EXHIBIT D

ANALYTICAL METHODS FOR CHLORINATED DIBENZO-p-DIOXINS (CDDs) AND CHLORINATED DIBENZOFURANS (CDFs)

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Exhibit D - Analytical Methods for CDDs/CDFs

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1.0 SCOPE AND APPLICATION

1.1 Method

The analytical method that follows is designed for the determination of tetra- through octa-chlorinated dibenzo-p-dioxins (CDDs) and dibenzofurans (CDFs) (see Table 1) in water, soil, sediment, sludge, tissue, and other sample matrices by high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS). The method is based on USEPA Method 1613, Revision B.

1.2 Quantitation Levels

The detection limits and quantitation levels in this method are usually dependent on the level of interferences rather than instrumental limitations. The levels listed in Exhibit C are the Contract Required Quantitation Limits (CRQL) that can be determined with no interferences present.

1.3 Qualitative Identification

The qualitative identification criteria (see Section 11.1) include requirements for retention times and limits on the ratio of the abundance of the two intense specified ions produced by each compound. In the instance where a signal is detected that meets all of the qualitative identification criteria except the ion abundance ratio, the method requires calculation of an "Estimated Maximum Possible Concentration" (EMPC). The presence of interference that coelute with the compounds of interest may cause the ion abundance ratio to fall outside the limits for qualitative identification and would also affect the quantitative results. The EMPC is a worst case estimate of the sample concentration that the signal would represent if it did meet all the identification criteria (see Section 11.2.6).

1.4 Caution

Because of the extreme toxicity of many of these compounds, the analyst must take the necessary precautions to prevent exposure to material known or believed to contain CDDs/CDFs.

2.0 SUMMARY OF METHOD

2.1 Extraction

2.1.1 Water samples (samples containing less than one percent solids): The stable isotopically labeled analogs of 15 of the 2,3,7,8-substituted CDDs/CDFs, are spiked into a one-liter sample and the sample is extracted by one of three procedures:

2.1.1.1 Samples containing no visible particles are extracted with methylene chloride in a separatory funnel or by the solid-phase extraction technique summarized in Section 2.1.1.3. The extract is concentrated for cleanup.

2.1.1.2 Samples containing visible particles are vacuum-filtered through a glass-fiber filter. The filter is extracted in a Soxhlet/Dean-Stark (SDS) extractor and the filtrate is extracted with methylene chloride in a separatory funnel. The methylene chloride extract is concentrated and combined with the SDS extract prior to cleanup.

2.1.1.3 The sample is vacuum-filtered through a glass-fiber filter on top of a solid-phase extraction (SPE) disk. The filter and disk are extracted in an SDS extractor and the extract is concentrated for cleanup.

2.1.2 Soil/sediment samples: The labeled compounds are spiked into a sample containing 10 g (dry weight) of soil/sediments. Samples containing coarse soil/sediments are ground or homogenized. The soil/sediments are then extracted in an SDS extractor.

2.1.3 Fish and other tissue: The sample is extracted by one of two procedures:

2.1.3.1 A 20-g aliquot of frozen sample is homogenized and a 10-g aliquot is spiked with the labeled compounds. The sample is mixed with sodium sulfate, allowed to dry overnight, and extracted for 12 to 24 hours using methylene chloride:hexane (1:1) in a Soxhlet extractor. The extract is evaporated to dryness and the lipid content is determined.

2.1.3.2 A 20-g aliquot is homogenized and a 10-g aliquot is placed in a bottle and spiked with the labeled compounds. After equilibration, 200 mL of hydrochloric acid and 200 mL of methylene chloride:hexane (1:1) are added and the bottle is agitated for 12 to 24 hours. The extract is evaporated to dryness and the lipid content is determined.

2.2 Cleanup and Analysis

2.2.1 After extraction, the cleanup standard, $^{37}Cl_4$-labeled 2,3,7,8-TCDD, is added to each extract to measure the efficiency of the cleanup process. Sample cleanups may include back-extraction with acid and/or base, gel permeation, alumina, silica gel, florisil, and

activated carbon chromatography. High-performance liquid chromatography (HPLC) can be used for further isolation of the 2,3,7,8- isomers or other specific isomers or congeners. Prior to the cleanup procedures above, tissue extracts are cleaned up using an anthropogenic isolation column, a batch silica gel adsorption, or sulfuric acid back extraction, depending on the tissue extraction procedure used.

2.2.2 After cleanup, the extract is concentrated to near dryness. Immediately prior to injection, the two internal standards, $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,3,7,8,9-HxCDD, are added to each extract. The former is used to determine the percent recoveries of tetra- and pentachlorinated CDD/CDF congeners, while the latter is used to determine the recoveries of the hexa-, hepta-, and octachlorinated CDD/CDF congeners.

2.2.3 An aliquot of the extract is injected into the high resolution gas chromatograph. The analytes are separated by the HRGC and detected by a high resolution ($ 10,000) mass spectrometer (HRMS). Two exact mass to charge ratio (m/z) ions are monitored for each analyte.

2.2.4 An individual CDD/CDF is identified by comparing the HRGC retention time and ion-abundance ratio of two exact m/z's with the corresponding retention time of an authentic standard and the theoretical or acquired ion-abundance ratio of the two exact m/z's. The non-2,3,7,8 substituted isomers and congeners are identified when retention times and ion-abundance ratios agree within pre-defined limits. Isomer specificity for 2,3,7,8-TCDD and 2,3,7,8-TCDF is achieved using HRGC columns that resolve these isomers from the other tetra-isomers.

2.3 Quantitative Analyses

2.3.1 Quantitative analysis is performed using selected ion current profile (SICP) areas, in one of the following ways.

2.3.1.1 For the fifteen 2,3,7,8-substituted CDDs/CDFs with labeled compound analogs, the HRGC/HRMS system is calibrated and the compound concentration is determined using the isotope dilution technique. The procedure used in determining the concentrations of 1,2,3,7,8,9-HxCDD and OCDF is detailed in Section 11.2.

2.3.1.2 For the labeled compounds and the cleanup standard the GC/MS system is calibrated and the concentration of each compound is determined using the internal standard technique.

2.3.1.3 For non-2,3,7,8-substituted isomers and for all isomers at a given level of chlorination (e.g., total TCDD), concentrations are determined using relative response factors from calibration of native analogs at the same level of chlorination.

2.3.2 The quality of the analysis is assured through reproducible calibration and testing of the extraction, cleanup, and HRGC/HRMS systems.

3.0 DEFINITIONS

See Exhibit G for a complete list of definitions.

4.0 INTERFERENCES

4.1 Sources

Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or elevated baselines causing misinterpretation of chromatograms. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Where possible, reagents are cleaned by extraction or solvent rinse.

4.2 Glassware Cleaning

Proper cleaning of glassware is extremely important because glassware may not only contaminate the samples, but may also remove the analytes of interest by adsorption on the glass surface.

4.2.1 Glassware should be rinsed with solvent and washed with a detergent solution as soon after use as is practical. Sonication of glassware containing a detergent solution may aid in cleaning. Glassware with removable parts, particularly separatory funnels with fluoropolymer stopcocks, must be disassembled prior to detergent washing.

4.2.2 After detergent washing, glassware should be rinsed immediately, first with methanol, then with hot tap water. The tap water rinse is followed by another methanol rinse, then acetone, and then methylene chloride.

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

4.2.4 Immediately prior to use, the Soxhlet apparatus should be pre-extracted with toluene for approximately three hours. Separatory funnels should be shaken with methylene chloride/toluene (80/20 mixture) for two minutes, drained, and then shaken with pure methylene chloride for two minutes.

4.3 Reagents and Materials

All materials used in the analysis shall be demonstrated to be free from interferences by running reference matrix method blanks initially and with each sample batch (samples started through the extraction process on a given 12-hour shift, to a maximum of 20 samples).

4.3.1 The reference matrix must simulate, as closely as possible, the sample matrix under test. The reference matrix should not contain the CDDs/CDFs in detectable amounts. See Section 12.1 for further guidance. USEPA retains the option to supply the contractor with a

reference matrix with the expected interferences for a particular project.

4.3.2 When a reference matrix that simulates the sample matrix under test is not available, reagent water (Section 7.6.1) can be used to simulate water samples; playground sand (Section 7.6.2) or white quartz sand (Section 7.3.2) can be used to simulate soils and corn oil (Section 7.6.3) can be used to simulate tissues.

4.4 Sample Cleanup

Interferences coextracted from samples will vary considerably from source to source, depending on the diversity of the site being sampled. Interfering compounds may be present at concentrations several orders of magnitude higher than the CDDs/CDFs. The most frequently encountered interferences are chlorinated biphenyls, methoxy biphenyls, hydroxydiphenyl ethers, benzylphenyl ethers, polynuclear aromatics, and pesticides. Because very low levels of CDDs/CDFs are measured by this method, the elimination of interferences is essential. The cleanup steps given must be used to reduce or eliminate these interferences and thereby permit reliable determination of the CDDs/CDFs at the levels shown in Table 2 and in Exhibit C.

4.5 Equipment

Each piece of reusable glassware should be numbered to associate that glassware with the processing of a particular sample. This will assist the laboratory in tracking possible sources of contamination for individual samples, identifying glassware associated with highly contaminated samples that may require extra cleaning, and determining when glassware should be discarded.

4.6 Lipids

The natural lipid content of tissue can interfere in the analysis of tissue samples for the CDDs/CDFs. The lipid contents of different species and portions of tissue can vary widely. Lipids are soluble to varying degrees in various organic solvents and may be present in sufficient quantity to overwhelm the column chromatographic cleanup procedures used for cleanup of sample extracts. Lipids must be removed by the lipid removal procedures, followed by alumina or Florisil, and carbon as minimum additional cleanup steps. If chlorodiphenyl ethers are detected, as indicated by the presence of peaks at the exact m/z's monitored for these interferents, alumina and/or Florisil cleanup must be employed to eliminate these interferences.

5.0 SAFETY

5.1 Toxicity

The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined; however, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level.

5.1.1 The 2,3,7,8-TCDD isomer has been found to be acnegenic, carcinogenic, and teratogenic in laboratory animal studies. It is soluble in water to approximately 200 ppt, and in organic solvents to 0.14%. On the basis of the available toxicological and physical properties of 2,3,7,8-TCDD, all of the CDDs/CDFs should be handled only by highly-trained personnel thoroughly familiar with handling and cautionary procedures and the associated risks.

5.1.2 It is recommended that the laboratory purchase dilute standard solutions of the analytes in this method. However, if primary solutions are prepared, they shall be prepared in a hood, and a NIOSH/MESA approved toxic gas respirator shall be worn when high concentrations are handled.

5.2 OSHA Requirements

The laboratory is responsible for maintaining a current awareness file of Occupational Safety and Health Administration (OSHA) regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDSs) should also be made available to all personnel involved in these analyses. It is also suggested that the laboratory perform personal hygiene monitoring of each analyst who uses this method and that the results of this monitoring be made available to the analyst.

5.3 Sample Handling

The CDDs/CDFs and samples suspected to contain these compounds are handled using essentially the same techniques employed in handling radioactive or infectious materials. Well-ventilated, controlled access laboratories are required. 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. The CDDs/CDFs are extremely toxic to laboratory animals. The contractor must develop a strict safety program for handling these compounds.

5.3.1 Facility — When finely divided samples (dusts, soils, dry chemicals) are handled, all operations (including removal of samples from sample containers, weighing, transferring, and mixing) should be performed in a glove box demonstrated to be leak-tight, or in a fume hood demonstrated to have adequate air flow. Gross losses to the laboratory ventilation system must not be allowed. Handling of the dilute solutions normally used in analytical and animal work presents no inhalation hazards except in the case of an accident.

5.3.2 Protective equipment — Disposable plastic gloves, apron or lab coat, safety glasses or mask, and a glove box or fume hood adequate for radioactive work should be used. During analytical operations that may give rise to aerosols or dusts, personnel should wear respirators equipped with activated carbon filters. Eye protection equipment (preferably full face shields) must be worn while working with exposed samples or pure analytical standards. Latex gloves are commonly used to reduce exposure of the hands. When handling samples suspected or known to contain high concentrations of the CDDs/CDFs, an additional set of gloves can also be worn beneath the latex gloves.

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

5.3.4 Personal hygiene — Hands and forearms should be washed thoroughly after each manipulation and before breaks (coffee, lunch, and shift).

5.3.5 Confinement — Isolated work areas posted with signs, segregated glassware and tools, and plastic absorbent paper on bench tops will aid in confining contamination.

5.3.6 Effluent vapors — The effluents of sample splitters from the gas chromatograph (GC) and from roughing pumps on the mass spectrometer (MS) should pass through either a column of activated charcoal or be bubbled through a trap containing oil or high-boiling alcohols to condense CDD/CDF vapors.

5.3.7 Waste Handling — Good technique includes minimizing contaminated waste. Plastic bag liners should be used in waste cans. Janitors and other personnel must be trained in the safe handling of waste.

5.4 Decontamination

5.4.1 Decontamination of personnel — Use any mild soap with plenty of scrubbing action.

5.4.2 Glassware, tools, and surfaces — Chlorothene NU Solvent is the least toxic solvent shown to be effective. Satisfactory cleaning may be accomplished by rinsing with Chlorothene, then washing with any detergent and water. If glassware is first rinsed with solvent, the dish water may be disposed of in the sewer. Given the cost of disposal, it is prudent to minimize solvent wastes.

5.4.3 Laundry — Clothing known to be contaminated 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 of the potential problem. The washer should be run through a cycle before being used again for other clothing.

5.4.4 Wipe tests — A useful method of determining cleanliness of work surfaces and tools is to wipe the surface with a piece of filter

paper. Extraction and analysis by GC with an electron capture detector (ECD) can achieve a limit of detection of 0.1 µg per wipe; analysis using this method can achieve an even lower detection limit. Less than 0.1 µg per wipe indicates acceptable cleanliness; anything higher warrants further cleaning. More than 10 µg on a wipe constitutes an acute hazard and requires prompt cleaning before further use of the equipment or work space, and indicates that unacceptable work practices have been employed.

5.4.5 Table or wrist-action shaker — The use of a table or wrist-action shaker for extraction of tissues presents the possibility of breakage of the extraction bottle and spillage of acid and flammable organic solvent. A secondary containment system around the shaker is suggested to prevent the spread of acid and solvents in the event of such a breakage. The speed and intensity of shaking action should also be adjusted to minimize the possibility of breakage.

6.0 EQUIPMENT AND SUPPLIES

6.1 Glassware Cleaning — Laboratory sink with overhead fume hood.

6.2 Equipment for Sample Preparation

6.2.1 Laboratory fume hood of sufficient size to contain the sample preparation equipment listed below.

6.2.2 Glove box (optional).

6.2.3 Tissue homogenizer — VirTis Model 45 Macro homogenizer (American Scientific Products H-3515, or equivalent) with stainless steel Macro-shaft and Turbo-shear blade.

6.2.4 Meat grinder — Hobart, or equivalent, with 3-5 mm holes in inner plate.

6.2.5 Equipment for determining percent solids

6.2.5.1 Oven — Capable of maintaining a temperature of 110 ±5°C.

6.2.5.2 Dessicator.

6.2.6 Balances

6.2.6.1 Analytical — Capable of weighing ±0.1 mg.

6.2.6.2 Top loading — Capable of weighing ±10 mg.

6.3 Extraction Apparatus

6.3.1 Water samples

6.3.1.1 pH meter, with combination glass electrode.

6.3.1.2 pH paper, wide range (Hydrion Papers, or equivalent).

6.3.1.3 Graduated cylinder, 1 L capacity.

6.3.1.4 Liquid/liquid extraction — Separatory funnels, 250 mL, 500 mL, and 2000 mL, with fluoropolymer stopcocks.

6.3.1.5 Solid-phase extraction

6.3.1.5.1 One liter filtration apparatus, including glass funnel, glass frit support, clamp, adapter, stopper, filtration flask, and vacuum tubing. For wastewater samples, the apparatus should accept 90 or 144 mm disks. For drinking water or other samples containing low solids, smaller disks may be used.

6.3.1.5.2 Vacuum source capable of maintaining 25 in. Hg, equipped with shutoff valve and vacuum gauge.

6.3.1.5.3 Glass-fiber filter — Whatman GMF 150 (or equivalent), 1 micron pore size, to fit filtration apparatus described in Section 6.3.1.5.1.

6.3.1.5.4 Solid-phase extraction disk containing octadecyl (C_{18}) bonded silica uniformly enmeshed in an inert matrix—Fisher Scientific 14-378F (or equivalent), to fit filtration apparatus described in Section 6.3.1.5.1.

6.3.2 Soxhlet/Dean-Stark (SDS) extractor — For filters and solid/sludge samples.

6.3.2.1 Soxhlet — 50 mm ID, 200 mL capacity with 500 mL flask (Cal-Glass LG-6900, or equivalent, except substitute 500 mL round-bottom flask for 300 mL flat-bottom flask).

6.3.2.2 Thimble — 43 x 123 to fit Soxhlet (Cal-Glass LG-6901-122, or equivalent).

6.3.2.3 Moisture trap — Dean Stark or Barret with fluoropolymer stopcock, to fit Soxhlet.

6.3.2.4 Heating mantle — Hemispherical, to fit 500 mL round-bottom flask (Cal-Glass LG-8801-112, or equivalent).

6.3.2.5 Variable transformer — Powerstat (or equivalent), 110 volt, 10 amp.

6.3.3 Apparatus for extraction of tissue.

6.3.3.1 Bottle for extraction (if digestion/extraction using HCl is used)— 500-600 mL wide-mouth clear glass, with fluoropolymer-lined cap.

6.3.3.2 Bottle for back-extraction — 100-200 mL narrow-mouth clear glass with fluoropolymer-lined cap.

6.3.3.3 Mechanical shaker — Wrist-action or platform-type rotary shaker that produces vigorous agitation (Sybron Thermolyne Model LE "Big Bill" rotator/shaker, or equivalent).

6.3.3.4 Rack attached to shaker table to permit agitation of four to nine samples simultaneously.

6.3.4 Beakers — 400-500 mL.

6.3.5 Spatulas — Stainless steel or glass rod.

6.4 Filtration Apparatus

6.4.1 Pyrex glass wool — Solvent extracted by SDS for three hours minimum. DO NOT BAKE

6.4.2 Glass funnel — 125-250 mL.

6.4.3 Glass-fiber filter paper — Whatman GF/D (or equivalent), to fit glass funnel referenced in Section 6.4.2.

6.4.4 Drying column — 15-20 mm ID Pyrex chromatographic column equipped with coarse-glass frit or glass-wool plug.

6.4.5 Buchner funnel — 15 cm.

6.4.6 Glass - fiber filter paper—to fit Buchner funnel referenced in Section 6.4.5.

6.4.7 Filtration flasks—1.5-2.0 L, with side arm.

6.4.8 Pressure filtration apparatus—Millipore YT30 142 HW, or equivalent.

6.5 Centrifuge Apparatus

6.5.1 Centrifuge — Capable of rotating 500 mL centrifuge bottles or 15 mL centrifuge tubes at 5,000 rpm minimum.

6.5.2 Centrifuge bottles — 500 mL, with screw-caps, to fit centrifuge.

6.5.3 Centrifuge tubes — 12-15 mL, with screw-caps, to fit centrifuge.

6.6 Cleanup Apparatus

6.6.1 Automated gel permeation chromatograph (Analytical Biochemical Labs, Inc., Columbia, MO, Model GPC Autoprep 1002, or equivalent).

6.6.1.1 Column — 600-700 mm long x 25 mm ID, packed with 70 g of SX-3 Bio-beads (Bio-Rad Laboratories, Richmond, CA, or equivalent).

6.6.1.2 Syringe — 10 mL, with Luer fitting.

6.6.1.3 Syringe filter holder — stainless steel, and glass-fiber or fluoropolymer filters (Gelman 4310, or equivalent).

6.6.1.4 UV detectors — 254 nm, preparative or semi-preparative flow cell (Isco, Inc., Type 6; Shimadzu, 5 mm path length; Beckman-Altex 152W, 8 µL micro-prep flow cell, 2 mm path; Pharmacia UV-1, 3 mm flow cell; LDC Milton-Roy UV-3, monitor #1203; or equivalent).

6.6.2 Reverse-phase high-performance liquid chromatograph.

6.6.2.1 Column oven and detector — Perkin-Elmer Model LC-65T (or equivalent) operated at 0.02 AUFS at 235 nm.

6.6.2.2 Injector — Rheodyne 7120 (or equivalent) with 50 µL sample loop.

6.6.2.3 Column — Two 6.2 mm x 250 mm Zorbax-ODS columns in series (DuPont Instruments Division, Wilmington, DE, or equivalent), operated at 50°C with 2.0 mL/min methanol isocratic effluent.

6.6.2.4 Pump — Altex 110A (or equivalent).

6.6.3 Pipets

6.6.3.1 Disposable, pasteur—150 mm long x 5-mm ID (Fisher Scientific 13-678-6A, or equivalent).

6.6.3.2 Disposable, serological—10 mL (6 mm ID).

6.6.4 Glass chromatographic columns

6.6.4.1 150 mm long x 8-mm ID, (Kontes K-420155, or equivalent) with coarse-glass frit or glass-wool plug and 250 mL reservoir.

6.6.4.2 200 mm long x 15 mm ID, with coarse-glass frit or glass-wool plug and 250 mL reservoir.

6.6.4.3 300 mm long x 25 mm ID, with 300 mL reservoir and glass or fluoropolymer stopcock.

6.6.5 Stirring apparatus for batch silica cleanup of tissue extracts.

6.6.5.1 Mechanical stirrer—Corning Model 320, or equivalent.

6.6.5.2 Bottle — 500-600 mL wide-mouth clear glass.

6.6.6 Oven — For baking and storage of adsorbents, capable of maintaining a constant temperature (±5°C) in the range of 105-250°C.

6.7 Concentration Apparatus

6.7.1 Rotary evaporator — Buchi/Brinkman-American Scientific No. E5045-10 or equivalent, equipped with a variable temperature water bath.

6.7.1.1 Vacuum source for rotary evaporator equipped with shutoff valve at the evaporator and vacuum gauge.

6.7.1.2 A recirculating water pump and chiller are recommended, as use of tap water for cooling the evaporator wastes large volumes of water and can lead to inconsistent performance as water temperatures and pressures vary.

6.7.1.3 Round-bottom flask — 100 mL and 500 mL or larger, with ground-glass fitting compatible with the rotary evaporator.

6.7.2 Kuderna-Danish (K-D) Concentrator

6.7.2.1 Concentrator tube — 10 mL, graduated (Kontes K-570050-1025, or equivalent) with calibration verified. Ground-glass stopper (size 19/22 joint) is used to prevent evaporation of extracts.

6.7.2.2 Evaporation flask — 500 mL (Kontes K-570001-0500, or equivalent), attached to concentrator tube with springs (Kontes K-662750-0012 or equivalent).

6.7.2.3 Snyder column—Three-ball macro (Kontes K-503000-0232, or equivalent).

6.7.2.4 Boiling chips

6.7.2.4.1 Glass or silicon carbide — Approximately 10/40 mesh, extracted with methylene chloride.

6.7.2.4.2 Fluoropolymer (optional) — Extracted with methylene chloride.

6.7.2.5 Water bath — Heated, with concentric ring cover, capable of maintaining a temperature within ±2°C, installed in a fume hood.

6.7.3 Nitrogen blowdown apparatus — Equipped with water bath controlled in the range of 30-60°C (N-Evap, Organomation Associates, Inc., South Berlin, MA, or equivalent), installed in a fume hood.

6.7.4 Sample vials

6.7.4.1 Amber glass — 2-5 mL with fluoropolymer-lined screw-cap.

6.7.4.2 Glass — 0.3 mL, conical, with fluoropolymer-lined screw or crimp cap.

6.8 Gas Chromatograph

Shall have splitless or on-column injection port for capillary column, temperature program with isothermal hold, and shall meet all of the performance specifications listed in Section 9 of this exhibit.

6.8.1 GC column for CDDs/CDFs and for isomer specificity for 2,3,7,8-TCDD—60 ±5 m long x 0.32 ±0.02 mm ID; 0.25 µm 5% phenyl, 94% methyl, 1% vinyl silicone bonded-phase fused-silica capillary column (J&W DB-5, or equivalent).

6.8.2 GC column for isomer specificity for 2,3,7,8-TCDF—30 ±5 m long x 0.32 ±0.02 mm ID; 0.25 µm bonded-phase fused-silica capillary column (J&W DB-225, or equivalent).

6.9 Mass Spectrometer

28-40 eV electron impact ionization, shall be capable of repetitively selectively monitoring 12 exact m/z's minimum at high resolution ($10,000) during a period of approximately one second, and shall meet all of the performance specifications listed in Section 9 of this exhibit.

6.10 GC/MS Interface

The mass spectrometer (MS) shall be interfaced to the GC such that the end of the capillary column terminates within 1 cm of the ion source, but does not intercept the electron or ion beams.

6.11 Data System

Capable of collecting, recording, and storing MS data.

7.0 REAGENTS AND STANDARDS

7.1 pH Adjustment and Back-Extraction

7.1.1 Potassium hydroxide—Dissolve 20 g reagent grade KOH in 100 mL reagent water.

7.1.2 Sulfuric acid—Reagent grade (specific gravity 1.84).

7.1.3 Hydrochloric acid, 6N. (1:1) Add 500 mL concentrated HCl to 400 mL reagent water and dilute to one liter (L).

7.1.4 Sodium chloride—solution. Dissolve 50g of reagent grade NaCl to reagent water and dilute to one liter (L).

7.2 Solution Drying and Evaporation

7.2.1 Solution drying—Sodium sulfate, reagent grade, granular, anhydrous (Baker 3375, or equivalent), rinsed with methylene chloride (20 mL/g), baked at 400°C for one hour minimum, cooled in a dessicator, and stored in a pre-cleaned glass bottle with screw-cap that prevents moisture from entering. If, after heating, the sodium sulfate develops a noticeable grayish cast (due to the presence of carbon in the crystal matrix), that batch of reagent is not suitable for use and should be discarded. Extraction with methylene chloride (as opposed to simple rinsing) and baking at a lower temperature may produce sodium sulfate that is suitable for use.

7.2.2 Tissue drying — Sodium sulfate, reagent grade, powdered, treated and stored as described above.

7.2.3 Prepurified nitrogen.

7.3 Extraction

7.3.1 Solvents — Acetone, toluene, cyclohexane, hexane, methanol, methylene chloride, and nonane; distilled in glass, pesticide quality, lot-certified to be free of interferences.

7.3.2 White quartz sand, 60/70 mesh — For Soxhlet/Dean-Stark extraction (Aldrich Chemical, Cat. No. 27-437-9, or equivalent). Bake at 450°C for four hours minimum.

7.4 GPC Calibration Solution

Prepare a solution in methylene chloride containing 300 mg/mL corn oil, 15 mg/mL bis(2-ethylhexyl) phthalate, 1.4 mg/mL pentachlorophenol, 0.1 mg/mL perylene, and 0.5 mg/mL sulfur.

7.5 Adsorbents for Sample Cleanup

7.5.1 Silica gel

7.5.1.1 Activated silica gel — 100-200 mesh, Supelco 1-3651 (or equivalent), rinsed with methylene chloride, baked at 180°C for a minimum of one hour, cooled in a dessicator, and stored in a pre-cleaned glass bottle with screw-cap that prevents moisture from entering.

7.5.1.2 Acid silica gel (30% w/w) — Thoroughly mix 44.0 g of concentrated sulfuric acid with 100.0 g of activated silica gel in a clean container. Break up aggregates with a stirring rod until a uniform mixture is obtained. Store in a bottle with a fluoropolymer-lined screw-cap.

7.5.1.3 Basic silica gel — Thoroughly mix 30 g of 1N sodium hydroxide with 100 g of activated silica gel in a clean container. Break up aggregates with a stirring rod until a uniform mixture is obtained. Store in a bottle with a fluoropolymer-lined screw-cap.

7.5.1.4 Potassium silicate

7.5.1.4.1 Dissolve 56 g of high purity potassium hydroxide (Aldrich, or equivalent) in 300 mL of methanol in a 750-1000 mL flat-bottom flask.

7.5.1.4.2 Add 100 g of silica gel and a stirring bar, and stir on a hot plate at 60-70°C for one to two hours.

7.5.1.4.3 Decant the liquid and rinse the potassium silicate twice with 100 mL portions of methanol, followed by a single rinse with 100 mL of methylene chloride.

7.5.1.4.4 Spread the potassium silicate on solvent-rinsed aluminum foil and dry for two to four hours in a hood.

7.5.1.4.5 Activate overnight at 200-250°C.

7.5.2 Alumina — Either one of two types of alumina, acid or basic, may be used in the cleanup of sample extracts. The same type of alumina must be used for all samples.

7.5.2.1 Acid alumina — Supelco 19996-6C (or equivalent). Activate by heating to 130°C for a minimum of 12 hours.

7.5.2.2 Basic alumina — Supelco 19944-6C (or equivalent). Activate by heating to 600°C for a minimum of 24 hours. Alternatively, activate by heating in a tube furnace at 650-700°C under an air flow rate of approximately 400 cc/minute. Do not heat over 700°C, as this can lead to reduced capacity for retaining the analytes. Store at 130°C in a covered flask. Use within five days of baking.

7.5.3 Carbon

7.5.3.1 Carbopak C — (Supelco 1-0258, or equivalent).

7.5.3.2 Celite 545 — (Supelco 2-0199, or equivalent).

7.5.3.3 Thoroughly mix 9.0 g Carbopak C and 41.0 g Celite 545. Activate the mixture at 130°C for a minimum of six hours. Store in a dessicator.

7.5.4 Anthropogenic isolation column — Pack the column in Section 6.6.4.3 from bottom to top with the following:

- 2 g silica gel (see Section 7.5.1.1);
- 2 g potassium silicate (see Section 7.5.1.4);
- 2 g granular anhydrous sodium sulfate (see Section 7.2.1);
- 10 g acid silica gel (see Section 7.5.1.2), and
- 2 g granular anhydrous sodium sulfate.

7.5.5 Florisil column

7.5.5.1 Florisil—60-100 mesh, Floridin Corp (or equivalent). Soxhlet extract in 500 g portions for 24 hours.

7.5.5.2 Insert a glass wool plug into the tapered end of a graduated serological pipet (se Section 6.6.3.2). Pack with 1.5 g (approximately 2 mL) of Florisil topped with approximately 1 mL of sodium sulfate (see Section 7.2.1) and a glass wool plug.

7.5.5.3 Activate in an oven at 130-150°C for a minimum of 24 hours and cool for 30 minutes. Use within 90 minutes of cooling.

7.6 Reference Matrices

Matrices in which the CDDs/CDFs and interfering compounds are not detected by this method.

7.6.1 Reagent water — Water demonstrated to be free from the analytes of interest and potentially interfering substances.

7.6.2 High-solids reference matrix — Playground sand or similar material. Prepared by extraction with methylene chloride and/or baking at 450°C for a minimum of four hours.

7.6.3 Tissue reference matrix — Corn or other vegetable oil. May be prepared by extraction with methylene chloride.

7.6.4 Other matrices — This method may be verified on any reference matrix that is free of the CDDs/CDFs, and in no case shall the background level of the CDDs/CDFs exceed the CRQLs in Exhibit C.

7.7 Solutions

7.7.1 Standard Solutions - Purchased as solutions or mixtures with certification to their purity, concentration, and authenticity, or prepared from materials of known purity and composition. If the chemical purity is 98% or greater, the weight may be used without correction to compute the concentration of the standard. When not being used, standards are stored in the dark at room temperature in screw-capped vials with fluoropolymer-lined caps. A mark is placed on the vial at the level of the solution so that solvent loss by evaporation can be detected. If solvent loss has occurred, the solution should be replaced.

7.7.2 Stock Solutions

7.7.2.1 Preparation — Prepare in nonane per the steps below or purchase as dilute solutions (Cambridge Isotope Laboratories (CIL), Woburn, MA, or equivalent). Observe the safety precautions outlined in Section 5 of this exhibit, paying close attention to the recommendation in Section 5.1.2.

7.7.2.2 Dissolve an appropriate amount of assayed reference material in solvent. For example, weigh 1-2 mg of 2,3,7,8-TCDD in a 10 mL ground-glass-stoppered volumetric flask and fill to the mark with nonane. After the TCDD is completely dissolved, transfer the solution to a clean 15 mL vial with a fluoropolymer-lined cap.

7.7.2.3 Stock standard solutions should be checked for signs of degradation prior to the preparation of calibration standards.

7.7.3 Native-Compound Stock Solution - All CDDs/CDFs — Using the solutions described in Section 7.7.2, prepare the native-compound stock solution to contain the CDDs/CDFs at the concentrations shown in Table 3.

7.7.4 Labeled-Compound Spiking Solution

7.7.4.1 Prepare this solution from CDDs/CDFs stock solutions, or from purchased mixtures. Prepare this solution to contain the labeled compounds in nonane at the concentrations shown in Table 3. This solution is diluted with acetone prior to use (see Section 7.7.4.2).

7.7.4.2 Dilute a sufficient volume of the labeled compound solution (see Section 7.7.4.1) by a factor of 50 with acetone to prepare a diluted spiking solution. Each sample requires 1 mL of the diluted solution, but no more solution shall be prepared than can be used in one day. The contractor shall provide a standard preparation log documenting the daily preparation of the labeled compound solution. Seal with Teflon tape and mark the meniscus of the solution to reduce and monitor evaporation of acetone.

7.8 Standards

7.8.1 Cleanup Standard - Prepare $^{37}Cl_4$-2,3,7,8-TCDD in nonane at the concentration shown in Table 3. The cleanup standard is added to all extracts prior to cleanup to measure the efficiency of the cleanup process.

7.8.2 Internal Standard(s) - Prepare the internal standard solution to contain $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,3,7,8,9-HxCDD in nonane at the concentration shown in Table 3. The internal standard solution is added to all extracts prior to analysis.

7.8.3 Calibration Standards (CS1 through CS5) — Combine the solutions described in Sections 7.7.3 through 7.8.2 to produce the five calibration solutions shown in Table 4 in nonane. These solutions permit the relative response (labeled to native) and relative response factor to be measured as a function of concentration. The CS3 standard is used for calibration verification.

7.8.4 Laboratory Control Sample Spiking Solution — Used for the LCS/LCSD. Dilute 10 µL of the native-compound stock solution (Section 7.7.3) to 2 mL with acetone for each sample matrix for each sample batch. One mL each are required for the LCS and LCSD with each matrix in each batch.

7.8.5 GC Retention Time Window Defining Solution and Isomer Specificity Check Standard — Used to define the beginning and ending retention times for the dioxin and furan isomers and to demonstrate isomer specificity of the GC columns employed for determination of 2,3,7,8-TCDD and 2,3,7,8-TCDF. The standard must contain the compounds listed in Table 5 (CIL EDF-4006, or equivalent), at a minimum.

7.9 Stability of Solutions

Standard solutions used for quantitative purposes (see Sections 7.7.4 through 7.8.5) should be analyzed periodically, and should be assayed against reference standards (see Section 7.7.2.2) before further use.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Sample Collection and Preservation

8.1.1 Grab and composite samples must be collected in amber glass containers following conventional sampling practices.

8.1.2 Fish and tissue samples collected in the field must be wrapped in aluminum foil, and must be maintained at a temperature of less than -10°C from the time of collection until receipt at the laboratory.

8.2 Procedure for Sample Storage

8.2.1 Maintain water and soil samples at 4°C ± 2 in the dark from the time of collection until extraction. If residual chlorine is present in aqueous samples, add 80 mg sodium thiosulfate per liter of water. EPA Methods 330.4 and 330.5 may be used to measure residual chlorine. If sample pH is greater than 9, adjust to pH 7-9 with sulfuric acid.

8.2.2 Fish and tissue samples received at the laboratory must be maintained at less than -10°C until prepared. Unused samples must be maintained at less than -10°C. Once thawed, tissue samples must be extracted within 24 hours.

8.2.3 Samples must be stored in an atmosphere demonstrated to be free of all potential contaminants.

8.2.4 Samples, sample extracts, and standards must be stored separately.

8.3 Contract Required Holding Times

8.3.1 Water and solid samples (except fish and tissue) shall be extracted within 30 days of the Validated Time of Sample Receipt (VTSR). Fish and tissue samples shall be extracted within one year of VTSR.

8.3.2 Analysis of sample extracts must be completed within 45 days of extraction.

8.3.3 Sample extracts can be stored up to 1 year from date of extraction in-case re-analysis is required.

9.0 CALIBRATION AND STANDARDIZATION

9.1 High Resolution Gas Chromatographic/High Resolution Mass Spectrometric (HRGC/HRMS) Conditions and Data Acquisition Parameters

9.1.1 High-Resolution Gas Chromatograph

9.1.1.1 Prior to analyzing the calibration solutions, blanks, samples, and QC samples, establish the HRGC operating conditions necessary to meet the minimum retention times for the internal standards and the relative retention times for the CDDs/CDFs. Once optimized, the same conditions must be used for the analysis of all standards, samples, blanks, and QC samples.

9.1.1.1.1 Suggested GC operating conditions:

Injector temperature:	270°C
Interface temperature:	290°C
Initial temperature:	200°C
Initial time:	2 minutes
Temperature program:	200 to 220°C, at 5°C/min
	220°C for 16 minutes
	220 to 235°C, at 5°C/min
	235°C for 7 minutes
	235 to 330°C, at 5°C/min

9.1.1.1.2 All portions of the column that connect the HRGC to the ion source shall remain at or above the interface temperature specified above during analysis to preclude condensation of less-volatile compounds.

9.1.2 High-Resolution Mass Spectrometer

9.1.2.1 Prior to analyzing the calibration solutions, blanks, samples, and QC samples, obtain an HRMS Selected Ion Current Profile (SICP), of each analyte at the specified two exact m/z's and at $ 10,000 resolving power by injecting an authentic standard of the CDDs/CDFs, either singly or as part of a mixture in which there is no interference between closely eluted components. The total cycle time for each Selected Ion Monitoring (SIM) descriptor must be # 1 second, including the sum of all the ion dwell times and voltage reset time.

9.1.2.2 The analysis time for CDDs/CDFs may exceed the long-term mass stability of the mass spectrometer. Because the instrument is operated in the high-resolution mode, mass drifts of a few ppm (e.g., 5 ppm in mass) can have serious adverse effects on instrument performance. Therefore, a mass-drift correction is mandatory and a lock-mass m/z from perfluorokerosene (PFK) is used for drift correction. The lock-mass m/z is dependent on the exact m/z's monitored within each descriptor. The level of PFK metered into the HRMS during analyses should be adjusted so that the amplitude of the most intense selected lock-mass m/z signal (regardless of the descriptor number) does not exceed 10% of the

full-scale deflection for a given set of detector parameters. Under those conditions, sensitivity changes that might occur during the analysis can be more effectively monitored.

9.1.2.3 If the HRMS has the capability to monitor resolution during the analysis, it is acceptable to terminate the analysis when the resolution falls below 10,000 to save re-analysis time. The GC run should be complete to allow elution of OCDD and OCDF.

9.2 HRGC/HRMS System Performance Check

9.2.1 Summary of HRGC/HRMS System Performance Check

9.2.1.1 The HRGC/HRMS system performance check consists of three parts: a) the HRMS system must be tuned to meet the minimum static resolving power, using a suitable calibrant such as PFK; the resolution of the HRGC system must be verified by the analyses of b) the Window Defining Mixture (WDM), and c) the isomer specificity check.

NOTE: The WDM and isomer specificity check solutions may be combined into a single solution [Column Performance Solution (CPS)], provided that the combined solution contains the isomers needed to determine that the criteria for analysis are met (see Table 5).

9.2.1.2 At the beginning of each 12-hour shift and prior to analysis of any samples, blanks, QC samples, and calibration standards, the Contractor must establish that the HRGC/HRMS system meets the static resolving power for PFK, that the beginning and ending retention times for the dioxin and furan isomers are defined, and that the isomer specificity is demonstrated.

9.2.1.3 The WDM and the isomer specificity check are also used to set the descriptor switching times such that isomers that elute from the HRGC 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 Contractor may use discretion in setting the switching times. However, <u>do not</u> set descriptor switching times such that a change in descriptors occurs at or near the expected retention times of any 2,3,7,8- substituted isomers.

9.2.2 HRMS System Tune

9.2.2.1 Frequency of HRMS System Tune

9.2.2.1.1 The PFK tune must be performed prior to each 12-hour period during which calibration standards, samples, blanks and QC samples are to be analyzed.

9.2.2.1.2 The 12-hour time period for HRGC/HRMS system performance check, does <u>not</u> begin until the HRMS system is tuned to meet the minimum required resolving power of 10,000 (10% valley) at m/z

304.9824 PFK or any other reference signal close to m/z 304 (from TCDF).

9.2.2.2 Procedure of HRMS System Tune

Using a PFK molecular leak, tune the instrument to meet the minimum requirement in Section 9.2.2.1.2. For each descriptor (see Table 8), monitor and record the resolution and exact m/z's of three to five reference peaks covering the mass range of the descriptor.

NOTE: Excessive PFK (or any other reference substance) may cause noise problems and contamination of the ion source resulting in an increase in time necessary to clean the source.

9.2.2.3 Technical Acceptance Criteria for HRMS System Tune

The HRMS static resolving power must be greater than or equal to 10,000, and the deviation between the exact m/z and the theoretical m/z for each exact m/z monitored must be less than 5 ppm.

9.2.2.4 Corrective Action for HRMS System Tune

9.2.2.4.1 If the technical acceptance criteria are not met, the instrument must be adjusted until the technical acceptance criteria are met.

9.2.2.4.2 Technical acceptance criteria must be met before any standards, samples, QC samples, and required blanks are analyzed. Any analysis conducted when the technical acceptance criteria have not been met will require re-analysis at no additional cost to USEPA.

9.2.3 Window Defining Mixture (WDM)

9.2.3.1 Frequency of WDM

9.2.3.1.1 The WDM must be analyzed as follows:

- After the HRMS PFK tune and before any initial calibration on each instrument and HRGC column used for analysis;
- Once at the beginning of each 12-hour period during which standards or samples are analyzed, and
- Whenever adjustments or instrument maintenance activities are performed that may affect retention times.

9.2.3.1.2 The 12-hour time period for the HRGC/HRMS system performance check and standards calibration (initial or continuing calibration criteria) begins at the moment of injection of the WDM that the laboratory submits as documentation of a compliant

instrument performance check. The time period ends after 12 hours have elapsed according to the system clock.

9.2.3.2 Procedure for WDM

9.2.3.2.1 Analyze a 1 or 2-µL aliquot of the WDM.

9.2.3.2.2 Adjust the descriptor switching times and the HRGC column conditions as needed to ensure that the isomers elute in the appropriate ion descriptors. Table 5 gives the elution order (first/last) of the window defining compounds.

9.2.3.3 Technical Acceptance Criteria for WDM

The analysis of the WDM is acceptable if the separation of all the 2,3,7,8-substituted isomers is demonstrated.

9.2.3.4 Corrective Action for WDM

9.2.3.4.1 If the technical acceptance criteria are not met, the instrument must be adjusted and the test repeated or the HRGC column must be replaced.

9.2.3.4.2 Technical acceptance criteria must be met before any standards, samples, QC samples, and required blanks are analyzed. Any analysis conducted when the technical acceptance criteria have not been met will require re-analysis at no additional cost to USEPA.

9.2.4 Isomer Specificity Check

9.2.4.1 Frequency of Isomer Specificity Check

9.2.4.1.1 The isomer specificity check must be analyzed as follows:

- After, or simultaneously with the WDM and before any initial calibration on each instrument and HRGC column used for analysis;
- Once at the beginning of each 12-hour period during which standards or samples are analyzed, and
- Whenever adjustments or instrument maintenance activities are performed that may affect retention times.

9.2.4.2 Procedure for Isomer Specificity Check

9.2.4.2.1 Analyze a 1 or 2-µL aliquot of the isomer specificity check solution.

9.2.4.2.2 Compute the percent valley between the HRGC peaks that elute most closely to the 2,3,7,8-TCDD and TCDF isomers.

9.2.4.3 Calculations for Isomer Specificity Check

Calculate the valley using the measurements made on the SICP for the appropriate ion for each isomer using the following equation:

EQ. 1

$$\text{Valley} = \left(\frac{X}{Y}\right) * 100$$

Where:

Y = The peak height of 2,3,7,8 TCDD or TCDF isomer; and

X = The height from the valley of least resolved adjacent isomer to baseline.

9.2.4.4 Technical Acceptance Criteria for Isomer Specificity Check

The isomer specificity check is acceptable if the height of the valley between the least resolved adjacent isomer and the 2,3,7,8-substituted isomers is less than or equal to 25 percent.

9.2.4.5 Corrective Action for Isomer Specificity Check

9.2.4.5.1 If the technical acceptance criteria are not met, the instrument must be adjusted and the test repeated or the HRGC column must be replaced.

9.2.4.5.2 Technical acceptance criteria must be met before any standards, samples, QC samples, and required blanks are analyzed. Any analysis conducted when the technical acceptance criteria have not been met will require re-analysis at no additional cost to USEPA.

9.3 Initial Calibration

9.3.1 Summary of Initial Calibration

9.3.1.1 Prior to the analysis of samples and required blanks, and after the HRGC/HRMS system performance check criteria have been met, each HRGC/HRMS system must be calibrated at a minimum of five concentrations to determine instrument sensitivity and the linearity of the HRGC/HRMS response for the target analytes.

9.3.1.2 Calibration by the isotope dilution – Isotope dilution calibration is used for the 15 2,3,7,8-substituted CDDs/CDFs for which labeled compounds are added to samples prior to extraction.

9.3.1.2.1 A calibration curve encompassing the concentration range is prepared for each compound to be determined.

9.3.1.2.2 The response of each CDD/CDF relative to its labeled analog is determined using the area responses of both the primary and secondary exact m/z's specified in Table 8, for each calibration.

9.3.1.3 Calibration by Internal Standard – The internal standard method is applied to the determination of the non 2,3,7,8-substituted CDD/CDF, the labeled compounds, and the cleanup standard.

9.3.1.3.1 The Relative Response Factor (RRF) is determined for each labeled compound, non 2,3,7,8-substituted CDD/CDF and the cleanup standard using equation 3 in Section 9.3.4.2.

9.3.1.4 Combined Calibration – By using calibration solutions containing the native CDDs/CDFs, the labeled compounds, and the internal standards, the single set of analyses can be used to produce calibration curves for the isotope dilution and internal standard methods.

9.3.2 Frequency of Initial Calibration

9.3.2.1 Each HRGC/HRMS system must be calibrated upon award of the contract, whenever the Contractor takes corrective action which may change or affect the initial calibration criteria (e.g., ion source cleaning or repairs, column replacement, etc.), or if the calibration verification technical acceptance criteria are not met.

9.3.2.2 If time still remains in the 12-hour time period after meeting the technical acceptance criteria for the initial calibration, samples may be analyzed. It is not necessary to analyze a calibration verification standard within this 12-hour time period, if the initial calibration standard that is the same concentration as the calibration verification standard meets the calibration verification technical acceptance criteria. Quantitate all sample and quality control sample results against the initial calibration standard that is the same concentration as the calibration verification standard.

9.3.3 Procedure for Initial Calibration

9.3.3.1 Inject a volume of calibration standards CS1 through CS5 identical to the volume and conditions chosen for the HRGC/HRMS system performance check.

9.3.3.2 Acquire Selected Ion Monitoring (SIM) mass spectral data for each analyte. The total cycle time must be # 1 second.

9.3.3.3 Compute the Relative Response (RR) and Relative Response Factor (RRF) for each native and labeled analyte at each concentration level.

9.3.3.4 Determine retention times, signal-to-noise (S/N) ratios, isotopic ratios, and isomer specificity for all calibration standards.

9.3.3.5 Determine linearity of the calibration standards, CS1-CS5, by calculating the percent relative standard deviation (%RSD) over the five-point calibration range.

9.3.4 Calculations for Initial Calibration

9.3.4.1 The response of each CDD/CDF relative to its labeled analog is determined using the area responses of both the primary and secondary exact m/z's (specified in Table 8), for each calibration standard, as follows:

EQ. 2

$$RR = \frac{(A1_N + A2_N)\ C_L}{(A1_L + A2_L)\ C_N}$$

Where:

RR = Relative Response

$A1_N$ and $A2_N$ = The areas of the primary and secondary m/z's for the native compound.

$A1_L$ and $A2_L$ = The areas of the primary and secondary m/z's for the labeled compound.

C_L = The concentration of the labeled compound in the calibration standard.

C_N = The concentration of the native compound in the calibration standard.

9.3.4.2 The Relative Response Factor (RRF) is determined for each labeled compound by the following equation:

EQ. 3

$$RRF = \frac{(A1_S + A2_S)\ C_{IS}}{(A1_{IS} + A2_{IS})\ C_S}$$

Where:

$A1_S$ and $A2_S$ = The areas of the primary and secondary m/z's for the compound to be calibrated.

$A1_{IS}$ and $A2_{IS}$ = The areas of the primary and secondary m/z's for the internal standard.

C_{IS} = The concentration of the internal standard.

C_S = The concentration of the compound in the calibration standard.

9.3.4.3 The mean Response Factor is determined by the following equation:

EQ. 4

$$\text{Mean RR} = \frac{\sum \text{RRi}}{5}$$

Where:

RRi = Relative Response for calibration standard of the initial calibration.

9.3.4.4 The mean Relative Response Factor is determined by the following equation:

EQ. 5

$$\text{Mean RRF} = \frac{\sum \text{RRFi}}{5}$$

Where:

RRFi = Relative Response Factor for calibration standard of the initial calibration.

9.3.4.5 The percent relative standard deviation (%RSD) is determined by the following equation:

EQ. 6

$$\text{\% RSD} = \frac{\text{Standard deviation of calibration response}}{\text{Mean value of calibration response}}$$

9.3.5 Technical Acceptance Criteria for Initial Calibration

9.3.5.1 All initial calibration standards must be analyzed at the concentration level and frequency described.

9.3.5.2 The isomer specificity shall be resolved with a valley of # 25 percent, in all calibration standards.

9.3.5.3 The isotopic ratios must be within the limits specified in Table 9.

9.3.5.4 The signal-to-noise (S/N) ratios for the HRGC/HRMS signal in every SICP must be $ 10.

9.3.5.5 The retention times of the isomers must fall within the appropriate retention time windows established by analysis of the WDM. The absolute retention time of $^{13}C_{12}$-1,2,3,4-TCDD shall exceed 25.0 minutes on the DB-5 column, and the retention time of $^{13}C_{12}$-1,2,3,4-TCDD shall exceed 15.0 minutes on the DB-225 column; otherwise, the GC temperature program shall be adjusted and this test repeated until the above-stated minimum retention time criteria are met.

9.3.5.6 The %RSD for the relative response (RR) must be ± 20% and the %RSD for the relative response factor (RRF) must be ± 35% over the five-point calibration range.

9.3.6 Corrective Action for Initial Calibration

9.3.6.1 If the initial calibration technical acceptance criteria are not met, inspect the system for problems. It may be necessary to change columns, adjust the system, and recalibrate until all the technical acceptance criteria are met.

9.3.6.2 All initial calibration technical acceptance criteria must be met before any samples, QC samples, and required blanks are analyzed. Any analysis conducted when the technical acceptance criteria have not been met will require re-analysis at no additional cost to USEPA.

9.4 Calibration Verification

9.4.1 Summary of Calibration Verification

9.4.1.1 Calibration verification consist of verification of the CS3 relative response (RR) and relative response factor (RRF) used for quantitation.

9.4.2 Frequency of Calibration Verification

9.4.2.1 A CS3 standard must be analyzed once at the beginning of each 12-hour period during which sample data are collected, but after the CPS. The 12-hour time period begins with the injection of the CPS.

9.4.3 Procedure for Calibration Verification

9.4.3.1 Inject 1 or 2-µL of the CS3 calibration standard and measure the SICP areas for the analytes and compute the ion abundance ratios at the exact m/z's. Compare the ratio to the theoretical ratio. Verify that the system meets the ion abundance ratios, the minimum signal-to-noise ratios and retention time criteria. Compute the relative response and relative response factor by the isotope dilution and internal standard methods, respectively. Determine the percent difference between the mean RR/RRF from the initial calibration and the Calibration Verification RR/RRF.

9.4.4 Calculations for Calibration Verification

9.4.4.1 Calculate the Relative Response (RR) and Relative Response Factor (RRF) according to Equations 2 and 3.

9.4.4.2 The percent difference (%D) between the initial calibration and the calibration verification response for each target and labeled analyte is determined by the following:

EQ. 7

$$\%D = \frac{Response_{ver} - Response_{INT}}{Response_{INT}}$$

Where:

$Response_{ver}$ = Response established during Calibration Verification.

$Response_{INT}$ = Mean response established during initial calibration.

9.4.5 Technical Acceptance Criteria for Calibration Verification

9.4.5.1 All CDDs/CDFs in the standard (both native and labeled) must be within their respective ion abundance ratios.

9.4.5.2 The absolute retention time of the internal standard $^{13}C_{12}$-1,2,3,4-TCDD must exceed 25.0 minutes on the DB-5 column, and the retention time must exceed 15.0 minutes on the DB-225 column. In addition, the absolute retention time of the internal standards must be within ± 15 seconds of the retention times obtained during initial calibration.

9.4.5.3 The relative retention times of the native and labeled CDDs/CDFs shall be within the limits defined in Table 2.

9.4.5.4 The %D between the calibration verification RR and the mean RR from the initial calibration must be within ± 20%. The %D between

the calibration verification RRF and the mean RRF from the initial calibration must be within ± 35%.

9.4.5.5 The peaks representing both native and labeled analytes in the CS3 standard must have a signal-to-noise (S\N) ratio greater than or equal to 10.0.

9.4.6 Corrective Action for Calibration Verification

9.4.6.1 If the calibration verification technical acceptance criteria are not met, inspect the system for problems. It may be necessary to change columns, adjust the system, recalibrate with fresh calibration standards.

9.4.6.2 Calibration Verification technical acceptance criteria must be met before any samples, QC samples, and required blanks are analyzed. Any analysis conducted when the technical acceptance criteria have not been met will require re-analysis at no additional cost to USEPA.

9.5 Analytical Sequence

Time	**Analysis**
Hour 0 Start of First 12-Hour Period	PFK HRMS Tune Window Defining Mixture (WDM) Isomer Specificity Check (ISC) Note: WDM can be combined with the ISC as a Column Performance Solution (CPS) CS3 CS1 (initial calibration) CS2 CS4 CS5 Blanks, LCSs, Samples (if time still remains on the 12-hour clock)
Beginning of Next 12-Hour Period	PFK HRMS Tune Column Performance Solution (CPS) CS3 Blanks, LCSs, Samples

10.0 PROCEDURE

10.1 Sample Preparation

10.1.1 Sample preparation involves modifying the physical form of the sample so that the CDDs/CDFs can be extracted efficiently. In general, the samples must be in a liquid form or in the form of finely divided solids in order for efficient extraction to take place. Table 10 lists the phases and suggested quantities for extraction of various sample matrices.

10.1.2 Multiphase/Insufficient Samples

10.1.2.1 If multiphase samples (e.g., a two-phase liquid sample) are received by the Contractor, the Contractor must contact SMO to apprise them of the type of sample received. SMO will contact the Region. If all phases of the sample are amenable to analysis, the Region may require the Contractor to do the following:

- Mix the sample and analyze an aliquot from the homogenized sample.
- Separate the phases of the sample and analyze each phase individually. SMO will provide EPA sample numbers for the additional phases.
- Separate the phases and analyze one or more of the phases, but not all of the phases. SMO will provide EPA sample numbers for the additional phases, if required.
- Not analyze the sample.

10.1.2.2 If an insufficient sample amount (less than the required amount) is received to perform the analyses, the Contractor must contact the Sample Management Office (SMO) to apprise them of the problem. SMO will contact the Region for instructions. The Region will either require that no sample analyses be performed, or a reduced volume be used for the sample analysis. No other changes in the analyses will be permitted. The Contractor must document the Region's decision in the SDG narrative.

10.1.3 Water Samples

Water samples visibly absent of particles are extracted directly using the separatory funnel (Section 10.2.1) or Solid Phase Extraction (SPE) technique (Section 10.2.2). Water samples containing visible particles and containing one percent suspended solids or less are extracted using the SPE technique followed by Soxhlet/Dean-Stark (SDS) extraction (Section 10.2.3) or are filtered using the procedure in Section 10.1.3.1.6. After filtration, the particles and filter are extracted using the SDS procedure (Section 10.2.3) and the filtrate is extracted using the separatory funnel procedure (Section 10.2.1). For aqueous samples containing greater

than one percent solids, a sample aliquot sufficient to provide 10 g of dry solids is used, as described in Section 10.1.4.2.

10.1.3.1 Preparation of Water Samples

10.1.3.1.1 Mark the original level of the sample on the sample bottle for reference. Weigh the sample plus bottle to ±1g.

10.1.3.1.2 Spike 1.0 mL of the diluted labeled compound spiking solution (Section 7.7.4.2) into the sample bottle. Cap the bottle and mix the sample by careful shaking. Allow the sample to equilibrate for 1 to 2 hours, with occasional shaking.

10.1.3.1.3 For each sample or sample batch (to a maximum of 20 samples) to be extracted during the same 12-hour shift, place three 1.0-L aliquots of reagent water in clean sample bottles or flasks.

10.1.3.1.4 Spike 1.0 mL of the diluted labeled compound spiking solution (Section 7.7.4.2) into the three reagent water aliquots. One of these aliquots will serve as the blank and two will serve as the LCS/LCSD. Spike the LCS/LCSD with 1.0 mL of the laboratory control sample spiking solution (see Section 7.8.4).

10.1.3.1.5 If SPE is to be used, add 5 mL of methanol to the sample, cap and shake the sample to mix thoroughly, and proceed to Section 10.2.2 for extraction. If SPE is not to be used, and the sample is visibly absent of particles, proceed to Section 10.2.1 for extraction. If SPE is not to be used and the sample contains visible particles, proceed to the following section for filtration of particles.

10.1.3.1.6 Filtration of particles

10.1.3.1.6.1 Assemble a Buchner funnel on top of a clean filtration flask. Apply vacuum to the flask, and pour the entire contents of the sample bottle through a glass fiber filter in the Buchner funnel, swirling the sample remaining in the bottle to suspend any particulate.

10.1.3.1.6.2 Rinse the sample bottle twice with approximately 5 mL portions of reagent water to transfer any remaining particulate onto the filter.

10.1.3.1.6.3 Rinse any particulate off the sides of the Buchner funnel with small quantities of reagent water.

10.1.3.1.6.4 Weigh the empty sample bottle to ±1g. Determine the weight of the sample by difference. Save the bottle for further use.

10.1.3.1.6.5 Extract the filtrates using the separatory funnel procedure in Section 10.2.1.

10.1.3.1.6.6 Extract the filter containing the particulate using the SDS procedure in Section 10.2.3.

10.1.4 Soil/sediment Samples

Mix samples thoroughly, especially composite samples. Discard any foreign objects such as sticks, leaves, and rocks. Also, decant and discard any standing water phase.

10.1.4.1 Determination of Percent Solids.

Weigh 5 to 10 g of sample to three significant figures into a tared beaker. This aliquot is used for determining the percent solids content of the sample, not for analysis of CDDs/CDFs. Dry for a minimum of 12 hours at 110 ± 5°C, and cool in a desiccator. Calculate percent solids as follows:

EQ.8

$$\% \text{ solids} = \frac{\text{weight of sample aliquot after drying}}{\text{weight of sample aliquot before drying}} * 100$$

10.1.4.2 Preparation of Soil/sediment Samples

10.1.4.2.1 Weigh a well-mixed aliquot of each sample (of the same matrix type) sufficient to provide 10 g of dry soil/sediments (based on the soil/sediments determination in Section 10.1.4.1) into a clean beaker or glass jar. Record sample weight to the nearest 0.01 g.

10.1.4.2.2 Spike 1.0 mL of the diluted labeled compound spiking solution (see Section 7.7.4.2) into the sample.

10.1.4.2.3 For each sample or sample batch (to a maximum of 20 samples) to be extracted during the same 12-hour shift, weigh three 10-g aliquots of the appropriate reference matrix into clean beakers or glass jars.

10.1.4.2.4 Spike 1.0 mL of the diluted labeled compound solution into each reference matrix aliquot. One aliquot will serve as the blank and two will serve as the LCS/LCSD. Spike the LCS/LCSD with 1.0 mL of the laboratory control sample spiking solution (see Section 7.8.4).

10.1.4.2.5 Stir or tumble and equilibrate the aliquots for 1 to 2 hours.

10.1.4.2.6 Decant excess water. If necessary to remove water, filter the sample through a glass fiber filter and discard the water liquid.

10.1.4.2.7 If particles greater than 1 mm are present in the sample, spread the sample on clean aluminum foil. After the sample is dry, grind to reduce the particle size.

10.1.4.2.8 Extract the sample and aliquots using the SDS procedure in Section 10.2.3.

10.1.5 Tissue Samples

Prior to processing tissue samples, the laboratory must determine the exact tissue to be analyzed. Common requests for analysis of fish tissue include whole fish—skin on, whole fish—skin removed, edible fish fillets (filleted in the field or by the laboratory), specific organs, and other portions. Once the appropriate tissue has been determined, the sample must be homogenized.

10.1.5.1 Homogenization.

10.1.5.1.1 Each analysis requires 10g of tissue (wet weight). Therefore, the laboratory should homogenize at least 20 g of tissue to allow for re-extraction of a second aliquot of the same homogenized sample, if re-analysis is required. When whole fish analysis is necessary, the entire fish is homogenized.

10.1.5.1.2 Homogenize the sample in a tissue homogenizer or grind in a meat grinder. Cut tissue that is too large to feed into the grinder into smaller pieces. To assure homogeneity, grind three times.

10.1.5.2 Preparation of Tissue Samples

10.1.5.2.1 Transfer approximately 10 g (wet weight) of homogenized tissue to a clean, tared, 400 to 500 mL beaker. For the alternate HCl digestion/extraction, transfer the tissue to a clean, tared 500 to 600 mL wide-mouth bottle. Record the weight to the nearest 0.01 g.

10.1.5.2.2 Prepare the blank and LCS/LCSD by adding approximately 10 g of the tissue reference matrix to 400 to 500-mL beakers. For the alternate HCl digestion/extraction, add the reference matrix to a 500 to 600-mL wide-mouth bottles. Record the weight to the nearest 0.01 g.

10.1.5.2.3 Spike 1.0 mL of the labeled compound spiking solution diluted in acetone (see Section 7.7.4.2) into the samples, blank and LCS/LCSD. Spike the LCS/LCSD with 1.0 mL of the laboratory control sample spiking solution (see Section 7.8.4).

10.1.5.2.4 Extract the aliquots using the Soxhlet extraction or HCL digestion/extraction and concentration procedures in Section 10.2.4 and 10.2.5.

10.1.6 Oily Samples

Prior to processing oily samples, the laboratory must determine the exact nature of the samples to be analyzed. Oily samples could be oily soils, oily sludges, wet fuel oil, or pure oil. Mix samples thoroughly, especially composite samples. Discard any foreign

objects such as sticks, leaves, and rocks. Decant and discard any standing water phase.

10.1.6.1 Weigh 2 grams of sample into a clean beaker or glass jar. Record the sample weight to the nearest 0.01 g.

10.1.6.2 Spike 1.0 mL of the diluted labeled compound spiking solution (see Section 10.1.3.1.2) into the sample.

10.1.6.3 For each sample or sample batch (to a maximum of 20 samples to be extracted during the same 12-hour shift), weigh 2-g aliquots of the appropriate reference matrix into clean beakers or glass jars.

10.1.6.4 Spike 1.0 mL of the diluted labeled compound spiking solution into each reference matrix aliquot. One aliquot will serve as the blank and two will serve as the LCS/LCSD. Spike the LCS/LCSD with 1.0 mL of the laboratory control sample spiking solution (see Section 7.8.4).

10.1.6.5 Stir or tumble and equilibrate the aliquots for 1 to 2 hours.

10.1.6.6 Decant excess water. If necessary to remove water, filter the sample through a glass fiber filter and discard the water liquid.

10.1.6.7 If particles > 1mm are present in the sample, spread the sample on clean aluminum foil. After the sample is dry, grind to reduce particle size.

10.1.6.8 Extract the sample and aliquots using the SDS procedure in Section 10.2.3.

10.2 Extraction

Extraction procedures include separatory funnel (Section 10.2.1) and SPE (see Section 10.2.2) for waters, Soxhlet/Dean-Stark (see Section 10.2.3) for soil/sediments, and Soxhlet extraction (Section 10.2.4) and HCl digestion (see Section 10.2.5) for tissues. Acid/ base back-extraction (see Section 10.2.6) is used for initial cleanup of extracts.

10.2.1 Separatory Funnel Extraction

10.2.1.1 Pour the spiked sample (see Section 10.1.3.1.2) or filtrate (Section 10.1.3.1.6.5) into a 2-L separatory funnel. Rinse the bottle or flask twice with 5 mL of reagent water and add these rinses to the separatory funnel.

10.2.1.2 Add 60 mL methylene chloride to the sample bottle (see Sections 10.1.3.1.1 or 10.1.3.1.6.5), 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. If an emulsion forms and is more than one-third the volume of the solvent layer, employ mechanical techniques to complete the phase separation. Drain the

methylene chloride extract through a solvent-rinsed glass funnel that is approximately one-half full with granular anhydrous sodium sulfate supported on clean glass fiber paper into a solvent-rinsed concentration device (see Section 10.3).

10.2.1.3 Extract the water sample two more times with 60 mL portions of methylene chloride. Drain each portion through the sodium sulfate into the concentrator. After the third extraction, rinse the separatory funnel with at least 20 mL of methylene chloride, and drain this rinse through the sodium sulfate into the concentrator. Repeat this rinse at least twice. Set aside the funnel with sodium sulfate if the extract is to be combined with the extract from the particulate.

10.2.1.4 Concentrate the extract using one of the macro-concentration procedures (see Section 10.3).

10.2.1.4.1 Adjust the final volume of the concentrated extract to approximately 10 mL with hexane, transfer to a 250-mL separatory funnel, and back-extract using the procedure in Section 10.2.6.

10.2.1.4.2 If the extract is from the water filtrate (see Section 10.1.3.1.6.5), set aside the K-D apparatus for addition of the SDS extract from the particulate (see Section 10.2.3.9).

10.2.2 Solid Phase Extraction (SPE)

10.2.2.1 Disk preparation.

10.2.2.1.1 Place a 90 mm glass fiber filter on top of a 90 mm SPE disk on the glass frit support of a clean filtration apparatus and wet with toluene. Clamp the filter and SPE disk between a 1-L glass reservoir and a 2-L vacuum filtration flask.

10.2.2.1.2 Rinse the sides of the filtration flask with approximately 15 mL of toluene using a squeeze bottle or syringe. Apply vacuum momentarily until a few drops appear at the drip tip. Release the vacuum and allow the filter/disk to soak for approximately one minute. Apply vacuum and draw all of the toluene through the filter/disk. Repeat the wash step with approximately 15 mL of acetone and allow the filter/disk to air dry.

10.2.2.1.3 Re-wet the filter/disk with approximately 15 mL of methanol, allowing the filter/disk to soak for approximately one minute. Pull the methanol through the filter/disk using the vacuum, but retain a layer of methanol (approximately 1 mm thick) on the filter. Do not allow the disk to go dry from this point until the end of the extraction.

10.2.2.1.4 Rinse the filter/disk with two 50 mL portions of reagent water by adding the water to the reservoir and pulling most through, leaving a layer of water on the surface of the filter.

10.2.2.2 Extraction

10.2.2.2.1 Add the spiked sample (see Section 10.1.3.1.2), blank (see Section 10.1.3.1.4) or LCS/LCSD to the reservoir and turn on the vacuum to begin the extraction. Adjust the vacuum to complete the extraction in no less than 10 minutes. For samples containing high particulate (suspended solids), filtration times may be 8 hours or longer.

10.2.2.2.2 If the filter clogs with particles and more rapid extraction is desired, replace the filter during the extraction by pulling the sample in the reservoir into the sample bottle to the level of the filter/disk (keeping the disk wet), removing the clamp and reservoir, and carefully removing the filter with tweezers. Place the filter in a clean glass Petri dish and cover. Label with the sample ID. Reassemble the apparatus with a clean filter and proceed with the extraction. Pull the sample through the filter/disk, leaving a layer of water on the surface of the filter.

10.2.2.2.3 Rinse the sample bottle with approximately 50 mL of reagent water to remove any soil/sediments, and pour into the reservoir. Pull through the filter/disk. Use additional reagent water rinses until all visible soil/sediments are removed.

10.2.2.2.4 After the sample and rinses have passed through the filter/disk, rinse the sides of the reservoir with small portions of reagent water. Allow the filter/disk to dry, then remove the filter and disk and place in the glass Petri dish. Extract the filter(s) and disk per the SDS extraction procedure in Section 10.2.3.

10.2.3 Soxhlet/Dean-Stark Extraction (SDS)

10.2.3.1 Charge a clean extraction thimble with 5.0 g of 100/200-mesh silica topped with 100 g of quartz sand.

NOTE: Do not disturb the silica layer throughout the extraction process.

10.2.3.2 Place the thimble in a clean extractor. Place 30 to 40 mL of toluene in the receiver and 200 to 250 mL of toluene in the flask.

10.2.3.3 Pre-extract the glassware by heating the flask until the toluene is boiling. When properly adjusted, 1 to 2 drops of toluene per second will fall from the condenser tip into the receiver. Extract the apparatus for a minimum of 3 hours.

10.2.3.4 After pre-extraction, cool and disassemble the apparatus. Rinse the thimble with toluene and allow to air dry.

10.2.3.5 Load the wet sample from Sections 10.1.3.1.6.6 and 10.1.4.2.8 and any non-water liquid into the thimble and manually mix into the

sand layer with a clean metal spatula, carefully breaking up any large lumps of sample. If the material to be extracted is the particulate matter from the filtration of an water sample, or the filter(s) and disk from the SPE extraction, add these items to the thimble also.

10.2.3.6 Re-assemble the pre-extracted SDS apparatus and add a fresh charge of toluene to the receiver and reflux flask. Apply power to the heating mantle to begin refluxing. Adjust the reflux rate to match the rate of percolation through the sand and silica beds until water removal lessens the restriction to toluene flow. Frequently check the apparatus for foaming during the first 2 hours of extraction. If foaming occurs, reduce the reflux rate until foaming subsides.

10.2.3.7 Drain the water from the receiver at 1 to 2 hours and 8 to 9 hours, or sooner if the receiver fills with water. Reflux the sample for a total of 16 to 24 hours. Cool and disassemble the apparatus. Record the total volume of water collected.

10.2.3.8 Remove the distilling flask. Drain the water from the Dean-Stark receiver and add any toluene in the receiver to the extract in the flask.

10.2.3.9 Concentrate the extract using the procedures in Section 10.3, as follows:

10.2.3.9.1 Extracts from the particulate portion of a water sample containing less than one percent solids (see Section 10.1.3.1.6.6).

10.2.3.9.1.1 Concentrate the extract to approximately 5 mL using the heating mantle or rotary evaporation procedures in Sections 10.3.2 or 10.3.1.

10.2.3.9.1.2 Quantitatively transfer the extract through the sodium sulfate (see Section 10.2.1.3) into the apparatus that was set aside (see Section 10.2.1.4.2) and reconcentrate to the level of the toluene.

10.2.3.9.1.3 Adjust to approximately 10 mL with hexane, transfer to a 250 mL separatory funnel, and proceed with back-extraction (see Section 10.2.6).

10.2.3.9.2 Extracts from soil/sediments (Section 10.1.4) or from the SPE filter(s) and disk—Concentrate to approximately 10 mL using the rotary evaporator or heating mantle (see Sections 10.3.1 or 10.3.2), transfer to a 250 mL separatory funnel, and proceed with back-extraction (see Section 10.2.6).

10.2.4 Soxhlet Extraction.

10.2.4.1 Add 30 to 40 g of powdered anhydrous sodium sulfate to each of the beakers (see Section 10.1.5.2.1) and mix thoroughly. Cover the

beakers with aluminum foil and allow to stand 12-24 hours. Remix prior to extraction to prevent clumping.

10.2.4.2 Assemble and pre-extract the Soxhlet apparatus per Section 10.2.3, however, use the methylene chloride:hexane (1:1) mixture for the pre-extraction and rinsing and omit the quartz sand. The Dean-Stark moisture trap may also be omitted, if desired.

10.2.4.3 Re-assemble the pre-extracted Soxhlet apparatus and add a fresh charge of methylene chloride:hexane to the reflux flask.

10.2.4.4 Transfer the sample/sodium sulfate mixture to the Soxhlet thimble and install the thimble in the Soxhlet apparatus.

10.2.4.5 Rinse the beaker with several portions of solvent mixture and add to the thimble. Fill the thimble/receiver with solvent. Extract for 18 to 24 hours.

10.2.4.6 After extraction, cool and disassemble the apparatus.

10.2.4.7 Quantitatively transfer the extract to a macro-concentration device and concentrate to near dryness. Set aside the apparatus for re-use.

10.2.4.8 Complete the removal of the solvent using the blowdown procedure (see Section 10.4.7) and a water bath temperature of 60°C. Weigh the receiver, record the weight, and return the receiver to the blowdown apparatus, concentrating the residue until a constant weight is obtained.

10.2.4.9 Percent Lipid determination

The lipid content is determined by extracting of tissue with the same solvent system (methylene chloride:hexane) that was used in USEPA's National Dioxin Study so that lipid contents are consistent with that study.

10.2.4.9.1 Redissolve the residue in the concentration device in hexane and spike 1.0 mL of the cleanup standard into the extract.

10.2.4.9.2 Transfer the residue/hexane to the anthropogenic isolation column or the narrow-mouth 100 to 200 mL bottle, retaining the boiling chips in the K-D receiver. Use several rinses to assure that all material is transferred to a maximum hexane volume of approximately 70 mL. If necessary, sonicate or heat the receiver slightly to assure that all material is re-dissolved. Allow the receiver to dry. Weigh the receiver and boiling chips.

10.2.4.9.3 Calculate the lipid content to the nearest three significant figures using the following equation:

EQ. 9

$$\text{Percent lipid} = \frac{\text{Weight of residue (g)}}{\text{Weight of tissue (g)}} * 100$$

10.2.4.9.4 It is not necessary to determine the lipid content of the blank, LCS and LCSD aliquots.

10.2.5 HCl Digestion/Extraction

10.2.5.1 Add 200 mL of 6-N HCl and 200 mL of methylene chloride:hexane (1:1) to the sample and QC aliquots (see Section 10.1.5.2).

10.2.5.2 Cap and shake each bottle 1 to 3 times. Loosen cap in a hood to vent excess pressure. Cap and shake each bottle for 10 to 30 seconds. Loosen cap in a hood to vent excess pressure.

10.2.5.3 Tightly cap and place on shaker. Adjust the shaker action and speed so that the acid, solvent, and tissue are in constant motion. However, take care to avoid such violent action that the bottle may be dislodged from the shaker. Shake for 12 to 24 hours.

10.2.5.4 After digestion, remove the bottles from the shaker. Allow the bottles to stand so that the solvent and acid layers separate.

10.2.5.5 Decant the solvent through a glass funnel with glass fiber filter containing approximately 10 g of granular anhydrous sodium sulfate into a macro-concentration apparatus (see Section 10.3). Extract the contents of the bottle with two 25 mL portions of hexane and pour through the sodium sulfate into the apparatus.

10.2.5.6 Concentrate the solvent to near dryness using a macro-concentration procedure (see Section 10.3).

10.2.5.7 Complete the removal of the solvent using the blowdown apparatus (see Section 10.4.7) and a water bath temperature of 60°C. Weigh the receiver, record the weight, and return the receiver to the blowdown apparatus, concentrating the residue until a constant weight is obtained.

10.2.5.8 Percent lipid determination

Determine the lipid content per the procedure described in Section 10.2.4.9.

10.2.5.9 Clean up the extract per Section 10.5.

10.2.6 Back extraction with base and acid

10.2.6.1 Spike 1.0 mL of the cleanup standard into the separatory funnels containing the sample and QC extracts (see Sections 10.2.1.4.1, or 10.2.3.9.1.3).

10.2.6.2 Partition the extract against 50 mL of potassium hydroxide solution (see Section 7.1.1). Shake for 2 minutes with periodic venting into a hood. Remove and discard the water layer. Repeat the base washing until no color is visible in the water layer, to a maximum of four washings. Minimize contact time between the extract and the base to prevent degradation of the CDDs/CDFs. Stronger potassium hydroxide solutions may be employed for back extraction, provided that the laboratory meets the specifications for labeled compound recovery.

10.2.6.3 Partition the extract against 50 mL of sodium chloride solution in the same way as with base. Discard the water layer.

10.2.6.4 Partition the extract against 50 mL of sulfuric acid in the same way as with base. Repeat the acid washing until no color is visible in the water layer, to a maximum of four washings.

10.2.6.5 Repeat the partitioning against sodium chloride solution and discard the water layer.

10.2.6.6 Pour each extract through a drying column containing 7 to 10 cm of granular anhydrous sodium sulfate. Rinse the separatory funnel with 30 to 50 mL of solvent and pour through the drying column. Collect each extract in a round-bottom flask. Reconcentrate the sample and QC aliquots per Sections 10.3 - 10.4, and clean up the samples and QC aliquots per Section 10.5.

10.3 Macro-Concentration

Extracts in toluene are concentrated using a rotary evaporator or a heating mantle. Extracts in methylene chloride or hexane are concentrated using a rotary evaporator, heating mantle, or Kuderna-Danish apparatus.

10.3.1 Rotary Evaporation

Concentrate the extracts in separate round-bottom flasks.

10.3.1.1 Assemble the rotary evaporator according to manufacturer's instructions, and warm the water bath to 45°C. On a daily basis, pre-clean 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 a contamination check if necessary. Between samples, three 2 to 3-mL aliquots of solvent should be rinsed down the feed tube into a waste beaker.

10.3.1.2 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.

10.3.1.3 Lower the flask into the water bath and adjust the speed of rotation and the temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of

concentration, the flow of solvent into the receiving flask will be steady, but no bumping or visible boiling of the extract will occur.

NOTE: If the rate of concentration is too fast, analyte loss may occur.

10.3.1.4 When the liquid in the concentration flask has reached an apparent volume of approximately 2 mL, remove the flask from the water bath and stop the rotation. Slowly and carefully admit air into the system. Be sure not to open the valve so quickly that the sample is blown out of the flask. Rinse the feed tube with approximately 2 mL of solvent.

10.3.1.5 Transfer the extract to a vial using three 2 to 3 mL rinses of solvent. Proceed to Section 10.2.6 for back-extraction with base and acid or to Section 10.4 for micro-concentration and solvent exchange.

10.3.2 Heating Mantle

Concentrate the extracts in separate round-bottom flasks.

10.3.2.1 Add one or two clean boiling chips to the round-bottom flask and attach a three-ball macro Snyder column. Pre-wet the column by adding approximately 1 mL of solvent through the top. Place the round-bottom flask in a heating mantle and apply heat as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood.

10.3.2.2 When the liquid has reached an apparent volume of approximately 10 mL, remove the round-bottom flask from the heating mantle and allow the solvent to drain and cool for at least 10 minutes. Remove the Snyder column and rinse the glass joint into the receiver with small portions of solvent.

10.3.2.3 Proceed to Section 10.2.6 for back-extraction with base and acid or to Section 10.4 for micro-concentration and solvent exchange.

10.3.3 Kuderna-Danish

Concentrate the extracts in separate 500 mL K-D flasks equipped with 10 mL concentrator tubes.

10.3.3.1 Add 1 to 2 clean boiling chips to the receiver. Attach a three-ball macro Snyder column. Prewet the column by adding approximately 1 mL of solvent (methylene chloride or hexane, as appropriate) through the top. Place the K-D apparatus in a hot water bath so that the entire lower rounded surface of the flask is bathed with steam.

10.3.3.2 Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20

minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood.

10.3.3.3 When the liquid has reached an apparent volume of 1 mL, remove the K-D apparatus from the bath and allow the solvent to drain and cool for at least 10-minutes. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride. A 5 mL syringe is recommended for this operation.

10.3.3.4 Remove the three-ball Snyder column, add a fresh boiling chip, and attach a two-ball micro Snyder column to the concentrator tube. Pre-wet the column by adding approximately 0.5 mL of solvent through the top. Place the apparatus in the hot water bath.

10.3.3.5 Adjust the vertical position and the water temperature as required to complete the concentration in 5 to 10 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood.

10.3.3.6 When the liquid reaches an apparent volume of 0.5 mL, remove the apparatus from the water bath and allow to drain and cool for at least 10 minutes.

10.3.3.7 Proceed to Section 10.2.6 for back-extraction with base and acid or to Section 10.4 for micro-concentration and solvent exchange.

10.4 Micro-Concentration and Solvent Exchange

10.4.1 Extracts to be subjected to GPC or HPLC cleanup are exchanged into methylene chloride. Extracts that are to be cleaned up using silica gel, alumina, carbon and/or florisil are exchanged into hexane.

10.4.2 Transfer the vial containing the sample extract to a nitrogen evaporation device. Adjust the flow of nitrogen so that the surface of the solvent is just visibly disturbed.

Note: A large vortex in the solvent may cause analyte loss.

10.4.3 Lower the vial into a 45°C water bath and continue concentrating.

10.4.4 When the volume of the liquid is approximately 100 µL, add 2 to 3 mL of the desired solvent (methylene chloride or hexane) and continue concentration to approximately 100 µL. Repeat the addition of solvent and concentrate once more.

10.4.5 If the extract is to be cleaned up by GPC or HPLC, adjust the volume of the extract to 5.0 mL with methylene chloride. Proceed with GPC cleanup (see Section 10.5.1).

10.4.6 If the extract is to be cleaned up by column chromatography (alumina, silica gel, Carbopak/Celite), bring the final volume to 1.0 mL with hexane. Proceed with column cleanups (see Sections 10.5.2 through 10.5.5).

10.4.7 For extracts to be concentrated for injection into the HRGC/HRMS, quantitatively transfer the extract to a 0.3 mL conical vial for final concentration, rinsing the larger vial with hexane and adding the rinse to the conical vial. Reduce the volume to approximately 100 µL. Add 10 µL of nonane to the vial, and evaporate the solvent to the level of the nonane. Seal the vial and label with the sample number. Store in the dark at room temperature until ready for HRGC/HRMS analysis.

10.4.8 For extracts to be concentrated to dryness for weight determination, blow dry until a constant weight is obtained.

10.5 Cleanup

Cleanup may not be necessary for relatively clean samples (e.g., treated effluents, groundwater, drinking water). If particular circumstances require the use of a cleanup procedure, the analyst may use any or all of the following procedures or any other appropriate procedure. Before using a cleanup procedure, the analyst must demonstrate that the requirements for the cleanup standard listed in Table 7 are met using the cleanup procedure. GPC (see Section 10.5.1) removes many high molecular weight interferences that cause GC column performance to degrade. It must be used for all soil and sediment extracts and may be used for water extracts that are expected to contain high molecular weight organic compounds (e.g., polymeric materials, humic acids). Acid, neutral, basic silica gel, alumina and florisil (see Sections 10.5.2, 10.5.3 and 10.5.7) are used to remove non-polar and polar interferences. Alumina and florisil are used to remove chlorodiphenyl ethers. Carbopak/Celite (see Section 10.5.4) is used to remove nonpolar interferences. HPLC (see Section 10.5.5) is used to provide specificity for the 2,3,7,8-substituted and other CDD and CDF isomers. The anthropogenic isolation column (see Section 10.5.6.1), acidified silica gel batch adsorption procedure (see Section 10.5.6.2), and sulfuric acid back extraction (see Section 10.5.6.3) are used for removal of lipids from tissue samples.

10.5.1 Sample Cleanup by Gel permeation chromatography (GPC)

10.5.1.1 Introduction

GPC is a size exclusion cleanup procedure using organic solvents and hydrophobic gels in the separation of natural (and synthetic) macromolecules. The packing gel is porous and is characterized by the range or uniformity (exclusion range) of that pore size. In the choice of gels, the exclusion range must be larger than the molecular size of the molecules to be separated.

10.5.1.2 Column packing

10.5.1.2.1 Place 70 to 75 g of SX-3 Bio-beads in a 400 to 500 mL beaker.

10.5.1.2.2 Cover the beads with methylene chloride and allow to swell overnight (for a minimum of 12 hours).

10.5.1.2.3 Transfer the swelled beads to the column and pump solvent through the column, from bottom to top, at 4.5 to 5.5 mL/min prior to connecting the column to the detector.

10.5.1.2.4 After purging the column with solvent for 1 to 2 hours, adjust the column head pressure to 7 to 10 psig and purge for 4 to 5 hours to remove air. Maintain a head pressure of 7 to 10 psig. Connect the column to the detector.

10.5.1.3 Calibration of GPC

10.5.1.3.1 Summary of Calibration

The GPC calibration procedure is based on monitoring the elution of standards with a UV detector connected to the GPC column.

10.5.1.3.2 Frequency of GPC Calibration

Each GPC system must be initially calibrated upon award of a contract, when the column is changed, when channeling occurs, and once every seven days when samples, including matrix spikes, duplicates, and blanks, are cleaned up using GPC.

10.5.1.3.3 Procedure for GPC Calibration

10.5.1.3.3.1 Load 5 mL of the GPC calibration solution into the sample loop.

10.5.1.3.3.2 Inject the calibration solution and record the signal from the detector. The elution pattern will be corn oil, bis (2-ethyl hexyl) phthalate, pentachlorophenol, perylene, and sulfur.

10.5.1.3.3.3 Set the "dump time" to allow >85% removal of the corn oil and >85% collection of the phthalate.

10.5.1.3.3.4 Set the "collect time" to the peak minimum between perylene and sulfur.

10.5.1.3.4 Technical Acceptance Criteria for GPC Calibration

10.5.1.3.4.1 The GPC system must be calibrated at the frequency described in Section 10.5.1.3.2.

10.5.1.3.4.2 Verify the calibration with the calibration solution after every 20 extracts.

10.5.1.3.4.3 Calibration is verified if the recovery of the pentachlorophenol is greater than 85%.

10.5.1.3.5 Corrective Action for GPC Calibration

10.5.1.3.5.1 If calibration does not meet the technical acceptance criteria, the system shall be re-calibrated using the calibration solution, and the previous 20 samples shall be re-extracted and cleaned up using the calibrated GPC system.

10.5.1.4 Sample Extract Cleanup by GPC

GPC requires that the column not be overloaded. The column specified in this method is designed to handle a maximum of 0.5 g of high molecular weight material in a 5 mL extract. If the extract is known or expected to contain more than 0.5 g, the extract is split into aliquots for GPC and the aliquots are combined after elution from the column. The residue content of the extract may be obtained gravimetrically by evaporating the solvent from a 50-µL aliquot.

10.5.1.4.1 Frequency of GPC Sample Cleanup

GPC cleanup must be performed once for each soil/sediment extract and for water extracts that contain high molecular weight contaminants that interfere with the analysis of the target analytes. In addition, GPC must be performed for all associated blanks, duplicates, and LCS/LCSD. If the cleanup procedure is inadequate, contact SMO.

10.5.1.4.2 Procedure for GPC Sample Cleanup

10.5.1.4.2.1 Filter the extract or load through the filter holder to remove any particulate. Load the 5.0 mL extract onto the column.

10.5.1.4.2.2 Elute the extract using the calibration data determined in Section 10.5.1.3. Collect the eluate in a clean 400 to 500-mL beaker.

10.5.1.4.2.3 Rinse the sample loading tube thoroughly with methylene chloride between extracts to prepare for the next sample.

10.5.1.4.2.4 If a particularly dirty extract is encountered, a 5.0 mL methylene chloride blank shall be run through the system to check for carry-over.

10.5.1.4.2.5 Concentrate the eluate per Sections 10.3 or 10.4 for further cleanup or for injection into the HRGC/HRMS.

10.5.2 Silica Gel Cleanup

10.5.2.1 Procedure for Silica Gel Cleanup

10.5.2.1.1 Place a glass-wool plug in a 15 mm ID chromatography column. Pack the column bottom to top with: 1 g silica gel; 4 g basic silica gel; 1 g silica gel; 8 g acid silica gel; 2 g silica gel; and 4 g granular anhydrous sodium sulfate. Tap the column to settle the adsorbents.

10.5.2.1.2 Pre-rinse the column with 50 to 100 mL of hexane. Close the stopcock when the hexane is within 1 mm of the sodium sulfate. Discard the eluate. Check the column for channeling. If channeling is present, discard the column and prepare another.

10.5.2.1.3 Apply the concentrated extract to the column. Open the stopcock until the extract is within 1 mm of the sodium sulfate.

10.5.2.1.4 Rinse the receiver twice with 1 mL portions of hexane and apply separately to the column. Elute the CDDs/CDFs with 100 mL hexane and collect the eluate.

10.5.2.1.5 Concentrate the eluate per Sections 10.3, or 10.4 for further cleanup or for injection into the HPLC or HRGC/HRMS.

10.5.2.1.6 For extracts of samples known to contain large quantities of other organic compounds (such as paper mill effluents), it may be advisable to increase the capacity of the silica gel column. This may be accomplished by increasing the strengths of the acid and basic silica gels. The acid silica gel may be increased in strength to as much as 44% w/w (7.9 g sulfuric acid added to 10 g silica gel). The basic silica gel may be increased in strength to as much as 33% w/w (50 mL 1 N NaOH added to 100 g silica gel), or the potassium silicate may be used.

NOTE: The use of stronger acid silica gel (44% w/w) may lead to charring of organic compounds in some extracts. The charred material may retain some of the analytes and lead to lower recoveries of CDDs/CDFs. Increasing the strengths of the acid and basic silica gel may also require different volumes of hexane than those specified above to elute the analytes off the column.

10.5.3 Alumina Cleanup

10.5.3.1 Procedure for Alumina Cleanup

10.5.3.1.1 Place a glass-wool plug in a 15 mm ID chromatography column.

10.5.3.1.2 If using acid alumina, pack the column by adding 6 g acid alumina. If using basic alumina, substitute 6 g basic alumina. Tap the column to settle the adsorbents.

10.5.3.1.3 Pre-rinse the column with 50 to 100 mL of hexane. Close the stopcock when the hexane is within 1 mm of the alumina.

10.5.3.1.4 Discard the eluate. Check the column for channeling. If channeling is present, discard the column and prepare another.

10.5.3.1.5 Apply the concentrated extract to the column. Open the stopcock until the extract is within 1 mm of the alumina.

10.5.3.1.6 Rinse the receiver twice with 1 mL portions of hexane and apply separately to the column. Elute the interfering compounds with 100 mL hexane and discard the eluate.

10.5.3.1.7 The choice of eluting solvents will depend on the choice of alumina (acid or basic) made in Section 10.5.3.1.2.

10.5.3.1.7.1 If using acid alumina, elute the CDDs/CDFs from the column with 20 mL methylene chloride:hexane (20:80 v/v). Collect the eluate.

10.5.3.1.7.2 If using basic alumina, elute the CDDs/CDFs from the column with 20 mL methylene chloride:hexane (50:50 v/v). Collect the eluate.

10.5.3.1.8 Concentrate the eluate per Sections 10.3 or 10.4 for further cleanup or for injection into the HPLC or HRGC/HRMS.

10.5.4 Carbon Column Cleanup

10.5.4.1 Procedure for Carbon Column Cleanup

10.5.4.1.1 Cut both ends from a 10 mL disposable serological pipet to produce a 10 cm column. Fire-polish both ends and flare both ends if desired. Insert a glass-wool plug at one end, then pack the column with 0.55 g of Carbopak/Celite to form an adsorbent bed approximately 2 cm long. Insert a glass-wool plug on top of the bed to hold the adsorbent in place.

10.5.4.1.2 Pre-rinse the column with 5 mL of toluene followed by 2 mL methylene chloride:methanol:toluene (15:4:1 v/v), 1 mL methylene chloride:cyclohexane (1:1 v/v), and 5 mL hexane. If the flow rate of eluate exceeds 0.5 mL/min, discard the column.

10.5.4.1.3 When the solvent is within 1 mm of the column packing, apply the sample extract to the column. Rinse the sample container twice with 1 mL portions of hexane and apply separately to the column. Apply 2 mL of hexane to complete the transfer.

10.5.4.1.4 Elute the interfering compounds with two 3 mL portions of hexane, 2 mL of methylene chloride: cyclohexane (1:1 v/v), and 2 mL of methylene chloride: methanol:toluene (15:4:1 v/v). Discard the eluate.

10.5.4.1.5 Invert the column and elute the CDDs/CDFs with 20 mL of toluene. If carbon particles are present in the eluate, filter through glass fiber filter paper.

10.5.4.1.6 Concentrate the eluate per Sections 10.3 or 10.4 for further cleanup or for injection into the HPLC or HRGC/HRMS.

10.5.5 Sample Cleanup by HPLC

10.5.5.1 Calibration of HPLC

10.5.5.1.1 Procedure for HPLC Calibration

10.5.5.1.2 Prepare a calibration standard containing the 2,3,7,8-substituted isomers and/or other isomers of interest at a concentration of approximately 500 pg/µL in methylene chloride.

10.5.5.1.3 Inject 30 µL of the calibration solution into the HPLC and record the signal from the detector. Collect the eluant for reuse. The elution order will be the tetra- through octa-isomers.

10.5.5.1.4 Establish the collect time for the tetra-isomers and for the other isomers of interest. Following calibration, flush the injection system with copious quantities of methylene chloride, including a minimum of five 50 µL injections while the detector is monitored, to ensure that residual CDDs/CDFs are removed from the system.

10.5.5.2 Technical Acceptance Criteria for HPLC Calibration

10.5.5.2.1 Verify the calibration with the calibration solution after every 20 extracts.

10.5.5.2.2 Calibration is verified if the recovery of the CDDs/CDFs from the calibration standard (Section 10.5.5.1.1) is within 75 to 125%.

10.5.5.3 Corrective Action for HPLC Calibration

10.5.5.3.1 If calibration does not meet the technical acceptance criteria, the system shall be re-calibrated using the calibration solution, and the previous 20 samples shall be re-extracted and cleaned up using the calibrated system.

10.5.5.4 Sample Extract Cleanup by HPLC

HPLC requires that the column not be overloaded. The column specified in this method is designed to handle a maximum of 30 µL of extract. If the extract cannot be concentrated to less than 30 µL, it is split into fractions and the fractions are combined after elution from the column.

10.5.5.4.1 Procedure for HPLC Sample Cleanup

10.5.5.4.1.1 Rinse the sides of the vial twice with 30 µL of methylene chloride and reduce to 30 µL with the evaporation apparatus.

10.5.5.4.1.2 Inject the 30 µL extract into the HPLC.

10.5.5.4.1.3 Elute the extract using the calibration data determined in Section 10.5.5.1.3. Collect the fraction(s) in a clean 20 mL concentrator tube containing 5 mL of hexane:acetone (1:1 v/v).

10.5.5.4.1.4 If an extract containing greater than 100 ng/mL of total CDD or CDF is encountered, a 30 µL methylene chloride blank shall be run through the system to check for carry-over.

10.5.5.4.1.5 Concentrate the eluate per Section 10.4 for injection into the HRGC/HRMS.

10.5.6 Cleanup of Tissue Lipids

Lipids are removed from the Soxhlet extract using either the anthropogenic isolation column (Section 10.5.6.1) or acidified silica gel (Section 10.5.6.2), or are removed from the HCl digested extract using sulfuric acid and base back-extraction (Section 10.5.6.3).

10.5.6.1 Anthropogenic Isolation Column Cleanup

10.5.6.1.1 Procedure for Anthropogenic Isolation Column Cleanup

10.5.6.1.1.1 Place a glass-wool plug in a 25 mm ID chromatography column. Pack the column bottom to top with: 2 g silica gel; 2 g potassium silicate; 2 g granular anhydrous sodium sulfate; 10 g acid silica gel; and 2 g granular anhydrous sodium sulfate. Tap the column to settle the adsorbents.

10.5.6.1.1.2 Pre-elute the column with 100 mL of hexane. Drain the hexane layer to the top of the column but do not expose the sodium sulfate.

10.5.6.1.1.3 Load the sample and rinses (see Section 10.2.4.9.2) onto the column by draining each portion to the top of the bed. Elute the CDDs/CDFs from the column into the apparatus used for concentration (see Section 10.2.4.7) using 200 mL of hexane.

10.5.6.1.1.4 Concentrate the cleaned up extract to constant weight per Section 10.4.8. If more than 500 mg of material remains, repeat the cleanup using a fresh anthropogenic isolation column.

10.5.6.1.1.5 Re-dissolve the extract in a solvent suitable for the additional cleanups to be used.

10.5.6.1.1.6 Spike 1.0 mL of the cleanup standard into the residue/solvent.

10.5.6.1.1.7 Clean up the extract using the procedures in Sections 10.5.1 – 10.5.6. Alumina and carbon are recommended as minimum additional cleanup steps.

10.5.6.1.1.8 Following cleanup, concentrate the extract to 10 uL as described in Section 10.4.8 and proceed with the analysis in Section 10.6.

10.5.6.2 Acidified Silica Gel Cleanup

This procedure is an alternate to the anthropogenic isolation column that is used for the removal of lipids from the Soxhlet/SDS extract.

10.5.6.2.1 Procedure for Acidified Silica Gel Cleanup

10.5.6.2.1.1 Adjust the volume of hexane in the bottle (see Section 10.2.4.9.2) to approximately 200 mL.

10.5.6.2.1.2 Spike 1.0 mL of the cleanup standard into the residue/solvent.

10.5.6.2.1.3 Drop the stirring bar into the bottle, place the bottle on the stirring plate, and begin stirring.

10.5.6.2.1.4 Add 30 to 100 g of acid silica gel to the bottle while stirring, keeping the silica gel in motion. Stir for 2 hours.

NOTE: 30 g of silica gel should be adequate for most samples and will minimize contamination from this source.

10.5.6.2.1.5 After stirring, pour the extract through approximately 10 g of granular anhydrous sodium sulfate contained in a funnel with glass fiber filter into a Kuderna-Danish concentrator. Rinse the bottle and sodium sulfate with hexane to complete the transfer.

10.5.6.2.1.6 Concentrate the extract to a volume suitable for the cleanups given in Sections 10.5.1 – 10.5.6.

10.5.6.3 Sulfuric Acid and Base Back Extraction Cleanup

Used with HCl digested extracts (see Section 10.2.5).

10.5.6.3.1 Procedure for Sulfuric Acid and Base Back Extraction Cleanup

10.5.6.3.1.1 Spike 1.0 mL of the cleanup standard into the residue/solvent (see Section 10.2.5.7).

10.5.6.3.1.2 Add 10 mL of concentrated sulfuric acid to the bottle. Immediately cap and shake 1 to 3 times. Loosen cap in a hood to vent excess pressure. Cap and shake the bottle so that the residue/solvent is exposed to the acid for a total time of approximately 45 seconds.

10.5.6.3.1.3 Decant the hexane into a 250 mL separatory funnel, making sure that no acid is transferred. Complete the quantitative transfer with several hexane rinses.

10.5.6.3.1.4 Back extract the solvent/residue with 50 mL of potassium hydroxide solution per Section 10.2.6, followed by two reagent water rinses.

10.5.6.3.1.5 Drain the extract through a filter funnel containing approximately 10 g of granular anhydrous sodium sulfate in a glass fiber filter into a K-D concentrator.

10.5.6.3.1.6 Concentrate the cleaned up extract to a volume suitable for the additional cleanups given in Sections 10.5.1 – 10.5.6. Gel permeation chromatography, Alumina, and Carbopak/Celite are recommended as minimum additional cleanup steps.

10.5.6.3.1.7 Following cleanup, concentrate the extract to 10 uL as described in Section 10.4, and proceed with analysis per Section 10.6.

10.5.7 Florisil Cleanup

10.5.7.1 Procedure for Florisil Cleanup

10.5.7.1.1 Pre-elute the activated Florisil column (Section 7.5.5) with 10 mL of methylene chloride followed by 10 mL of hexane:methylene chloride (98:2 v/v) and discard the solvents.

10.5.7.1.2 When the solvent is within 1 mm of the packing, apply the sample extract (in hexane) to the column. Rinse the sample container twice with 1 mL portions of hexane and apply to the column.

10.5.7.1.3 Elute the interfering compounds with 20 mL of hexane:methylene chloride (98:2) and discard the eluate.

10.5.7.1.4 Elute the CDDs/CDFs with 35 mL of methylene chloride and collect the eluate. Concentrate the eluate per Section 10.3 for further cleanup or for injection into the HPLC or GC/MS.

10.6 Sample Analyses by HRGC/HRMS

10.6.1 Sample extracts shall be analyzed only after the HRGC/HRMS system has met the WDM, isomer specificity check, HRGC/HRMS system performance check, initial calibration, and calibration verification requirements. The same instrument conditions must be employed for the analysis of samples as were used for calibration.

10.6.2 Establish the operating conditions given in Section 9.1.

10.6.3 Add 10 µL of the appropriate internal standard solution to the sample extract for a maximum final volume of 20 µL immediately prior to injection to minimize the possibility of loss by evaporation, adsorption, or reaction. If an extract is to be re-analyzed and evaporation has occurred, do <u>not</u> add more instrument recovery standard solution. Rather, bring the extract back to its previous

volume (e.g., 19 µL) with pure nonane or isooctane only (18 µL if 2 µL injections are used).

10.6.4 Inject 1 or 2 µL of the concentrated extract containing the internal standard solution using on-column or splitless injection. The volume injected must be identical to the volume used for calibration (see Section 9). Start the GC column initial isothermal hold upon injection. Start HRMS data collection after the solvent peak elutes. Stop data collection after the OCDD and OCDF have eluted.

10.6.5 Analysis of Complex Samples

Some samples may contain high levels (>10 ng/L or >1000 ng/kg) of the compounds of interest, interfering compounds, and/or polymeric materials. Some extracts will not concentrate to 10 µL (see Section 10.4.8); others may overload the HRGC column and/or mass spectrometer. Analyze a smaller aliquot of the sample (see Section 10.6.6) when the extract will not concentrate to 20 µL after all cleanup procedures have been exhausted.

10.6.6 Sample Dilution

If the SICP area at either quantitation m/z for any compound exceeds the calibration range of the system, a smaller sample aliquot is extracted.

10.6.6.1 For water samples containing 1% soil/sediments or less, dilute 100 mL, 10 mL, etc., of sample to 1 L with reagent water and re-prepare, extract, clean up, and analyze.

10.6.6.2 For samples containing greater than 1% soil/sediments, extract an amount of sample equal to 1/10, 1/100, etc., of the amount determined in Section 10.1.4.1. Re-prepare, extract, clean up, and analyze.

10.6.6.3 If a smaller size is not representative of the entire sample, the Contractor must contact SMO to apprise them of the type of sample received. SMO will contact the Region. The Region may require the Contractor to do the following:

- Dilute the sample extract by a factor of 10, adjust the concentration of the instrument internal standard to 100 pg/µL in the extract, and analyze an aliquot of this diluted extract by the internal standard method.
- Take a smaller aliquot with the chance of not obtaining a representative sample.
- Not analyze the sample.

10.6.7 Results are reported to three significant figures, as appropriate for the CDDs/CDFs and labeled compounds found in all standards, blanks, QC samples, and samples.

10.6.7.1 For aqueous samples report results in pg/L (parts-per-quadrillion).

10.6.7.2 For samples containing greater than 1% solids (soils, sediments, filter cake, compost) report results in ng/kg based on the dry weight of the sample. Report the percent solids so that the result may be corrected.

10.6.7.3 For tissues report results in ng/kg of wet tissue, not on the basis of the lipid content of the sample. Report the percent lipid content, so that the data user can calculate the concentration on a lipid basis if desired.

10.6.7.4 Samples — Report results for all peaks with S/N above 2.5 even if below the CRQL (See Exhibit C).

11.0 DATA ANALYSIS AND CALCULATIONS

11.1 Qualitative Identification

11.1.1 Identification of Target Analytes

A CDD or CDF (native or labeled) is identified when all of the criteria in Sections 11.1.1.1 through 11.1.1.4 are met.

11.1.1.1 The signals for the two exact m/z's must be present and must maximize within the same two seconds.

11.1.1.2 The signal-to-noise ratio (S/N) at each exact m/z must be greater than or equal to 2.5 for a sample extract, and greater than or equal to 10 for a calibration standard.

11.1.1.3 The ratio of the integrated areas of the two exact m/z's specified in Table 8 must be within the limits in Table 9, or within ± 10% of the ratio in the most recent midpoint (CS3) calibration standard.

11.1.1.4 The relative retention time of the peak representing a native 2,3,7,8-substituted CDD or CDF must be within the limit in Table 2. The retention time of peaks representing non-2,3,7,8-substituted CDDs/CDFs must be within the retention times established during the analysis of the WDM for the first and last eluting compounds.

11.1.2 HRGC/HRMS Confirmatory Analysis of CDDs/CDFs

Isomer specificity of 2,3,7,8-TCDF cannot be achieved on the DB-5 (or equivalent) HRGC column. Therefore, any sample in which 2,3,7,8-TCDF/ TCDD is positively identified at or greater than the CRQL by analysis on a DB-5 (or equivalent) HRGC column or if TCDF/TCDD is reported as an EMPC at or greater than the CRQL, must have a confirmatory analysis performed on a DB-225 or equivalent HRGC column. The operating conditions in Section 9.1 may be adjusted for analysis on the second HRGC column, but the HRGC/HRMS must meet the mass resolution and calibration specifications in Section 9. Confirmation analysis must meet the criteria given in Section 11.3.

NOTE: The confirmatory analysis is not required when the contractor chooses to use a column which meets isomer specificity requirements for both 2,3,7,8-TCDD and 2,3,7,8-TCDF. The column must meet all criteria established in Section 9.

11.2 Quantitative Determination: The relative ion abundance ratio criteria for native analytes and labeled standards must be met using peak areas to calculate ratios. If the ion abundance ratios are not met because of coeluting interferences, but all other criteria are met, the contractor can use peak heights to evaluate the ion ratio. If in the judgement of the analyst, the peak is a CDD/CDF, report the ion abundance ratios determined using peak heights, quantitate the peaks using peak heights

instead of areas (for both the native and labeled analytes), and flag the data on Form I-HR.

11.2.1 Isotope Dilution Method

11.2.1.1 By adding a known amount of labeled compounds to every sample prior to extraction, correction for recovery of the native compound can be made because the native compound and its labeled analog exhibit similar effects upon extraction, concentration, and gas chromatography.

11.2.1.2 Because of a potential interference, the labeled analog of the isomer OCDF is not added to the sample. Therefore, native OCDF is quantitated against labeled OCDD. As a result, the concentration of native OCDF is corrected for the recovery of the labeled OCDD. In instances where OCDD and OCDF behave differently during sample extraction, concentration, and cleanup procedures, this may decrease the accuracy of the OCDF results. However, given the low toxicity of this compound relative to the other dioxins and furans, the potential decrease in accuracy is not considered significant.

11.2.1.3 Because the labeled compound analog of 1,2,3,7,8,9-HxCDD is not added before extraction of the sample, it cannot be used to quantitate the native compound by strict isotope dilution procedures. Therefore, native 1,2,3,7,8,9-HxCDD is quantitated using the average of the responses of the labeled compound analogs of the other two 2,3,7,8-substituted HxCDDs: 1,2,3,4,7,8-HxCDD and 1,2,3,6,7,8-HxCDD. As a result, the concentration of native 1,2,3,7,8,9-HxCDD is corrected for the average recovery of the other two HxCDDs.

11.2.1.4 Any peaks representing non-2,3,7,8-substituted CDDs/CDFs are quantitated using an average of the response factors from all of the labeled standard 2,3,7,8- isomers at the same level of chlorination.

11.2.1.5 The relative response (RR) values are used in conjunction with the initial calibration data described in Section 9.3.4.1 to determine concentrations directly, so long as labeled compound spiking levels are constant, using the following equation:

EQ. 10

$$C_{EX}\ (ng/mL) = \frac{(A1_N + A2_N)\ C_L}{(A1_L + A2_L)\ RR}$$

Where:

C_{EX} = The concentration of the native compound in the extract.

The other terms are as defined in Section 9.3.4.1

11.2.2 Internal Standard Method

11.2.2.1 Compute the concentrations of the ^{13}C-labeled analogs and the ^{37}Cl-labeled cleanup standard in the extract using the relative response factors determined from the initial calibration data (see Section 9.3.4.2) and the following equation:

EQ. 11

$$C_{EX}\ (ng/mL) = \frac{(A1_S + A2_S)\ C_{IS}}{(A1_{IS} + A2_{IS})\ RRF}$$

Where:

C_{EX} = The concentration of the labeled compound in the extract.

The other terms are as defined in Section 9.3.4.2

11.2.2.1.1 There is only one m/z for the ^{37}Cl-labeled cleanup standard.

11.2.3 The concentration of a native compound in a soil/sediment or tissue sample is computed using the concentration of the compound in the extract and the weight of the sample (see Section 10.1.4.1), using the following equation:

EQ. 12

$$\text{Concentration in solid (ng/kg)} = \frac{(C_{EX} * V_{EX})}{W_S}$$

Where:

C_{EX} = The concentration of the native compound in the extract.

V_{EX} = The extract volume in mL.

W_S = The sample weight (dry weight) in kg.

11.2.4 The concentration of a native compound in a water matrix sample is computed using the concentration of the compound in the extract and the volume of water extracted (see Section 10.1.3),using the following equation:

EQ. 13

$$\text{Concentration in water phase (pg/L)} = \frac{(C_{EX} * V_{EX})}{V_S} * 1000$$

Where:

C_{EX} = The concentration of the native compound in the extract.

V_{EX} = The extract volume in mL.

V_S = The sample volume in liters.

11.2.5 Sample Specific Estimated Detection Limit. The sample specific Estimated Detection Limit (EDL) is 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. An EDL is calculated for each CDD/CDF that is not identified, regardless of whether or not other non-2,3,7,8-substituted CDD/CDF are present.

11.2.5.1 Use the expression for EDL below to calculate an EDL for each absent CDD/CDF (i.e., S/N < 2.5). The background level is determined by measuring the range of the noise (peak-to-peak) for the two quantitation ions (see Table 8) of a particular CDD/CDF, in the region of the SICP trace corresponding to the elution of the labeled compound or in the region of the SICP where the congener is expected to elute by comparison with the routine calibration data (for those congeners that do not have a ^{13}C-labeled compound), multiplying that noise height by 2.5, and relating the product to an estimated concentration that would produce that peak height. Use the following formula for aqueous samples:

EQ. 14

$$\text{Aqueous EDL (pg/L)} = \frac{2.5 * Q_{IS} * (H_{x1}+H_{x2}) * D}{V * (H_{IS1}+H_{IS2}) * RRF_n}$$

Use the following formula for soil samples:

EQ. 15

$$\text{Soil EDL (ng/Kg)} = \frac{2.5 * Q_{IS} * (H_{x1}+H_{x2}) * D}{W * (H_{IS1}+H_{IS2}) * RRF_n}$$

Where:

EDL = estimated detection limit for 2,3,7,8-substituted CDDs/CDFs.

Q_{IS} = Quantity (pg) of appropriate internal standard added prior added prior to sample extraction.

H_{x1}, H_{x2} = Peak heights of the noise for both quantitation ions of the CDD/CDF.

H_{IS1}, H_{IS2} = Peak heights of the internal standard quantitation ions.

D = Dilution Factor

V = Volume extracted in Liters

W = Weight extracted in grams

RRF_n = Relative Response Factor for the isomer of interest from CS3 standard.

11.2.6 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 a signal-to-noise (S/N) of at least 2.5 for both quantitation ions, but that do not meet all the identification criteria described in Section 11.

11.2.6.1 The EMPC of a native compound in a soil/sediment or tissue samples is computed using the concentration of the compound in the extract and the dilution factor (see Section 10.6.6), as follows:

EQ. 16

$$\text{EMPC (ng/Kg)} = \frac{(C_{EX} * D)}{W_S}$$

Where:

D = Dilution Factor.

W_S = Sample weight(dry weight) in Kg.

11.2.6.2 The concentration of a native compound in a water matrix sample is computed using the concentration of the compound in the extract and the dilution factor (see Section 10.6.6), as follows:

EQ. 17

$$\text{EMPC (pg/L)} = \frac{(C_{EX} * D)}{V_S}$$

Where:

D = Dilution Factor.

V_S = Sample volume in liters.

C_{EX} = The concentration of the native compound in the extract.

11.2.7 Percent Recovery of Labeled Compounds and Cleanup Standard

11.2.7.1 Compute the percent recovery of the labeled compounds and cleanup standard using the internal standard method and the following equation.

EQ. 18

$$\% \text{ Recovery} = \frac{\text{Measured Concentration}}{\text{Known Concentration}} * 100$$

11.2.7.2 The $^{13}C_{12}$-1,2,3,4-TCDD internal standard is used to quantitate the tetra and penta labeled compounds and the cleanup standard. The $^{13}C_{12}$-1,2,3,7,8,9-HxCDD internal standard is used to quantitate the HxCDD, HpCDF, and OCDD labeled compounds.

11.3 Technical Acceptance Criteria for Sample Analysis

11.3.1 The samples must be analyzed on a HRGC/HRMS system meeting the WDM, isomer specificity check, PFK, initial calibration, and blank technical acceptance criteria, and be bracketed by acceptable calibration verification. The sample must undergo cleanup procedures, when required.

11.3.2 The samples must be extracted and analyzed within the contract holding times.

11.3.3 The samples must have an associated method blank meeting the blank technical acceptance criteria.

11.3.4 The samples must meet the qualitative identification criteria in Table 9.

11.3.5 The labeled compounds of the sample must meet the acceptance criteria in Table 7.

11.3.6 If any target analyte concentration exceeds the upper limit of the initial calibration, the extract must be diluted and reanalyzed.

11.4 Corrective Action for Sample Analysis

11.4.1 Sample analysis technical acceptance criteria MUST be met before data are reported. Samples contaminated from laboratory sources or associated with a contaminated method blank (Refer to Section 12.1.4.2) will require re-extraction and re-analysis at no additional cost to USEPA. Any samples analyzed that do not meet the technical acceptance criteria will require re-extraction and/or re-analysis at no additional cost to USEPA.

11.4.2 If the sample analysis technical acceptance criteria are not met, check calculations, internal standard solution, and system performance. It may be necessary to recalibrate the system or take other corrective action procedures to meet the technical acceptance criteria, in which case, the affected samples must be re-analyzed at no additional cost to USEPA after the corrective action.

11.4.3 Corrective action for failure to meet system performance checks, initial calibration, and calibration verification must be completed before the re-analysis of samples.

12.0 QUALITY CONTROL

12.1 Method Blank

12.1.1 Summary of Method Blanks

A method blank is a volume of a clean reference matrix that is carried through the entire analytical procedure. The volume of the reference matrix must be approximately equal to the volume or weight of samples associated with the blank. The purpose of the method blank is to determine the level of contamination associated with the processing and analysis of samples.

12.1.2 Frequency of Method Blank Analyses

A method blank must be extracted each time samples are extracted. The number of samples extracted with each method blank shall not exceed 20 field samples (excluding laboratory control samples/laboratory control sample duplicate and PE samples). In addition, a method blank shall be extracted and cleaned up by the same procedures used to extract and clean up samples and shall be analyzed on each HRGC/HRMS system used to analyze associated samples.

12.1.3 Procedure for Method Blank Preparation

Extract, concentrate, cleanup, and analyze the blank according to the procedure for water, soil/sediment, and tissue samples. Analyze the method blank prior to analysis of samples from the same SDG.

12.1.4 Technical Acceptance Criteria for Method Blank Analyses

12.1.4.1 Acceptable method blanks must not contain any chemical interference or electronic noise at or above the CRQL at the m/z of the native compounds.

12.1.4.2 For all CDDs/CDFs, the method blanks must contain CDDs/CDFs at a concentration less than or equal to the CRQL. For OCDD/OCDF, the method blanks must contain concentrations less than three times the CRQL.

12.1.4.3 The method blank must meet the technical acceptance criteria for sample analyses.

12.1.5 Corrective Action for Method Blank Analyses

12.1.5.1 If a method blank does not meet the technical acceptance criteria for method blank analysis, the Contractor must consider the analytical system to be out-of-control. Samples associated with a contaminated blank must be re-extracted and re-analyzed at no additional cost to USEPA.

12.1.5.2 If contamination is the problem, the source of the contamination must be investigated and appropriate corrective measures must be taken and documented before further sample analysis proceeds. It

is the Contractor's responsibility to ensure that method interferences caused by contaminants in the solvent, reagents, glassware, and sample storage and processing hardware that lead to discrete artifacts and/or elevated baseline in the HRGC/HRMS are eliminated. Samples associated with a contaminated blank must be re-extracted and re-analyzed at no additional cost to USEPA.

12.2 Laboratory Control Sample (LCS)/Laboratory Control Sample Duplicate (LCSD)

12.2.1 Summary of LCS/LCSD

The LCS/LCSD are volumes of clean reference matrix that are spiked and carried through the entire analytical procedure. To evaluate the accuracy and precision of the method used for CDD/CDF analyses, USEPA has prescribed a mixture of CDD/CDF target compounds to be spiked into two aliquots of reference matrix and analyzed in accordance with the appropriate method.

12.2.2 Frequency of LCS/LCSD Analyses

A LCS/LCSD must be extracted and analyzed for every 20 field samples of a similar matrix in an SDG, whenever samples are extracted by the same procedure.

12.2.3 Procedure for Preparing LCS/LCSD

12.2.3.1 Prepare two aliquots of clean reference matrix. Spike each aliquot with 1.0 mL of the labeled compound spiking solution (Section 7.7.4.2) and 1.0 mL of the laboratory control sample spiking solution (Section 7.8.4).

12.2.3.2 Extract, concentrate, cleanup, and analyze the LCS/LCSD according to the procedure for water, soil/sediment, and tissue samples. Analyze the LCS/LCSD prior to analysis of samples from the same SDG.

12.2.4 Calculation for LCS/LCSD

12.2.4.1 Calculate the concentration of each analyte according to the procedure for calculations (see Section 11).

12.2.4.2 Compute the percent recovery of the LCS/LCSD analyte using the following equation:

EQ. 19

$$\% \text{ LCS Recovery} = \frac{\text{Spike Sample Result}}{\text{Spike Added}} * 100$$

12.2.4.3 Calculate the relative percent difference (%RPD) between the LCS and LCSD analyses by the following equation:

EQ. 20

$$\%RPD = \frac{|\text{Sample Result} - \text{Duplicate Results}|}{(\text{Sample Results} + \text{Duplicate Results})/2} * 100$$

12.2.5 Technical Acceptance Criteria for LCS/LCSD

12.2.5.1 All LCS/LCSD must be prepared and analyzed at the frequency described.

12.2.5.2 The LCS/LCSD must meet the technical acceptance criteria for sample analyses.

12.2.5.3 The limits for LCS/LCSD recovery are in Table 6.

12.2.5.4 The RPD for CDD/CDFs must not exceed 30%.

12.2.6 Corrective Action for LCS

If a LCS/LCSD does not meet the technical acceptance criteria for LCS/LCSD recovery or RPD, the Contractor must consider the analytical system to be out of control. Samples associated with a non-compliant LCS/LCSD must be re-extracted and re-analyzed at no additional cost to USEPA.

13.0 POLLUTION PREVENTION

13.1 The solvents used in this method pose little threat to the environment when managed properly.

13.2 Standards should be prepared in volumes consistent with laboratory use to minimize the volume of expired standards to be disposed.

14.0 WASTE MANAGEMENT

14.1 It is the laboratory's responsibility to comply with all Federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations.

14.2 Samples containing HCl to pH <2 are hazardous and must be neutralized before being poured down a drain or handled as hazardous waste.

14.3 The CDDs/CDFs decompose above 800°C. Low-level waste such as absorbent paper, tissues, animal remains, and plastic gloves may be burned in an appropriate incinerator. Gross quantities (milligrams) should be packaged securely and disposed through commercial or governmental channels that are capable of handling extremely toxic wastes.

14.4 Liquid or soluble waste should be dissolved in methanol or ethanol and irradiated with ultraviolet light with a wavelength shorter than 290 nm for several days. (Use F40 BL lamps or equivalent.) Analyze liquid wastes and dispose of the solutions when the CDDs/CDFs can no longer be detected.

14.5 For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel" and "Less is Better—Laboratory Chemical Management for Waste Reduction", available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

15.0 TABLES/DIAGRAMS/FLOWCHARTS

Table 1. Chlorinated Dibenzo-*p*-dioxins and Furans Determined by Isotope Dilution and Internal Standard High Resolution Gas Chromatography (HRGC)/High Resolution Mass Spectrometry (HRMS)

CDDs/CDFs [1]	CAS Registry	Labeled analog	CAS Registry
2,3,7,8-TCDD	1746-01-6	$^{13}C_{12}$-2,3,7,8-TCDD $^{37}Cl_4$-2,3,7,8-TCDD	76523-40-5 85508-50-5
Total TCDD	41903-57-5	–	–
2,3,7,8-TCDF	51207-31-9	$^{13}C_{12}$-2,3,7,8-TCDF	89059-46-1
Total-TCDF	55722-27-5	–	–
1,2,3,7,8-PeCDD	40321-76-4	$^{13}C_{12}$-1,2,3,7,8-PeCDD	109719-79-1
Total-PeCDD	36088-22-9	–	–
1,2,3,7,8-PeCDF	57117-41-6	$^{13}C_{12}$-1,2,3,7,8-PeCDF	109719-77-9
2,3,4,7,8-PeCDF	57117-31-4	$^{13}C_{12}$-2,3,4,7,8-PeCDF	116843-02-8
Total-PeCDF	30402-15-4	–	–
1,2,3,4,7,8-HxCDD	39227-28-6	$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	109719-80-4
1,2,3,6,7,8-HxCDD	57653-85-7	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	109719-81-5
1,2,3,7,8,9-HxCDD	19408-74-3	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	109719-82-6
Total-HxCDD	34465-46-8	–	–
1,2,3,4,7,8-HxCDF	70648-26-9	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	114423-98-2
1,2,3,6,7,8-HxCDF	57117-44-9	$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	116843-03-9
1,2,3,7,8,9-HxCDF	72918-21-9	$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	116843-04-0
2,3,4,6,7,8-HxCDF	60851-34-5	$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	116843-05-1
Total-HxCDF	55684-94-1	–	–
1,2,3,4,6,7,8-HpCDD	35822-46-9	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	109719-83-7
Total-HpCDD	37871-00-4	–	–
1,2,3,4,6,7,8-HpCDF	67562-39-4	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	109719-84-8
1,2,3,4,7,8,9-HpCDF	55673-89-7	$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	109719-94-0
Total-HpCDF	38998-75-3	–	–
OCDD	3268-87-9	$^{13}C_{12}$-OCDD	114423-97-1
OCDF	39001-02-0	not used	–

[1] Chlorinated dibenzo-*p*-dioxins and chlorinated dibenzofurans

TCDD = Tetrachlorodibenzo-*p*-dioxin
TCDF = Tetrachlorodibenzofuran
PeCDD = Pentachlorodibenzo-*p*-dioxin
PeCDF = Pentachlorodibenzofuran
HxCDD = Hexachlorodibenzo-*p*-dioxin
HxCDF = Hexachlorodibenzofuran
HpCDD = Heptachlorodibenzo-*p*-dioxin
HpCDF = Heptachlorodibenzofuran
OCDD = Octachlorodibenzo-*p*-dioxin
OCDF = Octachlorodibenzofuran

TABLE 2. Retention Time References, Quantitation References, Relative Retention Times, and Minimum Levels for CDDs and CDFs

CDD/CDF	Retention Time and Quantitation Reference	Relative Retention Time	CRQL		
			Water (pg/L; ppq)	Solid (ng/kg; ppt)	Extract (pg/µL;
Compounds using $^{13}C_{12}$-1,2,3,4-TCDD as the injection internal standard					
2,3,7,8-TCDF	$^{13}C_{12}$-2,3,7,8-TCDF	0.999–1.003	10	1	0.5
2,3,7,8-TCDD	$^{13}C_{12}$-2,3,7,8-TCDD	0.999–1.002	10	1	0.5
1,2,3,7,8-PeCDF	$^{13}C_{12}$-1,2,3,7,8-PeCDF	0.999–1.002	50	5	2.5
2,3,4,7,8-PeCDF	$^{13}C_{12}$-2,3,4,7,8-PeCDF	0.999–1.002	50	5	2.5
1,2,3,7,8-PeCDD	$^{13}C_{12}$-1,2,3,7,8-PeCDD	0.999–1.002	50	5	2.5
$^{13}C_{12}$-2,3,7,8-TCDF	$^{13}C_{12}$-1,2,3,4-TCDD	0.923–1.103			
$^{13}C_{12}$-2,3,7,8-TCDD	$^{13}C_{12}$-1,2,3,4-TCDD	0.976–1.043			
$^{37}Cl_4$-2,3,7,8-TCDD	$^{13}C_{12}$-1,2,3,4-TCDD	0.989–1.052			
$^{13}C_{12}$-1,2,3,7,8-PeCDF	$^{13}C_{12}$-1,2,3,4-TCDD	1.000–1.425			
$^{13}C_{12}$-2,3,4,7,8-PeCDF	$^{13}C_{12}$-1,2,3,4-TCDD	1.011–1.526			
$^{13}C_{12}$-1,2,3,7,8-PeCDD	$^{13}C_{12}$-1,2,3,4-TCDD	1.000–1.567			
Compounds using $^{13}C_{12}$-1,2,3,7,8,9-HxCDD as the injection internal standard					
1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	0.999–1.001	50	5	2.5
1,2,3,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	0.997–1.005	50	5	2.5
1,2,3,7,8,9-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	0.999–1.001	50	5	2.5
2,3,4,6,7,8-HxCDF	$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	0.999–1.001	50	5	2.5
1,2,3,4,7,8-HxCDD	$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	0.999–1.001	50	5	2.5
1,2,3,6,7,8-HxCDD	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	0.998–1.004	50	5	2.5
1,2,3,7,8,9-HxCDD[1]		1.000–1.019	50	5	2.5
1,2,3,4,6,7,8-HpCDF	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	0.999–1.001	50	5	2.5
1,2,3,4,7,8,9-HpCDF	$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	0.999–1.001	50	5	2.5
1,2,3,4,6,7,8-HpCDD	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	0.999–1.001	50	5	2.5
OCDF	$^{13}C_{12}$-OCDD	0.999–1.008	100	10	5.0
OCDD	$^{13}C_{12}$-OCDD	0.999–1.001	100	10	5.0
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.944–0.970			
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.949–0.975			
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.977–1.047			
$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.959–1.021			
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.977–1.000			
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.981–1.003			
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	1.043–1.085			
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	1.057–1.151			
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	1.086–1.110			
$^{13}C_{12}$-OCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	1.032–1.311			

[1]The retention time reference for 1,2,3,7,8,9-HxCDD is $^{13}C_{12}$-1,2,3,6,7,8-HxCDD. 1,2,3,7,8,9-HxCDD is quantified using the averaged responses of $^{13}C_{12}$-1,2,3,4,7,8-HxCDD and $^{13}C_{12}$-1,2,3,6,7,8-HxCDD.

TABLE 3. Concentration of Stock and Spiking Solutions Containing CDDs/CDFs and Labeled Compounds

CDD/CDF	Labeled compound stock solution[1] (ng/mL)	Labeled compound spiking solution[2] (ng/mL)	Native-Compound stock solution[3] (ng/mL)	Native-Compound spiking solution[4] (ng/mL)
2,3,7,8-TCDD	–	–	40	0.8
2,3,7,8-TCDF	–	–	40	0.8
1,2,3,7,8-PeCDD	–	–	200	4
1,2,3,7,8-PeCDF	–	–	200	4
2,3,4,7,8-PeCDF	–	–	200	4
1,2,3,4,7,8-HxCDD	–	–	200	4
1,2,3,6,7,8-HxCDD	–	–	200	4
1,2,3,7,8,9-HxCDD	–	–	200	4
1,2,3,4,7,8-HxCDF	–	–	200	4
1,2,3,6,7,8-HxCDF	–	–	200	4
1,2,3,7,8,9-HxCDF	–	–	200	4
2,3,4,6,7,8-HxCDF	–	–	200	4
1,2,3,4,6,7,8-HpCDD	–	–	200	4
1,2,3,4,6,7,8-HpCDF	–	–	200	4
1,2,3,4,7,8,9-HpCDF	–	–	200	4
OCDD	–	–	400	8
OCDF	–	–	400	8
$^{13}C_{12}$-2,3,7,8-TCDD	100	2	–	–
$^{13}C_{12}$-2,3,7,8-TCDF	100	2	–	–
$^{13}C_{12}$-1,2,3,7,8-PeCDD	100	2	–	–
$^{13}C_{12}$-1,2,3,7,8-PeCDF	100	2	–	–
$^{13}C_{12}$-2,3,4,7,8-PeCDF	100	2	–	–
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	100	2	–	–
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	100	2	–	–
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	100	2	–	–
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	100	2	–	–
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	100	2	–	–
$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	100	2	–	–
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	100	2	–	–
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	100	2	–	–
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	100	2	–	–
$^{13}C_{12}$-OCDD	200	4	–	–

TABLE 3. Concentration of Stock and Spiking Solutions Containing CDDs/CDFs and Labeled Compounds (Con’t)

CDD/CDF	Labeled compound stock solution[1] (ng/mL)	Labeled compound spiking solution[2] (ng/mL)	Native-Compound stock solution[3] (ng/mL)	Native-Compound spiking solution[4] (ng/mL)
Cleanup Standard[5]				
$^{37}Cl_4$-2,3,7,8-TCDD	0.8			
Internal Standards[6]				
$^{13}C_{12}$-1,2,3,4-TCDD	200			
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	200			

[1]Section 7.7.2, prepared in nonane and diluted to prepare spiking solution.

[2]Section 7.7.4, prepared in acetone from stock solution daily.

[3]Section 7.7.3, prepared in nonane and diluted to prepare spiking solution.

[4]Section 7.8.4, prepared in acetone from stock solution daily.

[5]Section 7.8.1, prepared in nonane and added to extract prior to cleanup.

[6]Section 7.8.2, prepared in nonane and added to the concentrated extract immediately prior to injection into the GC (Section 10.6.3).

TABLE 4. Concentration of CDDs/CDFs in Calibration and Calibration Verification Solutions

CDD/CDF	CS1 (ng/mL)	CS2 (ng/mL)	CS3[1] (ng/mL)	CS4 (ng/mL)	CS5 (ng/mL)
2,3,7,8-TCDD	0.5	2	10	40	200
2,3,7,8-TCDF	0.5	2	10	40	200
1,2,3,7,8-PeCDD	2.5	10	50	200	1000
1,2,3,7,8-PeCDF	2.5	10	50	200	1000
2,3,4,7,8-PeCDF	2.5	10	50	200	1000
1,2,3,4,7,8-HxCDD	2.5	10	50	200	1000
1,2,3,6,7,8-HxCDD	2.5	10	50	200	1000
1,2,3,7,8,9-HxCDD	2.5	10	50	200	1000
1,2,3,4,7,8-HxCDF	2.5	10	50	200	1000
1,2,3,6,7,8-HxCDF	2.5	10	50	200	1000
1,2,3,7,8,9-HxCDF	2.5	10	50	200	1000
2,3,4,6,7,8-HxCDF	2.5	10	50	200	1000
1,2,3,4,6,7,8-HpCDD	2.5	10	50	200	1000
1,2,3,4,6,7,8-HpCDF	2.5	10	50	200	1000
1,2,3,4,7,8,9-HpCDF	2.5	10	50	200	1000
OCDD	5.0	20	100	400	2000
OCDF	5.0	20	100	400	2000
$^{13}C_{12}$-2,3,7,8-TCDD	100	100	100	100	100
$^{13}C_{12}$-2,3,7,8-TCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8-PeCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8-PeCDF	100	100	100	100	100
$^{13}C_{12}$-2,3,4,7,8-PeCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	100	100	100	100	100
$^{13}C_{12}$-OCDD	200	200	200	200	200
Cleanup Standard					
$^{37}Cl_4$-2,3,7,8-TCDD	0.5	2	10	40	200
Internal Standards					
$^{13}C_{12}$-1,2,3,4-TCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	100	100	100	100	100

[1]Section 7.8.3, calibration verification solution.

Table 5. GC Retention Time Window Defining Solution and Isomer Specificity Check Standard (Section 7.8.5)

DB-5 Column GC Retention-Time Window Defining Solution

CDD/CDF	First Eluted	Last Eluted
TCDF	1,3,6,8-	1,2,8,9-
TCDD	1,3,6,8-	1,2,8,9-
PeCDF	1,3,4,6,8-	1,2,3,8,9-
PeCDD	1,2,4,7,9-	1,2,3,8,9-
HxCDF	1,2,3,4,6,8-	1,2,3,4,8,9-
HxCDD	1,2,4,6,7,9-	1,2,3,4,6,7-
HpCDF	1,2,3,4,6,7,8-	1,2,3,4,7,8,9-
HpCDD	1,2,3,4,6,7,9-	1,2,3,4,6,7,8-

DB-5 Column TCDD Isomer Specificity Check Standard

1,2,3,7 and 1,2,3,8-TCDD
2,3,7,8-TCDD
1,2,3,9-TCDD

DB-225 Column TCDF Isomer Specificity Check Standard

2,3,4,7-TCDF
2,3,7,8-TCDF
1,2,3,9-TCDF

Sp-2331 Column TCDD Isomer Specificity Check Standard
2,3,7,8-TCDD
1,4,7,8-TCDD
1,2,3,7-TCDD
1,2,3,8-TCDD

TABLE 6. Acceptance Criteria for Laboratory Control Sample (LCS)

CDD/CDF	Test conc (ng/mL)	LCS (% Recovery)
2,3,7,8-TCDD	10	67-158
2,3,7,8-TCDF	10	75-158
1,2,3,7,8-PeCDD	50	70–142
1,2,3,7,8-PeCDF	50	80-134
2,3,4,7,8-PeCDF	50	68-160
1,2,3,4,7,8-HxCDD	50	70-164
1,2,3,6,7,8-HxCDD	50	76-134
1,2,3,7,8,9-HxCDD	50	64-162
1,2,3,4,7,8-HxCDF	50	72-134
1,2,3,6,7,8-HxCDF	50	84-130
1,2,3,7,8,9-HxCDF	50	78-130
2,3,4,6,7,8-HxCDF	50	70–156
1,2,3,4,6,7,8-HpCDD	50	70–140
1,2,3,4,6,7,8-HpCDF	50	82-132
1,2,3,4,7,8,9-HpCDF	50	78-138
OCDD	100	78-144
OCDF	100	63-170

TABLE 7. Labeled Compound Recovery in Samples When All CDDs/CDFs are Tested

Compound	Test conc (ng/mL)	Labeled Compound Recovery (%)
$^{13}C_{12}$-2,3,7,8-TCDD	100	25-164
$^{13}C_{12}$-2,3,7,8-TCDF	100	24-169
$^{13}C_{12}$-1,2,3,7,8-PeCDD	100	25-181
$^{13}C_{12}$-1,2,3,7,8-PeCDF	100	24-185
$^{13}C_{12}$-2,3,4,7,8-PeCDF	100	21-178
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	100	32-141
$^{13}C_{12}$-1,2,3,6,7,8,-HxCDD	100	28-130
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	100	26-152
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	100	26-123
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	100	29-147
$^{13}C_{12}$-2,3,4,6,7,8,-HxCDF	100	28-136
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	100	23-140
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	100	28-143
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	100	26-138
$^{13}C_{12}$-OCDD	200	17-157
$^{37}Cl_4$-2,3,7,8-TCDD	10	35-197

TABLE 8. Descriptors, Exact m/z's, m/z Types, and Elemental Compositions of the CDDs and CDFs

Descriptor	Exact m/z[1]	m/z Type	Elemental Composition	Substance[2]
1	292.9825	Lock	$C_7\ F_{11}$	PFK
	303.9016	M	$C_{12}\ H_4\ ^{35}Cl_4\ O$	TCDF
	305.8987	M+2	$C_{12}\ H_4\ ^{35}Cl_3{}^{37}Cl\ O$	TCDF
	315.9419	M	$^{13}C_{12}\ H_4\ ^{35}Cl_4\ O$	TCDF[3]
	317.9389	M+2	$^{13}C_{12}\ H_4\ ^{35}Cl_3\ ^{37}Cl\ O$	TCDF[3]
	319.8965	M	$C_{12}\ H_4\ ^{35}Cl_4\ O_2$	TCDD
	321.8936	M+2	$C_{12}\ H_4\ ^{35}Cl_3\ ^{37}Cl\ O_2$	TCDD
	327.8847	M	$C_{12}\ H_4\ ^{37}Cl_4\ O_2$	TCDD[4]
	330.9792	QC	$C_7\ F_{13}$	PFK
	331.9368	M	$^{13}C_{12}\ H_4\ ^{35}Cl_4\ O_2$	TCDD[3]
	333.9339	M+2	$^{13}C_{12}\ H_4\ ^{35}Cl_3\ ^{37}Cl\ O_2$	TCDD[3]
	375.8364	M+2	$C_{12}\ H_4\ ^{35}Cl_5\ ^{37}Cl\ O$	HxCDPE
2	339.8597	M+2	$C_{12}\ H_3\ ^{35}Cl_4\ ^{37}Cl\ O$	PeCDF
	341.8567	M+4	$C_{12}\ H_3\ ^{35}Cl_3\ ^{37}Cl_2\ O$	PeCDF
	351.9000	M+2	$^{13}C_{12}\ H_3\ ^{35}Cl_4\ ^{37}Cl\ O$	PeCDF
	353.8970	M+4	$^{13}C_{12}\ H_3\ ^{35}Cl_3\ ^{37}Cl_2\ O$	PeCDF[3]
	354.9792	Lock	$C_9\ F_{13}$	PFK
	355.8546	M+2	$C_{12}\ H_3\ ^{35}Cl_4\ ^{37}Cl\ O_2$	PeCDD
	357.8516	M+4	$C_{12}\ H_3\ ^{35}Cl_3\ ^{37}Cl_2\ O_2$	PeCDD
	367.8949	M+2	$^{13}C_{12}\ H_3\ ^{35}Cl_4\ ^{37}Cl\ O_2$	PeCDD[3]
	369.8919	M+4	$^{13}C_{12}\ H_3\ ^{35}Cl_3\ ^{37}Cl_2\ O_2$	PeCDD[3]
	409.7974	M+2	$C_{12}\ H_3\ ^{35}Cl_6\ ^{37}Cl\ O$	HpCDPE
3	373.8208	M+2	$C_{12}\ H_2\ ^{35}Cl_5\ ^{37}Cl\ O$	HxCDF
	375.8178	M+4	$C_{12}\ H_2\ ^{35}Cl_4\ ^{37}Cl_2\ O$	HxCDF
	383.8639	M	$^{13}C_{12}\ H_2\ ^{35}Cl_6\ O$	HxCDF[3]
	385.8610	M+2	$^{13}C_{12}\ H_2\ ^{35}Cl_5\ ^{37}Cl\ O$	HxCDF[3]
	389.8157	M+2	$C_{12}\ H_2\ ^{35}Cl_5\ ^{37}Cl\ O_2$	HxCDD
	391.8127	M+4	$C_{12}\ H_2\ ^{35}Cl_4\ ^{37}Cl_2\ O_2$	HxCDD
	392.9760	Lock	$C_9\ F_{15}$	PFK
	401.8559	M+2	$^{13}C_{12}\ H_2\ ^{35}Cl_5\ ^{37}Cl\ O_2$	HxCDD[3]
	403.8529	M+4	$^{13}C_{12}\ H_2\ ^{35}Cl_4\ ^{37}Cl_2\ O_2$	HxCDD[3]
	430.9729	QC	$C_9\ F_{17}$	PFK
	445.7555	M+4	$C_{12}\ H_2\ ^{35}Cl_6\ ^{37}Cl_2\ O$	OCDPE
4	407.7818	M+2	$C_{12}\ H\ ^{35}Cl_6\ ^{37}Cl\ O$	HpCDF
	409.7789	M+4	$C_{12}\ H\ ^{35}Cl_5\ ^{37}Cl_2\ O$	HpCDF
	417.8253	M	$^{13}C_{12}\ H\ ^{35}Cl_7\ O$	HpCDF[3]
	419.8220	M+2	$^{13}C_{12}\ H\ ^{35}Cl_6\ ^{37}Cl\ O$	HpCDF[3]
	423.7766	M+2	$C_{12}\ H\ ^{35}Cl_6\ ^{37}Cl\ O_2$	HpCDD
	425.7737	M+4	$C_{12}\ H\ ^{35}Cl_5\ ^{37}Cl_2\ O_2$	HpCDD
	430.9729	Lock	$C_9\ F_{17}$	PFK
	435.8169	M+2	$^{13}C_{12}\ H\ ^{35}Cl_6\ ^{37}Cl\ O_2$	HpCDD[3]

TABLE 8. Descriptors, Exact m/z's, m/z Types, and Elemental Compositions of the CDDs and CDFs (Con't)

Descriptor	Exact m/z[1]	m/z Type	Elemental Composition	Substance[2]
	437.8140	M+4	$^{13}C_{12}$ H $^{35}Cl_5$ $^{37}Cl_2$ O_2	HpCDD[3]
	479.7165	M+4	C_{12} H $^{35}Cl_7$ $^{37}Cl_2$ O	NCDPE
5	441.7428	M+2	C_{12} $^{35}Cl_7$ ^{37}Cl O	OCDF
	442.9728	Lock	C_{10} F_{17}	PFK
	443.7399	M+4	C_{12} $^{35}Cl_6$ $^{37}Cl_2$ O	OCDF
	457.7377	M+2	C_{12} $^{35}Cl_7$ ^{37}Cl O_2	OCDD
	459.7348	M+4	C_{12} $^{35}Cl_6$ $^{37}Cl_2$ O_2	OCDD
	469.7779	M+2	$^{13}C_{12}$ $^{35}Cl_7$ ^{37}Cl O_2	OCDD[3]
	471.7750	M+4	$^{13}C_{12}$ $^{35}Cl_6$ $^{37}Cl_2$ O_2	OCDD[3]
	513.6775	M+4	C_{12} $^{35}Cl_8$ $^{37}Cl_2$ O	DCDPE

[1]Nuclidic masses used:

H = 1.007825 C = 12.00000 ^{13}C = 13.003355 F = 18.9984
O = 15.994915 ^{35}Cl = 34.968853 ^{37}Cl = 36.965903

[2]TCDD = Tetrachlorodibenzo-*p*-dioxin TCDF = Tetrachlorodibenzofuran
PeCDD = Pentachlorodibenzo-*p*-dioxin PeCDF = Pentachlorodibenzofuran
HxCDD = Hexachlorodibenzo-*p*-dioxin HxCDF = Hexachlorodibenzofuran
HpCDD = Heptachlorodibenzo-*p*-dioxin HpCDF = Heptachlorodibenzofuran
OCDD = Octachlorodibenzo-*p*-dioxin OCDF = Octachlorodibenzofuran
HxCDPE = Hexachlorodiphenyl ether HpCDPE = Heptachlorodiphenyl ether
OCDPE = Octachlorodiphenyl ether NCDPE = Nonachlorodiphenyl ether
DCDPE = Decachlorodiphenyl ether PFK = Perfluorokerosene

[3]Labeled compound

[4]There is only one m/z for $^{37}Cl_4$-2,3,7,8,-TCDD (cleanup standard).

TABLE 9. Theoretical Ion Abundance Ratios and Quality Control (QC) Limits

Number of Chlorine Atoms	m/z's Forming Ratio	Theoretical Ratio	QC Limit[1]	
			Lower	Upper
4[2]	M/(M+2)	0.77	0.65	0.89
5	(M+2)/(M+4)	1.55	1.32	1.78
6	(M+2)/(M+4)	1.24	1.05	1.43
6[3]	M/(M+2)	0.51	0.43	0.59
7	(M+2)/(M+4)	1.05	0.88	1.20
7[4]	M/(M+2)	0.44	0.37	0.51
8	(M+2)/(M+4)	0.89	0.76	1.02

[1]QC limits represent ±15% windows around the theoretical ion abundance ratios.

[2]Does not apply to $^{37}Cl_4$-2,3,7,8-TCDD (cleanup standard).

[3]Used for $^{13}C_{12}$-HxCDF only.

[4]Used for $^{13}C_{12}$-HpCDF only.

Table 10. Suggested Sample Quantities to be Extracted for Various Matrices[1]

Sample Matrix[2]	Example	Percent Solids	Phase	Quantity Extracted
Single-phase				
Aqueous	Drinking water	<1	[3]	1000 mL
	Groundwater			
	Treated wastewater			
Solid	Dry soil	>20	Solid	10 g
	Compost			
	Ash			
Organic	Waste Oil	<1	Organic	10 g
	Organic Polymer			
Tissue	Fish	—	Organic	10 g
Multi-phase				
Liquid/Solid				
Aqueous/Solid	Wet soil	1–30	Solid	10 g
	Untreated effluent			
	Digested municipal sludge			
Organic/solid	Industrial sludge	1–100	Both	10 g
	Oily waste			
Liquid/Liquid				
Aqueous/organic	In-process effluent	<1	Organic	10 g
	Untreated effluent			
Aqueous/organic/solid	Untreated effluent	>1	Organic & solid	10 g

[1]The quantity of sample to be extracted is adjusted to provide 10 g of solids (dry weight). One liter of aqueous samples containing one percent solids will contain 10 grams of solids. For aqueous samples containing greater than one percent solids, a lesser volume is used so that 10 grams of solids (dry weight) will be extracted.

[2]The sample matrix may be amorphous for some samples. In general, when the CDDs/CDFs are in contact with a multiphase system in which one of the phases is water, they will be preferentially dispersed in, or adsorbed on, the alternate phase because of their low solubility in water.

[3]Aqueous samples are filtered after spiking with the labeled compounds. The filtrate and the materials trapped on the filter are extracted separately, and the extracts are combined for cleanup and analysis.