for the quantitative determinations of ng/g and pg/g levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) residues in human, biological and environmental samples. Approximate minimum limits of detection were: human milk and adipose tissue - 0.5 to 4 ppt; beef, deer and elk adipose tissue - 2 to 5 ppt; fish - 2 to 20 ppt; water, soil and sediment - 0.02 to 5 ppt. The applications of this technique to the quantitative determination of TCDD, higher chlorinated dioxins, and other toxic compounds in needle biopsy samples of human tissue (ca. 100 milligrams) are also described. Variables that influence quantitative analyses, criteria for confirmation of toxic residues, detection limits, quality assurance programs for validation of results, and typical results are discussed in the text.

The GC-MS interface is a very critical part of this system. A versatile and unique GC-MS interface (1,2), which utilizes a positive helium atmosphere at the coupling point (atmospheric pressure to 1 x 10^{-6} Torr vacuum) and eliminates the need for a gas tight connection requiring graphite ferrules, was devised. The GC-MS interface has been in constant use for three years and has eliminated or minimized many of the problems associated with the use of glass capillary columns; e.g., vacuum leaks, and glass breakage. Additional advantages are: (1) direct GC-MS coupling for maximum transfer efficiency; (2) atmospheric pressure at the GC column exit to enhance column lifetime and efficiency; and (3) single valve isolation of the MS from the GC.

INTRODUCTION

Mass spectrometry (MS) was essentially created in 1898 when Wien[1] showed that a beam of positive ions could be deflected by electric and magnetic fields. Thompson[2] produced crude mass spectra in 1905 and documented the existence of two neon isotopes through the use of a single magnetic deflection instrument in 1912. Dempster[3] and Aston[4] in 1918 and 1919, respectively, designed more elaborate instruments for the measurement of relative abundance of isotopes. Reliable mass spectrometers became available in the 1940s and were used extensively then by the petroleum industry. During the 1960s, and especially in the 1970s, the field developed rapidly and has today become an indispensable tool for the qualitative and quantitative characterization of organic compounds.

While the mass spectrum is uniquely characteristic of the pure organic molecules being ionized, in most samples these compounds are encountered as only components of complex mixtures. Gas chromatography (GC) came into use in 1952 and was recognized to be an ideal separation tool for such complex mixtures. It was obvious that the combination GC and MS techniques would yield a very powerful analytical device. Unfortunately, the coupling of the instruments is quite difficult because of their incompatibility - the GC operating at atmospheric pressure, while the MS requires a high vacuum source. However, many types of GC-MS interfaces have been developed to meet the need; e.g., the frit separator introduced by Watson and Bieman[5], jet separators of Becker[6] and Ryhage[7], silcone membrane[8] and direct coupling.[9]

The GC-MS interface is a very critical part of this system described here. A versatile and unique GC-MS interface was developed[10] which utilizes a positive helium atmosphere at the coupling point (atmospheric pressure to less than 1×10^{-6} Torr vacuum) and eliminates the need for a gas tight connection requiring graphite ferrules. The interface has been in constant use in the authors' laboratory for three years and has eliminated most of the problems associated with the use of glass capillary columns; e.g., vacuum leaks and glass breakage. Additional advantages of the system are:

(1) direct GC-MS coupling insures maximum transfer efficiency;

(2) atmospheric pressure at the GC column exit enhances column lifetime and efficiency;

(3) single valve isolation of the MS from the GC;

(4) a polar and non-polar column is constantly ready for use and may be easily and rapidly (1 min) coupled to provide the desired chromatographic separation.

A direct-coupled capillary column GC-MS system is one of the most powerful tools in analytical chemistry. Polar and non-polar glass capillary GC columns provide optimal chromatographic resolution, the high sensitivity and maximum specificity. Nanogram- to picogram-per-gram (ng/g to pg/g) concentrations of toxic compounds present in complex mixtures may be unambiguously identified and quantitatively determined with this technique.

The application of HRMS techniques for the determination of parts-per-trillion (ppt) levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in various sample types has involved the following HRMS principles: (1) direct probe, single ion monitoring HRMS analysis;[11] (2) GC-HRMS double-ion monitoring;[12] and (3) the method of analysis described herein, which is based on glass capillary GC-HRMS techniques.[13] These techniques are quite versatile and may be easily and rapidly applied to the determination of other toxic compounds of interest; e.g., polychloroinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), etc.

It is imperative that the methodology utilized by U.S. Government regulatory agencies be validated. Therefore, great measures have been taken to address this requirement. The TCDD methodology utilized by the U.S. Environmental Protection Agency and its collaborating laboratories has been validated by scientifically acceptable methods which have included: (1) validation studies (blind samples fortified with known amounts of $^{37}Cl_4$-TCDD and 0.2 to 100 ppt TCDD); (2) the incorporation of quality assurance (QA) samples into the analysis of actual samples (10% to 20% of total); (3) multiple laboratory participation; and (4) the application of specific criteria for confirmation of TCDD residues.

Analyses for TCDD residues in human and environmental samples must be performed at the picogram-per-gram (ppt) concentration level because of its extreme toxicity and its occurrence as a trace contaminant (parts per billion) in specific chemical products. These analyses are complicated by the presence of naturally occurring compounds and chlorinated industrial pollutants such as PCBs, chlorinated benzylphenyl ethers, etc. Therefore,

extremely efficient and specific sample preparation procedures, coupled with highly sensitive and specific GC-HRMS detection techniques, are pre-requisites for TCDD analysis at the low ppt concentration range. The comprehensive sample preparation procedures and capillary column GC-HRMS methods of analysis for TCDD in various media are described elsewhere.[14]

The application of these techniques, and the results obtained for the quantitative determinations for TCDD in fresh water fish, fly ash from coal-fired power plants, and in 250 milligram samples of human tissue are discussed. Practical solutions to specific types of problems encountered in these quantitative analysis are described. The versatility of these techniques is shown in the determinations for polychlorinated dibenzofurans in ambient air samples collected near the incineration of PCBs.

EXPERIMENTAL

Instrumentation: A Varian MAT 311A mass spectrometer directly coupled to a Varian Model 2700 gas chromatograph (GC) was utilized for these analyses. The GC was equipped with a 30-m x 0.25-mm (i.d.) OV-101 WCOT glass capillary column capable of resolutions of 70,000 to more than 100,000 effective plates. The MS was equipped with a combination chemical ionization (CI) and electron impact (EI) ion source (operated in the EI mode), and an eight-channel hardware (manual control) multiple ion selection (MIS) device. Each MIS channel was individually controlled for selection of acceleration voltage, measurement of range output, signal bandwidth, compensation for background contamination, and integration rate. The intensities of the selected masses were monitored in a time-division multiplex system, alternatively set to each of the selected masses and recording their intensities simultaneously on an eight-channel Soltec recorder. The adjustable integration rate (0.01 to 1 sec) was sufficient to accurately reproduce capillary column peaks of 2-sec width at half peak-height. The electrostatic analyzer voltage (ESA) was monitored and used to calculate the exact acceleration voltage required for the specific masses to be monitored. The sensitivity by mass specific detection was dramatically increased compared to normal spectrum scanning because the specific masses were measured over a longer and integrated time basis.

The magnet current was tuned to perfluorokerosene (PFK) m/z 318.9793 and the MS adjusted for 7,000 to 8,500 mass resolution. The ESA voltage was monitored and used in calculating the exact acceleration voltages

required for TCDD masses 327.8847 ($C_{12}H_4O_2{}^{37}Cl_4$), 321.8936 ($C_{12}H_4O_2{}^{35}Cl_3{}^{37}Cl$) and 319.8965 ($C_{12}H_4O_2{}^{35}Cl_4$). The calculated values were introduced into MIS channels 2, 3, and 4.

COCL Loss Analysis: The exact acceleration voltage required for TCDD masses m/z 256.9327, m/z 258.9298, m/z 319.8965, m/z 321.8936, and m/z 327.8847 were introduced into respective MIS channels utilizing PFK m/z 254.9856 as the reference. The analysis was performed through adherence to the previously described MIS procedure time schedule. The GC-HRMS MIS five channel simultaneous responses for TCDD and ^{37}Cl-TCDD were recorded on an eight channel Soltec recorder.

Elemental Composition Analysis: The MS was adjusted for 10,000 mass resolution utilizing PFK m/z 318.9793 as the reference. The peak matching analysis in real time was initiated under the exact time schedule of events utilized in the GC-HRMS MIS analysis. The PFK reference mass and TCDD masses (m/z 319.8965 or 321.8936) are observed to be exactly superimposed on the MS oscilloscope at the correct retention time of TCDD. The presence of TCDD isomers eluting before or after TCDD may also be confirmed in this analysis.

GC-HRMS Operating Parameters: Injection port temperature - 260°C; GC transfer line into MS source - 255°C; column temperature - 70°C; programmed at 34°C/min to 270°C, beginning exactly 6 minutes after injection of sample; GC-MS interface isolation valve closed at 12 minutes; MS ion source temperature - 235°C; electron energy - 70 eV; filament emission - 1 mA; variable acceleration voltage - 3 KV maximum; mass resolution - 7,000 to 10,000; multiplier gain - 10^6; MIS analysis initiated 16 minutes after injection of sample; TCDD and ^{37}Cl-TCDD retention

time - 20 minutes ± 15 seconds. MIS technique, electric peak jumping mode; TCDD integration rate, 100 milliseconds; ^{37}Cl-TCDD integration rate 30 milliseconds.

Precision and Accuracy of GC-HRMS Technique: The reproducibility of MIS peak-height responses for TCDD quantification standards during daily operation was calculated to be ± 20% relative to 5 or 10 pg injected. The GC-HRMS peak matching accuracy in real time for known exact masses was determined to be ± 2 millimass units at 9,500 mass resolution with PFK as the reference. A recent modification to the MS provided an additional gain of 10 or 100 to the maximum MS sensitivity. Peak matching analysis in real time at 9,500 mass resolution can be performed on 1 pg TCDD with this modification.

GC-MS Interface: The platinum capillary coupling point (vacuum to atmosphere) is similar to the technique published by Neuner-Jehle et al.[15] It provides an excellent point for establishing the desired pressure drop, good mechanical stability, and presents an inert surface to most organic compounds.

The interface diagram is shown in Figure 1. A positive helium atmosphere is maintained in the interface and surrounds the atmosphere-to-vacuum coupling point. The platinum capillary tubing and glass capillary column are butted together inside a glass sleeve located within the helium atmosphere. The pressure of the helium atmosphere can be varied to accommodate capillary column flow rates of 0.5 to 5.0 mL/min helium. The chromatograph end of the interface contains the helium atmosphere and is open to the atmosphere. Therefore, it can be

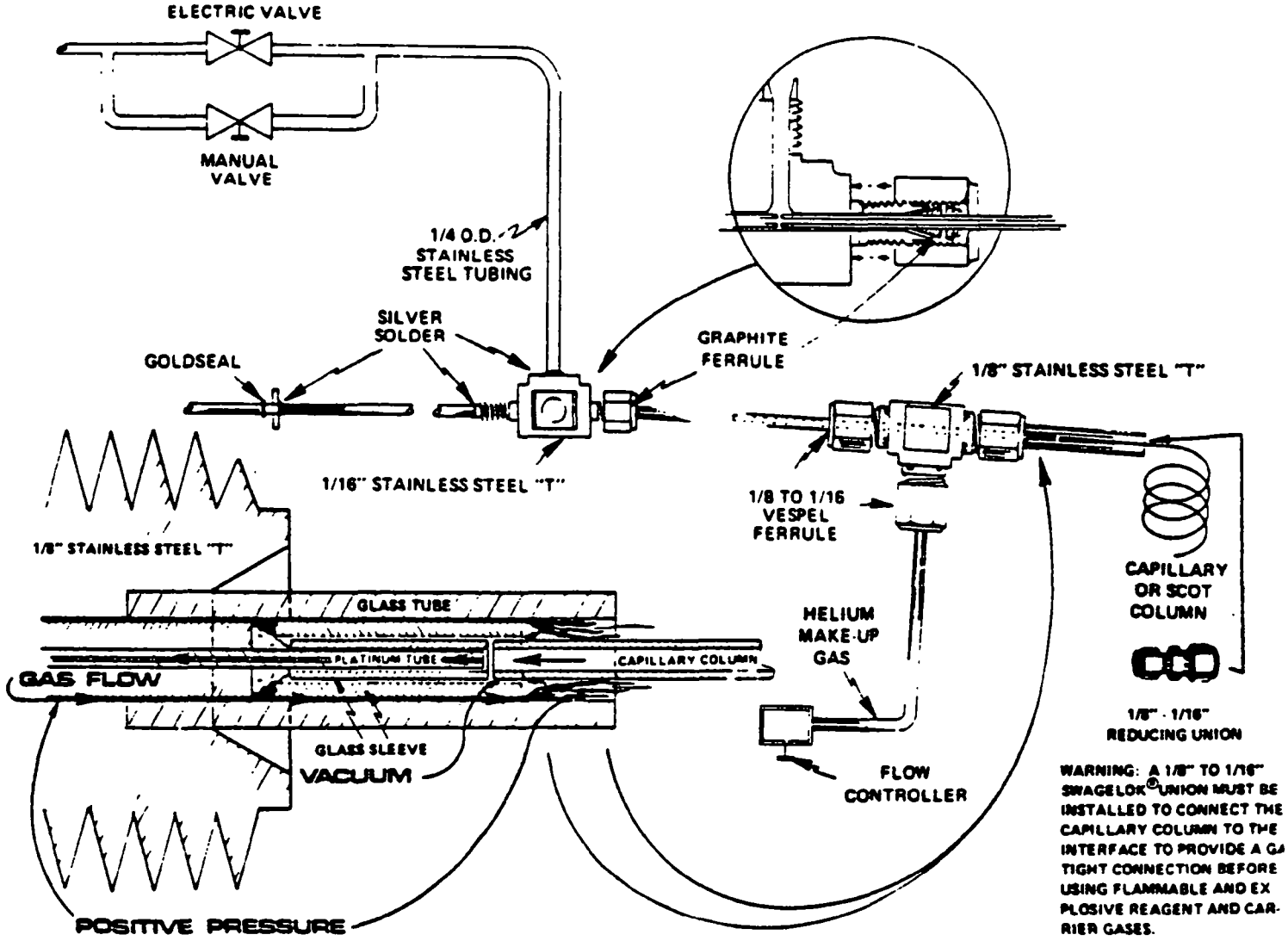

Figure 1. GC-MS Interface Diagram

considered a unique open system utilizing a helium atmosphere for connecting the glass capillary column (operating at atmospheric pressure) to the mass spectrometer ion source (operating at 10^{-6} Torr).

A bypass/vent line equipped with an electric and manual valve connects the interface to the MS rough vacuum pump. The activation of either valve effectively permits the solvent peak, or any unwanted peak to be diverted away from the ion source. For example, 2 µl of benzene injected into the capillary column at 100°C raises the source pressure to 2×10^{-6} Torr for thirty seconds if either valve is open. The electric valve is used during operation and the manual valve is used for standby operation and allows the ion source to be maintained at the desired pressure of 1×10^{-6} Torr for extended periods of time. After closure of the valves, the original source pressure of 9×10^{-6} Torr is reached in ca. 90 sec. This equilibration time can be reduced by relocating the valves and reducing the length of the bypass/vent line. The activation of either valve will reduce the source pressure from 9×10^{-6} Torr to less than 1×10^{-6} Torr in 4 sec. WARNING: A 1/8" to 1/16" Swagelok® union or equivalent must be installed to connect the capillary column to the interface to provide a gas tight connection before using flammable and explosive reagent and carrier gases.

Sample Preparation: The sample preparation procedure involved: (1) fortification of 10-g sample with 2.5 to 10 ng of ^{37}Cl-TCDD* for determination of extraction and clean-up efficiency; (2) saponification with hot alkali, followed by extraction with hexane; (3) washing with concentrated sulfuric acid; (4) chromatographic clean-up on alumina; and (5) concentration of the column extract to 60 µl for GC/HRMS analysis. In addition, a "neutral" clean-up procedure (acetonitrile partitioning) was utilized for the specific isolation of TCDD from highly contaminated sources for confirmation, but it has not been validated by multiple laboratory collaboration.

Fly ash samples (10 g) were fortified with 5 ng ^{37}Cl-TCDD and subjected to Soxhlet extraction with 100 mL benzene for 24 hours. The benzene was concentrated just to dryness on a steam bath. The residue was dissolved in 100 mL hexane and subjected to the previously described acid/base extraction and clean-up procedures.

Quality Assurance Program: The QA program was initiated prior to sample preparation and consisted of: (1) fortification of real and QA samples with 2.5 to 10 ng of ^{37}Cl-TCDD; (2) fortification of QA samples with 0 to 1250 pg (0-125 ppt) TCDD; and (3) submission of real samples and QA sample extracts (60 µl)to the GC/MS laboratories in a blind fashion (i.e., there was no way to distinguish between QA and actual samples). The efficiency, accuracy, precision and validity of TCDD analyses were dependent upon the incorporated QA program.

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*^{37}Cl-TCDD = labeled 2,3,7,8-$^{37}Cl_4$-TCDD, isotopic purity greater than 98%.

Criteria for Confirmation of TCDD Residues: The capillary column GC/HRMS MIS analysis for 2,3,7,8-TCDD residues had to satisfy the criteria (I-V) shown in Table 1 to be considered a confirmed positive sample. The supplemental criteria (A) and (B) were occasionally applied to 10 to 100 ppt TCDD analyses.

TABLE 1
CRITERIA UTILIZED FOR CONFIRMATION OF 2,3,7,8-TCDD RESIDUES

I. Correct capillary column GC/HRMS retention time of 2,3,7,8-TCDD.

II. Correct responses for the co-injection of samples fortified with ^{37}Cl-TCDD and TCDD standard.

III. Correct chlorine isotope ratio of the molecular ion (m/z 320 and m/z 322).

IV. Correct capillary column GC/HRMS multiple ion monitoring response for TCDD masses (simultaneous response for elemental composition of m/z 320, m/z 322, and m/z 328).

V. Response of m/z 320 and m/z 322 greater than 2.5 times noise level.

Supplemental criteria which were applied to highly contaminated sample extracts:

(A) COCl loss indicative of TCDD structure, and

(B) Capillary column GC/HRMS peak-matching analysis of m/z 320 and m/z 322 in real time to confirm the TCDD elemental compositions.

DISCUSSION AND RESULTS

The basic requirements for reliable quantitative trace organic analyses are accurate fortification and quantification standards and incorporated QA program, validated sample preparation procedures, and GC-HRMS detection methods. The analyses are improved by multiple laboratory participation, especially in the ppt concentration range. A primary fortification and quantification standard must be used by all participating laboratories to insure reasonable agreement in the quantitative results. The performance of a laboratory or a group of laboratories may be then determined by statistical evaluation of the analytical results.

Glass WCOT capillary columns provide total enhancement in the qualitative and quantitative aspects of GC-MS analyses. Unique problems can be encountered with WCOT capillary columns, compared with those associated with packed or SCOT columns, but they can be easily resolved or at least minimized. These problems are concerned with column load capacity, the size of the sample injected, dead space, deterioration of resolution caused by water or contamination, and other factors.

The direct-coupled GC-MS interface described in the Experimental section insures maximum transfer efficiency and can effectively by-pass 1 to 10 µl of benzene solvent away from the MS. Therefore, the MS can be optimized on 1- to 10-pg quantification standards and the sample can be diluted or concentrated to fall within the selected quantification range. This method provides the most efficient, accurate, and reliable analysis because the peak heights of the standard and internal standard

(co-injected with the sample) are constantly being evaluated during daily operation. Sample matrix effects can cause a significant decrease in sensitivity for the specific component because of gross amounts of contamination, co-eluting components, etc. The utilization of 1- to 10-pg internal quantification standards requires a minimum amount of sample and also corrects for any decrease in sensitivity due to sample matrix effects.

Injection of large sample volumes (1- to 10-µl) is confined to cold capillary columns (75°C or lower) in order to prevent damage to the column. Hundreds of 1- to 10-µl sample dilutions have been injected onto cold capillary columns without obvious deterioration of chromatographic resolution or column life in these specific types of analyses. A small amount (0.5 µl) of n-tetradecane is incorporated (co-injected) as a "keeper" in all sample injections and improves the chromatographic resolution and bandwidth of components.

Contamination, which may result in peak broading, can be minimized by the use of silanized glass wool in the injection port. Discarding the first 1 to 2 m of column and periodic cleaning the injection port also minimizes and/or resolves this type of problem. Trace amcunts of water in the helium carrier gas also can destroy the chromatographic resolution. The latter problem can be eliminated by use of a molecular seive, which should be frequently changed, and the column can be rejuvinated by repeated injections of methanol.

Quantification of TCDD: Peak height measurements for the mass m/z 328 of ^{37}Cl-TCDD internal standard and the m/z 320 and m/z 322 (TCDD)

mass of sample and the sample fortified with a ^{37}Cl-TCDD and TCDD quantification standard are used to determine: (1) the percent recovery of ^{37}Cl-TCDD (sample preparation efficiency); (2) the residue level of TCDD; and (3) the limit of detection for TCDD. Typical concentrations of quantification standards are 50 to 250 pg/µl for ^{37}Cl-TCDD and 1 to 5 pg/µl for TCDD. The ^{37}Cl-TCDD % recovery value is used to correct the TCDD residue value and the limit of detection for sample preparation procedure efficiency.

Limit of Detection: The minimum limit of detection is defined as the amount of TCDD that would provide clearly defined peak shapes for the masses m/z 320 and m/z 322 in the proper isotopic ratio and with a signal-to-noise ratio greater than 2.5:1. The sample weight and sample size used in analysis, % recovery, sample matrix effects and the noise present in the time frame of measurement will affect the minimum detection limit.

Quantitative Determinations for TCDD in Fish: The fish samples were collected in the state of Michigan (Tittabawassee, Grand and Saginaw rivers and Saginaw Bay) under the direction of EPA Region V. The species of fish were channel catfish, carp, yellow perch, small mouth bass, and sucker. A chain-of-custody procedure was maintained from time of collection through completion of analyses.

The edible portions (2.5 to 10 g) of the respective fish samples were fortified with 2.5 to 10 ng of ^{37}Cl-TCDD and subjected to acid/base extraction and the normal clean-up procedure. A typical GC-HRMS MIS analysis of an extract of ocean perch and the extract fortified with

^{37}Cl-TCDD and TCDD is shown in Figure 2. Unusual high mass intensities from high concentrations of chlorinated organic contaminants were encountered in samples of fish collected from polluted waters. These masses differed from the exact mass of TCDD and were detected at m/z 320 and m/z 322. The contaminant masses were not found in the analysis of fish taken from other locations. The high concentrations of co-extractable chlorinated components caused problems, such as capillary column overload, co-elution of components and decreased MS sensitivity. To minimize and/or cancel these effects, a very high MS sensitivity and small sample size were necessary.

A small number of highly contaminated fish extracts were subjected to the GC-HRMS analytical methods described previously and to the neutral clean-up procedure to provide additional confirmation for the presence of TCDD. The following criteria (Table 2) were used for verification of results.

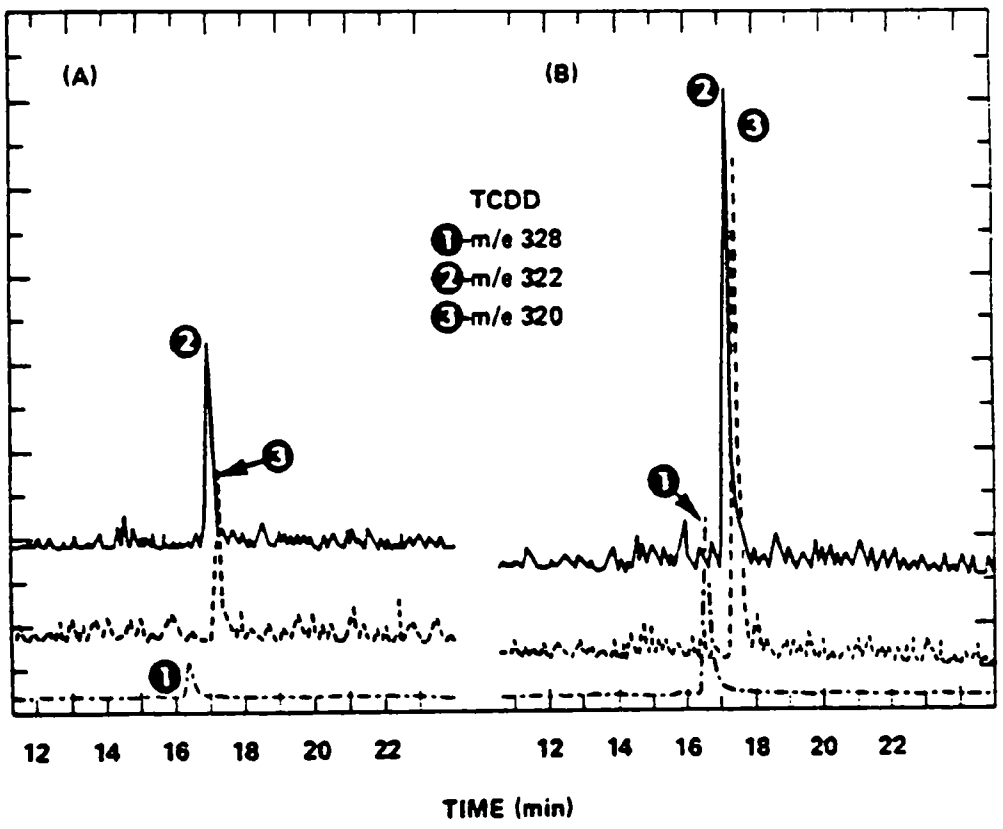


Figure 2. Capillary column GC-HRMS Analysis of a QA extract of ocean perch. (A) Sample. (B) Sample fortified with TCDD quantification standard. The experimental results were: 52% recovery of ^{37}Cl-TCDD; 34 ppt TCDD detected; 4 ppt TCDD detection limit. The sample had been fortified with 5 ng ^{37}Cl-TCDD and 37 ppt TCDD.

TABLE 2
SUPPLEMENTAL CRITERIA FOR CONFIRMATION OF RESULTS

(1) MIS simultaneous response of m/z 320, m/z 322, and m/z 324 to confirm the tetrachloro isotope ratio.

(2) MIS simultaneous responses of m/z 320, m/z 322, m/z 257, and m/z 259 to confirm the M+-COCl loss, which is indicative of TCDD structure.

(3) Capillary column GC-HRMS peak-matching analyses at 10,000 mass resolution in real time were used to confirm the elemental composition of TCDD masses m/z 319.8965 and m/z 321.8936. Three exact masses corresponding to TCDD isomers were also confirmed in these analyses. High concentrations of contaminant masses differing from the exact mass of TCDD were observed and measured to determine the elemental composition. Tentative identifications were assigned to a number of the components, PCBs (321.8677), chlorinated benyzlphenyl ethers (319.9329), etc.

Four highly contaminated samples were subjected to the neutral extraction and cleanup procedure. Capillary column GC-HRMS MIS analyses yielded positive ^{37}Cl-TCDD and TCDD responses which were essentially free from contamination. The neutral clean-up procedure was very effective for this specific isolation of TCDD from other chlorinated compound contamination; however, it has not been validated for quantitative TCDD analysis by multiple laboratory participation.

A summary of analytical results for the quantitative measurement of 2,3,7,8-TCDD in fish is given in Table 3.

TABLE 3
SUMMARY OF RESULTS FOR THE QUANTITATIVE DETERMINATION
OF TCDD RESIDUES IN FISH SAMPLES

1. Twenty-six of 35 samples contained detectable quantities of TCDD.

2. Eleven samples contained concentrations greater than 40 ppt.

3. Concentrations ranged from 4 ppt to 690 ppt.

4. The highest concentrations were detected in catfish and carp (bottom feeders).

5. The lowest concentrations were detected in perch and bass (game fish).

6. The highest concentration was detected in a catfish from the Tittabawassee River and the lowest concentration was detected in a perch from the Saginaw Bay (commercial fishing waters).

7. A river dilution effect was evident from results obtained for fish samples collected at specific points on the Tittabawassee River, which flows into the Saginaw Bay.

8. Three components which satisfied the analytical criteria for the other three TCDD isomers, with the exception of GC-HRMS retention time, were detected in several fish samples at very low concentrations.

Typical analytical results for quality assurance samples used in the analyses of the fish samples are shown in Table 4.

TABLE 4
TYPICAL ANALYTICAL RESULTS FOR QUALITY ASSURANCE SAMPLES
GENERATED DURING THE ANALYSIS OF FISH FOR 2,3,7,8-TCDD RESIDUES

Sample Weight (g) and ID[a]	^{37}Cl-TCDD % Recovery[b]	TCDD Detection Limit (ppt)[c]	TCDD Detected (ppt)[c,d]	TCDD Fortification Level (pg)	(ppt)
5(1)	62	2	22	100	20
5(1)	52	4	34	185	37
5(1)	82	3	ND[d]	0	0
5(4)	100	3	ND	0	0
5(3)	54	1	19	70	14
5(3)	100	2	ND	0	0
5(3)	78	2	ND	0	0
5(1)	92	2	19	55	11
5(1)	97	5	45	240	48
10(1)	100+	1	8	130	13
10(1)	100+	4	43	600	60
10(2)	100+	7	ND	0	0
10(2)	100+	3	ND	0	0
10(1)	100+	4	76	1250	125
10(4)	67	1	ND	0	0
10(1)	93	3	56	650	65
10(1)	84	4	73	620	62

[a]Key: (1) Ocean Perch, (2) Lake Trout, (3) Beef Liver, (4) Method Blank

[b]Each sample had been fortified with 5 or 10 ng ^{37}Cl-TCDD.

[c]Corrected for % recovery losses (^{37}Cl-TCDD Mean % Recovery, 86%; TCDD Mean % Accuracy, ± 15%).

[d]ND = Not Detected.

Evaluation of QA results shown in Table 4 indicate that reasonably accurate TCDD values were obtained in the presence of high concentrations of chlorinated contamination. The number of QA samples with zero fortification levels of TCDD were very important in these analyses because of the large number of positive fish extracts and the unusual and high concentrations of chlorinated contaminants. Accurate results were obtained for a positive fish sample fortified with a known amount of TCDD. The results shown in Table 3 were in reasonable agreement to those reported in the Dow Chemical report "Trace Chemistries of Fire"[17] for fish from similar locations. Kuehl et al.[16] used negative chemical ionization HRMS for the analysis of fish from various rivers and found a unique distribution of polychlorinated dibenzo-p-dioxins, including TCDD, in the Tittabawassee River. The estimated concentration of TCDD in fish from a similiar location on the Tittabawassee River was within experimental error to those reported in Table 3.

Based on results from three independent laboratories, it can be concluded that the Tibbawawasee River watershed and Saginaw Bay commercial fishing waters are contaminated with TCDD residues. The toxicological significance of the trace levels of TCDD in fish is not known. The source or sources of this contamination has been postulated but these theories need to be validated by other studies; e.g., additional monitoring studies on the Tittabawassee River and other similiar rivers in the U.S.

Quantitative Determinations for TCDD in Human Adipose Tissue: A preliminary validation study was performed to determine the feasibility

for the quantitative measurement of low ppt levels of TCDD in 250 milligram samples of human tissue (equivalent to a needle biopsy). The samples used were for quality assurance purposes and had been previously analyzed for TCDD. However, this fact was not known in the sample preparation laboratory prior to undertaking of the study. The results shown in Table 5 suggest that reasonably accurate results may be generated from 250 mg samples. One obvious major discrepancy is apparent in the case of the second sample listed. The small sample analysis indicated a concentration of 16 ppt TCDD instead of the 5 ppt previously detected in a 5 g sample of the sample tissue. Investigations are under way to determine the reasons for this discrepancy. These include confirmation of TCDD by multiple laboratory analysis and tracing the history of the sample.

TABLE 5
FEASIBILITY STUDY FOR THE QUANTITATIVE DETERMINATION
OF TCDD IN QA TISSUE SAMPLES

TCDD Detection Limit (ppt)[a]	TCDD Detected (ppt)[a]	TCDD Fortification Level[b]	
		(pg)	(ppt)
3	8	1	4
5	16	0	0
1	2	0.5	2[c]
1	3	2	8
1	6	1.6	6[c]

[a] ^{37}Cl-TCDD mean % recovery - 75%. Values are not corrected for % recovery losses.

[b] Each 0.250 g sample was fortified with 0.5 ng ^{37}Cl-TCDD.

[c] Standard solutions.

Two microliters of quantification standard, consisting of 12.5 pg/μl ^{37}Cl-TCDD and 0.5 pg/μl TCDD, were utilized in connection with the human fat analyses. An MIS response is shown in Figure 3. Capillary column resolution of components and a mass resolution of 8000 was sufficient to resolve 2,3,7,8-TCDD from other chlorinated compounds (e.g., DDE and PCBs). Future analyses will utilize 0.25 pg quantification standards and a mass resolution of 9,500 as a result of contamination encountered in this specific sample and the need to lower the detection limit.

Quantitative Search for TCDD in Fly Ash: Representative samples of fly ash from coal-fired power plants were subjected to a specific extraction (24 hour Soxhlet extraction utilizing benzene) and clean-up procedure prior to analysis for TCDD residues. The extraction of organic compounds from fly ash (carbon) is extremely difficult. The results shown in Table 6 show that TCDD was not detected at an average detection limit of 2 ppt. These results are in agreement with the findings reported by Kimble and Gross[18] and suggest that TCDDs are not formed in detectable quantities (>2 ppt) in highly efficient coal-fired combustion processes.

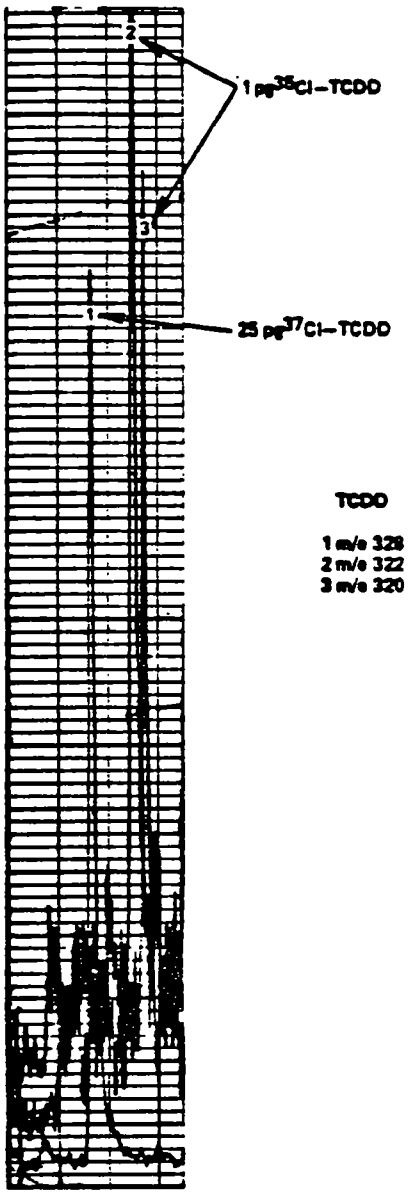


Figure 3. GC-HRMS Multiple Ion Selection Chromatogram for a Quantification Standard, 1 pg TCDD and 25 pg ^{37}Cl-TCDD.

TABLE 6
QUANTITATIVE DETERMINATIONS FOR TCDD IN FLY ASH[a]

^{37}Cl-TCDD % Recovery	TCDD Detection Limit[c] (ppt)	TCDD Detected[c] (ppt)
59	2.5	10[d]
67	2.2	ND
60	2.8	ND
80	1.3	ND
80	1.0	ND
79	1.5	ND
74	1.3	ND
71	2.2	ND

[a]Each 5 g sample was fortified with 2.5 ng ^{37}Cl-TCDD.

[b]Mean % recovery = 71%.

[c]Corrected for % recovery losses.

[d]QA sample fortified with 8 ppt TCDD.

Contamination: Contamination from chlorinated compounds is a constant problem in ppt TCDD analyses. Examples of the types of chlorinated compounds, their elemental compositions, exact masses, and the approximate mass resolution required to effect separation from TCDD masses are shown in Table 7.

TABLE 7

CONTAMINATION

COMPOUND	ELEMENTAL COMPOSITION	EXACT MASS	REQUIRED MASS RESOLUTION	TCDD ELEMENTAL COMPOSITION	TCDD EXACT MASS
TETRACHLORO-BENZYLPHENYL-ETHER	$C_{13}H_{O}Cl_4$	319.9329	9,000	$C_{12}H_4O_2{}^{35}Cl_4$	319.8965
DDE	$C_{14}H_8{}^{35}Cl_2{}^{37}Cl_2$	319.9321	9,000		
PCB HEPTACHLORO-BIPHENYL FRAGMENT	$C_{12}H_3{}^{35}Cl_5$	321.8678	12,000	$C_{12}H_4O_2{}^{35}Cl_3{}^{37}Cl$	321.8935
PCB PENTACHLORO-BIPHENYL	$C_{12}H_5{}^{35}Cl_3{}^{37}Cl_2$	327.8775	45,000	$C_{12}H_4O_2{}^{37}Cl_4$	327.8847

$$\text{RESOLUTION} = \frac{M}{\Delta M}$$

In general, PCBs cause the most serious problems because of distortion of the m/z 320 and m/z 322 chlorine isotope ratios. Fortunately, the sample preparation procedure is quite specific for removal of PCBs. In cases of gross PCB contamination, the MS mass resolution can be easily increased to ca. 14,000 to effect separation at m/z 322. In addition, the PCB m/z 326 mass can be utilized to calculate the contribution to

m/z 328, which is a mixture of PCB and TCDD mass ions. The minimum detection limit in this analysis is higher than normal, however.

Utilizing the criteria shown in Table 1 for confirmation of TCDD residues, the chlorinated compounds shown in Table 7 do not present serious problems in TCDD analysis except in rare cases of gross contamination. Neutral clean-up procedures, mass resolution of 14,000, and polar or non polar capillary columns effectively resolve this type of problem.

The occasional and unexpected sample containing high ppt to ppm levels of TCDD may cause serious contamination problems with other TCDD samples prepared in the laboratory. Erroneous or low ppt TCDD residues may be detected in succeeding samples as a result, even when extremely meticulous glassware cleaning procedures are exercised. Laboratory records, good quality assurance practices, and multiple laboratory participation should detect and eliminate these problems.

TCDD ISOMERS: Recent reports[17,20,21] have shown that chlorinated dioxins, including 2,3,7,8-TCDD and other TCDD isomers, may be formed in combustion processes. The toxicological properties of the various TCDD isomers are known to be significantly different; however, the mass spectra are almost identical.[19] Therefore, it is extremely important that TCDD isomers be resolved by utilization of glass capillary columns prior to their introduction into the MS to assure the most conclusive identification of a specific isomer. 2,3,7,8-TCDD and all other available isomers may be separated, detected and/or quantified by the technique described here. However, only a limited number of the 22 theoretical

TCDD isomers are commercially available. Compounds utilized in this laboratory for co-injection purposes are the 1,2,3,4-, 1,3,6,8-, 2,3,6,8-, and 2,3,7,8-tetrachlorodibenzo-p-dioxins. Confirmation of 2,3,7,8-TCDD in the presence of other TCDD isomers also requires analysis on a second capillary column of different polarity to differentiate between isomers.[20,21]

Most toxicological studies have been devoted to the 2,3,7,8-TCDD isomer and the other commercially available isomers. Therefore, the toxicological properties of all of the other TCDD isomers are not known. Unidentified TCDD isomers have been detected and confirmed in specific types of samples through the use of capillary column GC-HRMS techniques. The significance of these findings (ppt to ppm of unidentified TCDD isomers) should be determined in the future.

Analysis of PCB Incineration Emissions for Chlorinated Dibenzofurans: The ambient air samples were collected in the vicinity of a high temperature incineration during a test burn of PCBs. These samples were collected with the high-volume sampler developed by Lewis et al.[22] and were subjected to an extraction and cleanup procedure specific for PCB residues.[22] The concentrated extracts were then submitted to capillary column GC-HRMS analysis for polychlorinated dibenzofurnan (PCDFs). Theoretically, 135 PCDF isomers are possible. 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF) is the most toxic of the 38 TCDF isomers and is one of the major TCDF isomers formed in combustion of the PCBs, Aroclors 1254 and Aroclor 1260.[23] Only one TCDF isomer, 2,3,7,8-TCDF, was available to this laboratory. Therefore, the following procedures were utilized to rapidly screen the complex extracts for TCDF and PCDF residues.

The GC-HRMS parameters were established and optimized with 10- to 20-pg 2,3,7,8-TCDF standards and the procedures discussed earlier for TCDD. The criteria utilized for confirmation of TCDF residues and/or establishment of minimum detection limits were:

1. Capillary column GC-HRMS retention time of 2,3,7,8-TCDF.
2. Correct responses for the co-injection of sample and 2,3,7,8-TCDF standard.
3. Capillary column GC-HRMS multiple ion simultaneous response (m/z 303.9016 and m/z 305.8986).
4. Molecular ion chlorine isotope ratio (m/z 304 and m/z 306).
5. M/z 304 and m/z 306 MS response greater than 2.5 times noise level.

The capillary column GC-HRMS retention time of 2,3,7,8-TCDF was 13 minutes ±15 seconds. The MS mass resolution (8,500) was sufficient to resolve TCDF from contamination.

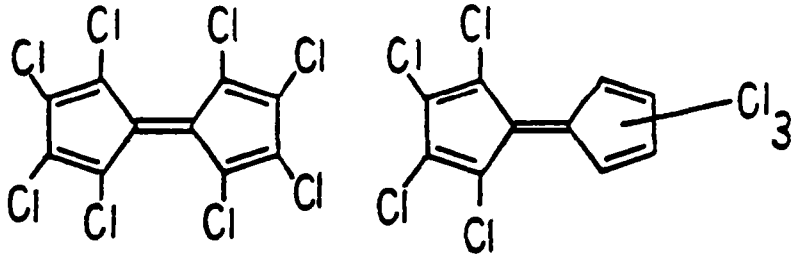
The sample extracts (300 µl each) were analyzed utilizing the capillary column GC-HRMS multiple ion selection technique. These analyses were performed on the TCDF molecular ion (m/z 303.9016, $C_{12}H_4O^{35}Cl_4$) and M + 2 ion (m/z 305.8986, $C_{12}H_4O^{35}Cl_3{}^{37}Cl$) using PFK m/z 292.9825 as the reference standard. The sequence for each sample analysis was: actual sample, then sample fortified with 10 or 20 pg of 2,3,7,8-TCDF. The isomers of TCDF and 2,3,7,8-TCDF were not detected at 1 to 10 pg/m^3 detection limits.

Capillary column GC-HRMS peak matching analysis in real time was utilized to monitor the concentrated extracts for the molecular ions of

tri- through octa-chlorodibenzofuran. These PCDF residues were not detected at an estimated detection limit of 100 pg/m^3.

The sample collection and analytical extraction efficiency is not known. Therefore the PCDF results must be considered as semi-quantitative.

Extremely high concentrations of chlorinated dicyclopentadienes (I and II), shown in Figure 4, were identified (in low resolution mass spectra and HRMS analysis) and determined to be major interfering compounds in the PCDF analysis. Analyses are being performed on the incinerator gases to confirm the PCDF results and the possible source of compounds I and II.



I II

Figure 4. Structures of Chlorinated Dicyclopentadines extracted from air.

CONCLUSION

Glass capillary columns provide a practical and effective solution to the separation and qualitative and quantitative characterization of trace level components in complex media. These validated results indicate that a specific portion of our environment and food chain are contaminated with low concentrations of TCDD residues. The toxicological significance of these results is not known at this time.

The future objectives of the dioxin program within this laboratory include: (1) the application and improvement of the capillary column GC-HRMS techniques and sample preparation procedures described here for the quantitative determination of tetra-, penta-, hexa-, hepta-, and octa-chlorinated dibenzo-p-dioxins in 100- to 200-mg needle biopsy samples of human or animal tissue (bioaccumulation and toxicological effects in animals may then be closely followed), and (2) application of capillary column GC-HRMS negative ionization (chemical ionization and electron impact) methods of analysis for the determination of ppt levels of chlorinated dibenzo-p-dioxins and other toxic compounds of interest to EPA.

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