# Determination of Chlorinated Dibenzo-p-dioxins and Dibenzofurans in Soils and Sediments by Gas Chromatography/Mass Spectrometry

Ann L. Alford-Stevens
James W. Eichelberger
Thomas A. Bellar
William L. Budde

December 15, 1986

Physical and Chemical Methods Branch
Environmental Monitoring and Support Laboratory
Office of Research and Development
U. S. Environmental Protection Agency
26 West St. Clair Street
Cincinnati, Ohio 45268

INDEX


| Section Number | Subject |
|---|---|
| 1 | Scope and Application |
| 2 | Summary of Method |
| 3 | Definitions |
| 4 | Interferences |
| 5 | Safety |
| 6 | Apparatus and Equipment |
| 7 | Reagents and Consumable Materials |
| 8 | Sample Collection, Preservation and Handling |
| 9 | Calibration |
| 10 | Quality Control |
| 11 | Procedures |
| 12 | Calculations |
| 13 | Confirmatory Analyses |
| 14 | Automated Identification and Measurement |
| 15 | Method Performance |
| 16 | References |

Tables

| | |
|---|---|
| 1 | GC Operating Conditions |
| 2 | Composition of Concentration Calibration Solutions |
| 3 | Composition of Retention Time Calibration Solution |
| 4 | Ions for Selected Ion Monitoring Data Acquisition |
| 5 | Relative Abundance Criteria for Ions Monitored to Identify and Measure CDDs and CDFs |
| 6 | Known Relative Abundances of Ions in CDD/CDF Molecular Ion Clusters |
| 7 | Precision of Triplicate Determinations of CDDs and CDFs in Composite Ash Extract |

DETERMINATION OF CHLORINATED DIBENZO-P-DIOXINS AND DIBENZOFURANS IN SOILS AND SEDIMENTS BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY

## 1. SCOPE AND APPLICATION

1.1 This method provides procedures for mass spectrometric analysis of extracts of soils and sediments to determine chlorinated dibenzo-p-dioxins (CDDs) and dibenzofurans (CDFs) containing four to eight chlorine atoms. The existence of 49 possibile CDD method analytes and 87 CDF method analytes makes impractical the listing of the Chemical Abstracts Service Registry Number for each method analyte. Because CDDs and CDFs are identified and measured as isomer groups, the non-specific CASRN for each level of chlorination is used to describe method analytes.

| Isomer Group | No. of Isomers | Formula | CASRN |
|---|---|---|---|
| $Cl_4$-CDDs | 22 | $C_{12}Cl_4H_4O_2$ | 41903-57-5 |
| $Cl_5$-CDDs | 14 | $C_{12}Cl_5H_3O_2$ | 36088-22-9 |
| $Cl_6$-CDDs | 10 | $C_{12}Cl_6H_2O_2$ | 55684-94-1 |
| $Cl_7$-CDDs | 2 | $C_{12}Cl_7HO_2$ | 37871-00-4 |
| $Cl_8$-CDDs | 1 | $C12Cl_8O_2$ | 3268-87-9 |
| $Cl_4$-CDFs | 38 | $C_{12}Cl_4H_4O$ | 55722-27-5 |
| $Cl_5$-CDFs | 28 | $C_{12}Cl_5H_3O$ | 30402-15-4 |
| $Cl_6$-CDFs | 16 | $C_{12}Cl_6H_2O$ | 34465-46-8 |
| $Cl_7$-CDFs | 4 | $C_{12}Cl_7HO$ | 38998-75-3 |
| $Cl_8$-CDFs | 1 | $C_{12}Cl_8O$ | 39001-02-0 |

1.2 Detection limits vary among method analytes and with sample matrix, sample preparation procedures, condition of the GC/MS system, and individual samples. The detector sensitivity decreases with increasing level of chlorination, and estimated method detection limits range from 1 ug/kg for each $Cl_4$-CDD or $Cl_4$-CDF to 3 ug/kg for $Cl_8$-CDD or $Cl_8$-CDF.

1.3 Because some CDDs and CDFs may be extremely toxic, safety procedures described in Section 5 should be followed to prevent exposure of laboratory personnel to materials containing these compounds.

## 2. SUMMARY OF METHOD

2.1 Four isotopically-labeled surrogate compounds (SCs) are added to each sample before sample extraction or other preparation procedures. These compounds are $^{13}C_{12}$-2,3,7,8-$Cl_4$-CDD, $^{13}C_{12}$-2,3,7,8-$Cl_4$-CDF, $^{13}C_{12}$-$Cl_8$-CDD, and $^{13}C_{12}$-$Cl_8$-CDF. After the SCs are added to a 10-g aliquot of soil or sediment sample, the wet soil or sediment is mixed with 20 g of anhydrous sodium sulfate and is extracted with a mixture of hexane and methanol while the sample aliquot and solvent are agitated continually in a glass jar. Column chromatographic procedures are used to help eliminate sample components that may interfere with detection and measurement of CDDs and CDFs. The sample extract is concentrated to 50 uL, and an internal standard (IS) is added before a 2-uL aliquot is analyzed. In this method, dibromo-octafluorobiphenyl (DBOFB, CASRN 10386-84-2) is used as the IS. Sample

extract components are separated with capillary column gas chromatography (GC) and selected ions are monitored with low resolution, electron ionization mass spectrometry (MS). An interfaced data system (DS) to control data acquisition and to store, retrieve, and manipulate mass spectral data is essential.

2.2 Analytes are identified and measured as isomer groups (i.e., by level of chlorination). Ten selected CDD and CDF congeners, one representing each level of chlorination, are used as concentration calibration standards. A concentration is measured for each CDD and CDF isomer group; total CDD and total CDF concentrations in each sample extract are obtained by summing appropriate isomer group concentrations. Method bias for SCs is calculated to indicate method performance with each sample.

## 3. DEFINITIONS

3.1 CONCENTRATION CALIBRATION SOLUTION (CAL) -- A solution of selected CDDs and CDFs used to calibrate the mass spectrometer response.

3.2 INTERNAL STANDARD (IS) -- A pure compound added to a sample extract in known amounts and used to calibrate concentration measurements of other compounds that are sample components. The IS must be a compound that is not a sample component.

3.3 LABORATORY DUPLICATE -- Two aliquots of the same environmental sample treated identically throughout a laboratory analytical procedure. Each aliquot is carried through the entire laboratory analytical method. Analytical results indicate precision associated with analytical procedures but not with sample collection, preservation or storage procedures.

3.4 LABORATORY PERFORMANCE CHECK SOLUTION -- A solution of selected method analytes, surrogate compounds, and internal standard used to evaluate the performance of the GC/MS/DS with respect to criteria defined in a method.

3.5 LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent blank solid material that is treated as a sample in all aspects in the laboratory, including addition of all method reagents, internal standards, and surrogate compounds, and exposure to all glassware, apparatus, and equipment. If a reagent blank solid material is not available, the entire method is followed, except that no solid material is added to the extraction jar.

3.6 LABORATORY SURROGATE SPIKE -- A surrogate compound concentration measured in a sample extract with the same procedures used to measure sample components. The known value is determined from standard gravimetric and/or volumetric techniques used during sample fortification.

3.8 PERFORMANCE EVALUATION (PE) SAMPLE -- A sample that contains known concentrations of method analytes and has been analyzed by multiple laboratories to determine statistically the accuracy and precision that can be expected when a method is performed by a competent analyst. Analyte concentrations are unknown to the analyst.

3.9 QUALITY CONTROL (QC) CHECK SAMPLE -- A sample containing known concentrations of analytes prepared by a laboratory other than the laboratory performing the analysis. The analyzing laboratory uses this sample to demonstrate that it can obtain acceptable identifications and measurements with

procedures to be used to analyze environmental samples containing the same or similar analytes. Analyte concentrations are known by the analyst.

3.10 RESPONSE FACTOR (RF) -- MS response to a known amount of a concentration calibration compound relative to a known amount of an internal standard.

3.11 SIGNAL-TO-NOISE RATIO (S/N) -- The ratio of the area of the analyte signal to the area of the random background signal; it is determined by integrating the signal for a characteristic ion in a region of the selected ion current profile where only random noise is observed and relating that area to the area measured for a positive response for the same ion. The same number of spectra must be integrated for both areas. (The ratio of peak heights may be used instead of peak areas.)

3.12 SURROGATE COMPOUNDS (SCs) -- Compounds that are not expected to be found in the sample, are added to each environmental sample to indicate method performance, and are measured with the same procedures used to measure sample components. In this method, four isotopically labeled dioxins and furans ($^{13}C_{12}$-$Cl_4$-TCDD, CASRN 80494-19-5; $^{13}C_{12}$-$Cl_4$-TCDF, CASRN unknown; $^{13}C_{12}$-$Cl_8$-CDD, CASRN 80479-48-7; and $^{13}C_{12}$-$Cl_8$-OCDF, CASRN unknown) are added to the soil/sediment sample aliquot before any sample extraction or other preparation procedures. Their concentrations are measured in each sample, and method bias is calculated for each to indicate acceptable method performance with each sample.

## 4. INTERFERENCES

4.1 Interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing equipment. High purity reagents and solvents must be used and all equipment must be scrupulously cleaned. All of these materials must be demonstrated to be free of interferences under the analytical conditions by routine analysis of laboratory reagent blanks.

4.2 Most frequently encountered interferences are other sample components that are extracted along with CDDs and CDFs. Because very low levels of CDDs and CDFs must be measured, elimination of interference is highly desirable. Column chromatographic procedures are used to remove some coextracted sample components; these procedures must be performed carefully to minimize loss of analytes during attempts to enrich their concentrations relative to other sample components. Capillary column GC RTs and the compound-specific characteristics of mass spectra eliminate many interferences. Sample components that produce the same quantitation and confirmation ions and have the same RT as a method analyte will interfere. These interferences will not produce false positives, however, unless the abundance of the confirmation ions relative to the quantitation ions is very similar to that of a method analyte.

4.3 Chlorinated diphenyl ethers are compounds that may cause false positive identifications of CDFs. Loss of two chlorine atoms from a chlorinated diphenyl ether produces an ion cluster with ions of the same masses and relative abundances as a molecular ion cluster produced by a CDF containing two less chlorine atoms than the chlorinated diphenyl ether. Because of the lack of standards of chlorinated diphenyl ethers, their RTs are unknown

with the GC column and analytical conditions used. To prevent false positives, CDF identification criteria include a check to determine the absence of at least one ion that would indicate the presence of an interfering chlorinated diphenyl ether.

4.4 Because one of the SCs, $^{13}C_{12}$-$Cl_4$-CDF, produces ions at m/z 320 and 322, this compound will interfere with any coeluting $Cl_4$-CDD. Such coelution has not been observed, but analysis of all 22 $Cl_4$-CDDs along with $^{13}C$-$Cl_4$-CDF with the analytical conditions to be used for samples is necessary to eliminate that possibility.

## 5. SAFETY

5.1 The toxicity or carcinogenicity of each chemical used in this method has not been precisely defined. Some CDDs and CDFs have been identified as suspected human or mammalian carcinogens. Therefore, each should be treated as a potential health hazard, and exposure should be reduced to the lowest feasible level. Each laboratory is responsible for maintaining current awareness of OSHA regulations regarding safe handling of the chemicals used in this method. A reference file of material data handling sheets should be made available to all personnel involved in analyses. Additional information on laboratory safety is available (1-3).

5.2 Each laboratory must develop a strict safety program for handling CDDs and CDFs. The following laboratory practices are recommended:

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

5.2.2 The effluents of sample splitters for the GC and roughing pumps on the GC/MS should pass through either a column of activated charcoal or through a trap containing oil or high-boiling alcohols.

5.3 The following precautions for safe handling of CDDs and CDFs in the laboratory are presented as guidelines only. Precautions for safe handling and use are necessarily general in nature because detailed, specific recommendations can be made only for particular exposures or circumstances. Assistance in evaluating the health hazards of particular laboratory conditions may be obtained from certain consulting laboratories and from state departments of health or labor, many of which have an industrial health service. Techniques used in handling radioactive and infectious materials are generally applicable to CDDs and CDFs.

5.3.1 Protective Equipment: Throw-away plastic gloves, apron or lab coat, safety glasses and lab hood adequate for radioactive work.

5.3.2 Training: Workers must be trained to remove contaminated gloves and clothing without contacting the exterior surfaces.

5.3.3 Personal Hygiene: Thorough washing of hands and forearms after each manipulation and before breaks (coffee, lunch, and shift) with a mild soap and plenty of scrubbing action.

5.3.4 Confinement: Isolated work area, posted with signs, segregated glassware and tools, plastic-backed absorbent paper on benchtops.

5.3.5 Waste: Contaminated waste should be minimized. Plastic bag liners should be used in waste cans. Janitors should not handle wastes.

5.3.6 Disposal of Wastes: Low-level waste such as absorbent paper and plastic gloves may be burned in a good incinerator. Water containing gross quantities (milligrams) of CDDs and CDFS should be packaged securely and disposed through commercial or governmental channels that are capable of handling high-level radioactive wastes or extremely toxic wastes. Liquids should be allowed to evaporate in a good hood and in a disposable container; residues may then be incinerated.

5.3.7 Glassware, Tools, and Surfaces -- Satisfactory cleaning may be accomplished by rinsing with solvents (such as acetone, methylene chloride, and trichloroethylene), followed by washing with detergent and water. Dish water may be disposed to the sewer. (Also see Sect. 6.5.)

5.3.8 Laundry: Clothing known to be contaminated should be disposed with the precautions described under Sect. 5.3.6. Lab coats or other clothing 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 a cycle before being used again for other clothing. Disposable garments may be used to avoid a laundry problem, but they must be properly disposed or incinerated.

5.3.9 Wipe Tests: A useful method to determine cleanliness of work surfaces and tools is to wipe the surface with a piece of filter paper, which is extracted and analyzed by GC or GC/MS. Less than 4 $ng/m^2$ of a CDD or CDF indicates acceptable laboratory cleanliness; anything higher warrants further cleaning. More than 10 ug on a 1-$m^2$ wipe sample indicates an acute hazard that requires prompt cleaning before further use of the equipment or work space and indicates that unacceptable work practices have been employed in the past.

5.3.10 Inhalation: Any procedure that may produce airborne contamination must be done with good ventilation. Gross losses to a ventilation system must not be allowed. Handling of the dilute solutions normally used in analytical and animal work presents no inhalation hazards except in case of an accident. Finely divided soils contaminated with CDDs and CDF may be hazardous because of the potential for inhalation. Such samples should be handled in a confined environment, such as a hood or glove box, or laboratory personnel should wear masks fitted with a particulate filter and charcoal sorbent.

5.3.11 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.

## 6. APPARATUS AND EQUIPMENT

### 6.1 COMPUTERIZED GC/MS SYSTEM

6.1.1 The GC must be capable of temperature programming and be equipped with all required accessories, such as syringes, gases, and a capillary column. The GC injection port must be designed for capillary columns. Manual splitless injections were used to acquire data used as the basis for quality control requirements. An automatic injector, however, is desirable, because it should provide more precise RTs and areas. On-column injection techniques are encouraged, because they minimize high mass descrimination and analyte degradation problems. If the low initial column temperature required for on-column injections cannot be reproduced, RTs will not be reproducible within acceptable limits. That may cause ion sets to be changed at inappropriate times during data acquistion. With on-column injection, an uncoated precolumn is recommended. Splitting injections are not appropriate.

6.1.2 Mass spectral data are obtained with electron ionization at a nominal electron energy of 70 eV. To ensure sufficient precision of mass spectral data, the required MS scan rate must allow acquisition of at least five data points for each monitored ion while a sample component elutes from the GC.

6.1.3 An interfaced DS is required to acquire, store, reduce, and output mass spectral data. The DS must be capable of searching a data file for specific ions and plotting ion abundances versus time or spectrum number to produce selected ion current profiles (SICPs) and extracted ion current profiles (EICPs). Also required is the capability to obtain chromatographic peak areas between specified times or spectrum numbers in SICPs or EICPs. Total data acquisition time per cycle should be $\geq$0.5 s and $\leq$1.0 s. The DS must be equipped with software capable of acquiring data for six groups of $\leq$12 ions, and the DS must allow automated and rapid changes of the set of ions being monitored. The times spent monitoring ions during sample analyses must be the same as the times used when CALs were analyzed.

6.2 GC COLUMN -- A 60 m X 0.32 mm ID fused silica capillary column coated with a 0.25 um or thicker film crosslinked phenyl methyl silicone (such as Durabond-5 (DB-5), J and W Scientific, Rancho Cordova, CA) or polydiphenyl vinyl dimethyl siloxane (such as SE-54, Alltech Associates, Deerfield, IL) is required. Operating conditions known to produce acceptable results with these columns are shown in Table 1, but conditions must be optimized at each laboratory with each GC and GC column.

### 6.3 MISCELLANEOUS EQUIPMENT

6.3.1 Nitrogen evaporation apparatus with variable flow rate from approximately 30 mL/min to 150 mL/min.

6.3.2 Mechanical shaker - A magnetic stirrer or a wrist-action or platform-type shaker that produces vigorous agitation. Agitation conditions must be determined and demonstrated.

6.3.3 Analytical balance capable of accurately weighing 0.01g.

6.3.4 Analytical balance capable of accurately weighing 0.0001 g.

6.3.5 Centrifuge capable of operating at 2000 rpm.

6.3.6 Water bath -- equipped with concentric ring cover and temperature controlled within ± 2°C. (A sand bath may be used.)

6.3.7 Stainless steel spatulas or spoons.

6.3.8 Stainless steel (or glass) pan large enough to hold contents of 1-pt sample containers.

6.3.9 Glove box.

6.3.10 Boiling Chips -- approximately 10/40 mesh. Heat at 400°C for 30 min or extract with methylene chloride in a Soxhlet apparatus.

6.4 GLASSWARE

6.4.1 Volumetric flasks - various standard sizes with ground glass stoppers.

6.4.2 Microsyringes - various standard sizes.

6.4.3 Extraction jars -- amber glass with Teflon-lined screw cap; minimum capacity of approximately 500 mL; must be compatible with mechanical shaker to be used.

6.4.4 Kuderna-Danish apparatus -- 500-mL evaporating flask, 10-mL graduated concentrator tubes with ground-glass stoppers, and 3-ball macro Snyder column.

6.4.5 Culture tubes -- 8-mL glass.

6.4.6 Mini-vials -- 1-mL amber borosilicate glass with conical-shaped reservoir and screw caps lined with Teflon-faced silicone disks.

6.4.7 Funnels -- glass; appropriate size to accommodate filter paper used to filter jar extract (volume of approximately 170 mL).

6.4.8 Chromatography columns -- 1 cm ID x 20 cm long and 1 cm ID X 30 cm long.

6.5 CAUTION: Reuse of glassware should be minimized to avoid contamination. All glassware that is reused must be scrupulously cleaned as soon as possible after use. Rinse glassware with the last solvent used in it. Wash with hot water containing detergent. Rinse with copious amounts of tap water and several portions of distilled water. Drain dry and heat in a muffle furnace at 450°C for a at least 4 h. Volumetric glassware should not be heated in a muffle furnace, and some thermally stable materials (such as PCBs) may not be removed by heating in a muffle furnace. In these cases, rinsing with high-purity acetone and hexane may be substituted for muffle furnace heating. After glassware is dry and cool, store inverted or capped with aluminum foil in a clean environment. Before using, rinse each piece with an appropriate solvent.

## 7. REAGENTS AND CONSUMABLE MATERIALS

### 7.1 COLUMN CHROMATOGRAPHY REAGENTS

7.1.1 Alumina, acidic -- Extract in a Soxhlet apparatus with methylene chloride for 21 h and activate by heating in a foil covered glass container for 24 h at 190°C.

7.1.2 Silica gel -- high purity grade, type 60, 70-230 mesh; Soxhlet extract with methylene chloride for 21 h and activate by heating in a foil-covered glass container for 24 h at 130°C.

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

7.1.4 Silica gel impregnated with sodium hydroxide -- Add one part (by weight) 1 N sodium hydroxide to two parts (by weight) silica gel (extracted and activated). Mix with a glass rod until free of lumps and store in a screw-capped glass bottle.

7.1.5 Sulfuric acid, concentrated -- ACS grade, specific gravity 1.84.

7.1.6 Sodium hydroxide, 1 N.

7.1.7 Graphitized carbon black (Carbopack C or equivalent), surface area of approximately 12 $m^2/g$, 80/100 mesh. Mix 3.6g of Carbopack C (or equivalent) with 16.4g of reagent grade Celite 545$^R$ (or equivalent) in a 40-mL vial and activate by heating in an oven at 130°C for 6h. Store in a desiccator.
CAUTION: Check each new batch of mixed Carbopack/Celite$^R$ to ensure recovery of $\geq$50% of each CDD and CDF in CAL #3.

7.2 FILTER PAPER -- pore size of $\leq$ 20 to 25 u; rinse with hexane before use.

7.3 GLASS WOOL -- silanized; extract with methylene chloride and hexane before use.

7.4 SODIUM SULFATE -- Granular, anhydrous; before use, extract with methylene chloride and dry for $\geq$ 4 h in a shallow tray placed in an oven heated to 120°C.

7.5 SOLVENTS -- High purity, distilled-in-glass hexane, methanol, methylene chloride, 2-propanol, and decane.

7.6 IS SOLUTION -- Prepare a 2.5 ng/uL solution of 4,4'-dibromooctaflurobiphenyl (DBOFB) in decane. Add a 50-uL aliquot to each sample extract just before concentration of the extract to final volume.

7.7 CALs -- Five decane solutions containing the ten calibration congeners, the four SCs, and the IS are needed. Compositions are shown in Table 2. Each of CALs #1-#5 contains a constant concentration of the IS and varying concentrations of calibration congeners and SCs. In a single solution, concentrations of calibration congeners and SCs increase with increasing number of chlorine atoms because the MS detector sensitivity decreases with

increasing number of chlorine atoms. Concentrations of CALs are equivalent to concentrations ranging from 1 to 50 ug/kg for $Cl_4$-analytes and from 3 to 150 ug/kg for $Cl_8$-analytes in 50-uL extracts of 10-g samples. Store concentration calibration solutions in small amber mini-vials.
CAUTION: Each time a vial containing a small volume of solution is warmed to room temperature and opened, a small volume of solvent in the vial headspace evaporates, significantly affecting concentration. Solutions should be stored with the smallest possible volume of headspace, and vials should be opened only when necessary.

7.9 SAMPLE FORTIFICATION SOLUTION - Prepare a methanol solution containing the each of the $Cl_4$ SCs at a concentration of 0.5 ng/uL, and each of the $Cl_8$ SCs at 1.5 ng/uL. A 100-uL aliquot of this solution is added to each sample aliquot.

7.10 LABORATORY PERFORMANCE CHECK SOLUTION - For both full-range data acquisition, and the SIM data acquisition option, CAL #3 is used as the laboratory performance check solution (LPC) to verify response factors and to demonstrate adequate GC resolution and MS performance.

7.11 RT CALIBRATION SOLUTION - Prepare a solution containing the first eluting CDF at each level of chlorination from $Cl_4$ through $Cl_8$ (Table 3). This solution will be used to determine when sets of monitored ions must be changed. Component concentrations are not critical but should be at least as great as those of analogous compounds in CAL #1 (Table 2).

7.12 TETRABUTYLAMMONIUM SULFITE REAGENT -- Dissolve 3.39 g of tetrabutylammonium hydrogen sulfate in 100 mL of distilled water. To remove impurities, extract this solution three times with 20-mL portions of hexane. Discard the hexane extracts, and add 25 g of sodium sulfite to the water solution. Store the resulting solution in an amber bottle with a Teflon-lined screw cap. The solution can be stored at room temperature for at least one month.

## 8. SAMPLE PRESERVATION AND STORAGE

### 8.1 SAMPLE PRESERVATION

8.1.1 When received, most samples will be contained in a 1-pt glass jar surrounded by vermiculite in a sealed metal paint can. Until a portion is to be removed for analysis, store the sealed paint cans in a locked limited-access area where ambient temperature is maintained above freezing but below 35°C. After a portion is removed for analysis, return the unused portion of sample to its original container and store as stated above. Do not freeze samples; they may contain sufficient water to break the sample jar if frozen.

8.1.2 To avoid photodecomposition, protect samples from light.

## 9. CALIBRATION

9.1 Initial calibration is required before any samples are analyzed and is required intermittently throughout sample analyses as dictated by results of continuing calibration checks. No samples are to be analyzed until acceptable initial calibration is demonstrated and documented. After

initial calibration is successfully performed, a continuing calibration check is required at the beginning and end of each 12-h period during which analyses are performed.

9.2. INITIAL CALIBRATION

9.2.1 Calibrate and tune the MS with standards and procedures prescribed by the manufacturer with any necessary modifications to meet USEPA requirements.

9.2.2 Determination of RTs -- Inject a 2-uL aliquot of the RT calibration solution (RT CAL, Table 3) and acquire SIM data with the six ion sets shown in Table 4.
CAUTION: Because CDDs and CDFs are mass deficient (i.e., accurate masses are less than unit nominal masses), accurate masses (Table 5) may need to be considered when establishing SIM data acquisition conditions to ensure correct mass assignments. Begin data acquisition with ion set 1 as soon as feasible after injection. As soon as the IS has eluted, collect data with ion set 2 until after the $Cl_4$-CDF elutes. Then collect data with ion set 3 until after the $Cl_5$-CDF elutes, ion set 4 until after the $Cl_6$-CDF elutes, ion set 5 until after the $Cl_7$-CDF elutes, and ion set 6 until after the $Cl_8$-CDF elutes. Use <u>exactly</u> the same GC conditions and SIM data acquisition conditions that will be used to collect data for samples. Acquire at least five scans for each solution component during elution of each GC peak. Total time per scan should be $\geq$0.5 s and $\leq$1.0 s.

9.2.3 Use the RTs (scan numbers) observed in Sect. 9.2.2 to determine when ions sets should be switched for subsequent analyses.

9.2.3.1 Begin data acquisition with ion set 1 as soon as feasible after injection.

9.2.3.2 Begin acquisition with ion set 2 soon after elution of the IS.

9.2.3.3 Stop data acquisition with ion set 2 and begin ion set 3 $\leq$15 s <u>before</u> elution of the $Cl_5$-CDF RT calibration congener.
CAUTION: This time is critical because of the small difference in RTs of late eluting $Cl_4$-analytes and early eluting $Cl_5$-analytes.

9.2.3.4 Begin acquisition with each remaining ion set $\leq$30 s before elution of each additional CDF RT calibration congener.

9.2.4 Optimize chromatographic conditions to obtain symmetrical peak shapes and reproducible peak areas. (A tailing IS peak indicates inadequate GC conditions and will prevent measurement of reproducible GC peak areas. Multiple analyses of the RT CAL may be necessary.

9.2.5 Inject a 2-uL aliquot of CAL #3 (Table 2) and acquire SIM data with the same conditions used to analyze the RT calibration solution and ensure that each calibration congener is observed.
CAUTION: GC operating conditions must be carefully reproduced for

each analysis to provide reproducible RTs; if not, ions will not be monitored at the appropriate times.

9.2.6 Performance Criteria

9.2.6.1 GC performance -- Baseline separation of $Cl_8$-CDD and $Cl_8$-CDF; symmetrical peak for each with peak width at half height equivalent to $\leq$5 s.

9.2.6.2 MS sensitivity -- S/N ratio of $\geq$15 for m/z 468 measured for $^{13}C_{12}$-$Cl_8$-CDD.

9.2.6.3 MS calibration -- For $^{13}C_{12}$-$Cl_8$-CDD, abundance of m/z 470 relative to m/z 472 is $\geq$77% and $\leq$97%; abundance of m/z 474 relative to m/z 472 is $\geq$56% and $\leq$76%. Abundance of m/z 457 relative to m/z 456 (produced by the IS) is 3-23%.

9.2.7 If all performance criteria are met, analyze one 2-uL aliquot of each of the other four CALs.

9.2.8 Response Factor Calculation --- Calculate response factors (RFs) for each CDD and CDF concentration calibration congener, and SC relative to the IS:

$$RF = A_x Q_{is} / A_{is} Q_x$$

where $A_x$ = integrated ion abundance of quantitation ions (Table 4) for a CDD or CDF calibration congener or SC,

$A_{is}$ = integrated ion abundance of the IS quantitation ions (Table 4),

$Q_{is}$ = injected quantity of IS,

$Q_x$ = injected quantity of CDD or CDF calibration congener or SC.

RF is a unitless number, units used to express quantities must be equivalent.

9.2.9 Response Factor Reproducibility -- For each concentration calibration congener and SC, calculate five RFs (one for each CAL) and the mean RF. If the relative standard deviation (RSD) exceeds 20%, analyze additional aliquots of appropriate CALS to obtain $\leq$20% RSD of RFs over the entire concentration range, or take action to improve GC/MS performance.

9.2.10 RT Reproducibility -- Absolute RTs of each calibration congener should not vary by more than $\pm$5 s from one analysis to the next and must not continue either to increase or decrease.

9.2.11 Calculate the mean RF for 2,3,7,8-$Cl_4$-CDD, 2,3,7,8-$Cl_4$-CDF, $Cl_8$-CDD, and $Cl_8$-CDF using the equation in Sect. 9.2.6, where the appropriate

$^{13}C_{12}$-labeled compound is used as the IS. (These RFs will be used to calculate concentrations of specific compounds with isotopic dilution procedures, Sect. 12.6)

9.2.12 Calculate the mean area measured for $Cl_8$-CDD, $Cl_8$-CDF and the IS.

9.3. CONTINUING CALIBRATION CHECK

9.3.1 With the following procedures, verify initial calibration at the beginning and end of each 12-h period during which analyses are to be performed.

9.3.2 Calibrate the MS with standards and procedures prescribed by the manufacturer.

9.3.3 Inject a 1-uL or 2-uL aliquot of CAL #3 and collect SIM data with the same conditions used during initial calibration.

9.3.4 Demonstrate acceptable performance for criteria described in Sect. 9.2.6.

9.3.5 Determine that neither the area measured for m/z 456 for $^{13}C_{12}$-$Cl_8$-CDF nor that for m/z 472 for $^{13}C_{12}$-$Cl_8$-CDD has decreased or increased by more than 30% from the area measured in the most recent previous analysis of a CAL or by more than 50% from the mean area measured during initial calibration.
Note: Variability in the ratio of these areas is an indication of chromatography or MS calibration problems. When sufficient data are available, guidelines will be added to this method.

9.3.6 RF Reproducibility -- For an acceptable continuing calibration check, the measured RF for each calibration congener/SC must be within ±20% of the mean value calculated (Sect. 9.2.9) during initial calibration. If not, remedial action must be taken; recalibration may be necessary.

9.3.7 RT Reproducibility -- Demonstrate and document acceptable reproducibility of absolute RTs of RT calibration congeners (Sect. 9.2.10).

9.3.8 Remedial actions must be taken if criteria are not met; possible remedies are:

9.3.8.1 Check and adjust GC and/or MS operating conditions.

9.3.8.2 Clean or replace injector liner.

9.3.8.3 Flush column with solvent according to manufacturers instructions.

9.3.8.4 Break off a short portion (approximately 0.33 m) of the column; check column performance by analysis of CAL #3, and ensure that RTs have not changed.

9.3.8.5 Replace GC column; performance of all initial calibration procedures is then required.

9.3.8.6 Adjust MS for greater or lesser resolution.

9.3.8.7 Calibrate MS mass scale.

9.3.8.8 Prepare and analyze new CALs.

## 10. QUALITY CONTROL

10.1 LABORATORY REAGENT BLANK (LRB) -- Perform all steps in the analytical procedure (Section 11) using all reagents, standards, SCs, equipment, apparatus, glassware, and solvents that would be used for a sample analysis, but omit an aliquot of soil/sediment sample. If available, substitute EPA-provided reagent blank solid material for an aliquot of soil/sediment sample.

10.1.1 An LRB must contain the same amounts of IS and SCs that are added to each sample before extraction.

10.1.2 Analyze an LRB before any samples are extracted and analyzed.

10.1.3 Before a new batch of solvents or reagents is used for sample extraction or for column chromatographic procedures, analyze an LRB. In addition, analyze a laboratory solvent blank (LSB), which is the same as an LRB except that no SC or IS is added; this demonstrates that reagents contain no impurities producing an ion current above the level of background noise for quantitation ions for those compounds.

10.1.4 Analyze an LRB along with each batch of $\leq$20 samples.

10.1.5 Acceptance criteria for an LRB have not been specifically defined. Until criteria are defined, analysts should exercise judgement and fully document results. Obviously, only ions produced by the IS and SCs are desirable in LRB data.

10.1.6 Corrective action for unacceptable LRB -- Check solvents, reagents, apparatus and glassware to locate and eliminate the source of contamination before any samples are extracted and analyzed. Purify or discard contaminated reagents and solvents. If the LRB that was extracted along with a batch of samples is contaminated, the entire batch of samples must be reextracted and reanalyzed.

10.2 CALIBRATION -- Included among initial and continuing calibration procedures are numerous quality control checks to ensure that valid data are acquired (See Sect. 9). Continuing calibration checks are accomplished with results from analysis of one solution, CAL #3.

10.2.1 If some criteria are not met for a continuing calibration check after a 12-h period during which samples were analyzed, those samples must be reanalyzed. Those criteria are: GC performance, MS calibration, and MS sensitivity. (See Sect. 9.2.6.)

10.2.2 When other criteria in Sect. 9.2 are not met, results for affected analytes must be labeled to alert the data user of the observed problem. Included among those criteria are: reproducibility of RTs and RFs for calibration congeners and SCs.

10.3 LABORATORY PERFORMANCE CHECK SOLUTION -- In this method, CAL #3 also serves as a laboratory performance check solution.

10.4 LABORATORY SURROGATE SPIKE

10.4.1 Measure the concentration and calculate method bias for each of the four SCs in every sample and blank.

10.5.2 Acceptance limits have not been established for SC measurements; report all results.

10.5 QUALITY CONTROL CHECK SAMPLE -- Not yet available.

10.6 LABORATORY DUPLICATE SAMPLE -- Select one sample from each batch of $\leq 20$ samples of similar type. If sufficient information is available, select a sample that is expected to produce positive results. After addition of SCs, extract and analyze (Sect. 11) these two aliquots. Relative difference (RD) of duplicate results for surrogate compound concentrations should be $\leq 30\%$. ($RD = [C_1 - C_2 / 0.5 (C_1 + C_2)] 100$ ) Insufficient data are currently available to provide guidance for acceptable RDs of measured concentrations of CDD and CDF isomer groups; report all results.

10.7 PERFORMANCE EVALUATION SAMPLE -- Not yet available; to be analyzed periodically when available.

## 11. PROCEDURES

11.1 SAMPLE HANDLING

11.1.1 CAUTION: Finely divided soils contaminated with CDDs or CDFs are hazardous because of the potential for inhalation or ingestion of particles. Such samples should be handled in a confined environment, (e.g., a closed hood or a glove box) whenever possible; otherwise, laboratory personnel should wear masks fitted with a particulate filter and charcoal sorbent.

11.2 PRE-EXTRACTION SAMPLE TREATMENT

11.2.1 Homogenization -- Although sampling personnel will attempt to collect homogeneous samples, examine each sample and judge if it needs further mixing. Stirring is recommended when possible.

11.2.2 Centrifugation -- If a sample contains an obvious aqueous liquid phase, centrifuge it to separate liquid and solid phases. Place the entire sample in a suitable centrifuge bottle and centrifuge for 30 min at 2000 rpm. Remove bottle from centrifuge. With a disposable pipet, remove liquid phase and discard.
CAUTION: This liquid may contain CDDs and CDFs and should be disposed as a potentially hazardous waste.
Mix solid layer with stainless steel spatula and remove a portion to be weighed and analyzed. Return the remaining solid portion to original sample bottle and store.

## 11.3 SAMPLE EXTRACTION

11.3.1 CAUTION: See Section 5 for safety guidelines and recommendations.

11.3.2 Note: Extremely wet samples may require centrifuging to remove water before addition of sodium sulfate; see Section 11.2.2.

11.3.3 Accurately weigh to three significant figures a 10-g (± 0.5 g) portion of the wet soil or sediment sample, and transfer it to the extraction jar.

11.3.4 Add 100 uL of the solution containing SCs to the soil or sediment in the extraction jar. Add small portions of the solutions at several sites on the surface of the soil or sediment.

11.3.5 Add 20 g of purified anhydrous sodium sulfate and mix thoroughly using a stainless steel spoon or spatula.

11.3.6 Allow the mixture of soil and sodium sulfate to set for two hours at ambient temperature; mix again, break all visible lumps, and allow to set for at least four more hours.

11.3.7 Mix again and add 20 mL of methanol; mix again and add 150 mL of hexane.

11.3.8 Place the extraction jar containing the soil, sodium sulfate and solvents in the shaker and shake for at least 3 h.

11.3.9 Remove the jar from the shaker and allow solids to settle. Decant the solvent through a glass funnel containing hexane-rinsed filter paper. Rinse the jar, solid sample residue, and filter the residue with four 5-mL portions of hexane.

11.3.10 For sediment samples, removal of elemental sulfur is recommended.

11.3.10.1 Concentrate the extract volume to approximately 10 mL with a Kuderna-Danish apparatus, and transfer the extract to a 50-mL clear glass bottle or vial with a Teflon-lined screw cap. Rinse the extract container with 1.0 mL of hexane, adding the rinse to the 50-mL bottle.

11.3.10.2 Add 1 mL of tetrabutylammonium-sulfite reagent (Sect. 7.12) and 1 mL 2-propanol, cap the bottle, and shake for at least 1 min. If the sample is colorless or if the initial color is unchanged, and if clear crystals (precipitated sodium sulfite) are observed, sufficient sodium sulfite is present. If the precipitated sodium sulfite disappears, add more crystalline sodium sulfite in approximately 100-mg portions until a solid residue remains after repeated shaking.

11.3.10.3 Add 5 mL of distilled water and shake for at least 1 min. Allow the sample to stand for 5-10 min and remove the hexane layer (top) for analysis. Dry the extract by passing it through a 10-cm column containing hexane-washed sodium sulfate. Rinse the sodium sulfate with about 30 mL of hexane and add this hexane to the extract.

11.3.11 Concentrate the extract volume to approximately 2-3 mL with a Kuderna-Danish apparatus. Note: Glassware used for more than one sample must be carefully cleaned between samples to prevent cross contamination.

11.3.12 Transfer the concentrated extract to an 8-mL glass culture tube. Rinse the evaporator flask with three 5-mL portions of hexane, and transfer each rinse to the culture tube. Between additions of hexane rinse, reduce the extract volume in the culture tube enough to allow addition of another 5-mL volume of rinse. To reduce the volume, place the culture tube in a water or sand bath adjusted to operate at 50°C and position the tube so that the surfaces of the extract and the water are at about the same level. Evaporate the solvent with a stream of nitrogen with the tip of the nitrogen delivery tube 2 cm above the solution.

11.3.13 After the final rinse has been added, reduce the extract volume to approximately 1 mL.

11.4 COLUMN CHROMATOGRAPHY

11.4.1 Column Preparation

11.4.1.1 Column 1: Place 1.0 g of silica gel into a 1 cm x 20 cm column and tap the column gently to settle the silica gel. Add 2 g sodium hydroxide-impregnated silica gel, 1 g silica gel 4.0 g of sulfuric acid-impregnated silica gel, and 2 g silica gel. Tap column gently after each addition.

11.4.1.2 Column 2: Place 6.0 g of alumina into a 1 cm x 30 cm column and tap the column gently to settle the alumina. Add a 1-cm layer of purified sodium sulfate to the top of the alumina.

11.4.1.3 Add hexane to columns 1 and 2 until the packing is free of channels and air bubbles. A small positive pressure (5 psi) of clean nitrogen can be used if needed.

11.4.1.4 Column 3 (carbon) -- Insert a small plug of glass wool into a silanized glass column approximately 7 cm long by 10 mm O.D. Apply suction with a vacuum aspirator, and add the Carbopack/Celite$^{R}$ mixture until a 2 cm column is obtained.

11.4.2 Quantitatively transfer the hexane sample extract from the culture tube to the top of the silica gel in Column 1. If extract tends to adhere to culture tube walls, the tube can be placed in a sonic bath to suspend undissolved material. Rinse the culture tube with two 0.5-mL portions of hexane and transfer rinses to Column 1.

11.4.3 With 90 mL of hexane, elute the extract from Column 1 directly into Column 2 containing alumina and sodium sulfate.

11.4.4 Add 20 mL of hexane to Column 2 and elute until the hexane level is just below the top of the sodium sulfate; discard the eluted hexane.

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

11.4.6 With a gentle stream of filtered dry nitrogen, reduce the volume of the eluate to about 1 to 2 mL.

11.4.7 Preelute column 3 (the Carbopack/Celite column) with:

11.4.7.1 2 mL of toluene,

11.4.7.2 1 mL of a mixture of 75% (by volume) methylene chloride, 20% methanol, and 5% benzene,

11.4.7.3 1 mL of 50% (by volume) cyclohexane and 50% methylene chloride, and

11.4.7.4 2 mL of hexane.

11.4.8 While the column is still wet with hexane, add the sample extract. Elute the column with the following sequence of solvents and discard eluates.

11.4.8.1 2 mL of hexane,

11.4.8.2 1 mL of 50% (by volume) cyclohexane and 50% methylene chloride, and

11.4.8.3 1 mL of 75% (by volume) methylene chloride, 20% methanol and 5% benzene,

11.4.9 Invert the column and elute (backflush) with 5 mL of toluene. Collect the entire eluate.

11.4.10 Reduce the eluate volume to approximately 1-2 mL. Transfer aliquots to a 1-mL amber minivial with conical reservoir. Concentrate and add additional aliquots with further concentration until entire eluate is transferred. Rinse eluate container with two 0.5-mL portions of hexane and transfer rinses to the minivial with further concentration as necessary.
CAUTION: Do not evaporate eluate (sample extract) to dryness.

11.4.11 With the final sample extract volume at approximately 1 mL, store the extract in a dark place at 4°C until just before GC/MS analysis.

11.5 GC/MS ANALYSIS

11.5.1 Remove the sample extract or blank from storage and allow it to warm to ambient laboratory temperature. Add a 50-uL aliquot of the 2.5 ng/uL solution of the IS. Immediately before GC/MS analysis, reduce the extract or blank volume to 50 uL with a stream of dry, filtered nitrogen. To prevent extract cooling, the extract container can be placed in a water bath at ambient temperature.

11.5.2 Inject a 2-uL aliquot of the extract into the GC operated under conditions previously used (Sect. 9) to produce acceptable results with the performance check solution.

11.5.3 Acquire mass spectral data using the same data acquisition time and MS operating conditions previously used to determine response factors.

11.5.4 Demonstrate acceptable GC/MS system performance.

11.5.4.1 For the IS, determine that the area measured in the sample extract has not increased or decreased by >30% from the area measured during the most recent previous analysis of a CAL or by >50% from the mean area measured during initial calibration. If either criterion is not met, remedial action must be taken to improve reproducibility and the sample extract must be reanalyzed.

11.5.4.2 Document that GC/MS system performance criteria in Sect. 9.2.6 are met.

## 11.6 IDENTIFICATION PROCEDURES

11.6.1 Using the ions shown in Table 4, obtain appropriate selected ion current profiles (SICPs) for IS quantitation and confirmation ions and for each ion monitored to detect CDDs/CDFs, the SCs, and the IS. The integrated ion current measured for each ion monitored to detect an analyte or SC ion must be at least 3 times background noise and must not have saturated the detector.

11.6.2 Internal Standard

11.6.2.1 The abundance of m/z 454 relative to m/z 456 must be ≥40% and ≤60%, and these ions must maximize within 1 s.

11.6.2.2 RT must be within ±10 s of that observed during the most recent acceptable calibration.

11.6.3 Surrogate compounds

11.6.3.1 Absolute RTs of SCs must be within ±5 s of that measured during the last previous continuing calibration check.

11.6.3.2 All ions monitored for each compound (Table 4) must be present, maximimize within 1 s, and meet relative abundance criteria in Table 5.

11.6.4 CDDs and CDFs

11.6.4.1 For each candidate for a particular CDD or CDF isomer group, the integrated peak areas measured for quantitation and confirmation ions must maximize within 1 s.

11.6.4.2 Use SICP data to calculate the relative abundance of measured quantitation and confirmation ions, and compare to criteria in Table 5. If criteria are not met, a coeluting or partially coeluting compound may be interfering. Examination of data from several scans may provide information that will allow application of additional background corrections to improve

the ion ratio. Known relative abundances of analyte ions are shown in Table 6.

11.6.4.3 Ions monitored to demonstrate the absence of a potentially interfering chlorinated diphenyl ether must be <5% abundance relative to appropriate CDF ions; all other ions for a CDD or CDF candidate must be present, maximize within 1 s, and meet relative abundance criteria in Table 5. For $Cl_8$-CDD and $Cl_8$-CDF, in addition to above criteria, the candidate spectrum must elute within 1 s of the relevant isotopically labeled SC.

11.6.4.4 Determine if 2,3,7,8-$Cl_4$-CDD and 2,3,7,8-$Cl_4$-CDF can be present among sample components. In addition to all other identification criteria, a candidate spectrum must maximize within 1 s of its isotopically-labeled homolog.

11.6.4.5 Determine if any $Cl_4$-analytes could have eluted after initiation of data acquistion with ion set 3. If $Cl_4$-CDD ions (320 and 322) or $Cl_4$-CDF ions (304 and 306) maximize within 1 s of each other at the apex of a peak and meet the relative abundance criteria in Table 5, estimate possible concentration with the equation in Sect. 12.3, and contact data user or use judgement as to whether the sample extract should be reanalyzed or data (including estimated concentration of possible late eluting $Cl_4$ analytes) should be reported without reanalysis.

## 12. CALCULATIONS

12.1 From appropriate SICPs of quantitation ions, obtain and record the spectrum number of the chromatographic peak apex and the area of the entire chromatographic peak.

12.2 For CDDs and CDFs, sum the areas for all isomers identified at each level of chlorination (e.g., sum all quantitation ion areas for $Cl_4$-CDDs).

12.3 Calculate the concentration of each SC and CDD/CDF isomer group using the formula:

$$C_x = (A_x \cdot Q_{is})/(A_{is} \cdot RF \cdot W)$$

where $C_x$ = concentration (nanograms per gram or micrograms per kilogram) of SC or CDD/CDF isomer group,

$A_x$ = the sum of the areas of quantitation ions for each SC <u>or</u> the sum of areas of quantitation ions for all CDD/CDF isomers at a particular level of chlorination,

$A_{is}$ = the sum of the area of the quantitation ions for the appropriate IS,

$Q_{is}$ = quantity (nanograms) of IS added to the sample extract before GC/MS analysis,

RF = mean response factor calculated during initial calibration for a SC or a calibration compound for a CDD/CDF isomer group, and

W = weight (grams) of sample extracted.

12.3.1 CAUTION: For these analyses with automated data interpretation a linear fit algorithm will produce erroneous concentration data.

12.3.2 If the calculated concentration of any analyte exceeds the concentration of CAL #5, the linear range may have been exceeded, and a smaller aliquot of that sample must be analyzed. Accurately weigh to two significant figures a 1-g aliquot of the wet soil/sediment. Add 100 uL of the solution containing SCs before sample extraction and other preparation procedures. Add 50 uL of the 2.5 ng/uL IS solution to the extract and analyze.

12.3.3 Report calculated concentrations to two significant figures if <10 ng/g; report to three significant figures if $\geq$10 ng/g.

12.4 Calculate the maximum possible concentration of 2,3,7,8-$Cl_4$-CDD and 2,3,7,8-$Cl_4$-CDF that could be present. Use the equation in Sect. 12.3, but terms are defined as:

$C_x$ = maximum possible concentration (nanograms per gram) of 2,3,7,8-$Cl_4$-CDD or 2,3,7,8-$Cl_4$-CDF,

$A_x$ = the sum of the quantitation ions for 2,3,7,8-$Cl_4$-CDD or 2,3,7,8-$Cl_4$-CDF from the candidate spectrum,

$A_{is}$ = the sum of the quantitation ions for the appropriate $^{13}C_{12}$-labeled analogue (SC),

$Q_{is}$ = the quantity (nanograms) of appropriate SC added to the sample before extraction,

RF = calculated mean response factor (Sect. 9.2.9) for 2,3,7,8-$Cl_4$-CDD relative to $^{13}C_{12}$-2,3,7,8-$Cl_4$-CDD or 2,3,7,8-$Cl_4$-CDF relative to $^{13}C_{12}$-2,3,7,8-$Cl_4$-CDF, and

W = weight (grams) of sample extracted.

12.5 For each SC, calculate the percent method bias using the equation:

$$B = 100\ (C_s - C_t)/\ C_t$$

where $C_s$ = measured concentration (in micrograms per kilogram or micrograms per liter),

$C_t$ = theoretical concentration (i.e., the quantity added to the sample aliquot/weight or volume of sample aliquot).

Note: The bias value retains a positive or negative sign.

12.6 When $Cl_8$-CDD or $Cl_8$-CDF is identified as a sample component, calculate its concentration using the mean RF relative to its $^{13}C$-labeled analog (determined during initial calibration, Sect. 9.2.12). Because this concentration is calculated with a $^{13}C$-labeled analogue, the calculated value is corrected for losses during sample preparation and analysis. For each compound, this concentration should be essentially the same as that calculated relative to the IS and corrected with the appropriate bias value. If not, the cause of the discrepancy should be sought; a likely cause is deterioration of chromatographic performance.

## 13. CONFIRMATORY ANALYSES

13.1 For each sample extract in which 2,3,7,8-$Cl_4$-CDD may be present, analyze a second 2-uL aliquot with a 60-m X 0.32 mm SP-2330 or 50 m X 0.32 mm CP-SIL 88 column coated with a film of appoximately 0.25 um with helium flow at 28-29 cm/sec at approximately 250°C. A solution containing 1,4,7,8-, 2,3,7,8-, and 1,2,3,7/1,2,3,8-$Cl_4$-CDDs should be analyzed. With both columns, 2,3,7,8-isomer elutes between the 1,4,7,8-peak and the combined 1,2,3,7/1,2,3,8-peak. GC conditions should be optimized to separate 2,3,7,8-$Cl_4$-CDD from 1,4,7,8- and and 1,2,3,7/1,2,3,8 with a valley $\leq$25%. (For this confirmatory analysis, detection and measurement of the IS, DBOFB, is not necessary.)

13.1.1 Confirmation requires elution of the candidate sample component within 1 s of the $^{13}C_{12}$-$Cl_4$-CDD SC and measured ion abundances meeting all criteria in Table 5.

13.1.2 If identification is confirmed, calculate and report concentration as specified in Sect. 12.4.
Note: Because this concentration is obtained with an isotope dilution procedure, the value obtained accounts for 2,3,7,8-$Cl_4$-CDD losses during sample preparation and analysis. Labeled and unlabeled analogues are presumed to behave in the same way.

13.2 For each sample extract in which 2,3,7,8-$Cl_4$-CDF may be present, analyze a 2-uL aliquot with a 60-m X 0.32 mm SP-2330 column.

13.2.1 If the candidate sample component elutes within 1 s of the $^{13}C_{12}$-2,3,7,8-$Cl_4$-CDF and all criteria in Table 5 are met, the candidate may be 2,3,7,8-$Cl_4$-CDF or 1,2,6,9-$Cl_4$-CDF, which are not resolved with the chromatographic condititions.

13.2.2 If criteria in Sect. 13.2.1 are met, calculate and report the maximum possible concentration of 2,3,7,8-$Cl_4$-CDF as specified in Sect. 12.4.

## 14. AUTOMATED IDENTIFICATION AND MEASUREMENT

Special software can be used for automated identification and measurement of CDDs and CDFs. Unprocessed GC/MS data are handled without human interaction with the software operating on the dedicated computer. A concentration for each CDD/CDF isomer group is calculated automatically. Contact EMSL-Cincinnati for further information.

## 15. METHOD PERFORMANCE

Because no soil or sediment samples containing known concentrations of a variety of CDDs and CDFs are available to assess method performance, accuracy data are not available. To assess precision of concentration measurements, triplicate 2-uL aliquots of a composite extract of an ash from a municipal incinerater was analyzed with identification and measurement procedures described in this method. RSDs of measured concentrations ranged from 2-14%, and SC recoveries ranged from 29-74%. This low recovery for $^{13}C$-$Cl_8$-CDD indicates that better column chromatography procedures are needed to achieve adequate sensitivity for the higher chlorinated congeners. Because $^{13}C_{12}$-$Cl_8$-CDF was not available when this sample extract was prepared, no data for this SC are available. Because triplicate aliquots of a composite extract were analyzed, data reported here reflect precision of measured concentrations but do not reflect variability associated with sample preparation.

## 16. REFERENCES


1. "Carcinogens -- Working with Carcinogens", Department of Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, August 1977.

2. "OSHA Safety and Health Standards, General Industry", 29 CFR 1910, Occupational Safety and Health Administration, OSHA 2206, Revised January 1976.

3. "Safety in Academic Chemistry Laboratories", American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.

Table 1. GC Operating Conditions[a]

| | |
|---|---|
| Column type: | DB-5 or SE-54 |
| Film thickness: | 0.25 um |
| Column dimensions: | 60 m X 0.32 mm |
| Helium linear velocity: | 28-29 cm/sec at 250°C |
| Injector temperature | 290°C |
| Transfer line temperature | 290°C |
| Temperature program (splitless injection): | Inject at 80°C and hold 1 min; increase as rapidly as possible to 160°C and hold for 4 min; increase from 160°C to 300°C at 3°C/min; hold until after elution of $Cl_8$-CDD. (Begin data acquisition about 10 min after injection.) |

[a] These operating conditions produced acceptable results in one laboratory but different conditions may be required in other laboratories and may vary with instrument and GC column.

Table 2. Composition of Concentration Calibration Solutions

| Compound | Concentration (ng/uL) | | | | |
|---|---|---|---|---|---|
| | CAL 1 | CAL 2 | CAL 3 | CAL 4 | CAL 5 |
| CDD and CDF Cal. Congeners | | | | | |
| $Cl_4$-CDD (2,3,7,8)[a] | 0.2 | 1 | 2 | 4 | 10 |
| $Cl_5$-CDD (1,2,3,7,8) | 0.3 | 1.5 | 3 | 6 | 15 |
| $Cl_6$-CDD (1,2,3,4,7,8) | 0.4 | 2 | 4 | 8 | 20 |
| $Cl_7$-CDD (1,2,3,4,6,7,8) | 0.5 | 2.5 | 5 | 10 | 25 |
| $Cl_8$-CDD | 0.6 | 3 | 6 | 12 | 30 |
| $Cl_4$-CDF (2,3,7,8) | 0.2 | 1 | 2 | 4 | 10 |
| $Cl_5$-CDF (1,2,3,7,8) | 0.3 | 1.5 | 3 | 6 | 15 |
| $Cl_6$-CDF (2,3,4,6,7,8) | 0.4 | 2 | 4 | 8 | 20 |
| $Cl_7$-CDF (1,2,3,4,6,7,8) | 0.5 | 2.5 | 5 | 10 | 25 |
| $Cl_8$-CDF | 0.6 | 3 | 6 | 12 | 30 |
| Surrogate Compounds | | | | | |
| $^{13}C_{12}$-$Cl_4$-CDD (2,3,7,8) | 0.2 | 0.4 | 0.6 | 0.8 | 1 |
| $^{13}C_{12}$-$Cl_8$-CDD | 0.6 | 1.2 | 1.8 | 2.4 | 3 |
| $^{13}C_{12}$-$Cl_4$-CDF (2,3,7,8) | 0.2 | 0.4 | 0.6 | 0.8 | 1 |
| $^{13}C_{12}$-$Cl_8$-CDF | 0.6 | 1.2 | 1.8 | 2.4 | 3 |
| Internal Standard | | | | | |
| 4,4'-dibromooctafluoro-biphenyl | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |

[a]Chlorine substitution positions.

Table 3. Composition of Retention Time (RT) Calibration Solution

| Compound (Chlorine Sub.) | RRT[a] of RT Congener |
|---|---|
| $Cl_4$-CDF (1,3,6,8) | 1.974 |
| $Cl_5$-CDF (1,3,4,6,8) | 2.302 |
| $Cl_6$-CDF (1,2,3,4,6,8) | 2.665 |
| $Cl_7$-CDF (1,2,3,4,6,7,8) | 3.022 |
| $Cl_8$-CDF (1,2,3,4,6,7,8,9) | 3.431 |

[a]Retention time relative to the IS, dibromooctafluorobiphenyl, which elutes about 12 min after injection with GC conditions described in Table 1. These are provided for information only; RRTs will vary with particular GC conditions used to analyze the solution.

Table 4. Ion Sets for Selected Ion Monitoring Data Acquisition

| Set | Ion | Compound Monitored/Purpose |
|---|---|---|
| 1 | 257 | Set ion range lower limit |
| | 454 | IS quantitation ion |
| | 456 | IS quantitation ion |
| | 457 | MS resolution check |
| | 458 | IS confirmation ion |
| | 514 | Set ion range upper limit |
| 2 | 257 | $Cl_4$-CDD confirmation ion |
| | 259 | $Cl_4$-CDD confirmation ion |
| | 304 | $Cl_4$-CDF quantitation ion |
| | 306 | $Cl_4$-CDF quantitation ion |
| | 308 | $Cl_4$-CDF confirmation ion |
| | 316 | $^{13}C$-$Cl_4$-CDF quantitation ion |
| | 318 | $^{13}C$-$Cl_4$-CDF quantitation ion |
| | 320 | $Cl_4$-CDD quant. ion and $^{13}C$-$Cl_4$-CDF confirm. ion |
| | 322 | $Cl_4$-CDD quantitation ion |
| | 332 | $^{13}C$-$Cl_4$-CDD quantitation ion |
| | 334 | $^{13}C$-$Cl_4$-CDD quantitation ion |
| | 376 | $Cl_4$-CDF confirmation ion |
| 3 | 293 | $Cl_5$-CDD confirmation ion |
| | 304 | $Cl_4$-CDF check ion |
| | 306 | $Cl_4$-CDF check ion |
| | 320 | $Cl_4$-CDD check ion |
| | 322 | $Cl_4$-CDD check ion |
| | 338 | $Cl_5$-CDF quantitation ion |
| | 340 | $Cl_5$-CDF quantitation ion |
| | 342 | $Cl_5$-CDF confirmation ion |
| | 354 | $Cl_5$-CDD confirmation ion |
| | 356 | $Cl_5$-CDD quantitation ion |
| | 358 | $Cl_5$-CDD quantitation ion |
| | 410 | $Cl_5$-CDF confirmation ion |
| 4 | 327 | $Cl_6$-CDD confirmation ion |
| | 372 | $Cl_6$-CDF confirmation ion |
| | 374 | $Cl_6$-CDF quantitation ion |
| | 376 | $Cl_6$-CDF quantitation ion |
| | 388 | $Cl_6$-CDD confirmation ion |
| | 390 | $Cl_6$-CDD quantitation ion |
| | 392 | $Cl_6$-CDD quantitation ion |
| | 446 | $Cl_6$-CDF confirmation ion |
| 5 | 361 | $Cl_7$-CDD confirmation ion |
| | 406 | $Cl_7$-CDF confirmation ion |
| | 408 | $Cl_7$-CDF quantitation ion |
| | 410 | $Cl_7$-CDF quantitation ion |
| | 412 | $Cl_7$-CDF confirmation ion |
| | 422 | $Cl_7$-CDD confirmation ion |
| | 424 | $Cl_7$-CDD quantitation ion |
| | 426 | $Cl_7$-CDD quantitation ion |
| | 480 | $Cl_7$-CDF confirmation ion |
| 6 | 440 | $Cl_8$-CDF confirmation ion |
| | 442 | $Cl_8$-CDF quantitation ion |
| | 444 | $Cl_8$-CDF quantitation ion |
| | 446 | $Cl_8$-CDF confirmation ion |
| | 456 | $^{13}C$-$Cl_8$-CDF quant. ion and $Cl_8$-CDD confirm. ion |
| | 458 | $^{13}C$-$Cl_8$-CDF quant. ion and $Cl_8$-CDD quant. ion |
| | 460 | $^{13}C$-$Cl_8$-CDF confirm. ion and $Cl_8$-CDD quant. ion |
| | 462 | $Cl_8$-CDD quantitation ion |
| | 470 | $^{13}C$-$Cl_8$-CDD confirm. ion |
| | 472 | $^{13}C$-$Cl_8$-CDD quant. ion |
| | 474 | $^{13}C$-$Cl_8$-CDD quant. ion |
| | 514 | $Cl_8$-CDF confirm. ion |

**SUMMARY**

| Set | No. of Ions | Compounds Monitored |
|---|---|---|
| 1 | 6 | IS |
| 2 | 12 | $Cl_4$-analytes |
| 3 | 12 | $Cl_5$-analytes[a] |
| 4 | 8 | $Cl_6$-analytes |
| 5 | 9 | $Cl_7$-analytes |
| 6 | 12 | $Cl_8$-analytes |

[a]Set 3 includes four ions to check that $Cl_4$-analytes did not elute after data acquisition with ion set 3 was initiated.

Table 5. Relative Abundance Criteria for Ions Monitored to Identify and Measure CDDs and CDFs

| Analyte Isomer Group | Nominal m/z | Exact m/z | Acceptance Criteria |
|---|---|---|---|
| $Cl_4$-CDF | 304 | 303.9016 | ≥66% and ≤86% |
| | 306 | 305.8987 | 100% |
| | 308 | 307.8957 | ≥39% and ≤59% |
| | 376 | 375.8364 | Absent or <5% |
| $Cl_5$-CDF | 338 | 337.8626 | ≥51% and ≤71% |
| | 340 | 339.8597 | 100% |
| | 342 | 341.8567 | ≥55% and ≤75% |
| | 410 | 409.7974 | Absent or <5% |
| $Cl_6$-CDF | 372 | 371.8236 | ≥41% and ≤61% |
| | 374 | 373.8207 | 100% |
| | 376 | 375.8178 | ≥72% and ≤92% |
| | 446 | 445.7554 | Absent or <5% |
| $Cl_7$-CDF | 406 | 405.7847 | ≥34% and ≤54% |
| | 408 | 407.7817 | 100% |
| | 410 | 409.7788 | ≥87% and ≤100% |
| | 412 | 411.7758 | ≥42% and ≤62% |
| | 480 | 479.7164 | Absent or <5% |
| $Cl_8$-CDF | 440 | 439.7457 | ≥24% and ≤44% |
| | 442 | 441.7428 | ≥78% and ≤98% |
| | 444 | 443.7398 | 100% |
| | 446 | 445.7369 | ≥55% and ≤75% |
| | 514 | 513.6774 | Absent or <5% |
| $^{13}C_{12}$-$Cl_4$-CDF | 316 | 315.9418 | ≥67% and ≤87% |
| | 318 | 317.9389 | 100% |
| | 320 | 319.9360 | ≥39% and ≤59% |
| $^{13}C_{12}$-$Cl_8$-CDF | 456 | 455.7800 | 100% |
| | 458 | 457.7771 | ≥55% and ≤75% |
| | 460 | 459.7741 | ≥16% and ≤36% |

Table 5 (cont.). Relative Abundance Criteria for Ions Monitored to Identify and Measure CDDs and CDFs

| Analyte Isomer Group | m/z | Accurate m/z | Acceptance Criteria |
|---|---|---|---|
| $Cl_4$-CDD | 320 | 319.8965 | ≥67% and ≤87% |
| | 322 | 321.8936 | 100% |
| | 257 | 256.9327 | >10% and ≤40% |
| | 259 | 258.9298 | ≥10% and ≤40% |
| $Cl_5$-CDD | 354 | 353.8575 | ≥52% and ≤72% |
| | 356 | 355.8546 | 100% |
| | 358 | 357.8516 | ≥55% and ≤75% |
| | 293 | 292.8908 | ≥10% and ≤40% |
| $Cl_6$-CDD | 388 | 387.8186 | ≥41% and ≤61% |
| | 390 | 389.8156 | 100% |
| | 392 | 391.8127 | ≥71% and ≤91% |
| | 327 | 326.8518 | Present and <40% |
| $Cl_7$-CDD | 422 | 421.7796 | ≥34% and ≤54% |
| | 424 | 423.7766 | 100% |
| | 426 | 425.7737 | ≥87% and ≤100% |
| | 361 | 360.8128 | Present and <40% |
| $Cl_8$-CDD | 456 | 455.7406 | ≥24% and ≤44% |
| | 458 | 457.7377 | ≥78% and ≤98% |
| | 460 | 459.7347 | 100% |
| | 462 | 461.7318 | ≤55% and ≥75% |
| $^{13}C_{12}$-$Cl_4$-CDD | 332 | 331.9367 | ≥66% and ≤86% |
| | 334 | 333.9338 | 100% |
| $^{13}C_{12}$-$Cl_8$-CDD | 470 | 469.7779 | ≥77% and ≤97% |
| | 472 | 471.7749 | 100% |
| | 474 | 473.7720 | ≥55% and ≤75% |

Table 6. Known Relative Abundances of Ions in CDD/CDF Molecular Ion Clusters

| Isomer Group | m/z CDDs | m/z CDFs | Relative Abundance |
|---|---|---|---|
| $Cl_4$ | 320 | 304 | 77 |
| | 321 | 305 | 10 |
| | 322 | 306 | 100 |
| | 323 | 307 | 13 |
| | 324 | 308 | 49 |
| | 325 | 309 | 7 |
| | 326 | 310 | 10 |
| | 327 | 311 | 1 |
| | 328 | 312 | <1 |
| $Cl_5$ | 354 | 338 | 62 |
| | 355 | 339 | 8 |
| | 356 | 340 | 100 |
| | 357 | 341 | 13 |
| | 358 | 342 | 65 |
| | 359 | 343 | 9 |
| | 360 | 344 | 21 |
| | 361 | 345 | 3 |
| | 362 | 346 | 3 |
| | 363 | 347 | <1 |
| | 364 | 348 | <1 |
| $Cl_6$ | 388 | 372 | 51 |
| | 389 | 373 | 7 |
| | 390 | 374 | 100 |
| | 391 | 375 | 13 |
| | 392 | 376 | 81 |
| | 393 | 377 | 11 |
| | 394 | 378 | 35 |
| | 395 | 379 | 5 |
| | 396 | 380 | 9 |
| | 397 | 381 | 1 |
| | 398 | 382 | 1 |
| | 399 | 383 | <1 |
| $Cl_7$ | 422 | 406 | 44 |
| | 423 | 407 | 6 |
| | 424 | 408 | 100 |
| | 425 | 409 | 13 |
| | 426 | 410 | 97 |
| | 427 | 411 | 13 |
| | 428 | 412 | 52 |
| | 429 | 413 | 7 |
| | 430 | 414 | 17 |
| | 431 | 415 | 2 |
| | 432 | 416 | 3 |
| | 433 | 417 | <1 |
| | 434 | 418 | <1 |
| $Cl_8$ | 456 | 440 | 34 |
| | 457 | 441 | 5 |
| | 458 | 442 | 88 |
| | 459 | 443 | 12 |
| | 460 | 444 | 100 |
| | 461 | 445 | 13 |
| | 462 | 446 | 65 |
| | 463 | 447 | 9 |
| | 464 | 448 | 26 |
| | 465 | 449 | 4 |
| | 466 | 450 | 7 |
| | 467 | 451 | 1 |
| | 468 | 452 | 1 |
| | 469 | 453 | <1 |
| | 470 | 454 | <1 |

Table 7. Precision of Triplicate Determinations of CDDs and CDFs in Composite Ash Extract

| Analyte | Conc. ng/g (RSD) | | No. of Isomers |
|---|---|---|---|
| CDDs | | | |
| $Cl_4$-CDDs | 37.8 | (14) | 7 |
| $Cl_5$-CDDs | 101 | (4.1) | 5 |
| $Cl_6$-CDDs | 84.5 | (4.3) | 3 |
| $Cl_7$-CDDs | 65.9 | (2.7) | 2 |
| $Cl_8$-CDD | 59.5 | (5.0) | 1 |
| Total CDDs | 349 | | 18 |
| CDFs | | | |
| $Cl_4$-CDFs | 194 | (2.1) | 7 |
| $Cl_5$-CDFs | 108 | (2.8) | 3 |
| $Cl_6$-CDFs | 24.3 | (1.6) | 2 |
| $Cl_7$-CDFs | 2.93 | [a] | 1 |
| $Cl_8$-CDF | | | |
| Total CDFs | 329 | | 13 |
| Surrogate Compounds | | | |
| $^{13}C$-$Cl_4$-CDD | 27.8 | (2.4)[b] | |
| $^{13}C$-$Cl_8$-CDD | 14.9 | (1.6)[b] | |
| $^{13}C$-$Cl_4$-CDF | 36.8 | (3.3)[b] | |

[a]Identified in two of three replicates. [b]Recovery of 56% of $^{13}C$-$Cl_4$-CDD, 29% of $^{13}C$-$Cl_8$-CDD, and 74% of $^{13}C$-$Cl_4$-CDF.