United States
Environmental Protection
Agency

Environmental Monitoring
Systems Laboratory
Las Vegas NV 89114

Research and Development

EPA/600/S4-86/004 Apr. 1986

EPA

Project Summary

Protocol for the Analysis of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin by High-Resolution Gas Chromatography/High-Resolution Mass Spectrometry

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An analytical protocol for the determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and total TCDDs in soil, sediment, and aqueous samples using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) was developed using the best features of several candidate methods and input from experts in the field. After extensive peer review, the protocol was tested on TCDD-contaminated soil and TCDD-spiked aqueous samples. The results of these tests led to ruggedness testing and refinements of the chromatographic cleanup procedures and corresponding changes in the protocol. A final, single-laboratory evaluation of the refined protocol consisting of triplicate analyses of five solid and five aqueous samples showed that the method is useful for the determination of 2,3,7,8-TCDD and total TCDDs at concentrations ranging from 10 to 200 pg/g (ppt) in soils and 100 to 2,000 pg/L (ppq) in aqueous samples. Absolute recoveries ranged from 40 to 120 percent with better than 50 percent precision. An attempt to reach a quantitation limit for 2,3,7,8-TCDD of 5 ppt (or less) for solid samples and 50 ppq (or less) for aqueous samples was not successful. Based on the data generated during this study and on the evaluation of several options, sections of the protocol were modified at the EPA's Environmental Monitoring Systems Laboratory, Las Vegas, Nevada, to lower the quantitation limit for TCDD to 2 ppt in soil/sediment samples and to 20 ppq in aqueous samples.

This Project Summary was developed by EPA's Environmental Monitoring Systems Laboratory, Las Vegas, NV, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Methods for the determination of low levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) and total TCDDs in soil/sediment samples and aqueous samples by HRGC/HRMS are required to support the National Dioxin Program. A project was therefore initiated with the objective to provide an analytical protocol, based on the best features of several candidate methods, for the determination of 2,3,7,8-TCDD and total TCDDs in soils and sediments from 10 ppt to 200 ppt and in water from 100 ppq to 2 ppt. This protocol, after incorporation of technical comments from EPA and other experts, was to be written in the format used in the Invitation for Bid for the Superfund Contract Laboratory Program (CLP) and then was to be tested and improved in a single laboratory. Based on the results of the evaluation study, the protocol was to be modified to allow (1) detection and quantitation of 2,3,7,8-TCDD concentrations of 2 pg/g (2 ppt) to

1.2 ng/g (1.2 ppb) in soil/sediment samples and 20 pg/L (20 ppq) to 12 ng/L (12 ppt) in aqueous samples, and (2) estimation of quantities of total TCDDs present in the samples.

Procedure

Protocol Development

A protocol for HRGC/HRMS determination of 2,3,7,8-TCDD and total TCDDs in soil/sediment samples and aqueous samples was written based on methods published by EPA (including Method 613 and the Region 7 low-resolution protocol) and in the open literature, and on input from experts in the field. The resulting protocol, which was written in the format used for the Superfund CLP and which includes the stringent quality assurance/quality control features required by this program, was extensively reviewed and refined before being tested in the laboratory. The protocol, as written, is designed to allow quantitation of 2,3,7,8-TCDD at 10 ppt for soil/sediment samples and 100 ppq for aqueous samples.

Preliminary Protocol Evaluation

During a preliminary protocol evaluation, low recoveries of the internal standard $^{13}C_{12}$-2,3,7,8-TCDD were obtained, and the accuracy and precision of duplicate sample analyses were poor. The data seemed to indicate that the problems resulted from poor chromatographic separation in the extract cleanup columns. As part of the method, the sample extract volume was reduced to 1.0 mL (benzene), the concentrate was eluted through an acidic silica column with hexane, and the total collected eluate was added to an acidic alumina column. The alumina column was then eluted with hexane/20-percent methylene chloride, the eluate was concentrated and cleaned further using a Carbopak C/Celite column, and the TCDDs were eluted from the Carbopak C/Celite column with 2 mL toluene.

A systematic evaluation of the individual cleanup steps specified in the original protocol led to the following conclusions:

- The eluate from the acidic silica column must be concentrated (to 0.5 mL) before transfer to the alumina column.
- The hexane/methylene chloride eluate from the acidic alumina column need not be concentrated prior to application to the Carbopak C/Celite column.
- The optimum toluene volume for the elution of the TCDDs from the Carbopak C/Celite column is approximately 6 mL.
- Reverse elution of the Carbopak C/Celite column with toluene further improves the recovery.

Appropriate changes were incorporated in the modified protocol.

Evaluation of the improved column cleanup procedures using 1-mL portions of benzene spiked with eight TCDD isomers gave overall recoveries of 84 percent or better.

Description Of The Method (Figure 1)

Sample Extraction

Soil/Sediment Samples

A 10-g soil or sediment sample is spiked with 500 pg $^{13}C_{12}$-2,3,7,8-TCDD (internal) standard), is mixed with anhydrous sodium sulfate, and is extracted with benzene in a Soxhlet apparatus for 24 hours. The extract is then concentrated to 1 mL.

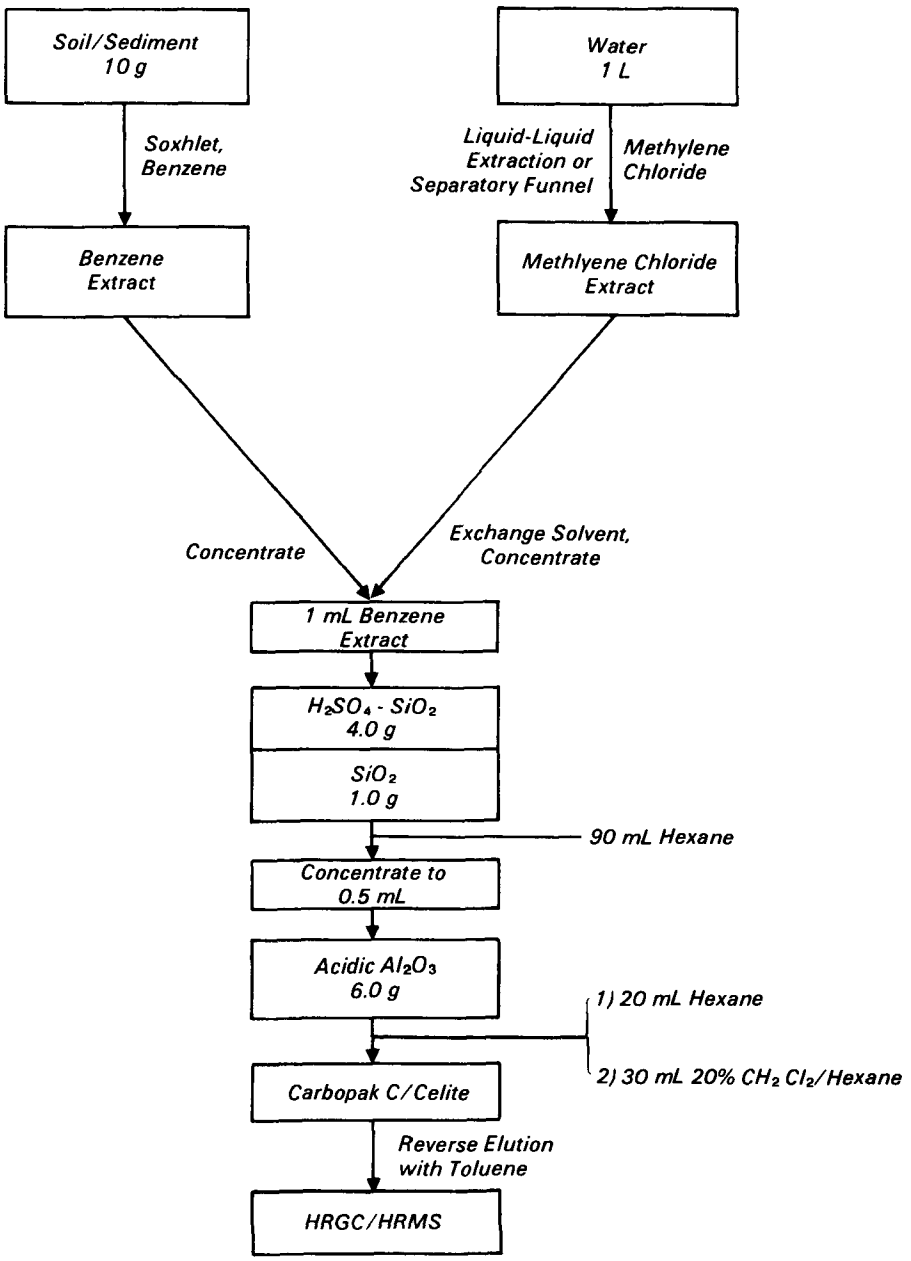

Figure 1. Extraction, cleanup, and analysis steps in the refined protocol.

Aqueous Samples

A 1-L aqueous sample is spiked with 500 pg $^{13}C_{12}$-2,3,7,8-TCDD (internal) standard) and is extracted with methylene chloride either in a continuous liquid-liquid extractor for 24 hours or in a separatory funnel. The extract is dried, the solvent is exchanged for benzene, and the benzene extract is concentrated to 1 mL.

Extract Cleanup

The concentrated benzene extract is passed through a column with acid-impregnated (H_2SO_4) silica gel using hexane as eluant, the eluate is concentrated to 0.5 mL and is transferred to a column with acidic alumina which is then eluted with 20 percent methylene chloride in hexane. The eluate is added to the top of a column containing a mixture of activated Carbopak C and Celite 545® and is eluted sequentially with hexane, cyclohexane/methylene chloride, and methylene chloride/methanol/benzene. The column is then inverted, the TCDD fraction is eluted with 6 mL toluene, the toluene volume is reduced, and 50 µL tridecane is added.

Extract Analysis

The sample extracts and calibration standards were analyzed with a Carlo Erba Mega Series GC that was coupled to a Kratos MS 50 TC double-focusing mass spectrometer. The GC/MS interface was a direct connection of the GC column to the ion source via a heated interface oven. A Finnigan 2300 Incos data system was used for data acquisition and processing. The HRGC/HRMS operating conditions are summarized in Table 1.

The mass spectrometer was tuned daily to yield a resolution of at least 10,000 (10 percent valley) and optimal response at m/z 254.986. This step was followed by calibration of an accelerating voltage scan beginning at m/z 254. Other voltage scans from the same data file were then used to establish and document both the resolution at m/z 316.983 and the mass measurement accuracy at m/z 330.979.

Just before analysis, a recovery standard spiking solution was added to the extract (5 µL of a 100-pg/µL solution of $^{13}C_{12}$-1,2,3,4-TCDD in isooctane).

Final Protocol Evaluation

The refined protocol was evaluated on five solid samples and five aqueous samples.

Solid Samples

Four soil samples and one fly ash sample were analyzed. The four soil samples were known to contain endogenous 2,3,7,8-TCDD. Each sample was fortified with 500 pg of $^{13}C_{12}$-2,3,7,8-TCDD in 1.5 mL acetone and was analyzed in triplicate as specified in the protocol. One of each triplicate was spiked with other TCDD isomers at varying concentrations before analysis.

Aqueous Samples

Distilled water, influent and effluent wastewater from a sewage treatment facility, industrial wastewater (pH <1), and an aqueous extract from a highly contaminated soil sample were evaluated. The industrial wastewater and the soil extract were known to contain endogenous 2,3,7,8-TCDD. Each sample was fortified with 500 pg of $^{13}C_{12}$-2,3,7,8-TCDD in 1.5 mL acetone and was analyzed in triplicate as specified in the protocol. Except for the soil extract, one of each triplicate was spiked with 2,3,7,8-TCDD and other TCDD isomers at varying concentrations before analysis.

Results And Discussion

The results of the evaluation are summarized in Tables 2 and 3 for 2,3,7,8-TCDD. Results from the analysis of standards demonstrate that the method is capable of achieving quantitation limits of 12.5 pg/g (ppt) for soil/sediment samples and 125 pg/L (ppq) for aqueous samples.

The relative response factors (RRF) determined for native 2,3,7,8-TCDD versus the internal standard $^{13}C_{12}$-2,3,7,8-TCDD, and the RRF of the internal standard versus the recovery standard $^{13}C_{12}$-1,2,3,4-TCDD over the five-point concentration calibration curve demonstrate that the HRGC/HRMS method maintains a linear response for 2,3,7,8-TCDD from 12.5 to 200 ppt for soil/sediment samples and 125 to 2,000 ppq for aqueous samples.

The results of the analyses of spiked aqueous samples demonstrate that in-

Table 1. *HRGC/HRMS Operating Conditions*

Mass Spectrometer	
Accelerating voltage	*8,000 V*
Trap current	*500 µA*
Electron energy	*70 eV*
Electron multiplier voltage	*2,000 V*
Source temperature	*280°C*
Resolving power	*10,000 (10% valley definition)*
Ions monitored	*Nominal dwell times (sec)*
258.930	*0.15*
319.897	*0.15*
321.894	*0.15*
331.937	*0.15*
333.934	*0.15*
280.9825 (lock mass)	*0.10*
Overall SIM cycle time = 1 sec	
Gas Chromatograph	
Column coating	*CP-Sil 88*
Film thickness	*0.2 µm*
Column dimensions	*50 m x 0.22 mm ID*
Helium linear velocity	*~ 25 cm/sec*
Helium head pressure	*1.75 kg/cm² (25 psi)*
Injection type	*Splitless, 45 sec*
Split flow	*30 mL/min*
Purge flow	*6 mL/min*
Injector temperature	*270°C*
Interface temperature	*240°C*
Injection size	*2 µL*
Initial temperature	*200°C*
Initial time	*1 min*
Temperature program	*200°C to 240° at 4°C/min*

ternal standard (isotope dilution) quantitation provides an accurate measurement of 2,3,7,8-TCDD. The accuracy of the 2,3,7,8-TCDD measurement for triplicate analysis of four out of five aqueous samples spiked at various concentrations was quite good.

The results of the analyses of samples spiked with additional TCDD isomers demonstrate that the internal standard quantitation gives good estimates of total TCDD values.

The overall results of the analyses demonstrate that the requirements for absolute recovery of the internal standard (40 to 120 percent) and the precision of replicate analyses (RPD < 50 percent) could be achieved for most of the samples tested.

Significant problems were encountered with the fly ash, the soil extract and the industrial wastewater samples.

The triplicate analyses of the fly ash sample resulted in absolute recoveries of less than 10 percent for the internal standard in each aliquot analyzed. This low recovery may be associated with the total fixed carbon content of the fly ash material. Previous work with fly ash from coal-fired power plants has demonstrated low recoveries of analytes from materials with high carbon content.

The soil extract contained a large amount of suspended particulate in each of the three replicates, and the interference and TCDD responses observed in these replicates were probably due to direct extraction of the suspended soil particulate material. Centrifugation prior to analysis resulted in absolute recoveries of 78 and 96 percent of the internal standard. The triplicate analysis of the industrial wastewater sample resulted in absolute internal standard recoveries of 23, 20, and 29 percent. Additional experiments indicate that this sample matrix (which may have contained miscellaneous industrial solvents) had a considerable impact on the extraction efficiency and on the effectiveness of the cleanup procedure. The influence of the sample pH (<1 for the industrial wastewater sample) on extraction efficiency is not known.

Analysis of 2 μL of the 1.0 pg/μL standard did not yield satisfactory results.

Table 2. *Precision of the HRGC/HRMS Analysis for 2,3,7,8-TCDD of Soil and Fly Ash Samples*

Sample Matrix	Estimated Endogenous 2,3,7,8-TCDD level (ppt)*		2,3,7,8-TCDD Detected (ppt)		$^{13}C_{12}$-2,3,7,8-TCDD Absolute Recovery (%)
Soil (B5)	50		18.2		73
			15.1		85
			12.9		48
		Avg. conc.	15.4	Avg. rec.	69
		RPD*	34	RPD	54
			34.3		73
Soil (H1)	70		36.6		46
			30.3		56
		Avg. conc.	33.7	Avg. rec.	58
		RPD	19	RPD	47
Soil (B1)	360		937		95
			785		75
			1,280		80
		Avg. conc	1,000	Avg. rec.	83
		RPD	50	RPD	24
Soil (H3)	1,700		2,020		79
			2,260		99
			1,800		86
		Avg conc.	2,030	Avg. rec.	88
		RPD	23	RPD	23
Fly Ash	—		1,720		4
			1,020		7
			1,160		5
		Avg. conc.	1,300	Avg. rec.	5.3
		RPD	54	RPD	57

*Relative percent difference.

Modification Of The Protocol

Examination of the results from the single-laboratory protocol evaluation study showed that the minimum amount of 2,3,7,8-TCDD which could be quantified under the conditions described above was 5 pg. To adapt the protocol to quantitation limits of 2 ppt for soil/sediment samples and 20 ppq for aqueous samples (without increasing the sample sizes, and while still overlapping the ppb low-resolution method without necessitating a second extraction for samples containing higher levels of TCDD) the protocol was modified as follows:

- The following calibration solutions will be used:

  HRCC1: 2 pg/μL 2,3,7,8-TCDD and $^{13}C_{12}$-1,2,3,4-TCDD
  10 pg/μL $^{13}C_{12}$-2,3,7,8-TCDD

  HRCC2: 10 pg/μL 2,3,7,8-TCDD and $^{13}C_{12}$-1,2,3,4-TCDD
  10 pg/μL $^{13}C_{12}$-2,3,7,8-TCDD

  HRCC3: 50 pg/μL 2,3,7,8-TCDD and $^{13}C_{12}$-1,2,3,4-TCDD
  10 pg/μL $^{13}C_{12}$-2,3,7,8-TCDD

  HRCC4: 100 pg/μL 2,3,7,8-TCDD and $^{13}C_{12}$-1,2,3,4-TCDD
  10 pg/μL $^{13}C_{12}$-2,3,7,8-TCDD

- The final extract volume will be 10 μL. The decision to select such a small final volume was necessary to comply with the above requirements. It is realized that handling such small volumes requires special technical skills of the operator.

- The fortification level of the internal standard $^{13}C_{12}$-2,3,7,8-TCDD was raised from 500 pg/sample to 1,000 pg/sample. By diluting a 2-μL aliquot of the remaining concentrate by a factor of 12 with a solution of the recovery standard (10 pg/μL of $^{13}C_{12}$-1,2,3,4-TCDD in tridecane), this allows analysis of (1) soil/sediment samples containing between 100 ppt and 1.2 ppb of any TCDD isomer and (2) aqueous samples containing between 1 ppt and 12 ppt of any TCDD isomer. Recoveries will be reported using the data generated from the first injection. Thus, the decision to dilute an aliquot of the 10-μL final

Table 3. *Accuracy and Precision of the HRGC/HRMS Analysis for 2,3,7,8-TCDD From Laboratory Aqueous Matrix Spikes*

Sample Matrix	2,3,7,8-TCDD Spike level (ppq)		2,3,7,8-TCDD Detected (ppq)		2,3,7,8-TCDD Recovery (%)		$^{13}C_{12}$-2,3,7,8-TCDD Absolute Recovery (%)
Distilled water	250		234		93.6		82
	250		265		106		42
	250		246		103		69
		Avg. conc.	248	Avg. rec.	101	Avg. rec.	64
		RPD*	12.5	RPD	9.3	RPD	63
Effluent wastewater	1,000		1,090, 1,030		109,103		61,66
	1,000		1,010		101		91
	1,000		1,050		105		80
		Avg. conc.	1,050	Avg. rec.	105	Avg. rec.	75
		RPD	7.6	RPD	7.6	RPD	40
Influent wastewater	500		534		107		77
	500		508		102		75
	500		530		106		71
		Avg. conc.	524	Avg. rec.	105	Avg. rec.	74
		RPD	5.0	RPD	4.8	RPD	8.1
Industrial wastewater	500		1,290		258		23
	500		1,520		304		20
	500		1,430		286		29
		Avg. conc.	1,410	Avg. rec.	283	Avg. rec.	24
		RPD	16	RPD	16	RPD	38
Industrial wastewater	___		604		___		60
	___		628		___	Avg. rec.	57
		Avg. conc.	616			RPD	58
		RPD	3.9				5.2
Soil extract	___		27,100		___		78
	___		28,100		___		96
		Avg. conc.	27,600			Avg. rec.	87
		RPD	3.6			RPD	25

*Relative percent difference.

extract will not be based on the concentration of 2,3,7,8-TCDD or total TCDD in the sample but on the concentration of the most abundant TCDD isomer or group of coeluting TCDD isomers in the 10-μL final extract volume. This will eliminate unnecessary dilutions of the sample extract, and will eliminate analyses for soil/sediment samples containing between 100 ppt and 250 ppt and for aqueous samples containing between 1 ppt and 2.5 ppt of a TCDD isomer or group of coeluting TCDD isomers but for which the recoveries were low.

The original and the refined protocol are included in the full report.

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The complete report, entitled "Protocol for the Analysis of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin by High-Resolution Gas Chromatography/High-Resolution Mass Spectrometry," (Order No. PB 86-161 361/AS; Cost: $16.95, subject to change) will be available only from:

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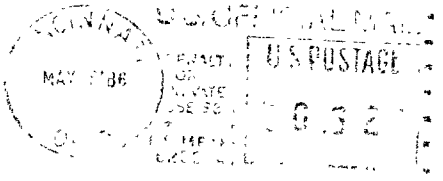

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