THE ANALYSIS OF POLYCHLORINATED DIBENZO-*p*-DIOXINS AND POLYCHLORINATED DIBENZOFURANS BY HIGH RESOLUTION GAS CHROMATOGRAPHY/LOW RESOLUTION MASS SPECTROMETRY (HRGC/LRMS)

1.0 SCOPE AND APPLICATION

1.1 This method is appropriate for the detection and quantitative measurement of 2,3,7,8-tetrachlorinated dibenzo-*p*-dioxin (2,3,7,8-TCDD), 2,3,7,8-tetrachlorinated dibenzofuran (2,3,7,8-TCDF), and the 2,3,7,8-substituted penta-, hexa-, hepta-, and octachlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) (Figure 1) in water (at part-per-trillion concentrations), soil, fly ash, and chemical waste samples, including stillbottoms, fuel oil, and sludge matrices (at part-per-billion concentrations). The following compounds can be determined by this method (see Sec. 1.4 for a discussion of "total" concentrations).

Compound	CAS Registry No.
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)	1746-01-6
1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin (PeCDD)	40321-76-4
1,2,3,4,7,8-Hexachlorodibenzo-*p*-dioxin (HxCDD)	39227-28-6
1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin (HxCDD)	57653-85-7
1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin (HxCDD)	19408-74-3
1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin (HpCDD)	35822-46-9
1,2,3,4,5,6,7,8-Octachlorodibenzo-*p*-dioxin (OCDD)	3268-87-9
2,3,7,8-Tetrachlorodibenzofuran (TCDF)	51207-31-9
1,2,3,7,8-Pentachlorodibenzofuran (PeCDF)	57117-41-6
2,3,4,7,8-Pentachlorodibenzofuran (PeCDF)	57117-31-4
1,2,3,4,7,8-Hexachlorodibenzofuran (HxCDF)	70648-26-9
1,2,3,6,7,8-Hexachlorodibenzofuran (HxCDF)	57117-44-9
1,2,3,7,8,9-Hexachlorodibenzofuran (HxCDF)	72918-21-9
2,3,4,6,7,8-Hexachlorodibenzofuran (HxCDF)	60851-34-5
1,2,3,4,6,7,8-Heptachlorodibenzofuran (HpCDF)	67562-39-4
1,2,3,4,7,8,9-Heptachlorodibenzofuran (HpCDF)	55673-89-7
1,2,3,4,5,6,7,8-Octachlorodibenzofuran (OCDF)	39001-02-0
Total Tetrachlorodibenzo-*p*-dioxin (TCDD)	41903-57-5
Total Pentachlorodibenzo-*p*-dioxin (PeCDD)	36088-22-9
Total Hexachlorodibenzo-*p*-dioxin (HxCDD)	34465-46-8
Total Heptachlorodibenzo-*p*-dioxin (HpCDD)	37871-00-4
Total Tetrachlorodibenzofuran (TCDF)	55722-27-5
Total Pentachlorodibenzofuran (PeCDF)	30402-15-4
Total Hexachlorodibenzofuran (HxCDF)	55684-94-1
Total Heptachlorodibenzofuran (HpCDF)	38998-75-3

1.2 The analytical method requires the use of high resolution gas chromatography and low resolution mass spectrometry (HRGC/LRMS) on sample extracts that have been subjected to specified cleanup procedures. The calibration range is dependent on the compound and the sample size. The sample size varies by sample matrix. Table 2 lists the quantitation limits for the various matrices.

1.3 This method requires the calculation of the 2,3,7,8-TCDD toxicity equivalence according to the procedures given in the U.S. Environmental Protection Agency "Update of Toxicity Equivalency Factors (TEFs) for Estimating Risks Associated with Exposures to Mixtures of Chlorinated Dibenzo-*p*-Dioxins and Dibenzofurans (CDDs/CDFs)" February 1989 (EPA 625/3-89/016). If the toxicity equivalence is greater than or equal to 0.7 ppb (soil or fly ash), 7 ppt (aqueous), or 7 ppb (chemical waste), analysis on a column capable of resolving all 2,3,7,8-substituted PCDDs/PCDFs is necessary. If the expected concentrations of the PCDDs and PCDFs are below the quantitation limits in Table 2, use of Method 8290 may be more appropriate.

1.4 This method contains procedures for reporting the total concentration of all PCDDs/PCDFs in a given level of chlorination (i.e., Total TCDD, Total PeCDF, etc.), although complete chromatographic separation of all 210 possible PCDDs/PCDFs is not possible under the instrumental conditions described here.

1.5 This method is restricted for use only by analysts experienced with residue analysis and skilled in HRGC/LRMS. Each analyst must demonstrate the ability to generate acceptable results with this method.

1.6 Because of the extreme toxicity of these compounds, the analyst must take necessary precautions to prevent the exposure of laboratory personnel or others to materials known or believed to contain PCDDs or PCDFs. Typical infectious waste incinerators are not satisfactory devices for disposal of materials highly contaminated with PCDDs or PCDFs. A laboratory planning to use these compounds should prepare a disposal plan. Additional safety instructions are outlined in Sec. 11.0.

2.0 SUMMARY OF THE METHOD

2.1 This procedure uses a matrix-specific extraction, analyte-specific cleanup, and high-resolution capillary column gas chromatography/low resolution mass spectrometry (HRGC/LRMS) techniques.

2.2 If interferants are encountered, the method provides selected cleanup procedures to aid the analyst in their elimination. The analysis flow chart is shown at the end of this procedure.

2.3 A specified amount of water, soil, fly ash, or chemical waste samples is spiked with internal standards and extracted according to a matrix-specific extraction procedure. Aqueous samples are filtered, and solid samples that show an aqueous phase are centrifuged before extraction. The extraction procedures and solvents are:

2.3.1 Soil, fly ash, or chemical waste samples are extracted with the combination of a Dean-Stark water trap and a Soxhlet extractor using toluene.

2.3.2 Water samples are extracted with a separatory funnel or liquid-liquid extractor using methylene chloride.

2.4 The extracts are spiked with $^{37}Cl_4$-2,3,7,8-TCDD and submitted to an acid-base washing treatment, dried and concentrated. The extracts are cleaned up by column chromatography on alumina, silica gel, and activated carbon on Celite 545® and concentrated again.

2.5 An aliquot of the concentrated extract is injected into an HRGC/LRMS system capable of performing the selected ion monitoring.

2.6 The identification of the target compounds is based on their ordered elution and comparison to standard solutions (Table 1) from an appropriate GC column and MS identification. Isomer specificity for all 2,3,7,8-substituted PCDDs/PCDFs cannot be achieved on a single column. The use of both DB-5 and SP2331 (or equivalent) columns is advised. No analyses can proceed unless all the criteria for retention times, peak identification, signal-to-noise and ion abundance ratios are met by the GC/MS system after the initial calibration and calibration verification.

2.7 A calculation of the toxicity equivalent concentration (TEQ) of each sample is made using international consensus toxicity equivalence factors (TEFs), and the TEQ is used to determine if the concentrations of target compounds in the sample are high enough to warrant confirmation of the results on a second GC column.

3.0 INTERFERENCES

3.1 Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines which may cause misinterpretation of chromatographic data. All of these materials must be demonstrated to be free from interferents under the conditions of analysis by running laboratory method blanks.

3.2 The use of high purity reagents and pesticide grade solvents helps to minimize interference problems. Purification of solvents by distillation, in all glass systems, may be required.

3.3 Interferants co-extracted from the sample will vary considerably from source to source, depending upon the industrial process being sampled. PCDDs and PCDFs are often associated with other interfering chlorinated compounds such as PCBs and polychlorinated diphenyl ethers (PCDPEs) which may be found at concentrations several orders of magnitude higher than that of the analytes of interest. Retention times of target analytes must be verified using reference standards. While certain cleanup techniques are provided as part of this method, unique samples may require additional cleanup techniques to achieve the sensitivity specified in this method.

3.4 High resolution capillary columns are used to resolve as many isomers as possible; however, no single column is known to resolve all of the 210 isomers. The columns employed by the laboratory in these analyses must be capable of resolving all 17 of the 2,3,7,8-substituted PCDDs/PCDFs sufficiently to meet the method specifications.

4.0 APPARATUS AND MATERIALS

4.1 Gas chromatograph/mass spectrometer system:

4.1.1 Gas chromatograph - An analytical system with a temperature-programmable gas chromatograph and all necessary accessories including syringes, analytical columns, and gases. The GC injection port shall be designed for capillary columns; a splitless or an on-column injection technique is recommended. A 2-µL injection volume is assumed throughout

this method; however, with some GC injection ports, other volumes may be more appropriate. A 1-µL injection volume may be used if adequate sensitivity and precision can be demonstrated.

4.1.2 GC column - Fused silica capillary columns are needed. The columns shall demonstrate the required separation of all 2,3,7,8-specific isomers whether a dual column or a single column analysis is chosen. Column operating conditions shall be evaluated at the beginning and end of each 12 hour period during which samples or concentration calibration solutions are analyzed.

4.1.2.1 Isomer specificity for all 2,3,7,8-substituted PCDDs/PCDFs cannot be achieved on the 60 m DB-5 column. Problems have been associated with the separation of 2,3,7,8-TCDD from 1,2,3,7-TCDD and 1,2,6,8-TCDD, and separation of 2,3,7,8-TCDF from 1,2,4,9-, 1,2,7,9-, 2,3,4,6-, 2,3,4,7-, and 2,3,4,8-TCDF. Because of the toxicologic concern associated with 2,3,7,8-TCDD and 2,3,7,8-TCDF, additional analyses may be necessary for some samples, as described in Sec. 7.15.8. In instances where the toxicity equivalent concentration (TEQ) is greater than 0.7 ppb (solids), 7 ppt (aqueous), or 7 ppb (chemical waste), the reanalysis of the sample extract on a 60 m SP-2330 or SP-2331 GC, or DB-225 column (or equivalent column) may be required in order to determine the concentrations of the individual 2,3,7,8-substituted isomers. For the DB-225 column, problems are associated with the separation of 2,3,7,8-TCDF from 2,3,4,7-TCDF and a combination of 1,2,3,9- and 2,3,4,8-TCDF.

4.1.2.2 For any sample analyzed on a DB-5 or equivalent column in which 2,3,7,8-TCDF is reported as an Estimated Maximum Possible Concentration (Sec. 7.15.7) that is above the quantitation limit for the matrix, analysis of the extract is recommended on a second GC column which provides better specificity for 2,3,7,8-TCDF.

4.1.2.3 Analysis on a single column is acceptable if the required separation of all the 2,3,7,8-specific isomers is demonstrated, and the minimum acceptance criteria outlined in Sec. 7.12 are met. See Sec. 7.14.5 for the specifications for the analysis of the 2,3,7,8-specific isomers using both dual columns and single columns.

4.2 Mass spectrometer - A low resolution instrument is employed, utilizing 70 volts (nominal) electron energy in the electron impact ionization mode. The system must be capable of selected ion monitoring (SIM). The recommended configuration is for at least 18 ions per cycle, with a cycle time of 1 sec or less, and a minimum integration time of 25 msec per m/z. Other cycle times and integration times may be employed, provided that the analyst can demonstrate acceptable performance for the calibration standards and window defining mixes. The integration time used to analyze samples shall be identical to the time used to analyze the initial and continuing calibration solutions and quality control samples.

4.2.1 Interfaces - GC/MS interfaces constructed of all glass or glass-lined materials are necessary. Glass can be deactivated by silanizing with dichlorodimethylsilane. Inserting a fused silica column directly into the MS source is recommended. Care must be taken not to expose the end of the column to the electron beam.

4.2.2 Data system - An interfaced data system is necessary to acquire, store, reduce and output mass spectral data.

4.3 Miscellaneous equipment

4.3.1 Nitrogen evaporation apparatus (N-Evap* Analytical Evaporator Model 111, Organomation Association Inc., Northborough, MA, or equivalent).

4.3.2 Balance capable of accurately weighing ±0.01 g.

4.3.3 Water bath - Equipped with concentric ring cover and temperature controlled within ±2°C.

4.3.4 Stainless steel (or glass) pan large enough to hold contents of 1 pint sample containers.

4.3.5 Glove box - For use in preparing standards from neat materials and in handling soil/sediment samples containing fine particulates that may pose a risk of exposure.

4.3.6 Rotary evaporator, R-110, Buchi/Brinkman - American Scientific No. E5045-10 or equivalent.

4.3.7 Centrifuge - Capable of operating at 400 x G with a 250-300 mL capacity.

4.3.8 Drying oven.

4.3.9 Vacuum oven - Capable of drying solvent-washed solid reagents at 110°C.

4.3.10 Mechanical shaker - A magnetic stirrer, wrist-action or platform-type shaker that produces vigorous agitation. Used for pre-treatment of fly ash samples.

4.4 Miscellaneous laboratory glassware

4.4.1 Extraction jars - Amber glass with polytetrafluoroethylene (PTFE)-lined screw cap; minimum capacity of approximately 200 mL; must be compatible with mechanical shaker to be used.

4.4.2 Kuderna-Danish (K-D) Apparatus - 500-mL evaporating flask, 10-mL graduated concentrator tubes with ground glass stoppers, three-ball macro-Synder column.

NOTE: The use of a solvent vapor recovery system (Kontes K-545000-1006 or K-547300-0000, Ace Glass 6614-30, or equivalent) is recommended for the purpose of solvent recovery during the concentration procedures requiring the use of Kuderna-Danish evaporative concentrators. Incorporation of this apparatus may be required by State or local municipality regulations that govern air emissions of volatile organics. EPA recommends the incorporation of this type of reclamation system as a method to implement an emissions reduction program. Solvent recovery is a means to conform with waste minimization and pollution prevention initiatives.

4.4.3 Disposable Pasteur pipets, 150 mm long x 5 mm ID.

4.4.4 Disposable serological pipets, 10-mL for preparation of the carbon column described in Sec. 7.10.

4.4.5 Vials - 0.3-mL and 2-mL amber borosilicate glass with conical shaped reservoir and screw caps lined with PTFE-faced silicone disks.

4.4.6 Funnels - Glass; appropriate size to accommodate filter paper (12.5 cm).

4.4.7 Chromatography columns - 300 mm x 10.5 mm glass chromatographic column fitted with PTFE stopcock.

4.4.8 Soxhlet apparatus, 500-mL flask, all glass - Complete with glass extractor body, condenser, glass extraction thimbles, heating mantle, and variable transformer for heat control.

NOTE: Extraction thimbles must be of sufficient size to hold 100 g of sand, 5 g of silica gel, and at least 10 g of solid sample, with room to mix the sand and sample in the thimble.

4.4.9 Dean-Stark water separator apparatus, with a PTFE stopcock. Must fit between Soxhlet extractor body and condenser.

4.4.10 Concentrator tubes - 15-mL conical centrifuge tubes.

4.4.11 Separatory funnels - 125-mL and 2-L separatory funnels with a PTFE stopcock.

4.4.12 Continuous liquid-liquid extractor - 1-L sample capacity, suitable for use with heavier than water solvents.

4.4.13 PTFE boiling chips - wash with hexane prior to use.

4.4.14 Buchner funnel - 15 cm.

4.4.15 Filtration flask - For use with Buchner funnel, 1-L capacity.

4.5 Filters

4.5.1 Filter paper - Whatman No. 1 or equivalent.

4.5.2 Glass fiber filter - 15 cm, for use with Buchner funnel.

4.5.3 0.7 µm, Whatman GFF, or equivalent material compatible with toluene. Rinse with toluene.

4.6 Glass wool, silanized - Extract with methylene chloride and hexane before use.

4.7 Laboratory glassware cleaning procedures - Reuse of glassware should be minimized to avoid the risk of using contaminated glassware. All glassware that is reused shall be scrupulously cleaned as soon as possible after use, applying the following procedure.

4.7.1 Rinse glassware with the last solvent used in it.

4.7.2 Wash with hot water containing detergent.

4.7.3 Rinse with copious amounts of tap water and several portions of organic-free reagent water. Drain dry.

4.7.4 Rinse with pesticide grade acetone and hexane.

4.7.5 After glassware is dry, store inverted or capped with aluminum foil in a clean environment.

4.7.6 Do not bake reusable glassware as a routine part of cleaning. Baking may be warranted after particularly dirty samples are encountered, but should be minimized, as repeated baking may cause active sites on the glass surface that will irreversibly adsorb PCDDs/PCDFs.

CAUTION: The analysis for PCDDs/PCDFs in water samples is for much lower concentrations than in soil/sediment, fly ash, or chemical waste samples. Extreme care must be taken to prevent cross-contamination between soil/sediment, fly ash, chemical waste and water samples. Therefore, it is strongly recommended that separate glassware be reserved for analyzing water samples.

4.8 Pre-extraction of glassware - All glassware should be rinsed or pre-extracted with solvent immediately before use. Soxhlet-Dean-Stark (SDS) apparatus and continuous liquid-liquid extractors should be pre-extracted for approximately three hours immediately prior to use, using the same solvent and extraction conditions that will be employed for sample extractions. The pooled waste solvent for a set of extractions may be concentrated and analyzed as a method of demonstrating that the glassware was free of contamination.

It is recommended that each piece of reusable glassware be numbered in such a fashion that the laboratory can associate all reusable glassware with the processing of a particular sample. This will assist the laboratory in:

1) Tracking down possible sources of contamination for individual samples,

2) Identifying glassware associated with highly contaminated samples that may require extra cleaning, and

3) Determining when glassware should be discarded.

5.0 REAGENTS

5.1 Solvents - all solvents must be pesticide grade, distilled-in-glass.

5.1.1 Hexane, C_6H_{14}

5.1.2 Methanol, CH_3OH

5.1.3 Methylene chloride, CH_2Cl_2

5.1.4 Toluene, $C_6H_5CH_3$

5.1.5 Isooctane, $(CH_3)_3CCH_2CH(CH_3)_2$

5.1.6 Cyclohexane, C_6H_{12}

5.1.7 Acetone, CH_3COCH_3

5.1.8 Tridecane, $CH_3(CH_2)_{11}CH_3$

5.1.9 Nonane, C_9H_{20}

5.2 White quartz sand - 60/70 mesh, for use in the Soxhlet-Dean-Stark (SDS) extractor. Bake at 450°C for 4 hours minimum.

5.3 Sodium sulfate (granular, anhydrous), Na_2SO_4 - Purify by heating at 400°C for 4 hours in a shallow tray, or by extracting with methylene chloride. If, after heating, the sodium sulfate develops a noticeable grayish cast (due to the presence of carbon in the crystal matrix) that batch of sodium sulfate is not suitable for use and should be discarded. Extraction with methylene chloride may produce sodium sulfate that is suitable for use in such instances, but following extraction, a reagent blank must be analyzed that demonstrates that there is no interference from the sodium sulfate.

5.4 Potassium hydroxide, KOH - ACS reagent grade, prepare a 20% (w/v) solution in organic-free reagent water.

5.5 Sulfuric acid, H_2SO_4, concentrated - ACS reagent grade, specific gravity 1.84.

5.6 Sodium chloride, NaCl - ACS reagent grade, prepare a 5% (w/v) solution in organic-free reagent water.

5.7 Hydrochloric acid, HCl, concentrated - ACS reagent grade, specific gravity 1.17. Prepare a 1N solution in organic-free reagent water for pretreatment of fly ash samples.

5.8 Column chromatography reagents

This section describes the column chromatography reagents employed in this method for cleanup of sample extracts. The quality of two of these reagents, the alumina and silica gel, is critical to a successful analysis. Prior to employing the reagents in Secs. 5.8.1., 5.8.4., 5.8.5., and 5.8.6., the analyst should demonstrate that they meet the performance requirements in Sec. 7.9.2.

5.8.1 Alumina, acidic - Supelco 19996-6C (or equivalent). Soxhlet extract with methylene chloride for 18 hours and activate by heating to 130°C for a minimum of 12 hours.

5.8.2 Charcoal carbon - Activated carbon, Carbopak C (Supelco) or equivalent, prewashed with methanol and dried *in vacuo* at 110°C. (Note: AX-21 [Anderson Development Company] carbon is no longer available, but existing stocks may be utilized).

5.8.3 Celite 545 (Supelco) or equivalent.

5.8.4 Silica gel - High-purity grade, type 60, 70-230 mesh. Soxhlet extract with methylene chloride for 21 hours and activate by heating in a foil covered glass container for 24 hours at 190°C.

5.8.5 Silica gel impregnated with 2% (w/w) sodium hydroxide - Add one part by weight of 1 M NaOH solution to two parts silica gel (extracted and activated) in a screw-cap bottle and mix with a glass rod until free of lumps.

5.8.6 Silica gel impregnated with 40% (w/w) sulfuric acid. Add two parts by weight concentrated sulfuric acid to three parts silica gel (extracted and activated), mix with a glass rod until free of lumps, and store in a screw-cap glass bottle.

5.9 Calibration solutions (Table 1) - Prepare five tridecane (or nonane) solutions (CC1-CC5) containing 10 unlabeled and 7 carbon-labeled PCDDs/PCDFs at known concentrations for use in instrument calibration. One of these five solutions (CC3) is used as the calibration verification solution and contains 7 additional unlabeled 2,3,7,8-isomers. The concentration ranges are homologue-dependent, with the lowest concentrations associated with tetra- and pentachlorinated dioxins and furans (0.1 to 2.0 ng/µL), and the higher concentrations associated with the hexa- through octachlorinated homologues (0.5 to 10.0 ng/µL). Commercially-available standards containing all 17 unlabeled analytes in each solution may also be utilized.

5.10 Internal standard solution (Table 3) - Prepare a solution containing the five internal standards in tridecane (or nonane) at the nominal concentrations listed in Table 3. Mix 10 µL with 1.0 mL of acetone before adding to each sample and blank.

5.11 Recovery standard solution (Table 3) - Prepare a solution in hexane containing the recovery standards, $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,3,7,8,9-HxCDD, at concentrations of 5.0 ng/µL, in a solvent other than tridecane or nonane.

5.12 Calibration verification solution - Prepare a solution containing standards to be used for identification and quantitation of target analytes (Table 4).

5.13 Cleanup standard - Prepare a solution containing $^{37}Cl_4$-2,3,7,8-TCDD at a concentration of 5 ng/µL (5 µg/mL) in tridecane (or nonane). Add this solution to all sample extracts prior to cleanup. The solution may be added at this concentration, or diluted into a larger volume of solvent. The recovery of this compound is used to judge the efficiency of the cleanup procedures.

5.14 Matrix spiking standard - Prepare a solution containing ten of the 2,3,7,8-substituted isomers, at the concentrations listed in Table 5 in tridecane (or nonane). Use this solution to prepare the spiked sample aliquot. Dilute 10 µL of this standard to 1.0 mL with acetone and add to the aliquot chosen for spiking.

5.15 Window defining mix - Prepare a solution containing the first and last eluting isomer of each homologue (Table 6). Use this solution to verify that the switching times between the descriptors have been appropriately set.

5.16 Column performance solutions

Chromatographic resolution is verified using a test mixture of PCDDs/PCDFs specific to each column show below.

DB-5 test mix:	1,2,3,7-TCDD/1,2,3,8-TCDD
	2,3,7,8-TCDD
	1,2,3,9-TCDD
DB-225 test mix:	2,3,4,7-TCDF
	2,3,7,8-TCDF
	1,2,3,9-TCDF

SP-2331 test mix:	2,3,7,8-TCDD
	1,4,7,8-TCDD
	1,2,3,7-TCDD
	1,2,3,8-TCDD

The concentrations of these isomers should be approximately 0.5 ng/µL in tridecane (or nonane).

If the laboratory employs a column that has a different elution order than those specified here, the laboratory must ensure that the isomers eluting closest to 2,3,7,8-TCDD are represented in the column performance solution.

6.0 SAMPLE COLLECTION, HANDLING, AND PRESERVATION

6.1 See the introductory material to this chapter, Organic Analytes.

6.2 Sample collection

6.2.1 Sample collection personnel should, to the extent possible, homogenize samples in the field before filling the sample containers. This should minimize or eliminate the necessity for sample homogenization in the laboratory. The analyst should make a judgment, based on the appearance of the sample, regarding the necessity for additional mixing. If the sample is clearly not homogeneous, the entire contents should be transferred to a glass or stainless steel pan for mixing with a stainless steel spoon or spatula before removal of a sample portion for analysis.

6.2.2 Grab and composite samples must be collected in glass containers. Conventional sampling practices must be followed. The bottle must not be prewashed with sample before collection. Sampling equipment must be free of potential sources of contamination.

6.2.3 If residual chlorine is present in aqueous samples, add 80 mg sodium thiosulfate per liter of sample. If sample pH is greater than 9, adjust to pH 7-9 with sulfuric acid.

6.3 Storage and holding times - All samples should be stored at 4°C in the dark, extracted within 30 days and completely analyzed within 45 days of extraction. Whenever samples are analyzed after the holding time expiration date, the results should be considered to be minimum concentrations and should be identified as such.

NOTE: The holding times listed in Sec. 6.3 are recommendations. PCDDs and PCDFs are very stable in a variety of matrices, and holding times under the conditions listed in Sec. 6.3 may be as high as a year for certain matrices. Sample extracts, however, should always be analyzed within 45 days of extraction.

7.0 PROCEDURE

Four types of extraction procedures are employed in these analyses, depending on the sample matrix.

1) Chemical waste samples are extracted by refluxing with a Dean-Stark water separator.

2) Fly ash samples and soil/sediment samples are extracted in a combination of a Soxhlet extractor and a Dean-Stark water separator.

3) Water samples are filtered and then the filtrate is extracted using either a separatory funnel procedure or a continuous liquid-liquid extraction procedure.

4) The filtered particulates are extracted in a combination of a Soxhlet extractor and a Dean-Stark water separator.

Sec. 7.1 provides general information on the use of the Soxhlet-Dean-Stark apparatus. The four matrix-specific extraction procedures are described in Secs. 7.2 - 7.5.

7.1 General considerations for use of the Soxhlet-Dean-Stark (SDS) apparatus - The following procedures apply to use of the SDS apparatus for extracting matrices covered by this protocol.

The combination of a Soxhlet extractor and a Dean-Stark trap is used for the removal of water and extraction of PCDDs/PCDFs from samples of fly ash, soil/sediment, and the particulate fraction of water samples.

For soil/sediment samples, the results of these analyses are reported based on the wet weight of the sample. However, use of the SDS allows the water content of a sample to be determined from the same aliquot of sample that is also extracted for analysis. The amount of water evolved from the sample during extraction is used to approximate the percent solids content of the sample. The percent solids data may be employed by the data user to approximate the dry weight concentrations. The percent solids determination does not apply to the extraction of particulates from the filtration of water samples or to the extraction of fly ash samples which are treated with an HCl solution prior to extraction.

7.1.1 The extraction of soil/sediment, fly ash, and particulates from water samples will require the use of a Soxhlet thimble. See Sec. 4.6 for a discussion of pre-extraction of glassware such as the SDS. Prior to pre-extraction, prepare the thimble by adding 5 g of 70/230 mesh silica gel to the thimble to produce a thin layer in the bottom of the thimble. This layer will trap fine particles in the thimble. Add 80-100 g of quartz sand on top of the silica gel, and place the thimble in the extractor.

7.1.2 Pre-extract the SDS for three hours with toluene, then allow the apparatus to cool and remove the thimble. Mix the appropriate weight of sample with the sand in the thimble, being careful not to disturb the silica gel layer.

7.1.3 If the sample aliquot to be extracted contains large lumps, or is otherwise not easily mixed in the thimble, the sand and sample may be mixed in another container. Transfer approximately 2/3 of the sand from the thimble to a clean container, being careful not to disturb the silica gel layer when transferring the sand. Thoroughly mix the sand with the sample with a clean spatula, and transfer the sand/sample mixture to the thimble.

7.1.4 If a sample with particularly high moisture content is to be extracted, it may be helpful to leave a small conical depression in the material in the thimble. This will allow the water to drain through the thimble more quickly during the early hours of the extraction. As the moisture is removed during the first few hours of extraction, the depression will collapse, and the sample will be uniformly extracted.

7.2 Chemical waste extraction (including oily sludge/wet fuel oil and stillbottom/oil).

7.2.1 Assemble a flask, a Dean-Stark trap, and a condenser, and pre-extract with toluene for three hours (see Sec. 4.6). After pre-extraction, allow the apparatus to cool, and discard the used toluene, or pool it for later analysis to verify the cleanliness of the glassware.

7.2.2 Weigh about 1 g of the waste sample to two decimal places into a tared pre-extracted 125-mL flask. Add 1 mL of the acetone-diluted internal standard solution (Sec. 5.10) to the sample in the flask. Attach the pre-extracted Dean-Stark water separator and condenser to the flask, and extract the sample by refluxing it with 50 mL of toluene for at least three hours.

Continue refluxing the sample until all the water has been removed. Cool the sample, filter the toluene extract through a rinsed glass fiber filter into a 100-mL round bottom flask. Rinse the filter with 10 mL of toluene; combine the extract and rinsate. Concentrate the combined solution to approximately 10 mL using a K-D or rotary evaporator as described in Secs. 7.6.1 and 7.6.2. Transfer the concentrated extract to a 125-mL separatory funnel. Rinse the flask with toluene and add the rinse to the separatory funnel. Proceed with acid-base washing treatment per Sec. 7.7, the micro-concentration per Sec. 7.8, the chromatographic procedures per Secs. 7.9 and 7.10, and a final concentration per Sec. 7.11.

7.2.3 Prepare an additional two 1-g aliquots of the sample chosen for spiking. After weighing the sample in a tared pre-extracted flask (Sec. 7.2.2), add 1.0 mL of the acetone-diluted matrix spiking standard solution (Sec. 5.14) to each of the two aliquots. After allowing the matrix spiking solution to equilibrate to approximately 1 hour, add the internal standard solution and extract the aliquots as described in Sec. 7.2.2.

7.3 Fly ash sample extraction

7.3.1 Weigh about 10 g of the fly ash to two decimal places, and transfer to an extraction jar. Add 1 mL of the acetone-diluted internal standard solution to the sample.

7.3.2 Add 150 mL of 1 N HCl to the fly ash sample in the jar. Seal the jar with the PTFE-lined screw cap, place on a mechanical shaker, and shake for 3 hours at room temperature.

7.3.3 Rinse a Whatman #1 (or equivalent) filter paper with toluene, and then filter the sample through the filter paper in a Buchner funnel into a 1 L receiving flask. Wash the fly ash with approximately 500 mL of organic-free reagent water.

7.3.4 Mix the fly ash with the sand in the pre-extracted thimble (Sec. 7.1.2). Place the filter paper from Sec. 7.3.3 on top of the sand. Place the thimble in a SDS extractor, add 200 mL toluene, and extract for 16 hours. The solvent should cycle completely through the system 5-10 times per hour. Cool and filter the toluene extract through a rinsed glass fiber filter into a 500-mL round-bottom flask. Rinse the filter with 10 mL of toluene. Concentrate the extract as described in Secs. 7.6.1 or 7.6.2. Transfer the concentrated extract to a 125-mL separatory funnel. Rinse the flask with toluene and add the rinse to the separatory funnel. Proceed with acid-base washing treatment per Sec. 7.7, the micro-concentration per Sec. 7.8, the chromatographic procedures per Secs. 7.9 and 7.10 and a final concentration per Sec. 7.11.

NOTE: A blank should be analyzed using a piece of filter paper handled in the same manner as the fly ash sample.

7.3.5 Prepare an additional two 10-g aliquots of the sample chosen for spiking for use as the matrix spike and matrix spike duplicate. Transfer each aliquot to a separate extraction jar and add 1.0 mL of the acetone-diluted matrix spiking standard solution (Sec. 5.14) to each of the two aliquots. After allowing the matrix spiking solution to equilibrate to approximately 1 hour, add the internal standard solution and extract the aliquots as described in Sec. 7.3.1.

7.4 Soil/sediment sample extraction

NOTE: Extremely wet samples may require centrifugation to remove standing water before extraction.

7.4.1 Weigh about 10 grams of the soil to two decimal places and transfer to a pre-extracted thimble (Sec. 7.1.2). Mix the sample with the quartz sand, and add 1 mL of the acetone-diluted internal standard solution (Sec. 5.10) to the sample/sand mixture. Add small portions of the solution at several sites on the surface of the sample/sand mixture.

7.4.2 Place the thimble in the SDS apparatus, add 200 to 250 mL toluene, and reflux for 16 hours. The solvent should cycle completely through the system 5-10 times per hour.

7.4.3 Estimate the percent solids content of the soil/sediment sample by measuring the volume of water evolved during the SDS extraction procedure. For extremely wet samples, the Dean-Stark trap may need to be drained one or more times during the 16-hour extraction. Collect the water from the trap, measure its volume to the nearest 0.1 mL. Assume a density of 1.0 g/mL, and calculate the percent solids content according to the formula below:

$$\text{Percent solids} = \frac{\text{Wet weight of sample} - \text{Weight of water}}{\text{Wet weight of sample}} \times 100$$

7.4.4 Concentrate this extract as described in Secs. 7.6.1 or 7.6.2. Transfer the concentrated extract to a 125 mL separatory funnel. Rinse the flask with toluene and add the rinse to the separatory funnel. Proceed with acid-base washing treatment per Sec. 7.7, the micro concentration per Sec. 7.8, the chromatographic procedures per Secs. 7.9 and 7.10 and a final concentration per Sec. 7.11.

7.4.5 Prepare an additional two 10-g aliquots of the sample chosen for spiking for use as the matrix spike and matrix spike duplicate. After transferring each aliquot to a separate pre-extracted Soxhlet thimble, add 1.0 mL of the acetone-diluted matrix spiking standard solution (Sec. 5.14) to each of the two aliquots. After allowing the matrix spiking solution to equilibrate to approximately 1 hour, add the internal standard solution (Sec. 5.10) and extract the aliquots as described in Sec. 7.4.1.

7.5 Aqueous sample extraction

7.5.1 Allow the sample to come to ambient temperature, then mark the water meniscus on the side of the 1-L sample bottle for determination of the exact sample volume.

7.5.2 Add 1 mL of the acetone-diluted internal standard solution (Sec. 5.10) to the sample bottle. Cap the bottle, and mix the sample by gently shaking for 30 seconds.

7.5.3 Filter the sample through a 0.7-µm filter that has been rinsed with toluene. Collect the aqueous filtrate in a clean flask. If the total dissolved and suspended solids

contents are too much to filter through the 0.7-µm filter, centrifuge the sample, decant, and then filter the aqueous phase. Alternatively, other filter configurations, including stacked filters of decreasing pore sizes, may be employed. Procedures for extraction of the particulate fraction are given in Sec. 7.5.4. The aqueous portion may be extracted using either the separatory funnel technique (Sec. 7.5.5.1) or a pre-extracted continuous liquid-liquid extractor (Sec. 7.5.5.2).

NOTE: Organic-free reagent water used as a blank must also be filtered in a similar fashion, and subjected to the same cleanup and analysis as the water samples.

7.5.4 Particulate fraction

7.5.4.1 Combine the particulate on the filter and the filter itself, and if centrifugation was used, the solids from the centrifuge bottle(s), with the quartz sand in the pre-extracted Soxhlet thimble. Place the filter on top of the particulate/sand mixture, and place the thimble into a pre-extracted SDS apparatus.

7.5.4.2 Add 200 to 250 mL of toluene to the SDS apparatus and reflux for 16 hours. The solvent should cycle completely through the system 5-10 times per hour.

7.5.4.3 Allow the Soxhlet to cool, remove the toluene and concentrate this extract as described in Secs. 7.6.1. or 7.6.2.

7.5.5 Aqueous filtrate

The aqueous filtrate may be extracted by either a separatory funnel procedure (Sec. 7.5.5.1) or a continuous liquid-liquid extraction procedure (Sec. 7.5.5.2).

7.5.5.1 Separatory funnel extraction - Pour the filtered aqueous sample into a 2-L separatory funnel. Add 60 mL methylene chloride to the sample bottle, seal, and shake 60 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. Drain the methylene chloride extract into a 500-mL K-D concentrator (mounted with a 10-mL concentrator tube) by passing the extract through a funnel packed with a glass wool plug and half-filled with anhydrous sodium sulfate. Extract the water sample two more times using 60 mL of fresh methylene chloride each time. Drain each extract through the funnel into the K-D concentrator. After the third extraction, rinse the sodium sulfate with at least 30 mL of fresh methylene chloride. Concentrate this extract as described in Secs. 7.6.1 or 7.6.2.

7.5.5.2 Continuous liquid-liquid extraction - A continuous liquid-liquid extractor may be used in place of a separatory funnel when experience with a sample from a given source indicates that a serious emulsion problem will result or an emulsion is encountered using a separatory funnel. The following procedure is used for a continuous liquid-liquid extractor.

7.5.5.2.1 Pre-extract the continuous liquid-liquid extractor for three hours with methylene chloride and reagent water. Allow the extractor to cool, discard the methylene chloride and the reagent water, and add the filtered

aqueous sample to the continuous liquid-liquid extractor. Add 60 mL of methylene chloride to the sample bottle, seal and shake for 30 seconds.

7.5.5.2.2 Transfer the solvent to the extractor. Repeat the sample bottle rinse with an additional 50 to 100 mL portion of methylene chloride and add the rinse to the extractor. Add 200 to 500 mL methylene chloride to the distilling flask and sufficient reagent water to ensure proper operation. Extract for 16 hours. Allow to cool, then detach the flask and dry the sample by running it through a rinsed funnel packed with a glass wool plug and 5 g of anhydrous sodium sulfate into a 500-mL K-D flask. Concentrate the extract according to Secs. 7.6.1 or 7.6.2.

7.5.6 Combination of extracts - The extracts from both the particulate fraction (Sec. 7.5.4) and the aqueous filtrate (Sec. 7.5.5) must be concentrated using the procedures in Sec. 7.6.1 and then combined together prior to the acid-base washing treatment in Sec. 7.7.

7.5.7 Determine the original aqueous sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1-L graduated cylinder. Record the sample volume to the nearest 5 mL.

7.5.8 Prepare an additional two 1-L aliquots of the sample chosen for spiking for use as the matrix spike and matrix spike duplicate. Add 1.0 mL of the acetone-diluted matrix spiking standard solution (Sec. 5.14) to each of the two aliquots in the original sample bottles. After allowing the matrix spiking solution to equilibrate to approximately 1 hour, add the internal standard solution and filter and extract the aliquots as described in Sec. 7.5.2.

7.6 Macro-concentration procedures (all matrices)

Prior to cleanup, extracts from all matrices must be concentrated to approximately 10 mL. In addition, as noted above, the concentrated extracts from the aqueous filtrate and the filtered particulates must be combined prior to cleanup. Two procedures may be used for macro-concentration, rotary evaporator, or Kuderna-Danish (K-D). Concentration of toluene by K-D involves the use of a heating mantle, as toluene boils above the temperature of a water bath. The two procedures are described below.

7.6.1 Concentration by K-D

7.6.1.1 Add one or two clean boiling chips to the flask and attach a three-ball Snyder column. Pre-wet the column by adding approximately 1 mL of toluene through the top.

7.6.1.2 Attach the solvent recovery system condensor, place the round bottom flask in a heating mantle and apply heat as required to complete the concentration in 15-20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood.

7.6.1.3 When the apparent volume of liquid reaches 10 mL, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes.

7.6.2 Concentration by rotary evaporator

7.6.2.1 Assemble the rotary evaporator according to manufacturer's instructions, and warm the water bath to 45°C. On a daily basis, preclean the rotary evaporator by concentrating 100 mL of clean extraction solvent through the system. Archive both the concentrated solvent and the solvent in the catch flask for contamination check if necessary. Between samples, three 2-3 mL aliquots of toluene should be rinsed down the feed tube into a waste beaker.

7.6.2.2 Attach the round bottom flask containing the sample extract to the rotary evaporator. Slowly apply vacuum to the system and begin rotating the sample flask. Lower the sample flask into the water bath and adjust the speed of rotation to complete the concentration in 15-20 minutes. At the proper rate of concentration, the flow of condensed solvent into the receiving flask will be steady, but no bumping or visible boiling will occur.

7.6.2.3 When the apparent volume of the liquid reaches 10 mL, shut off the vacuum and the rotation. Slowly admit air into the system, taking care not to splash the extract out of the sample flask.

7.7 Micro-concentration procedures (all matrices)

When further concentration is required, either a micro-Snyder column technique or a nitrogen evaporation technique is used to adjust the extract to the final volume required.

7.7.1 Micro-Snyder column technique

7.7.1.1 Add another one or two clean boiling chips to the concentrator tube and attach a two-ball micro-Snyder column. Prewet the column by adding about 0.5 mL of toluene to the top of the column.

7.7.1.2 Place the round bottom flask in a heating mantle and apply heat as required to complete the concentration in 5-10 minutes. At the proper rate of distillation the balls of the column will actively chatter, but the chambers will not flood.

7.7.1.3 When the apparent volume of liquid reaches 0.5 mL, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes. Remove the Snyder column and rinse the flask and its lower joints with about 0.2 mL of solvent and add to the concentrator tube. Adjust the final volume to 1.0 mL with solvent.

7.7.2 Nitrogen blowdown technique

7.7.2.1 Place the concentrator tube in a warm water bath (approximately 35°C) and evaporate the solvent volume to the required level using a gentle stream of clean, dry nitrogen (filtered through a column of activated carbon).

CAUTION: Do not use plasticized tubing between the carbon trap and the sample.

7.7.2.2 The internal wall of the tube must be rinsed down several times with the appropriate solvent during the operation. During evaporation, the solvent level in the tube must be positioned to prevent water from condensing into the sample (i.e., the

solvent level should be below the level of the water bath). Under normal operating conditions, the extract should not be allowed to become dry.

7.7.2.3 When the apparent volume of liquid reaches 0.5 mL, remove the concentrator tube from the water bath. Adjust the final volume to 1.0 mL with solvent.

7.8 Acid-base cleanup procedure (all matrices)

7.8.1 The concentrated extracts from all matrices are subjected to a series of cleanup procedures generally beginning with an acid-base wash, and continuing on with silica gel chromatography, alumina chromatography, and carbon chromatography. The acid-base wash may not be necessary for uncolored extracts, but all the other cleanup procedures should be employed, regardless of the color of the extract. Begin the cleanup procedures by quantitatively transferring each concentrated extract to a separate 125-mL separatory funnel.

7.8.2 Prior to cleanup, all extracts are spiked with the $^{37}Cl_4$-2,3,7,8-TCDD cleanup standard (Sec. 5.13). The recovery of this standard is used to monitor the efficiency of the cleanup procedures. Spike 5 µL of the cleanup standard (or a larger volume of diluted solution containing 25 ng of $^{37}Cl_4$-2,3,7,8-TCDD) into each separatory funnel containing an extract, resulting in a concentration of 0.25 ng/µL in the final extract analyzed by GC/MS.

CAUTION: Concentrated acid and base produce heat when mixed with aqueous solutions, and may cause solutions to boil or splatter. Perform the following extractions carefully, allowing the heat and pressure in the separatory funnel to dissipate before shaking the stoppered funnel.

7.8.3 Partition the concentrated extract against 40 mL of concentrated sulfuric acid. Shake for 2 minutes. Remove and discard the acid layer (bottom). Repeat the acid washing until no color is visible in the acid layer. (Perform acid washing a maximum of 4 times.)

7.8.4 Partition the concentrated extract against 40 mL of 5 percent (w/v) sodium chloride. (Caution: Acid entrained in the extract may produce heat when mixed with the sodium chloride solution). Shake for two minutes. Remove and discard the aqueous layer (bottom).

7.8.5 Partition the concentrated extract against 40 mL of 20 percent (w/v) potassium hydroxide (KOH). (Caution: Allow heat to dissipate before shaking). Shake for 2 minutes. Remove and discard the base layer (bottom). Repeat the base washing until color is not visible in the bottom layer (perform base washing a maximum of four times). Strong base (KOH) is known to degrade certain PCDDs/PCDFs; therefore, contact time should be minimized.

7.8.6 Partition the concentrated extract against 40 mL of 5 percent (w/v) sodium chloride. (Caution: Base entrained in the extract may produce heat when mixed with the sodium chloride solution). Shake for 2 minutes. Remove and discard the aqueous layer (bottom). Dry the organic layer by pouring it through a funnel containing a rinsed filter half-filled with anhydrous sodium sulfate. Collect the extract in an appropriate size (100- to 250-mL) round bottom flask. Wash the separatory funnel with two 15-mL portions of hexane, pour through the funnel and combine the extracts.

7.8.7 Concentrate the extracts of all matrices to 1.0 mL of hexane using the procedures described in Sec. 7.7. Solvent exchange is accomplished by concentrating the extract to

approximately 100 µL, adding 2-3 mL of hexane to the concentrator tube and continuing concentration to a final volume of 1.0 mL.

7.9 Silica gel and alumina column chromatographic procedures

7.9.1 Silica gel column - Insert a glass wool plug into the bottom of a gravity column (1 cm x 30 cm glass column) fitted with a PTFE stopcock. Add 1 g silica gel and tap the column gently to settle the silica gel. Add 2 g sodium hydroxide-impregnated silica gel, 1 g silica gel, 4 g sulfuric acid-impregnated silica gel, and 2 g silica gel (Sec. 5.8). Tap the column gently after each addition. A small positive pressure (5 psi) of clean nitrogen may be used if needed.

7.9.2 Alumina column - Insert a glass wool plug onto the bottom of a gravity column (1 cm x 30 cm glass column) fitted with a PTFE stopcock. Add 6 g of the activated acid alumina (Sec. 5.8.1). Tap the top of the column gently.

NOTE: Check each new batch of silica gel and alumina by combining 50 µL of the continuing calibration solution (CC3) with 950 µL of hexane. Process this solution through both columns in the same manner as a sample extract (Secs. 7.9.5 through 7.9.9). Concentrate the continuing calibration solution to a final volume of 50 µL. Proceed to Sec. 7.14. If the recovery of any of the analytes is less than 80%, the batch of alumina or silica gel may not be appropriate for use.

7.9.3 Add hexane to each column until the packing is free of air bubbles. A small positive pressure (5 psi) of clean dry nitrogen may be used if needed. Check the columns for channeling. If channeling is present, discard the column. Do not tap a wetted column.

7.9.4 Assemble the two columns such that the eluate from the silica gel column drains directly into the alumina column. Alternatively, the two columns may be eluted separately.

7.9.5 Apply the concentrated extract (in hexane) from Sec. 7.8.7 to the top of the silica gel column. Rinse the vial with enough hexane (1-2 mL) to complete the quantitative transfer of the sample to the surface of the silica.

7.9.6 Using 90 mL of hexane, elute the extract from Column 1 directly onto Column 2 which contains the alumina. Do not allow the alumina column to run dry.

7.9.7 Add 20 mL of hexane to Column 2, and elute until the hexane level is just below the top of the alumina. Do not discard the eluted hexane, but collect in a separate flask and store it for later use, as it may be useful in determining where the labeled analytes are being lost if recoveries are less than 50%.

7.9.8 Add 20 mL of 20% methylene chloride/80% hexane (v/v) to Column 2 and collect the eluate.

7.9.9 Concentrate the extract to approximately 2 to 3 mL using the procedures in Sec. 7.7.

CAUTION: Do not concentrate the eluate to dryness. The sample is now ready to be transferred to the carbon column.

7.10 Carbon column chromatographic procedure

7.10.1 Thoroughly mix 9.0 g activated carbon (Carbopak C, Sec. 5.8.2) and 41.0 g Celite 545 to produce a 18% w/w mixture. Activate the mixture at 130°C for 6 hours, and store in a desiccator.

NOTE: Check each new batch of the carbon/Celite mixture by adding 50 µL of the calibration verification solution to 950 µL of hexane. Process the spiked solution in the same manner as a sample extract (Secs. 7.10.3 through 7.10.5). Concentrate the calibration verification solution to 50 µL and proceed with Sec. 7.14. If the recovery of any of the analytes is less than 80%, this batch of carbon/Celite mixture may not be used.

7.10.2 Prepare a 4-inch long glass column by cutting off each end of a 10-mL disposable serological pipet. Fire polish both ends and flare if desired. Insert a glass wool plug at one end of the column, and pack it with 1 g of the Carbon/Celite mixture. Insert an additional glass wool plug in the other end.

CAUTION: It is very important that the column be packed properly to ensure that carbon fines are not carried into the eluate. PCDDs/PCDFs will adhere to the carbon fines and greatly reduce recovery. If carbon fines are carried into the eluate in Sec. 7.10.5, filter the eluate, using a 0.7 µm filter (pre-rinsed with toluene), then proceed to Sec. 7.11.

7.10.3 Rinse the column with:

- 4 mL Toluene
- 2 mL of Methylene Chloride/Methanol/Toluene (75:20:5 v/v)
- 4 mL of Cyclohexane/Methylene Chloride (50:50 v/v)

Discard all the column rinsates.

7.10.4 While the column is still wet, transfer the concentrated eluate from Sec. 7.9.10 to the prepared carbon column. Rinse the eluate container with two 0.5-mL portions of hexane and transfer the rinses to the carbon column. Elute the column with the following sequence of solvents.

10 mL of Cyclohexane/Methylene Chloride (50:50 v/v).
5 mL of Methylene Chloride/Methanol/Toluene (75:20:5 v/v).

NOTE: The above two eluates may be collected and combined, and used as a check on column efficiency.

7.10.5 Once the solvents have eluted through the column, turn the column over, and elute the PCDD/PCDF fraction with 20 mL of toluene, and collect the eluate.

7.11 Final concentration

7.11.1 Evaporate the toluene fraction from Sec. 7.10.5 to approximately 1.0 mL, using the procedures in Secs. 7.6 and 7.7. Transfer the extract to a 2.0-mL conical vial using a toluene rinse.

CAUTION: Do not evaporate the sample extract to dryness.

7.11.2 Add 100 µL tridecane (or nonane) to the extract and reduce the volume to 100 µL using a gentle stream of clean dry nitrogen (Sec. 7.7). The final extract volume should be 100 µL of tridecane (or nonane). Seal the vial and store the sample extract in the dark at ambient temperature until just prior to GC/MS analysis.

7.12 Chromatographic conditions (recommended)

7.12.1 Establish the GC operating conditions necessary to achieve the resolution and sensitivity required for the analyses, using the following conditions as guidance for the DB-5 (or equivalent) column:

Helium Linear Velocity:	35 - 40 cm/sec at 240°C
Initial Temperature:	170°C
Initial Time:	10 minutes
Temperature Program:	increase to 320°C at 8°C/minute
Hold Time	until OCDF elutes
Total Time	40-45 minutes

On the DB-5 column,the chromatographic resolution is evaluated using the CC3 calibration standard during both the initial calibration and the calibration verification. The chromatographic peak separation between the $^{13}C_{12}$-2,3,7,8-TCDD peak and the $^{13}C_{12}$-1,2,3,4-TCDD peak must be resolved with a valley of $\leq$ 25 percent, where:

$$\text{Valley} = \left(\frac{x}{y}\right) \times 100$$

y = the peak height of any TCDD isomer
x = measured as shown in Figure 2

The resolution criteria must be evaluated using measurements made on the selected ion current profile (SICP) for the appropriate ions for each isomer. Measurements are not made from total ion current profiles.

Optimize the operating conditions for sensitivity and resolution, and employ the same conditions for both calibration and sample analyses.

7.12.2 When an SP-2331 (or equivalent) GC column is used to confirm the results for 2,3,7,8-TCDF, the chromatographic resolution is evaluated before the analysis of any calibration standards by the analysis of a commercially-available column performance mixture (Sec. 5.16) that contains the TCDD isomers that elute most closely with 2,3,7,8-TCDD on this GC column (1,4,7,8-TCDD and the 1,2,3,7/1,2,3,8-TCDD pair). Analyze a 2-µL aliquot of this solution, using the column operating conditions and descriptor switching times previously established. The GC operating conditions for this column should be modified from those for the DB-5 (or equivalent) column, focusing on resolution of the closely-eluting TCDD and TCDF isomers.

NOTE: The column performance mixture may be combined with the window defining mix into a single analysis, provided that the combined solution contains the isomers needed to determine that criteria for both analyses can be met.

The chromatographic peak separation between unlabeled 2,3,7,8-TCDD and the peaks representing all other unlabeled TCDD isomers should be resolved with a valley of $\leq$ 25 percent, where:

$$\text{Valley} = \left(\frac{x}{y}\right) \times 100$$

y = the peak height of any TCDD isomer
x = measured as shown in Figure 2

The resolution criteria must be evaluated using measurements made on the selected ion current profile (SICP) for the appropriate ions for each isomer. Measurements are not made from total ion current profiles.

Further analyses may not proceed until the GC resolution criteria have been met.

7.13 GC/MS Calibration

Calibration of the GC/MS system involves three separate procedures, mass calibration of the MS, establishment of GC retention time windows, and calibration of the target analytes. These three procedures are described in Secs. 7.13.1 to 7.13.3. Samples should not be analyzed until acceptable descriptor switching times, chromatographic resolution, and calibrations are achieved and documented. The sequence of analyses is shown in Figure 3.

NOTE: The injection volume for all sample extracts, blanks, quality control samples and calibration solutions must be the same.

7.13.1 Mass calibration - Mass calibration of the MS is recommended prior to analyzing the calibration solutions, blanks, samples and QC samples. It is recommended that the instrument be tuned to greater sensitivity in the high mass range in order to achieve better response for the later eluting compounds. Optimum results using FC-43 for mass calibration may be achieved by scanning from 222-510 amu every 1 second or less, utilizing 70 volts (nominal) electron energy in the electron ionization mode. Under these conditions, m/z 414 and m/z 502 should be 30-50% of m/z 264 (base peak).

7.13.2 Retention time windows - Prior to the calibration of the target analytes, it is necessary to establish the appropriate switching times for the SIM descriptors (Table 7). The switching times are determined by the analysis of the Window Defining Mix, containing the first and last eluting isomers in each homologue (Table 8). Mixes are available for various columns.

The ions in each of the four recommended descriptors are arranged so that there is overlap between the descriptors. The ions for the TCDD, TCDF, PeCDD, and PeCDF isomers are in the first descriptor, the ions for the PeCDD, PeCDF, HxCDD and HxCDF isomers are in the second descriptor, the ions for the HxCDD, HxCDF, HpCDD and HpCDF isomers are in the third, and the ions for the HpCDD, HpCDF, OCDD and OCDF isomers are in the fourth descriptor. The descriptor switching times are set such that the isomers that elute from the GC during a given retention time window will also be those isomers for which the ions are monitored. For the homologues that overlap between descriptors, the laboratory may use discretion in setting the switching times. However, do not set descriptor switching times such that a change in descriptors occurs at or near the expected retention time of any of the 2,3,7,8-substituted isomers.

7.13.3 Calibration of target analytes - Two types of calibration procedures, initial calibration and calibration verification, are necessary (Secs. 7.13.3.1 and 7.13.3.2). The initial calibration is needed before any samples are analyzed for PCDDs/PCDFs, and intermittently throughout sample analysis, as dictated by the results of the calibration verification. The calibration verification is necessary at the beginning of each 12-hour time period during which sample are analyzed.

7.13.3.1 Initial Calibration - Once the Window Defining Mix has been analyzed and the descriptor switching times have been verified (and after the analysis of the column performance solution, if using a GC column other than DB-5), analyze the five concentration calibration solutions (CC1-CC5), described in Table 1, prior to any sample analysis.

7.13.3.1.1 The relative ion abundance criteria for PCDDs/PCDFs presented in Table 9 should be met for all PCDD/PCDF peaks, including the labeled internal and recovery standards, in all solutions. The lower and upper limits of the ion abundance ratios represent a ±15% window around the theoretical abundance ratio for each pair of selected ions. The $^{37}Cl_4$-2,3,7,8-TCDD cleanup standard contains no ^{35}Cl, thus the ion abundance ratio criterion does not apply to this compound.

7.13.3.1.2 If the laboratory uses a GC column other than those described here, the laboratory must ensure that the isomers eluting closest to 2,3,7,8-TCDD on that column are used to evaluate GC column resolution

7.13.3.2 Calculate the relative response factors (RFs) for the seventeen unlabeled target analytes relative to their appropriate internal standards (RF_n) (Table 10), according to the formulae below. For the seven unlabeled analytes and the $^{37}Cl_4$-2,3,7,8-TCDD cleanup standard that are found only in the CC3 solution, only one RF is calculated for each analyte. For the other 10 unlabeled analytes, calculate the RF of each analyte in each calibration standard.

Calculate the RFs for the five labeled internal standards and the cleanup standard relative to the appropriate recovery standard (RF_{is}) (Table 10), in each calibration standard, according to the following formulae:

$$RF_n = \frac{(A_n^1 + A_n^2) \times Q_{is}}{(A_{is}^1 + A_{is}^2) \times Q_n}$$

$$RF_{is} = \frac{(A_{is}^1 + A_{is}^2) \times Q_{rs}}{(A_{rs}^1 + A_{rs}^2) \times Q_{is}}$$

where:

A_n^1 and A_n^2 = integrated areas of the two quantitation ions of the isomer of interest (Table 8)

A_{is}^1 and A_{is}^2 = integrated areas of the two quantitation ions of the appropriate internal standard (Table 8)

A_{rs}^1 and A_{rs}^2 = integrated areas of the two quantitation ions of the appropriate recovery standard (Table 8)

Q_n = nanograms of unlabeled target analyte injected

Q_{is} = nanograms of appropriate internal standard injected

Q_{rs} = nanograms of appropriate recovery standard injected.

There is only one quantitation ion for the ^{37}Cl cleanup standard. Calculate the relative response factor as described for RF_{is}, using one area for the cleanup standard, and the sum of the areas of the ions from the recovery standard.

The RF_n and xRF_{is} are dimensionless quantities; therefore, the units used to express the Q_n, Q_{is}, and Q_{rs} must be the same.

7.13.3.3 Calculate the relative response factors for the unlabeled PCDDs/PCDFs relative to the recovery standards (RF_{rs}), where:

$$RF_{rs} = RF_n \times RF_{is}$$

This relative response factor is necessary when the sample is diluted to the extent that the S/N ratio for the internal standard is less than 10.0.

7.13.3.4 Relative Response Factor Criteria - Calculate the mean RF and percent relative standard deviation (%RSD) of the five RFs (CC1 to CC5) for each unlabeled PCDD/PCDF and labeled internal standards present in all five concentration calibration solutions. No mean RF or %RSD calculations are possible for the 2,3,7,8-substituted isomers or the cleanup standard found only in the CC3 solution.

$$\%RSD = \frac{\text{Standard deviation}}{\text{Mean RF}} \times 100$$

The %RSD of the five RFs (CC1-CC5) for the unlabeled PCDDs/PCDFs and the internal standards should not exceed 15.0%.

7.13.3.5 The response factors to be used for determining the total homologue concentrations are described in Sec. 7.15.2.

7.13.3.6 Calibration Verification - The calibration verification consists of two parts: evaluation of the chromatographic resolution, and verification of the RF values to be used for quantitation. At the beginning of each 12-hour period, the chromatographic resolution is verified in the same fashion as in the initial calibration, through the analysis of the CC3 solution on the DB-5 (or equivalent) column, or through the analysis of the column performance solution on the SP-2331 (or equivalent) column.

Prepare the CC3 solution by combining the volumes of the solutions listed in Table 4 to yield a final volume of 1.0 mL at the concentrations listed for the CC3 solution

in Table 1. Alternatively, use a commercially-prepared solution that contains the target analytes at the CC3 concentrations listed in Table 1.

For the DB-5 (or equivalent) column, begin the 12-hour period by analyzing the CC3 solution. Inject a 2-µL aliquot of the calibration verification solution (CC3) into the GC/MS. The identical GC/MS/DS conditions used for the analysis of the initial calibration solutions must be used for the calibration verification solution. Evaluate the chromatographic resolution using the QC criteria in Sec. 7.12.1.

For the SP-2331 (or equivalent) column, or other columns with different elution orders, begin the 12-hour period with the analysis of a 2-µL aliquot of the appropriate column performance solution. Evaluate the chromatographic resolution using the QC criteria in Sec. 7.12.2. If this solution meets the QC criteria, proceed with the analysis of a 2-µL aliquot of the CC3 solution. The identical GC/MS/DS conditions used for the analysis of the initial calibration solutions must be used for the calibration verification solution.

Calculate the RFs for the seventeen unlabeled target analytes relative to their appropriate internal standards (RF_n) and the response factors for the five labeled internal standards and the cleanup standard relative to the appropriate recovery standard (RF_{is}), according to the formulae in Sec. 7.13.3.2.

Calculate the RFs for the unlabeled PCDDs/PCDFs relative to the recovery standards (RF_{rs}), using the formula in Sec. 7.13.3.3.

Do not proceed with sample analyses until the calibration verification criteria have been met for:

1) GC Column Resolution Criteria - The chromatographic resolution on the DB-5 (or equivalent) and /or the SP-2331 (or equivalent) column must meet the QC criteria in Sec. 7.12. In addition, the chromatographic peak separation between the 1,2,3,4,7,8-HxCDD and the 1,2,3,6,7,8-HxCDD in the CC3 solution shall be resolved with a valley of $\leq$ 50 percent (Figure 2).

2) Ion Abundance Criteria - The relative ion abundances listed in Table 9 must be met for all PCDD/PCDF peaks, including the labeled internal and recovery standards.

3) Instrument Sensitivity Criteria - For the CC3 solution, the signal-to-noise (S/N) ratio shall be greater than 2.5 for the unlabeled PCDD/PCDF ions, and greater than 10.0 for the labeled internal and recovery standards.

4) Response Factor Criteria - The measured RFs of each analyte and internal standard in the CC3 solution must be within ±30.0% of the mean RFs established during initial calibration for the analytes in all five calibration standards, and within ±30.0% of the single-point RFs established during initial calibration for those analytes present in only the CC3 standard (see Sec. 7.13.3.2).

$$\% \text{ Difference} = \frac{(RF_i - RF_c)}{RF_i} \times 100$$

where:

RF_i = Relative response factor established during initial calibration.

RF_c = Relative response factor established during calibration verification.

7.13.3.7 In order to demonstrate that the GC/MS system has retained adequate sensitivity during the course of sample analyses, the lowest standard from the initial calibration is analyzed at the end of each 12-hour time period during which samples are analyzed. This analysis must utilize the same injection volume and instrument operating conditions as were used for the preceding sample analyses.

The results of this analysis must meet the acceptance criteria for retention times, ion abundances, and S/N ratio that are listed in Sec 7.13.3.6 for the continuing calibration standard. Response factors do not need to be evaluated in this end-of-shift standard. If this analysis fails either the ion abundance or S/N ratio criteria, then any samples analyzed during that 12-hour period that indicated the presence of any PCDDs/PCDFs below the method quantitation limit or where estimated maximum possible concentrations were reported must be reanalyzed. Samples with positive results above the method quantitation limit need not be reanalyzed.

7.14 GC/MS analysis of samples

7.14.1 Remove the extract of the sample or blank from storage. Gently swirl the solvent on the lower portion of the vial to ensure complete dissolution of the PCDDs/PCDFs.

7.14.2 Transfer a 50-µL aliquot of the extract to a 0.3-mL vial, and add sufficient recovery standard solution to yield a concentration of 0.5 ng/µL. Reduce the volume of the extract back down to 50 µL using a gentle stream of dry nitrogen.

7.14.3 Inject a 2-µL aliquot of the extract into the GC/MS instrument. Reseal the vial containing the original concentrated extract. Analyze the extract by GC/MS, and monitor all of the ions listed in Table 7. The same MS parameters used to analyze the calibration solutions must be used for the sample extracts.

7.14.4 Dilution of the sample extract is necessary if the concentration of any PCDD/PCDF in the sample has exceeded the calibration range, or the detector has been saturated. An appropriate dilution will result in the largest peak in the diluted sample falling between the mid-point and high-point of the calibration range.

7.14.4.1 Dilutions are performed using an aliquot of the original extract, of which approximately 50 µL remain from Sec. 7.14.2. Remove an appropriate size aliquot from the vial and add it to a sufficient volume of tridecane (or nonane) in a clean 0.3-mL conical vial. Add sufficient recovery standard solution to yield a concentration of 0.5 ng/µL. Reduce the volume of the extract back down to 50 µL using a gentle stream of dry nitrogen.

7.14.4.2 The dilution factor is defined as the total volume of the sample aliquot and clean solvent divided by the volume of the sample aliquot that was diluted.

7.14.4.3 Inject 2 µL of the diluted sample extract into the GC/MS, and analyze according to Secs. 7.14.1 through 7.14.3.

7.14.4.4 Diluted samples in which the MS response of any internal standard is greater than or equal to 10% of the MS response of that internal standard in the most recent calibration verification standard are quantitated using the internal standards.

Diluted samples in which the MS response of any internal standard is less than 10% of the MS response of that internal standard in the most recent calibration verification standard are quantitated using the recovery standards (see Sec. 7.15.3).

7.14.5 Identification Criteria - For a gas chromatographic peak to be unambiguously identified as a PCDD or PCDF, it must meet all of the following criteria.

7.14.5.1 Retention times - In order to make a positive identification of the 2,3,7,8-substituted isomers for which an isotopically labeled internal or recovery standard is present in the sample extract, the absolute retention time (RT) at the maximum peak height of the analyte must be within -1 to +3 seconds of the retention time of the corresponding labeled standard.

In order to make a positive identification of the 2,3,7,8-substituted isomers for which a labeled standard is *not* available, the relative retention time (RRT) of the analyte must be within 0.05 RRT units of the RRT established by the calibration verification. The RRT is calculated as follows:

$$\text{RRT} = \frac{\text{retention time of the analyte}}{\text{retention time of the corresponding internal standard}}$$

For non-2,3,7,8-substituted compounds (tetra through hepta), the retention time must be within the retention time windows established by the window defining mix for the corresponding homologue (Sec. 7.13.2).

In order to assure that retention time shifts do not adversely affect the identification of PCDDs/PCDFs, the absolute retention times of the two recovery standards added to every sample extract immediately prior to analysis may not shift by more than ±10 seconds from their retention times in the calibration verification standard.

7.14.5.2 Peak identification - All of the ions listed in Table 8 for each PCDD/PCDF homologue and labeled standards must be present in the SICP. The ion current response for the two quantitation ions and the M-$[COCL]^+$ ions for the analytes must maximize simultaneously (±2 seconds). This requirement also applies to the internal standards and recovery standards. For the cleanup standard, only one ion is monitored.

7.14.5.3 Signal-to-noise ratio - The integrated ion current for each analyte ion listed in Table 8 must be at least 2.5 times background noise and must not have saturated the detector (Figure 4). The internal standard ions must be at least 10.0 times background noise and must not have saturated the detector. However, if the M-$[COCL]^+$ ion does not meet the 2.5 times S/N requirement but meets all the other criteria listed in Sec. 7.14.5 and, in the judgement of the GC/MS Interpretation Specialist the peak is a PCDD/PCDF, the peak may be reported as positive and the data flagged on the report form.

7.14.5.4 Ion abundance ratios - The relative ion abundance criteria listed in Table 9 for unlabeled analytes and internal standards must be met using peak areas to calculate ratios.

7.14.5.4.1 If interferences are present, and ion abundance ratios are not met using peak areas, but all other qualitative identification criteria are met (RT, S/N, presence of all 3 ions), then use peak heights to evaluate the ion ratio.

7.14.5.4.2 If, in the judgement of the analyst, the peak is a PCDD/PCDF, then report the ion abundance ratios determined using peak heights, quantitate the peaks using peak heights rather than areas for both the target analyte and the internal standard, and flag the result on the report form.

7.14.5.5 Polychlorinated diphenyl ether (PCDPE) interferences.

The identification of a GC peak as a PCDF cannot be made if a signal having S/N greater than 2.5 is detected at the same retention time (±2 seconds) in the corresponding PCDPE channel (Table 8). If a PCDPE is detected, an Estimated Maximum Possible Concentration (EMPC) should be calculated for this GC peak according to Sec. 7.15.7, regardless of the ion abundance ratio, and reported.

7.14.6 When peaks are present that do not meet all of the identification criteria in Sec. 7.14.5 and the reporting of an estimated maximum possible concentration according to Sec. 7.15.7 will not meet the specific project objectives, then the analyst may need to take additional steps to resolve the potential interference problems. However, this decision generally is project-specific and should not be applied without knowledge of the intended application of the results. These steps may be most appropriate when historical data indicate that 2,3,7,8-substituted PCDDs/PCDFs have been detected in samples from the site or facility, yet the results from a specific analysis are inconclusive. The additional steps may include the use of additional or repeated sample cleanup procedures or the use of HRGC/MS/MS (e.g., tandem mass spectrometry).

7.15 Calculations

7.15.1 For GC peaks that have met all the identification criteria outlined in Sec. 7.14.5, calculate the concentration of the individual PCDD or PCDF isomers using the formulae:

ALL MATRICES OTHER THAN WATER:

$$C_n\ (\mu g/kg) = \frac{Q_{is} \times (A_n^1 + A_n^2)}{W \times (A_{is}^1 + A_{is}^2) \times RF_n}$$

WATER:

$$C_n\ (ng/L) = \frac{Q_{is} \times (A_n^1 + A_n^2)}{V \times (A_{is}^1 + A_{is}^2) \times RF_n}$$

where:

A_n^1 and A_n^2 = integrated ion abundances (peak areas) of the quantitation ions of the isomer of interest (Table 8).

A_{is}^1 and A_{is}^2 = integrated ion abundances (peak areas) of the quantitation ions of the appropriate internal standard (Table 8).

C_n = concentration of unlabeled PCDD/PCDF found in the sample.

W = weight of sample extracted, in grams.

V = volume of sample extracted, in liters.

Q_{is} = nanograms of the appropriate internal standard added to the sample prior to extraction.

RF_n = calculated relative response factor from calibration verification (see Sec. 7.13.3.6).

NOTE: In instances where peak heights are used to evaluate ion abundance ratios due to interferences (Sec. 7.14.5.4), substitute peak heights for areas in the formulae above.

For solid matrices, the units of ng/g that result from the formula above are equivalent to µg/kg. Using isotope dilution techniques for quantitation the concentration data are recovery corrected, and therefore, the volume of the final extract and the injection volume are implicit in the value of Q_{is}.

7.15.1.1 For homologues that contain only one 2,3,7,8-substituted isomer (TCDD, PeCDD, HpCDD, and TCDF), the RF of the 2,3,7,8-substituted isomer from the calibration verification will be used to quantitate both the 2,3,7,8-substituted isomers and the non-2,3,7,8-isomers.

7.15.1.2 For homologues that contain *more than* one 2,3,7,8-substituted isomer (HxCDD, PeCDF, HxCDF, and HpCDF), the RF used to calculate the concentration of each 2,3,7,8-substituted isomers will be the RF determined for that isomer during the calibration verification.

7.15.1.3 For homologues that contain one or more non-2,3,7,8-substituted isomer, the RF used to calculate the concentration of these isomers will be the lowest of the RFs determined during the calibration verification for the 2,3,7,8-substituted isomers in that homologue. This RF will yield the highest possible concentration for the non-2,3,7,8-substituted isomers.

NOTE: The relative response factors of given isomers within any homologue may be different. However, for the purposes of these calculations, it will be assumed that every non-2,3,7,8-substituted isomer for a given homologue has the same relative response factor. In order to minimize the effect of this assumption on risk assessment, the 2,3,7,8-substituted isomer with the lowest RF was chosen as representative of each homologue. All relative response factor calculations for the non-2,3,7,8--substituted isomers in a given homologue are based on that isomer.

7.15.2 In addition to the concentrations of specific isomers, the total homologue concentrations are also reported. Calculate the total concentration of each homologue of PCDDs/PCDFs as follows:

Total concentration = sum of the concentrations of every positively identified isomer of each PCDD/PCDF homologue.

The total must include the non-2,3,7,8-substituted isomers as well as the 2,3,7,8-substituted isomers that are also reported separately. The total number of GC peaks included in the total homologue concentration should be reported.

7.15.3 If the area of any internal standard in a diluted sample is less than 10% of the area of that internal standard in the calibration verification standard, then the unlabeled PCDD/PCDF concentrations in the sample shall be estimated using the recovery standard, using the formulae that follow. The purpose is to ensure that there is an adequate MS response for quantitation in a diluted sample. While use of a smaller aliquot of the sample might require smaller dilutions and therefore yield a larger area for the internal standard in the diluted extract, this practice leads to other concerns about the homogeneity of the sample and the representativeness of the aliquot taken for extraction.

ALL MATRICES OTHER THAN WATER:

$$C_n\ (\mu g/kg) = \frac{Q_{rs} \times (A_n^1 + A_n^2) \times D}{W \times (A_{rs}^1 + A_{rs}^2) \times RF_{rs}}$$

WATER:

$$C_n\ (ng/L) = \frac{Q_{rs} \times (A_n^1 + A_n^2) \times D}{V \times (A_{rs}^1 + A_{rs}^2) \times RF_{rs}}$$

where:

D = the dilution factor (Sec. 7.14.4.2).

A_n^1, A_n^2, A_{rs}^1, A_{rs}^2, Q_{rs}, RF_{rs}, W, and V are defined in Secs. 7.13.3.2 and 7.15.1.

7.15.4 Report results for soil/sediment, fly ash, and chemical waste samples in micrograms per kilogram (µg/kg) and water samples in nanograms per liter (ng/L).

7.15.5 Calculate the percent recovery, R_{is}, for each internal standard and the cleanup standard in the sample extract, using the formula:

$$R_{is}(\%) = \frac{(A_{is}^1 + A_{is}^2) \times Q_{rs}}{(A_{rs}^1 + A_{rs}^2) \times RF_{is} \times Q_{is}} \times 100$$

where:

A_{is}^1, A_{is}^2, A_{rs}^1, A_{rs}^2, Q_{is}, Q_{rs}, and RF_{is} are defined in Secs. 7.13.3.2 and 7.15.1.

NOTE: When calculating the recovery of the $^{37}Cl_4$-2,3,7,8-TCDD cleanup standard, only one m/z is monitored for this standard; therefore, only one peak area will be used in the numerator of this formula. Use both peak areas of the $^{13}C_{12}$-1,2,3,4-TCDD recovery standard in the denominator.

7.15.5.1 The $^{13}C_{12}$-1,2,3,4-TCDD is used to quantitate the TCDD and TCDF internal standards and the cleanup standard, and the $^{13}C_{12}$-1,2,3,7,8,9-HxCDD is used to quantitate the HxCDD, HpCDF and OCDD internal standards (Table 10).

7.15.5.2 If the original sample, prior to any dilutions, has any internal standard with a percent recovery of less than 25% or greater than 150%, re-extraction and reanalysis of that sample is necessary.

7.15.6 Sample specific estimated detection limit - The sample specific estimated detection limit (EDL) is the estimate made by the laboratory of the concentration of a given analyte required to produce a signal with a peak height of at least 2.5 times the background signal level. The estimate is specific to a particular analysis of the sample, and will be affected by sample size, dilution, etc.

7.15.6.1 An EDL is calculated for each 2,3,7,8-substituted isomer that is not identified, regardless of whether or not non-2,3,7,8-substituted isomers in that homologue are present. The EDL is also calculated for 2,3,7,8-substituted isomers giving responses for both the quantitation ions that are less than 2.5 times the background level.

7.15.6.2 Use the formulae below to calculate an EDL for each absent 2,3,7,8-substituted PCDD/PCDF. The background level (H_n) is determined by measuring the height of the noise at the expected retention times of both the quantitation ions of the particular 2,3,7,8-substituted isomer.

ALL MATRICES OTHER THAN WATER:

$$\text{EDL } (\mu g/kg) = \frac{2.5 \times Q_{is} \times (H_n^1 + H_n^2) \times D}{W \times (H_{is}^1 + H_{is}^2) \times RF_n}$$

WATER:

$$\text{EDL } (ng/L) = \frac{2.5 \times Q_{is} \times (H_n^1 + H_n^2) \times D}{V \times (H_{is}^1 + H_{is}^2) \times RF_n}$$

where:

H_n^1 and H_n^2 = The peak heights of the noise for both of the quantitation ions of the 2,3,7,8-substituted isomer of interest

H_{is}^1 and H_{is}^2 = The peak heights of both the quantitation ions of the appropriate internal standards

D = dilution factor (Sec. 7.14.4.2).

Q_{is}, RF_{is}, W and V are defined in Secs. 7.13.3.2 and 7.15.1.

7.15.6.3 If none of the isomers within a homologue are detected, then the EDL for the "total" homologue concentration is the lowest EDL for any of the 2,3,7,8-substituted isomers that were not detected. Do not add together the EDLs for the various isomers. If a 2,3,7,8-substituted isomer is reported in the homologue, then no EDL for the "total" is calculated.

7.15.7 Estimated maximum possible concentration - An estimated maximum possible concentration (EMPC) is calculated for 2,3,7,8-substituted isomers that are characterized by a response with an S/N of at least 2.5 for both the quantitation ions, and meet all of the identification criteria in Sec. 7.4.5 except the ion abundance ratio criteria in Sec. 7.14.5.4 or when a peak representing a PCDPE has been detected (7.15.5.5). An EMPC is a worst-case estimate of the concentration. Calculate the EMPC according to the following formulae:

ALL MATRICES OTHER THAN WATER:

$$EMPC_n\ (\mu g/kg) = \frac{Q_{is} \times (A_n^1 + A_n^2) \times D}{W \times (A_{is}^1 + A_{is}^2) \times RF_n}$$

WATER:

$$EMPC_n\ (ng/L) = \frac{Q_{is} \times (A_n^1 + A_n^2) \times D}{V \times (A_{is}^1 + A_{is}^2) \times RF_n}$$

where:

A_x^1 and A_x^2 = Areas of both the quantitation ions.

A_{is}^1, A_{is}^2, Q_{is}, RF, D, W, and V are defined in Secs. 7.13.3.2 and 7.15.1.

7.15.8 Toxic equivalent concentration (TEQ) calculation - The 2,3,7,8-TCDD toxic equivalent concentration of PCDDs/PCDFs present in the sample is calculated according to the method recommended by the Chlorinated Dioxins Workgroup (CDWG) of the EPA and the Centers for Disease Control (CDC). This method assigns a 2,3,7,8-TCDD toxicity equivalency factor (TEF) to each of the seventeen 2,3,7,8-substituted PCDDs/PCDFs shown in Table 11 *("Update of Toxicity Equivalency Factors [TEFs] for Estimating Risks Associated with Exposures to Mixtures of Chlorinated Dibenzo-p-Dioxins and -Dibenzofurans [CDDs/CDFs]" March 1989 [EPA 625/3-89/016])*.

7.15.8.1 The 2,3,7,8-TCDD TEQ of the PCDDs/PCDFs present in the sample is calculated by summing the product of the concentration for each of the compounds listed in Table 11 and the TEF for each compound. The principal purpose of making this calculation is to provide the data user with a single value, normalized to the toxicity of 2,3,7,8-TCDD, that can more readily be used in decisions related to mixtures of these highly toxic compounds.

7.15.8.1.1 The exclusion of homologues such as mono-, di-, tri- and the non-2,3,7,8-substituted isomers in the higher homologues does not mean that they are not toxic. Their toxicity, as estimated at this time, is much less than the toxicity of the compounds listed in Table 11. Hence, only the 2,3,7,8-substituted isomers are included in the TEF calculations. The procedure for calculating the 2,3,7,8-TCDD toxic equivalence cited above is not claimed by the CDWG to be based on a thoroughly established scientific foundation. Rather, the procedure represents a "consensus recommendation on science policy."

7.15.8.1.2 When calculating the TEQ of a sample, include only those 2,3,7,8-substituted isomers that were detected in the sample and met all of the qualitative identification criteria in Sec. 7.14.5. Do not include EMPC or EDL values in the TEQ calculation.

7.15.8.2 The TEQ of a sample is also used in this analytical procedure to determine when second column confirmation may be necessary. The need for second column confirmation is based on the known difficulties in separating 2,3,7,8-TCDF from other isomers. Historical problems have been associated with the separation of 2,3,7,8-TCDF from 1,2,4,9-, 1,2,7,9-, 2,3,4,6-, 2,3,4,7- and 2,3,4,8-TCDF. Because of the toxicological concern associated with 2,3,7,8-TCDF, additional analyses may be required for some samples as described below. If project-specific requirements do not include second column confimation or specify a different approach to confirmation, then this step may be omitted and the project-specific requirements take precedence.

7.15.8.2.1 If the TEQ calculated in Sec. 7.15.8.1 is greater than 0.7 ppb for soil/sediment or fly ash, 7 ppb for chemical waste, or 7 ppt for an aqueous sample, and 2,3,7,8-TCDF is either detected or reported as an EMPC, then better isomer specificity may be required than can be achieved on the DB-5 column. The TEQ values listed here for the various matrices are equivalent to 70% of the historical "Action Level" set by the CDC for soil concentrations of 2,3,7,8-TCDD at Superfund sites. As such, it provides a conservative mechanism for determining when the additional specificity provided by a second column confirmation may be required.

7.15.8.2.2 The sample extract may be reanalyzed on a 60 m SP-2330 or SP-2331 GC column (or equivalent) in order to achieve better GC resolution, and therefore, better identification and quantitation of 2,3,7,8-TCDF. Other columns that provide better specificity for 2,3,7,8-TCDF than the DB-5 column may also be used.

7.15.8.2.3 Regardless of the GC column used, for a gas chromatographic peak to be identified as a 2,3,7,8-substituted PCDD/PCDF isomer during the second column confirmation, it must meet the ion abundance, signal-to-noise, and retention time criteria listed in Sec. 7.14.5.

7.15.8.2.4 The second column confirmation analysis may be optimized for the analysis of 2,3,7,8-TCDF, and need not be used to confirm the results for any other 2,3,7,8-substituted PCDDs/PCDFs identified during the original analysis.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One and Method 8000 for specific quality control (QC) procedures. Each laboratory should maintain a formal quality assurance program. The labortory should also maintain records to document the quality of the data generated.

8.2 Quality control procedures necessary to evaluate the GC/MS system operation include evaluation of chromatographic resolution, retention time windows, calibration verification and chromatographic analysis of samples. Performance criteria are given in the following sections of Method 8280A:

8.2.1 GC resolution criteria for the DB-5 or equivalent column are given in Sec. 7.12.1.

8.2.2 GC resolution criteria for SP-2331 or equivalent column are given in Sec. 7.12.2.

8.2.3 Initial calibration criteria are given in Sec. 7.13.3.1.

8.2.4 Relative response factor criteria for the initial calibration criteria are given in Sec. 7.13.3.4.

8.2.5 Calibration verification criteria are given in Sec. 7.13.3.6.

8.2.6 Ion abundance criteria are given in Secs. 7.13.3.1, 7.13.3.6, and 7.14.5.4.

8.2.7 Instrument sensitivity criteria are given in Sec. 7.13.3.6.

8.2.8 Relative response factor criteria for the calibration verification are given in Sec. 7.13.3.6.

8.2.9 Identification criteria are given in Sec. 7.14.5.

8.2.10 Criteria for Isotopic Ratio Measurements for PCDDs/PCDFs are given in 7.13.3.1, 7.13.3.6, and Table 9.

8.3 Initial Demonstration of Proficiency - Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes, by generating data of acceptable accuracy and precision for target analytes in a clean matrix. The laboratory must also repeat the following operations whenever new staff are trained or significant changes in instrumentation are made. See Method 8000, Sec. 8.0 for information on how to accomplish this demonstration.

8.4 Sample Quality Control for Preparation and Analysis - The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, accuracy, and detection limit). At a minimum, this includes the analysis of QC samples including a method blank, a matrix spike, a duplicate, and a laboratory control sample (LCS) in each analytical batch.

8.4.1 Documenting the effect of the matrix should include the analysis of at least one matrix spike and one duplicate unspiked sample or one matrix spike/matrix spike duplicate pair. The decision on whether to prepare and analyze duplicate samples or a matrix spike/matrix spike duplicate must be based on a knowledge of the samples in the sample batch. If samples are expected to contain target analytes, then laboratories may use one matrix spike and a duplicate analysis of an unspiked field sample. If samples are not expected to contain target

analytes, laboratories should use a matrix spike and matrix spike duplicate pair. Consult Sec. 8 of Method 8000 for information on developing acceptance criteria for the MS/MSD.

8.4.2 A Laboratory Control Sample (LCS) should be included with each analytical batch. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume. The LCS is spiked with the same analytes at the same concentrations as the matrix spike. When the results of the matrix spike analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix. Consult Sec. 8 of Method 8000 for information on developing acceptance criteria for the LCS.

8.4.3 The analysis of method blanks is critical to the provision of meaningful sample results.

8.4.3.1 Method blanks should be prepared at a frequency of at least 5%, that is, one method blank for each group of up to 20 samples prepared at the same time, by the same procedures.

8.4.3.2 When sample extracts are subjected to cleanup procedures, the associated method blank must also be subjected to the same cleanup procedures.

8.4.3.4 As described in Chapter One, the results of the method blank should be:

8.4.3.4.1 Less than the MDL for the analyte.

8.4.3.4.2 Less than 5% of the regulatory limit associated with an analyte.

8.4.3.4.3 Or less than 5% of the sample result for the same analyte, whichever is greater.

8.4.3.4.4 If the method blank results do not meet the acceptance criteria above, then the laboratory should take corrective action to locate and reduce the source of the contamination and to re-extract and reanalyze any samples associated with the contaminated method blank.

8.4.4 The laboratory should not subtract the results of the method blank from those of any associated samples. Such "blank subtraction" is inappropriate and often leads to negative sample results. If the method blank results do not meet the acceptance criteria in 8.4.3 and reanalysis is not practical, then the data user should be provided with the sample results, the method blank results, and a discussion of the corrective actions undertaken by the laboratory.

8.5 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

9.0 METHOD PERFORMANCE

Method performance data are currently not available.

10.0 REFERENCES

1. "Update of Toxicity Equivalency Factors (TEFs) for Estimating Risks Associated with Exposures to Mixtures of Chlorinated Dibenzo-*p*-Dioxins and Dibenzofurans (CDDs/CDFs)", March 1989 (EPA 6251/3-89/016).

2. "Method 8290: Polychlorinated Dibenzodioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) by High Resolution Gas Chromatography/High Resolution Mass Spectrometry (HRGC/HRMS)", Test Methods for Evaluating Solid Waste, Physical/Chemical Methods (EPA OSW SW-846).

3. "Statement of Work for Analysis of Polychlorinated Dibenzo-*p*-dioxins (PCDD) and Polychlorinated Dibenzofurans, Multi-Media, Multi-Concentration, DFLM01.1", September 1991.

4. Method 613: 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin, 40 CFR Part 136, Guidelines Establishing Test Procedures for the Analysis of Pollutants Under the Clean Water Act, October 26, 1984.

11.0 RECOMMENDED SAFETY AND HANDLING PROCEDURES FOR PCDDs/PCDFs

11.1 The following safety practices are excerpts from EPA Method 613, Sec. 4 (July 1982 version) and amended for use in conjunction with this method. The 2,3,7,8-TCDD isomer has been found to be acnegenic, carcinogenic, and teratogenic in laboratory animal studies. Other PCDDs and PCDFs containing chlorine atoms in positions 2,3,7,8 are known to have toxicities comparable to that of 2,3,7,8-TCDD. The analyst should note that finely divided dry soils contaminated with PCDDs and PCDFs are particularly hazardous because of the potential for inhalation and ingestion. It is recommended that such samples be processed in a confined environment, such as a hood or a glove box. Laboratory personnel handling these types of samples should wear masks fitted with charcoal filters to prevent inhalation of dust.

11.2 The toxicity or carcinogenicity of each reagent used in this method is not precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be kept to a minimum. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals listed in this method. A reference file of material safety data sheets should be made available to all personnel involved in the chemical analysis of samples suspected to contain PCDDs and/or PCDFs.

11.3 Each laboratory must develop a strict safety program for the handling of PCDDs and PCDFs. The laboratory practices listed below are recommended.

11.3.1 Contamination of the laboratory will be minimized by conducting most of the manipulations in a hood.

11.3.2 The effluents of sample splitters for the gas chromatograph and roughing pumps on the HRGC/HRMS system should pass through either a column of activated charcoal or be bubbled through a trap containing oil or high boiling alcohols.

11.3.3 Liquid waste should be dissolved in methanol or ethanol and irradiated with ultraviolet light at a wavelength less than 290 nm for several days (use F 40 BL lamps, or equivalent). Using this analytical method, analyze the irradiated liquid wastes and dispose of the solutions when 2,3,7,8-TCDD and -TCDF congeners can no longer be detected.

11.4 The following precautions were issued by Dow Chemical U.S.A. for safe handling of 2,3,7,8-TCDD in the laboratory and amended for use in conjunction with this method. The following 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. Assistance in evaluating the health hazards of particular plant conditions may be obtained from certain consulting laboratories and from State Departments of Health or of Labor, many of which have an industrial health service. The 2,3,7,8-TCDD isomer is extremely toxic to certain kinds of laboratory animals. However, it has been handled for years without injury in analytical and biological laboratories. Many techniques used in handling radioactive and infectious materials are applicable to 2,3,7,8-TCDD.

11.4.1 Protective Equipment - Disposable plastic gloves, apron or lab coat, safety glasses and laboratory hood adequate for radioactive work. However, PVC gloves should not be used.

11.4.2 Training - Workers must be trained in the proper method of removing contaminated gloves and clothing without contacting the exterior surfaces.

11.4.3 Personal Hygiene - Thorough washing of hands and forearms after each manipulation and before breaks (coffee, lunch, and shift).

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

11.4.5 Waste - Good technique includes minimizing contaminated waste. Plastic bag liners should be used in waste cans.

11.4.6 Disposal of Hazardous Wastes - Refer to the November 7, 1986 issue of the Federal Register on Land Ban Rulings for details concerning the handling of dioxin containing wastes.

11.4.7 Decontamination of Personnel - apply a mild soap with plenty of scrubbing action. Glassware, tools and surfaces - Chlorothene NU Solvent (Trademark of the Dow Chemical Company) is the least toxic solvent shown to be effective. Satisfactory cleaning may be accomplished by rinsing with Chlorothene, then washing with a detergent and water. Dish water may be disposed to the sewer after percolation through a charcoal bed filter. It is prudent to minimize solvent wastes because they require special disposal through commercial services that are expensive.

11.4.8 Laundry - Clothing known to be contaminated should be disposed with the precautions described under "Disposal of Hazardous Wastes". Laboratory coats or other clothing worn in 2,3,7,8-TCDD work area 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 launderer knows the problem. The washer should be run through one full cycle before being used again for other clothing.

11.4.9 Wipe Tests - A useful method for determining cleanliness of work surfaces and tools is to wipe the surface with a piece of filter paper, extract the filter paper and analyze the extract.

11.4.10 Inhalation - Any procedure that may generate airborne contamination must be carried out 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 significant inhalation hazards except in case of an accident.

11.4.11 Accidents - Remove contaminated clothing immediately, taking precautions not to contaminate skin or other articles. Wash exposed skin vigorously and repeatedly until medical attention is obtained.

11.5 It is recommended that personnel working in laboratories where PCDD/PCDF are handled be given periodic physical examinations (at least annually). Such examinations should include specialized tests, such as those for urinary porphyrins and for certain blood parameters which, based upon published clinical observations, are appropriate for persons who may be exposed to PCDDs/PCDFs. Periodic facial photographs to document the onset of dermatologic problems are also advisable.

TABLES IN METHOD 8280

FIGURES IN METHOD 8280

TABLE 1

CALIBRATION SOLUTIONS

	Analyte	Concentration of Standard in ng/µL				
		CC1	CC2	CC3	CC4	CC5
	2,3,7,8-TCDD	0.1	0.25	0.5	1.0	2.0
	2,3,7,8-TCDF	0.1	0.25	0.5	1.0	2.0
	1,2,3,7,8-PeCDF	0.1	0.25	0.5	1.0	2.0
	1,2,3,7,8-PeCDD	0.1	0.25	0.5	1.0	2.0
*	2,3,4,7,8-PeCDF			0.5		
*	1,2,3,4,7,8-HxCDF			1.25		
	1,2,3,6,7,8-HxCDF	0.25	0.625	1.25	2.5	5.0
*	1,2,3,4,7,8-HxCDD			1.25		
	1,2,3,6,7,8-HxCDD	0.25	0.625	1.25	2.5	5.0
*	1,2,3,7,8,9-HxCDD			1.25		
*	2,3,4,6,7,8-HxCDF			1.25		
*	1,2,3,7,8,9-HxCDF			1.25		
*	1,2,3,4,7,8,9-HpCDF			1.25		
	1,2,3,4,6,7,8-HpCDF	0.25	0.625	1.25	2.5	5.0
	1,2,3,4,6,7,8-HpCDD	0.25	0.625	1.25	2.5	5.0
	OCDD	0.5	1.25	2.5	5.0	10.0
	OCDF	0.5	1.25	2.5	5.0	10.0
	$^{13}C_{12}$-2,3,7,8-TCDD	0.5	0.5	0.5	0.5	0.5
	$^{13}C_{12}$-2,3,7,8-TCDF	0.5	0.5	0.5	0.5	0.5
	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	0.5	0.5	0.5	0.5	0.5
	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	1.0	1.0	1.0	1.0	1.0
	$^{13}C_{12}$-OCDD	1.0	1.0	1.0	1.0	1.0
	$^{13}C_{12}$-1234-TCDD	0.5	0.5	0.5	0.5	0.5
	$^{13}C_{12}$-123789-HxCDD	0.5	0.5	0.5	0.5	0.5
	$^{37}Cl_4$-2378-TCDD			0.25		

* These compounds are only ***required*** in the CC3 solution. Therefore, do not perform % RSD calculations on these analytes.

TABLE 2

QUANTITATION LIMITS FOR TARGET COMPOUNDS

Analyte	CAS Number	Water (ng/L)	Soil (µg/kg)	Fly Ash (µg/kg)	Chemical Waste* (µg/kg)
2,3,7,8-TCDD	1746-01-6	10	1.0	1.0	10
2,3,7,8-TCDF	51207-31-9	10	1.0	1.0	10
1,2,3,7,8-PeCDF	57117-41-6	25	2.5	2.5	25
1,2,3,7,8-PeCDD	40321-76-4	25	2.5	2.5	25
2,3,4,7,8-PeCDF	57117-31-4	25	2.5	2.5	25
1,2,3,4,7,8-HxCDF	70648-26-9	25	2.5	2.5	25
1,2,3,6,7,8-HxCDF	57117-44-9	25	2.5	2.5	25
1,2,3,4,7,8-HxCDD	39227-28-6	25	2.5	2.5	25
1,2,3,6,7,8-HxCDD	57653-85-7	25	2.5	2.5	25
1,2,3,7,8,9-HxCDD	19408-74-3	25	2.5	2.5	25
2,3,4,6,7,8-HxCDF	60851-34-5	25	2.5	2.5	25
1,2,3,7,8,9-HxCDF	72918-21-9	25	2.5	2.5	25
1,2,3,4,6,7,8-HpCDF	67562-39-4	25	2.5	2.5	25
1,2,3,4,6,7,8-HpCDD	35822-46-9	25	2.5	2.5	25
1,2,3,4,7,8,9-HpCDF	55673-89-7	25	2.5	2.5	25
OCDD	3268-87-9	50	5.0	5.0	50
OCDF	39001-02-0	50	5.0	5.0	50

* “Chemical waste” includes the matrices of oils, still bottoms, oily sludge, wet fuel oil, oil-laced soil, and surface water heavily contaminated with these matrices.

TABLE 3

INTERNAL STANDARD, RECOVERY STANDARD, AND CLEANUP STANDARD SOLUTIONS

INTERNAL STANDARD SOLUTION

Internal Standards	Concentration
$^{13}C_{l2}$-2,3,7,8-TCDD	5 ng/µL
$^{13}C_{l2}$-2,3,7,8-TCDF	5 ng/µL
$^{13}C_{l2}$-1,2,3,6,7,8-HxCDD	5 ng/µL
$^{13}C_{l2}$-1,2,3,4,6,7,8-HpCDF	10 ng/µL
$^{13}C_{l2}$-OCDD	10 ng/µL

RECOVERY STANDARD SOLUTION

Recovery Standards	Concentration
$^{13}C_{l2}$-1,2,3,4-TCDD	5 ng/µL
$^{13}C_{l2}$-1,2,3,7,8,9-HxCDD	5 ng/µL

CLEANUP STANDARD SOLUTION

Cleanup Standards	Concentration
$^{37}Cl_4$-2,3,7,8-TCDD	5 ng/µL

TABLE 4

CALIBRATION VERIFICATION SOLUTION

Volume	Solution
500 µL	CC4 (Table 1)
125 µL	Supplemental Calibration solution (below)
50 µL	Internal Standard solution (Table 3)
50 µL	Recovery Standard solution (Table 3)
50 µL	Cleanup Standard solution (Table 3)
225 µL	Tridecane (or nonane)

This solution will yield a final volume of 1.0 mL at the concentrations specified for the CC3 solution in Table 1.

Supplemental Calibration Solution Prepared from Commercially-Available Materials

Analyte	Concentration (ng/µL)
2,3,4,7,8-PeCDF	4
1,2,3,7,8,9-HxCDD	10
1,2,3,4,7,8-HxCDD	10
1,2,3,4,7,8-HxCDF	10
1,2,3,7,8,9-HxCDF	10
2,3,4,6,7,8-HxCDF	10
1,2,3,4,7,8,9-HpCDF	10

TABLE 5

MATRIX SPIKING SOLUTION

Analyte	Concentration (ng/µL)
2,3,7,8-TCDD	2.5
2,3,7,8-TCDF	2.5
1,2,3,7,8-PeCDF	6.25
1,2,3,7,8-PeCDD	6.25
1,2,3,6,7,8-HxCDF	6.25
1,2,3,6,7,8-HxCDD	6.25
1,2,3,4,6,7,8-HpCDF	6.25
1,2,3,4,6,7,8-HpCDD	6.25
OCDD	12.5
OCDF	12.5

This solution is prepared in tridecane (or nonane) and diluted with acetone prior to use (see Sec. 5.16).

TABLE 6

PCDD/PCDF ISOMERS IN THE WINDOW DEFINING MIX FOR A 60 m DB-5 COLUMN

Homologue	First Eluted	Last Eluted	Approximate Concentration (µg/mL)
TCDD	1,3,6,8-	1,2,8,9-	1.0
TCDF	1,3,6,8-	1,2,8,9-	1.0
PeCDD	1,2,4,7,9-	1,2,3,8,9-	1.0
PeCDF	1,3,4,6,8-	1,2,3,8,9-	1.0
HxCDD	1,2,4,6,7,9-	1,2,3,4,6,7-	1.0
HxCDF	1,2,3,4,6,8-	1,2,3,4,8,9-	1.0
HpCDD	1,2,3,4,6,7,9-	1,2,3,4,6,7,8-	1.0
HpCDF	1,2,3,4,6,7,8-	1,2,3,4,7,8,9-	1.0

TABLE 7

RECOMMENDED SELECTED ION MONITORING DESCRIPTORS

Descriptor 1	Descriptor 2	Descriptor 3	Descriptor 4
243	277	311	345
259	293	327	361
277	311	345	379
293	327	361	395
304	338	374	408
306	340	376	410
316	342	390	420
318	354	392	422
320	356	402	424
322	358	404	426
328	374	408	442
332	376	410	444
334	390	420	458
340	392	422	460
342	402	424	470
356	404	426	472
358	410	446	480
376	446	480	514

The ions at m/z 376 (HxCDPE), 410 (HpCDPE), 446 (OCDPE), 480 (NCDPE) and 514 (DCDPE) represent the polychlorinated diphenyl ethers.

The ions in each of the four recommended descriptors are arranged so that there is overlap between the descriptors. The ions for the TCDD, TCDF, PeCDD, and PeCDF isomers are in the first descriptor, the ions for the PeCDD, PeCDF, HxCDD and HxCDF isomers are in the second descriptor, the ions for the HxCDD, HxCDF, HpCDD and HpCDF isomers are in the third, and the ions for the HpCDD, HpCDF, OCDD and OCDF isomers are in the fourth descriptor.

TABLE 8

IONS SPECIFIED FOR SELECTED ION MONITORING FOR PCDDs/PCDFs

Analyte	Quantitation Ions		M-[COCl]$^+$
TCDD	320	322	259
PeCDD	356	358	293
HxCDD	390	392	327
HpCDD	424	426	361
OCDD	458	460	395
TCDF	304	306	243
PeCDF	340	342	277
HxCDF	374	376	311
HpCDF	408	410	345
OCDF	442	444	379
Internal Standards			
$^{13}C_{l2}$-2,3,7,8-TCDD	332	334	---
$^{13}C_{l2}$-1,2,3,6,7,8-HxCDD	402	404	---
$^{13}C_{l2}$-OCDD	470	472	---
$^{13}C_{l2}$-2,3,7,8-TCDF	316	318	---
$^{13}C_{l2}$-1,2,3,4,6,7,8-HPCDF	420	422	---
Recovery Standards			
$^{13}C_{l2}$-1,2,3,4-TCDD	332	334	---
$^{13}C_{l2}$-1,2,3,7,8,9-HxCDD	402	404	---
Cleanup Standard			
$^{37}Cl_4$-2,3,7,8-TCDD	328	(1)	265
Polychlorinated diphenyl ethers			
HxCDPE	376	---	---
HpCDPE	410	---	---
OCDPE	446	---	---
NCDPE	480	---	---
DCDPE	514	---	---

(1) There is only one quantitation ion monitored for the cleanup standard.

TABLE 9

CRITERIA FOR ISOTOPIC RATIO MEASUREMENTS FOR PCDDs/PCDFs

Analyte	Selected Ions	Theoretical Ion Abundance	Control Limits
TCDD	320/322	0.77	0.65 - 0.89
PeCDD	356/358	1.55	1.32 - 1.78
HxCDD	390/392	1.24	1.05 - 1.43
HpCDD	424/426	1.04	0.88 - 1.20
OCDD	458/460	0.89	0.76 - 1.02
TCDF	304/306	0.77	0.65 - 0.89
PeCDF	340/342	1.55	1.32 - 1.78
HxCDF	374/376	1.24	1.05 - 1.43
HpCDF	408/410	1.04	0.88 - 1.20
OCDF	442/444	0.89	0.76 - 1.02
Internal Standards			
$^{13}C_{l2}$-1,2,3,4-TCDD	332/334	0.77	0.65 - 0.89
$^{13}C_{l2}$-1,2,3,6,7,8-HxCDD	402/404	1.24	1.05 - 1.43
$^{13}C_{l2}$-OCDD	470/472	0.89	0.76 - 1.01
$^{13}C_{l2}$-2,3,7,8-TCDF	316/318	0.77	0.65 - 0.89
$^{13}C_{l2}$-1,2,3,4,6,7,8-HPCDF	420/422	1.04	0.88 - 1.20
Recovery Standards			
$^{13}C_{l2}$-,1,2,3,4-TCDD	332/334	0.77	0.65 - 0.89
$^{13}C_{l2}$-1,2,3,7,8,9-HxCDD	402/404	1.24	1.05 - 1.43

TABLE 10

RELATIONSHIP OF INTERNAL STANDARDS TO ANALYTES, AND RECOVERY STANDARDS TO INTERNAL STANDARDS, CLEANUP STANDARD, AND ANALYTES

INTERNAL STANDARDS VS. ANALYTES

Internal Standard	Analyte
$^{13}C_{12}$-TCDD	2,3,7,8-TCDD
	1,2,3,7,8-PeCDD
$^{13}C_{12}$-HxCDD	1,2,3,6,7,8-HxCDD
	1,2,3,7,8,9-HxCDD
	1,2,3,4,7,8-HxCDD
	1,2,3,4,6,7,8-HpCDD
$^{13}C_{12}$-OCDD	1,2,3,4,6,7,8,9-OCDD
	1,2,3,4,6,7,8,9-OCDF
$^{13}C_{12}$-TCDF	2,3,7,8-TCDF
	1,2,3,7,8-PeCDF
	2,3,4,7,8-PeCDF
$^{13}C_{12}$-HpCDF	1,2,3,6,7,8-HxCDF
	1,2,3,7,8,9-HxCDF
	1,2,3,4,7,8-HxCDF
	2,3,4,6,7,8-HxCDF
	1,2,3,4,5,8,9-HpCDF
	1,2,3,4,7,8,9-HpCDF

TABLE 10 (cont.)

RECOVERY STANDARDS VS. ANALYTES, INTERNAL STANDARDS, AND CLEANUP STANDARD

Recovery Standard	Analyte, Internal Standard
$^{13}C_{12}$-1,2,3,4-TCDD	2,3,7,8-TCDD
	1,2,3,7,8-PeCDD
	2,3,7,8-TCDF
	1,2,3,7,8-PeCDF
	2,3,4,7,8-PeCDF
	$^{13}C_{12}$-2,3,7,8-TCDD
	$^{13}C_{12}$-2378-TCDF
	$^{37}Cl_4$-2378-TCDD
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	1,2,3,6,7,8-HxCDD
	1,2,3,7,8,9-HxCDD
	1,2,3,4,7,8-HxCDD
	1,2,3,6,7,8-HxCDF
	1,2,3,7,8,9-HxCDF
	1,2,3,4,7,8-HxCDF
	2,3,4,6,7,8-HxCDF
	1,2,3,4,5,8,9-HpCDF
	1,2,3,4,7,8,9-HpCDF
	1,2,3,4,6,7,8,9-OCDD
	1,2,3,4,6,7,8,9-OCDF
	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD
	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF
	$^{13}C_{12}$-OCDD

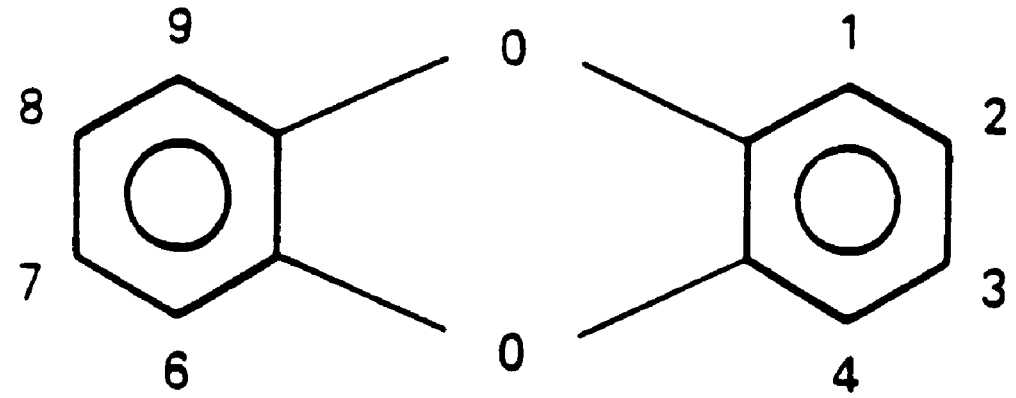
TABLE 11

2,3,7,8-TCDD TOXICITY EQUIVALENCY FACTORS (TEFs) FOR THE PCDDs/PCDFs

Compound	Toxicity Equivalency Factor (TEF)
Mono-, di-, and trichloro dibenzo-*p*-dioxins	0.0
2,3,7,8-tetrachloro-dibenzo-*p*-dioxin	1.0
All other tetrachloro-dibenzo-*p*-dioxins	0.0
1,2,3,7,8-pentachloro-dibenzo-*p*-dioxin	0.5
All other pentachloro-dibenzo-*p*-dioxins	0.0
1,2,3,4,7,8-hexachloro-dibenzo-*p*-dioxin	0.1
1,2,3,6,7,8-hexachloro-dibenzo-*p*-dioxin	0.1
1,2,3,7,8,9-hexachloro-dibenzo-*p*-dioxin	0.1
All other hexachloro-dibenzo-*p*-dioxins	0.0
1,2,3,4,6,7,8-heptachloro-dibenzo-*p*-dioxin	0.01
All other heptachloro-dibenzo-*p*-dioxins	0.0
Octachloro-dibenzo-*p*-dioxin	0.001
All mono-, di-, and trichloro dibenzofurans	0.0
2,3,7,8-tetrachlorodibenzofuran	0.1
All other tetrachlorodibenzofurans	0.0
1,2,3,7,8-pentachlorodibenzofuran	0.05
2,3,4,7,8-pentachlorodibenzofuran	0.5
All other pentachlorodibenzofurans	0.0
1,2,3,4,7,8-hexachlorodibenzofuran	0.1
1,2,3,6,7,8-hexachlorodibenzofuran	0.1
1,2,3,7,8,9-hexachlorodibenzofuran	0.1
2,3,4,6,7,8-hexachlorodibenzofuran	0.1
All other hexachlorodibenzofurans	0.0
1,2,3,4,6,7,8-heptachlorodibenzofuran	0.01
1,2,3,4,7,8,9-heptachlorodibenzofuran	0.01
All other heptachlorodibenzofurans	0.0
Octachlorodibenzofuran	0.001

FIGURE 1

GENERAL STRUCTURES OF PCDDs (top) AND PCDFs (bottom)



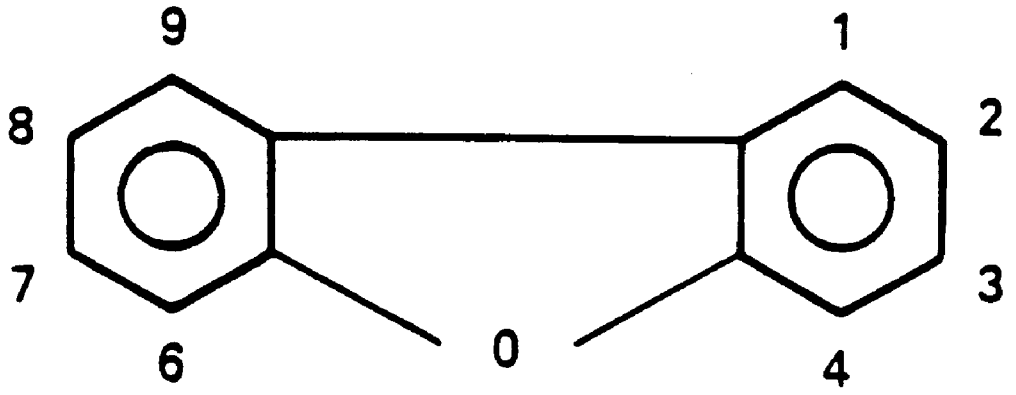
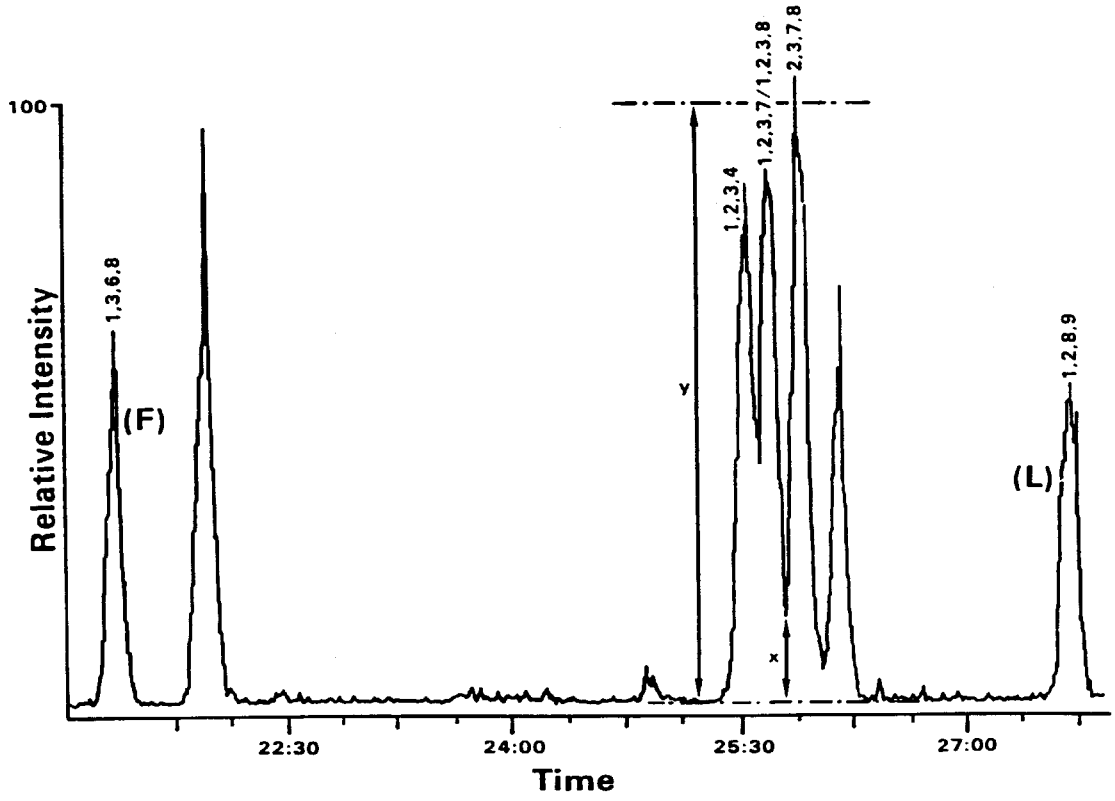


FIGURE 2

VALLEY BETWEEN 2,3,7,8-TCDD AND OTHER CLOSELY ELUTING ISOMERS ON A DB-5 GC COLUMN



Selected ion current profile for m/z 322 (TCDDs) produced by MS analysis of GC performance check solution on a 60 m x 0.32 mm DB-5 fused silica capillary column with 0.25 µm film thickness.

Injector temp:	270°C
Starting temp:	200°C for 2 min
	200 to 220°C @ 5°/min and held for 16 min
	220 to 235°C @ 5°/min and held for 7 min
	235 to 330°C @ 5°/min and held for 5 min
Splitless valve time:	45 sec
Total time:	60 min

FIGURE 3

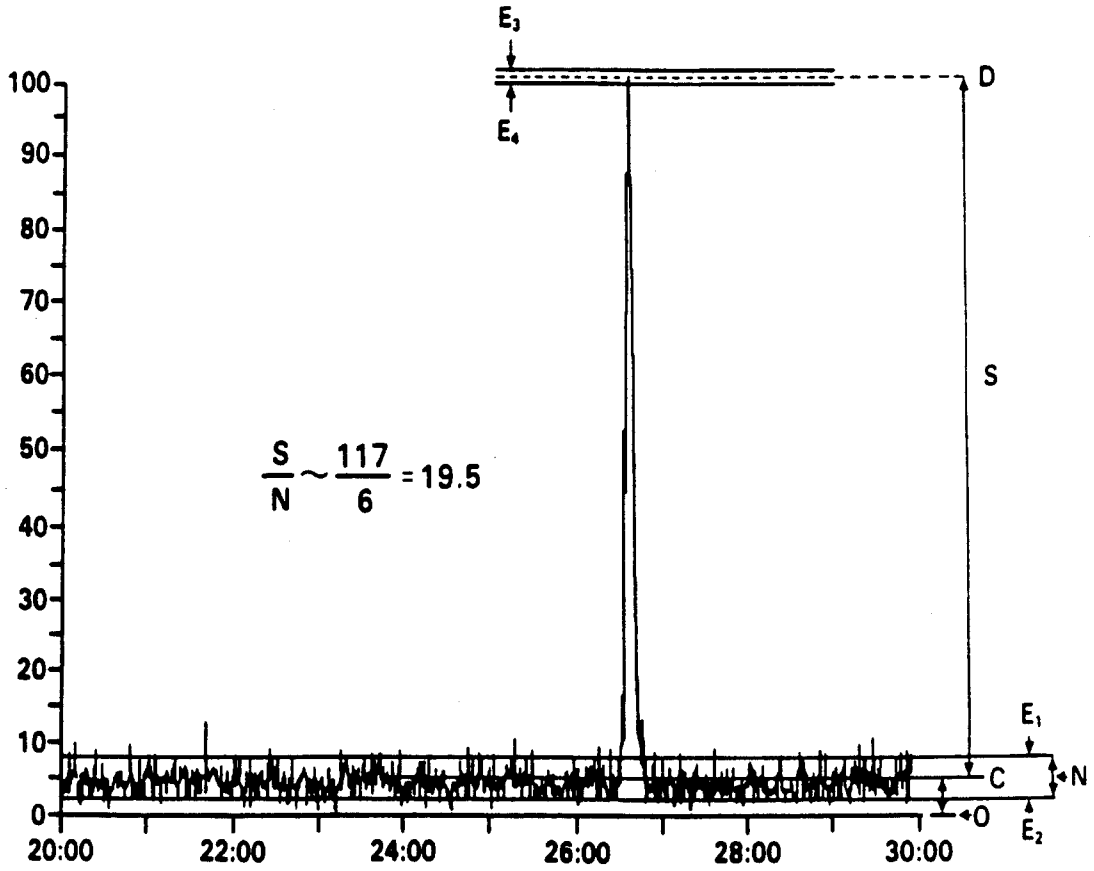
EXAMPLE OF THE ANALYTICAL SEQUENCE FOR CALIBRATING AN SP-2331 COLUMN

Time	Analysis
	Window Defining Mix
	Column Performance Solution (SP-2331)
Hour 0	CC3
	CC1 (Initial Calibration)
	CC2
	CC4
	CC5
	Blanks and Samples
	CC1 (must be injected within the 12-hour period.)
Hour 12	Column Performance Solution (SP-2331)
	CC3
	Blanks and Samples
	CC1 (must be injected within the 12-hour period.)
Hour 24	Column Performance Solution (SP-2331)
	CC3
	Blanks and Samples
	CC1 (must be injected within the 12-hour period.)

NOTE: When a column other than SP-2331 is employed, the column performance solution need not be analyzed.

FIGURE 4

MEASUREMENT OF THE SIGNAL-TO-NOISE RATIO



Manual Determination of S/N

The peak height (S) is measured between the mean noise (lines C and D). These mean signal values are obtained by tracing the line between the baseline average noise extremes, E1 and E2, and between the apex average noise extremes, E3 and E4, at the apex of the signal.

NOTE: It is imperative that the instrument interface amplifier electronic zero offset be set high enough so that negative going baseline noise is recorded.

THE ANALYSIS OF POLYCHLORINATED DIBENZO-*p*-DIOXINS AND POLYCHLORINATED DIBENZOFURANS BY HIGH RESOLUTION GAS CHROMATOGRAPHY/LOW RESOLUTION MASS SPECTROMETRY (HRGC/LRMS)

Complex waste sample

↓

(1) Add internal standards
(2) Perform matrix-specific extraction.

↓

Extract sample

↓

(1) Wash with conc. H_2SO_4
(2) Wash with 5% NaCl
(3) Wash with 20% KOH
(4) Wash with 5% NaCl
(5) Dry extract
(6) Solvent exchange
(7) Silica Gel column
(8) Alumina column

↓

60% CH_2Cl_2/hexane fraction

↓

(1) Concentrate eluate
(2) Perform carbon column cleanup
(3) Add recovery standard(s)

↓

Analyze by GC/MS