

EXHIBIT D

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

Exhibit D - Analytical Methods for Chlorinated Dibenzo-P-Dioxins (CDDs) and
Chlorinated Dibenzofurans (CDFs)

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Exhibit D CDDs/CDFs -- Section 1
Scope and Application

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 chlorinated dibenzofurans (CDFs) (see Table 1) in water, soil, sediment, sludge, tissue, ash, oil and oil matrices by High Resolution Gas Chromatography/High Resolution Mass Spectrometry (HRGC/HRMS). The method is based on USEPA Method 1613, Revision B and SW-846 Method 8290 (Revision 0). Modifications may be requested in individual task orders.

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 (CRQLs) 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 (RTs) 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 interferences 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 chlorinated dibenzo-p-dioxins/chlorinated dibenzofurans (CDDs/CDFs), are spiked into a 1 L 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 (SPE) 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 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, $^{37}\text{Cl}_4$ -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.

Exhibit D CDDs/CDFs -- Section 2
Summary of Method (Con't)

- 2.2.2 After cleanup, the extract is concentrated to near dryness. Immediately prior to injection, the two internal standards, $^{13}\text{C}_{12}$ -1,2,3,4-TCDD and $^{13}\text{C}_{12}$ -1,2,3,7,8,9-HxCDD, are added to each extract. The former is used to determine the Percent Recoveries (%R) of tetra- and pentachlorinated CDD/CDF congeners, while the latter is used to determine the recoveries of the hexachlorinated, heptachlorinated, and octachlorinated CDD/CDF congeners.
- 2.2.3 An aliquot of the extract is injected into the High Resolution Gas Chromatograph (HRGC). The analytes are separated by the HRGC and detected by a High Resolution ($\geq 10,000$) Mass Spectrometer (HRMS). Two exact mass-to-charge (m/z) ratio ions are monitored for each analyte.
- 2.2.4 An individual CDD/CDF is identified by comparing the HRGC Retention Time (RT) and ion abundance ratio of two exact mass-to-charge (m/z) ratios with the corresponding RT of an authentic standard and the theoretical or acquired ion abundance ratio of the two exact m/z ratios. The non-2,3,7,8-substituted isomers and congeners are identified when RTs 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 octachlorinated dibenzofuran (OCDF) is detailed in Section 11.2.
- 2.3.1.2 For the labeled compounds and the cleanup standard, the Gas Chromatograph/Mass Spectrometer (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 (RRFs) 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 3 hours. Separatory funnels should be shaken with methylene chloride/toluene (80/20 mixture) for 2 minutes, drained, and then shaken with pure methylene chloride for 2 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 testing. 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 testing is not available, reagent water (see Section 7.6.1) can be used to simulate water samples; playground sand (see Section 7.6.2) or white quartz sand (see Section 7.3.2) can be used to simulate soils, and corn oil (see 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 mass-to-charge (m/z) ratios monitored for these interferences, 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 chlorinated dibenzo-p-dioxins/chlorinated dibenzofurans (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 an Occupational Safety and Health Administration/Mining Safety and Health Administration (OSHA/MSHA) approved toxic gas respirator shall be worn when high concentrations are handled.

5.2 Occupational Safety and Health Administration (OSHA) Requirements

The laboratory is responsible for maintaining a current awareness file of 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, and 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.

Exhibit D CDDs/CDFs -- Section 5
Safety (Con't)

- 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 are 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. Traps should be replaced on a routine basis to ensure they continue to be effective.
- 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 – Chloroethene NU Solvent is the least toxic solvent shown to be effective. Satisfactory cleaning may be accomplished by rinsing with Chloroethene, then washing with any detergent and water. If glassware is first rinsed with solvent and the residual solvent is allowed to evaporate under the exhaust hood, the dishwasher 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 an empty 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 (%S)
 - 6.2.5.1 Oven – Capable of maintaining a temperature of 110°C (±5°C).
 - 6.2.5.2 Desiccator.
 - 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 (Hydriion Papers, or equivalent).
 - 6.3.1.3 Graduated cylinder (Class A), 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 (SPE)
 - 6.3.1.5.1 1 L 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 inches 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.

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Equipment and Supplies (Con't)

- 6.3.1.5.4 SPE 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 4 to 9 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 Borosilicate glass wool -- Solvent extracted by SDS for 3 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 borosilicate 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 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 (GPC) (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 μ L 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 (HPLC)

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 μ L 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 drying and storage of adsorbents, capable of maintaining a constant temperature ($\pm 5^\circ\text{C}$) in the range of 105-250°C.

6.6.7 Oven or Tube Furnace -- for baking and activating adsorbents, capable of maintaining a constant temperature ($\pm 5^\circ\text{C}$) in the range of 400 - 700°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.

Exhibit D CDDs/CDFs -- Section 6
Equipment and Supplies (Con't)

- 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 $\pm 2^{\circ}\text{C}$, installed in a fume hood.
- 6.7.3 Nitrogen blowdown apparatus -- Equipped with water bath controlled in the range of $30\text{--}60^{\circ}\text{C}$ (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.
- 6.7.5 Volumetric flasks 10 mL, Class A, -- for the preparation of stock standard solutions.
- 6.7.6 Glass gas tight syringes (various sizes from 5 μL up to 2.0 mL) for preparation of standard solutions and spiking solutions (Laboratory Control Sample spiking solution, labeled reference compounds solution and internal standard solution).

6.8 Gas Chromatograph (GC)

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. The specified analytical columns are:

- 6.8.1 GC column for chlorinated dibenzo-p-dioxins/chlorinated dibenzofurans (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-25, or equivalent).

6.9 Mass Spectrometer (MS)

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

6.10 Gas Chromatograph/Mass Spectrometer (GC/MS) Interface

The 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 1 L.

7.1.4 Sodium chloride – solution. Dissolve 50g of reagent grade NaCl to reagent water and dilute to 1 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 desiccator, 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 Pre-purified 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 (SDS) extraction (Aldrich Chemical, Cat. No. 27-437-9, or equivalent). Bake at 450°C for 4 hours minimum.

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Reagents and Standards (Con't)

7.4 Gel Permeation Chromatography (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 desiccator, 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 1 to 2 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 2 to 4 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 Carbowpak 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 Carbowpak C and 41.0 g Celite 545. Activate the mixture at 130°C for a minimum of 6 hours. Store in a desiccator.

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 (see 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 chlorinated dibenzo-p-dioxins/chlorinated dibenzofurans (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 4 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 Contract Required Quantitation Limits (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.

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Reagents and Standards (Con't)

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 polytetrafluoroethylene (PTFE) tape and mark the meniscus of the solution to reduce and monitor evaporation of acetone.

7.8 Standards

- 7.8.1 Cleanup standard – Prepare $^{37}\text{Cl}_4$ -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 $^{13}\text{C}_{12}$ -1,2,3,4-TCDD and $^{13}\text{C}_{12}$ -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 (RR) (labeled to native) and Relative Response Factor (RRF) to be measured as a function of concentration. The Mid-Point Calibration Standard (CS3) is used for calibration verification.
- 7.8.4 Laboratory Control Sample (LCS) spiking solution – used for the LCS. Dilute 10 μL of the native compound stock solution (see Section 7.7.3) to 2 mL with acetone for each sample matrix for each sample batch. One mL is required for the LCS with each matrix in each batch.

Exhibit D CDDs/CDFs -- Sections 7 & 8
Sample Collection, Preservation, and Storage

- 7.8.5 Gas Chromatograph (GC) Retention Time (RT) Window Defining Mixture (WDM) and isomer specificity check standard – Used to define the beginning and ending RTs 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 4°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 ($\pm 2^\circ\text{C}$) 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. Standard Method (SM) 4500-Cl E (18th, 19th, or 20th Editions) and SM 4500-Cl E (2000) 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 Holding Times

- 8.3.1 All samples should be extracted, analyzed, and completed within 35 days of the last sample receipt date in the SDG per contract requirement.
- 8.3.2 The technical holding time for water or soil samples, stored in the dark at 4°C ($\pm 2^\circ\text{C}$) is one year. The technical holding time for tissue samples stored in the dark at less than -10°C is one year. The technical holding time for sample extracts stored in the dark at less than -10°C is one year.

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 (HRGC)

9.1.1.1 Prior to analyzing the calibration solutions, blanks, samples, and Quality Control (QC) samples, establish the HRGC operating conditions necessary to meet the minimum Retention Times (RTs) for the internal standards and the Relative Retention Times (RRTs) for the chlorinated dibenzo-p-dioxins/chlorinated dibenzofurans (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 Gas Chromatograph (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 (HRMS)

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 mass-to-charge (m/z) ratios and at $\geq 10,000$ 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 (MS). 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 resolving power to save re-analysis time. The GC run should be complete to allow elution of octachlorinated dibenzo-p-dioxin (OCDD) and octachlorinated dibenzofuran (OCDF).

9.2 High Resolution Gas Chromatograph/High Resolution Mass Spectrometer
(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; b) the resolution of the HRGC system must be verified by the analyses of the descriptor switching times set using the Window Defining Mixture (WDM), and c) the resolution verified by the analysis of 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 and end 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 mass resolution requirement, that the beginning and ending RTs for the dioxin and furan isomers are defined, and that the isomer specificity is demonstrated. (See Section 9.2.1.4 for the requirement for the MS calibration check at end of sequence.)
- 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 RT window will also be those isomers for which the ions are monitored.
- 9.2.1.4 The HRMS System Tune, the WDM, and the Isomer Specificity Check must also be analyzed at the end of each 12-hour shift or analytical sequence. These analyses may also be used to establish the beginning of the next 12-hour shift or analytical sequence.

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 the HRGC/HRMS system performance check does not begin until the HRMS system is tuned to meet the minimum required resolving power of 10,000 (10 Percent 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 ratios of three to five reference peaks covering the mass range of the descriptor.

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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 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.

9.2.2.4.2 If the technical acceptance criteria at the end of the 12-hour shift or analytical sequence are not met, all sample analyzed in that shift or analytical sequence having positive hits will be reanalyzed at no additional cost to USEPA.

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

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 RTs.

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 to ensure that all congeners in each homologous series elute in the appropriate descriptor window. Table 5 gives the elution order (first/last) of the window defining compounds on the DB-5 column. Particular caution should be exercised for the switching time between the last tetrachlorinated congener (i.e., 1,2,8,9-TCDD) and the first pentachlorinated congener (i.e., 1,3,4,6,8-PeCDF), as these two compounds elute within 15 seconds of each other on the 60 m DB-5 column. Alternatively, the tetra and penta homologue groups may be analyzed in a single descriptor. See also WDM corrective actions under Section 9.2.3.4.

9.2.3.3 Technical Acceptance Criteria for WDM

The analysis of the WDM is acceptable if the criteria given in Table 5 are met.

- 9.2.3.3.1 The retention times for the switching of SIM ions characteristic of one homologous series to the next higher homologous series must be indicated in the SICP.
- 9.2.3.3.2 All congeners included in the WDM standard must be present in the SICP.
- 9.2.3.3.3 If the GC/MS system is not capable of detecting both 1,2,8,9-TCDD and 1,3,4,6,8-PeCDF within one analysis, corrective action must be taken (see Section 9.2.3.2.2, above, for alternate corrective action).
- 9.2.3.3.4 The presence in the appropriate descriptors and chromatographic separation of all 2,3,7,8-substituted congeners must be demonstrated.
- 9.2.3.4 Corrective Action for WDM
- 9.2.3.4.1 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.
- 9.2.3.4.2 If the technical acceptance criteria at the end of the 12-hour shift or analytical sequence are not met, all sample analyzed in that shift or analytical sequence having positive hits will be reanalyzed at no additional cost to USEPA.
- 9.2.3.4.3 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 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 μ L 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:

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EQ. 1 Percent Valley

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

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%.

9.2.4.5 Corrective Action for Isomer Specificity Check

9.2.4.5.1 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.

9.2.4.5.2 If the technical acceptance criteria at the end of the 12-hour shift or analytical sequence are not met, all samples analyzed in that shift or analytical sequence having positive hits will be reanalyzed at no additional cost to USEPA.

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

9.3 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 fifteen 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 ratios 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 QC sample results against the mean Relative Response (RRF) and the mean Relative Response Factor (RRF) from the initial calibration.

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 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 RRF for each native and labeled analyte respectively 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 native CDD/CDF relative to its labeled analog is determined using the area responses of both the primary and secondary exact m/z ratios (specified in Table 8), for each calibration standard, as follows:

EQ. 2 Relative Response

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

Where:

RR = Relative Response of native compound to its labeled analog.

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

A1_L and A2_L = The areas of the primary and secondary m/z ratios 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.

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9.3.4.2 The RRF is determined for each labeled compound by the following equation:

EQ. 3 Relative Response Factor

$$RRF = \frac{(A1_s + A2_s) C_{IS}}{(A1_{IS} + A2_{IS}) C_s}$$

Where:

RRF = Relative Response Factor of each labeled analog to its internal standard.

A1_s and A2_s = The areas of the primary and secondary m/z ratios for the labeled compound.

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

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

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

EQ. 4 Mean Relative Response

$$\overline{RR} = \frac{\sum_{j=1}^5 RR}{5}$$

Where:

\overline{RR} = The mean Relative Response of the five initial calibration standards.

j = The injection number (or calibration solution number);
j = 1 to 5.

9.3.4.4 The mean RRF is determined by the following equation:

EQ. 5 Mean Relative Response Factor

$$\overline{RRF} = \frac{\sum_{j=1}^5 RRF}{5}$$

Where:

\overline{RRF} = The mean Relative Response factor of the five initial calibration standards.

j = As defined in Equation 4.

9.3.4.5 The Percent Relative Standard Deviation (%RSD) is determined for both the native CDD/CDF and the labeled analogs by the following equation:

EQ. 6 Percent Relative Standard Deviation

$$\%RSD = \frac{\text{Standard deviation of calibration response}}{\text{Mean value of calibration response}} \times 100$$

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 $\leq 25\%$ 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 S/N ratios for the HRGC/HRMS signal in every SICP must be ≥ 10 .
- 9.3.5.5 The RTs of the isomers must fall within the appropriate RT windows established by analysis of the WDM. The Absolute RT of $^{13}\text{C}_{12}$ -1,2,3,4-TCDD shall exceed 25 minutes on the DB-5 column, and the retention time of $^{13}\text{C}_{12}$ -1,2,3,4-TCDD shall exceed 15 minutes on the DB-225 column; otherwise, the GC temperature program shall be adjusted and this test repeated until the above-stated minimum RT criteria are met.
- 9.3.5.6 The %RSD for the RR must be $\pm 20\%$ and the %RSD for the RRF must be $\pm 35\%$ 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 consists of verification of the Mid-Point Calibration Standard (CS3) RR and RRF.

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 Column Performance Solution (CPS). The 12-hour time period begins with the injection of the CPS.
- 9.4.2.2 The CS3 standard must also be analyzed at the end of each 12-hour shift or analytical sequence. This analysis may also be used to establish the beginning of the next 12-hour shift or analytical sequence.

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 ratios. Compare the ratio to the theoretical ratio. Verify that the system meets the ion abundance ratios, the minimum S/N ratios and RT criteria. Compute the RR and RRF by the isotope dilution and internal standard methods, respectively. Determine the Percent Difference (%D) between the mean RR/RRF from the initial calibration and the calibration verification RR/RRF.

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9.4.4 Calculations for Calibration Verification

9.4.4.1 Calculate the RR and 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 Percent Difference

$$\%D = \frac{\text{Response}_{\text{ver}} - \text{Response}_{\text{INT}}}{\text{Response}_{\text{INT}}} \times 100$$

Where:

Response_{ver} = Response established during calibration verification.

Response_{INT} = Mean response established during initial calibration according to Equations 4 and 5.

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 RT of the internal standard ¹³C₁₂-1,2,3,4-TCDD must exceed 25 minutes on the DB-5 column, and the RT must exceed 15 minutes on the DB-225 column.

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

9.4.5.4 The Percent Difference (%D) between the calibration verification RR and the mean RR from the initial calibration must be within ±25%. The Percent Difference 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 S/N ratio greater than or equal to 10.

9.4.6 Corrective Action for Calibration Verification

9.4.6.1 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.

9.4.6.2 If the technical acceptance criteria at the end of the 12-hour shift or analytical sequence are not met, all samples analyzed in that shift or analytical sequence will be reanalyzed at no additional cost to USEPA.

9.4.6.3 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, or recalibrate with fresh calibration standards.

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, Laboratory Control Samples (LCSs), Samples (if time still remains on the 12-hour clock) Ending CPS Ending PFK HRMS Tune
Beginning of Next 12-Hour Period	PFK HRMS Tune CPS CS3 Blanks, LCSs, Samples Ending CPS Ending PFK HRMS Tune

10.0 PROCEDURE

10.1 Sample Preparation

10.1.1 Sample preparation involves modifying the physical form of the sample so that the chlorinated dibenzo-p-dioxins/chlorinated dibenzofurans (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 the Sample Management Office (SMO) to apprise them of the type of sample received. SMO will then contact USEPA for a resolution. If all phases of the sample are amenable to analysis, USEPA may require the Contractor to do any of 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; or
- 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 SMO to apprise them of the problem. SMO will then contact USEPA for a resolution. USEPA 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 USEPA's decision in the Sample Delivery Group (SDG) Narrative.

10.1.3 Water Samples

Water samples visibly absent of particles are extracted directly using the separatory funnel (see Section 10.2.1) or Solid Phase Extraction (SPE) technique (see Section 10.2.2). Water samples containing visible particles and containing 1% suspended solids or less are prepared according to Section 10.1.3.1 and extracted using the SPE technique, followed by Soxhlet/Dean-Stark (SDS) extraction (see 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 (see Section 10.2.3) and the filtrate is extracted using the separatory funnel procedure (see 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 ± 1 g.

10.1.3.1.2 Spike 1 mL of the diluted labeled compound spiking solution (see 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 two 1 L aliquots of reagent water in clean sample bottles or flasks.
- 10.1.3.1.4 Spike 1 mL of the diluted labeled compound spiking solution (see Section 7.7.4.2) into the two reagent water aliquots. One of these aliquots will serve as the blank and the other will serve as the Laboratory Control Sample (LCS). Spike the LCS with 1 mL of the LCS 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 ± 1 g. 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 (%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 $110 \pm 5^\circ\text{C}$, and cool in a desiccator. Calculate Percent Solids as follows:

EQ.8 Percent Solids

$$\% \text{Solids} = \frac{\text{Weight of Sample Aliquot After Drying}}{\text{Weight of Sample Aliquot Before Drying}} \times 100$$

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 two 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 the other will serve as the LCS. Spike the LCS with 1.0 mL of the LCS 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 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 by adding approximately 10 g of the tissue reference matrix to a 400 to 500 mL beaker. For the alternate HCl digestion/extraction, add the reference matrix to a 500 to 600 mL wide mouth bottle. 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. Spike the LCS with 1 mL of the LCS 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 Sections 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 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 mL of the diluted labeled compound spiking solution into each reference matrix aliquot. One aliquot will serve as the blank and the other will serve as the LCS. Spike the LCS with 1 mL of the LCS 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 liquid.

10.1.6.7 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 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 (see Section 10.2.1) and SPE (see Section 10.2.2) for waters, SDS (see Section 10.2.3) for soil/sediments, and Soxhlet extraction (see 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 (see 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 for 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 filter 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 Kuderna-Danish (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 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. Re-assemble 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 (SDS) Extraction
- 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 a 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.

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- 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 1% solids (see Section 10.1.3.1.6.6).
 - 10.2.3.9.2 Concentrate the extract to approximately 5 mL using the heating mantle or rotary evaporation procedures in Sections 10.3.2 or 10.3.1.
 - 10.2.3.9.3 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.
 - 10.2.3.9.4 Adjust to approximately 10 mL with hexane, transfer to a 250 mL separatory funnel, and proceed with back extraction (see Section 10.2.6).
 - 10.2.3.9.5 Extracts from soil/sediments (see 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 to form a dry free flowing powder. 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 D-S 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 quantitatively transfer the extract to a macro-concentration device.
 - 10.2.4.7 Disassemble the apparatus and set aside for re-use.
 - 10.2.4.7.1 Complete the removal of the solvent using the blowdown procedure (see Section 10.4) 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.
 - 10.2.4.8 Percent Lipids (% Lipids) 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.
 - 10.2.4.8.1 Redissolve the residue in the concentration device in hexane and spike 1.0 mL of the cleanup standard into the extract.

10.2.4.8.2 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.

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

EQ. 9 Percent Lipids

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

10.2.4.8.4 It is not necessary to determine the lipid content of the blank and LCS 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 Quality Control (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 Lipids Determination

Determine the lipid content per the procedure described in Section 10.2.4.8.

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 10.2.3.9.4).

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- 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 and 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 K-D 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 1 to 2 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 (K-D) Apparatus

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. Pre-wet 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 Gel Permeation Chromatography (GPC) or High Performance Liquid Chromatograph (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. If the GPC or HPLC system does not have a 5.0 mL loop, adjust the volume of the extract to fit an injection volume of at least 2.0 mL. 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, Carbpak/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 High Resolution Gas Chromatograph/High Resolution Mass Spectrometer (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

- 10.5.1 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 removes many high molecular weight interferences that cause GC column performance to degrade. It should 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). If other cleanup techniques prove adequate, they may be used in lieu of GPC. Acid, neutral, basic silica gel, alumina and Florisil (see Sections 10.5.3, 10.5.4, 10.5.7, and 10.5.8) are used to remove non-polar and polar interferences. Alumina and Florisil are used to remove chlorodiphenyl ethers. Carbpak/Celite (see Sections 10.5.4 and 10.5.8) is used to remove nonpolar

interferences. HPLC (see Section 10.5.6) is used to provide specificity for the 2,3,7,8-substituted and other CDD and CDF isomers. The anthropogenic isolation column (see Section 10.5.7.1), acidified silica gel batch adsorption procedure (see Section 10.5.7.2), and sulfuric acid back extraction (see Section 10.5.7.3) are used for removal of lipids from tissue samples.

10.5.2 Sample Cleanup by GPC

10.5.2.1 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.

10.5.2.2 Column Packing

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

10.5.2.2.2 Cover the beads with methylene chloride and allow to swell for a minimum of 2 hours).

10.5.2.2.3 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.

10.5.2.2.4 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.

10.5.2.3 Calibration of GPC

10.5.2.3.1 Summary of Calibration

The GPC calibration procedure is based on monitoring the elution of standards with an Ultraviolet (UV) detector connected to the GPC column.

10.5.2.3.2 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.

10.5.2.3.3 Procedure for GPC Calibration

10.5.2.3.3.1 Load 5 mL of the GPC calibration solution into the sample loop. If the autosampler injection loop does not have the 5 mL capacity, adjust the concentration of the GPC calibration solution so that an injection loop volume of 2 mL contains the same mass of each GPC calibration solution component.

10.5.2.3.3.2 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.

10.5.2.3.3.3 Set the "dump time" to allow greater than 85% removal of the corn oil and greater than 85% collection of the phthalate.

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

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10.5.2.3.5 Technical Acceptance Criteria for GPC Calibration

10.5.2.3.5.1 The GPC system must be calibrated at the frequency described in Section 10.5.2.3.2.

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

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

10.5.2.3.6 Corrective Action for GPC Calibration

10.5.2.3.6.1 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.

10.5.2.4 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.

10.5.2.4.1 Frequency of GPC Sample Cleanup

GPC cleanup may be performed for soil/sediment and water extracts that contain high molecular weight contaminants that interfere with the analysis of the target analytes. In this case GPC must also be performed for all associated blanks, and LCS.

10.5.2.4.2 Procedure for GPC Sample Cleanup

10.5.2.4.2.1 Filter the extract or load through the filter holder to remove any particulate. Load the concentrated sample extract into the sample injection loop and inject it onto the GPC column.

10.5.2.4.2.2 Elute the extract using the calibration data determined in Section 10.5.2.3. Collect the eluate in a clean 400 to 500 mL beaker.

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

10.5.2.4.2.4 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.

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

10.5.3 Silica Gel Cleanup

10.5.3.1 Procedure for Silica Gel Cleanup

10.5.3.1.1 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.

10.5.3.1.2 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.

- 10.5.3.1.3 Apply the concentrated extract to the column. Open the stopcock until the extract is within 1 mm of the sodium sulfate.
- 10.5.3.1.4 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.
- 10.5.3.1.5 Concentrate the eluate per Sections 10.3 or 10.4 for further cleanup or for injection into the HPLC or HRGC/HRMS.
- 10.5.3.1.6 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.

10.5.4 Alumina Cleanup

10.5.4.1 Procedure for Alumina Cleanup

- 10.5.4.1.1 Place a glass wool plug in a 15 mm ID chromatography column.
- 10.5.4.1.2 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.
- 10.5.4.1.3 Pre-rinse the column with 50 to 100 mL of hexane. Close the stopcock when the hexane is within 1 mm of the alumina.
- 10.5.4.1.4 Discard the eluate. Check the column for channeling. If channeling is present, discard the column and prepare another.
- 10.5.4.1.5 Apply the concentrated extract to the column. Open the stopcock until the extract is within 1 mm of the alumina.
- 10.5.4.1.6 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.
- 10.5.4.1.7 The choice of eluting solvents will depend on the choice of alumina (acid or basic) made in Section 10.5.4.1.2.
 - 10.5.4.1.7.1 If using acid alumina, elute the CDDs/CDFs from the column with 20 mL methylene chloride:hexane (20:80 v/v). Collect the eluate.
 - 10.5.4.1.7.2 If using basic alumina, elute the CDDs/CDFs from the column with 20 mL methylene chloride:hexane (50:50 v/v). Collect the eluate.
- 10.5.4.1.8 Concentrate the eluate per Sections 10.3 or 10.4 for further cleanup or for injection into the HPLC or HRGC/HRMS.

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10.5.5 Carbon Column Cleanup

10.5.5.1 Procedure for Carbon Column Cleanup

- 10.5.5.1.1 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 Carbowpak/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.
- 10.5.5.1.2 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.
- 10.5.5.1.3 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.
- 10.5.5.1.4 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.
- 10.5.5.1.5 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.
- 10.5.5.1.6 Concentrate the eluate per Sections 10.3 or 10.4 for further cleanup or for injection into the HPLC or HRGC/HRMS.

10.5.6 Sample Cleanup by HPLC

10.5.6.1 Calibration of HPLC

10.5.6.1.1 Procedure for HPLC Calibration

- 10.5.6.1.2 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/ μ L in methylene chloride.
- 10.5.6.1.3 Inject 30 μ L of the calibration solution into the HPLC and record the signal from the detector. Collect the eluant for re-use. The elution order will be the tetrachlorinated through octachlorinated isomers.
- 10.5.6.1.4 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 μ L injections while the detector is monitored, to ensure that residual CDDs/CDFs are removed from the system.

10.5.6.2 Technical Acceptance Criteria for HPLC Calibration

- 10.5.6.2.1 Verify the calibration with the calibration solution after every 20 extracts.
- 10.5.6.2.2 Calibration is verified if the recovery of the CDDs/CDFs from the calibration standard (Section 10.5.6.1.2) is within 75 to 125%.

10.5.6.3 Corrective Action for HPLC Calibration

- 10.5.6.3.1 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.

10.5.6.4 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 μ L of extract. If the extract cannot be concentrated to less than 30 μ L, it is split into fractions and the fractions are combined after elution from the column.

10.5.6.4.1 Procedure for HPLC Sample Cleanup

10.5.6.4.1.1 Rinse the sides of the vial twice with 30 μ L of methylene chloride and reduce to 30 μ L with the evaporation apparatus.

10.5.6.4.1.2 Inject the 30 μ L extract into the HPLC.

10.5.6.4.1.3 Elute the extract using the calibration data determined in Section 10.5.6.1. Collect the fraction(s) in a clean 20 mL concentrator tube containing 5 mL of hexane:acetone (1:1 v/v).

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

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

10.5.7 Cleanup of Tissue Lipids

Lipids are removed from the Soxhlet extract using either the anthropogenic isolation column (see Section 10.5.7.1) or acidified silica gel (see Section 10.5.7.2), or are removed from the HCl digested extract using sulfuric acid and base back extraction (see Section 10.5.7.3).

10.5.7.1 Anthropogenic Isolation Column Cleanup

10.5.7.1.1 Procedure for Anthropogenic Isolation Column Cleanup

10.5.7.1.1.1 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.

10.5.7.1.1.2 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.

10.5.7.1.1.3 Load the sample and rinses (see Section 10.2.4.8.2) 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 10.2.4.6) using 200 mL of hexane.

10.5.7.1.1.4 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.

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

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

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

10.5.7.1.1.8 Following cleanup, concentrate the extract to 10 μ L as described in Section 10.4.8, and proceed with the analysis in Section 10.6.

10.5.7.2 Acidified Silica Gel Cleanup

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

10.5.7.2.1 Procedure for Acidified Silica Gel Cleanup

10.5.7.2.1.1 Adjust the volume of hexane in the bottle (see Section 10.2.4.8.1) to approximately 200 mL.

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

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

10.5.7.2.1.4 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.

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

10.5.7.2.1.6 Concentrate the extract to a volume suitable for the cleanups given in Sections 10.5.

10.5.7.3 Sulfuric Acid and Base Back Extraction Cleanup

Used with HCl digested extracts (see Section 10.2.5).

10.5.7.3.1 Procedure for Sulfuric Acid and Base Back Extraction Cleanup

10.5.7.3.1.1 Spike 1.0 mL of the cleanup standard into the residue/solvent (see Section 10.2.4.8.1).

10.5.7.3.1.2 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.

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

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

10.5.7.3.1.5 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.

10.5.7.3.1.6 Concentrate the cleaned up extract to a volume suitable for the additional cleanups given in Sections 10.5.1 through 10.5.6. GPC, alumina, and Carboxpak/Celite are recommended as minimum additional cleanup steps.

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

10.5.8 Florisil Cleanup

10.5.8.1 Procedure for Florisil Cleanup

- 10.5.8.1.1 Pre-elute the activated Florisil column (see 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.
- 10.5.8.1.2 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.
- 10.5.8.1.3 Elute the interfering compounds with 20 mL of hexane:methylene chloride (98:2) and discard the eluate.
- 10.5.8.1.4 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 Gas Chromatograph/Mass Spectrometer (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 Window-Defining Mixture (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 not 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 octachlorinated dibenzo-p-dioxin (OCDD) and octachlorinated dibenzofuran (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 10.4.7); others may overload the HRGC column and/or MS. 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 of the 2,3,7,8 substituted PCDD/PCDF given in Table 1 of Exhibit C (except OCDD and OCDF) exceeds the calibration range of the system, the Contractor shall do the following:

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- 10.6.6.1 Add sufficient amount of the 15 labeled standards to the original sample extract such that the concentration upon dilution is equal to the concentration of the labeled standard in the original sample extract. For the original analysis, the spike must be added prior to dilution. Dilute the respiked sample extract in order to bring the high concentration compound(s) within calibration range. Do not exceed a 100x dilution. This reanalysis is not billable. If the SICP area at either quantitation m/z for any of the 2,3,7,8-substituted PCDD/PCDF given in Table 1 of Exhibit C (except OCDD and OCDF) still exceeds the calibration range of the system, the Contractor shall do the following:
- 10.6.6.1.1 For water samples containing 1% soil/sediments or less, dilute 100 mL, 10 mL, etc., of sample up to 1 L with reagent water and re-prepare, extract, clean up and analyze. This reextraction/reanalysis is not billable.
- 10.6.6.1.2 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. The Contractor has the option to reextract an aliquot of sufficient size to ensure that it is representative of the entire sample. Either immediately following extraction, or after extraction and clean-up, prior to GC/MS analysis, spike and dilute the sample so that it has a concentration of internal standard equivalent to that present in the calibration standard. **For a reextraction/reanalysis, the spike may be performed before or after the dilution.** Then, analyze the diluted extract. This reextraction/reanalysis is not billable. The exact procedure followed in this step must be documented in the case narrative. Results and supporting raw data should be submitted for all analytical runs, and the initial, less dilute, data will be used by the Region to evaluate initial labeled compound recovery. The contractor must not correct results for recovery of the 15 labeled standards.
- 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 one percent 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 Lipids 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 signal-to-noise (S/N) ratios above 2.5, even if below the Contract Required Quantitation Limit (CRQL) (See Exhibit C).

11.0 DATA ANALYSIS AND CALCULATIONS

11.1 Qualitative Identification

11.1.1 Identification of Target Analytes

A chlorinated dibenzo-p-dioxin (CDD) and chlorinated dibenzofuran (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 mass-to-charge (m/z) ratios must be present and must maximize within the same 2 seconds.
- 11.1.1.2 The signal-to-noise (S/N) ratio 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 $\pm 10\%$ of the ratio in the most recent Midpoint Calibration Standard (CS3).
- 11.1.1.4 The Relative Retention Time (RRT) 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 (RT) of peaks representing non-2,3,7,8-substituted CDDs/CDFs must be within the RTs established during the analysis of the Window Defining Mix (WDM) for the first and last eluting compounds.

11.1.2 High Resolution Gas Chromatography/High Resolution Mass Spectrometry (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 is positively identified at or above the Contract Required Quantitation Limit (CRQL) by analysis on a DB-5 (or equivalent) HRGC column, or TCDF is reported as an Estimated Maximum Possible Concentration (EMPC) at or above 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 judgment 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

The dilution results are not corrected based on labeled standard recovery in the original sample, however the laboratory may note, for completeness of documentation in the data package, that no correction was performed.

This is applicable where this practice differs from the laboratory's Standard Operating Procedures.

- 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 (GC).
- 11.2.1.2 Because of a potential interference, the labeled analog of the isomer octachlorinated dibenzofuran (OCDF) is not added to the sample. Therefore, native OCDF is quantitated against labeled octachlorinated dibenzo-p-dioxin (OCDD). As a result, the concentration of native OCDF is corrected for the recovery of the 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 mean Relative Response (\overline{RR}) values from the initial calibration data described in Section 9.3.4.3 are used to determine concentrations directly, so long as labeled compound spiking levels are constant, using the following equation:

EQ. 10 Extract Concentration by Relative Response

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

Where:

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

C_L = The concentration of the labeled compound in the extract.

\overline{RR} is defined in Section 9.3.4.3. 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 1,2,3,7,8,9-HxCDD, OCDF, ^{13}C -labeled analogs, and the ^{37}Cl -labeled cleanup standard in the extract using the mean Relative Response Factors (RRFs) determined from the initial calibration data (see Section 9.3.4.4) and the following equation:

EQ. 11 Extract Concentration by Relative Response Factor

$$C_{EX}(\text{ng/mL}) = \frac{(A1_s + A2_s) C_{IS}}{(A1_{IS} + A2_{IS}) RRF}$$

Where:

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

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

\overline{RRF} is defined in Section 9.3.4.4. 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 Concentration in Solid

$$\text{Concentration in Solid (ng/kg)} = \frac{(C_{EX} \times V_{EX})}{W_s}$$

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 Aqueous Concentration

$$\text{Concentration in Water Phase (pg/L)} = \frac{(C_{EX} \times V_{EX})}{V_s} \times 1000$$

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 (EDL)

The 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 CDDs/CDFs are present.

The EDL is not adjusted with the theoretical ion balance. However, the laboratory may note, for completeness of documentation in the data package, that no correction was performed. This is applicable where this practice differs from the laboratory's Standard Operating Procedures.

- 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 Selected Ion Current Profile (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 ¹³C-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 Aqueous Estimated Detection Limit

$$\text{Aqueous EDL (pg/L)} = \frac{2.5 \times Q_L \times (H_{x1} + H_{x2}) \times D}{V \times (H_{L1} + H_{L2}) \times RR}$$

Use the following formula for soil samples:

EQ. 15 Soil Estimated Detection Limit

$$\text{Soil EDL (ng/Kg)} = \frac{2.5 \times Q_L \times (H_{x1} + H_{x2}) \times D}{W \times (H_{L1} + H_{L2}) \times RR}$$

Where:

EDL = Estimated Detection Limit for 2,3,7,8-substituted CDDs/CDFs.

Q_L = Quantity (pg) of appropriate labeled standard added prior to sample extraction.

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

H_{L1}, H_{L2} = Peak heights of the labeled standard ions.

D = Dilution Factor.

V = Volume extracted in liters.

W = Weight extracted in grams.

\overline{RR} = The mean Relative Response for the isomer of interest from the initial calibration (See Section 9.3.4.3).

NOTE: If interferences can be shown to be present (e.g., PCBs), then descriptor ions other than those listed in Table 8 may be used for quantitation. This deviation shall be adequately documented in the Case Narrative.

11.2.6 Estimated Maximum Possible Concentration (EMPC)

An EMPC is calculated for 2,3,7,8-substituted isomers that are characterized by a response with an S/N of at least 2.5 for both quantitation ions, but that do not meet all the identification criteria described in Section 11.

The EMPC is not adjusted with the theoretical ion balance. However, the laboratory may note, for completeness of documentation in the data package, that no correction was performed. This is applicable where this practice differs from the laboratory's Standard Operating Procedures.

- 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 Solid Estimated Maximum Possible Concentration

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

Where:

D = Dilution Factor.

W_s = Sample weight (dry weight) in kg.

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

- 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 Aqueous Estimated Maximum Possible Concentration

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

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 (%R) 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 Percent Recovery

$$\%R = \frac{\text{Measured Concentration}}{\text{Known Concentration}} \times 100$$

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

- 11.2.8 CDD/CDF Toxicity Equivalence Factor Adjusted Concentration

The concentration of each of the 2,3,7,8-substituted CDD and CDF isomers is multiplied by a Toxicity Equivalence Factor* (TEF) to arrive at an equivalent toxicity concentration of 2,3,7,8-TCDD. Calculate the adjusted TEF concentration for each species using the TEF associated with the species. The 2005 World Health Organization (WHO) TEFs for mammal and the 1998 WHO TEFs for fish and bird are used.

11.2.8.1 Toxicity Equivalence Summary

Add all 17 TEF-adjusted concentrations for each species together to calculate the Toxicity Equivalence Summary **for each of the three sets of TEFs: mammal, fish, and bird using the applicable TEFs for each.**

EQ. 19 TEF Adjusted Concentration

$$C_{\text{TEF Adj}} = C_{\text{isomer}} \times \text{TEF}$$

Where:

$C_{\text{TEF Adj}}$ = TEF adjusted concentration

C_{isomer} = Concentration of 2,3,7,8-substituted CDD and CDF isomer

TEF = Toxicity Equivalence Factor

EQ. 20 Toxicity Equivalence Summary

$$\sum C_{\text{TEF Adj}} = \text{Toxicity Equivalence Summary}$$

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, perfluorokerosene (PFK), initial calibration, calibration verification, and blank technical acceptance criteria. 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 2 and 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, see Section 10.6.6.

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 System performance checks, initial calibration, and calibration verification must be acceptable before the re-analysis of samples.

12.0 QUALITY CONTROL (QC)

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 (LCSS) and Performance Evaluation (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 High Resolution Gas Chromatography/High Resolution Mass Spectrometry (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 Sample Delivery Group (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 Contract Required Quantitation Limit (CRQL) at the mass-to-charge (m/z) ratio of the native compounds.

12.1.4.2 For all chlorinated dibenzo-p-dioxins/chlorinated dibenzofurans (CDDs/CDFs) [except octachlorinated dibenzo-p-dioxin/octachlorinated dibenzofuran (OCDD/OCDF)], the concentration of the native compounds in the method blank must be less than or equal to the CRQL. The concentration of OCDD/OCDF in the method blank must be less than three times the CRQL, if detected.

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 with native compound concentrations above the CRQLs and 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)

12.2.1 Summary of LCS

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

12.2.2 Frequency of LCS Analysis

A LCS 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

12.2.3.1 Prepare an aliquot of clean reference matrix. Spike the aliquot with 1.0 mL of the labeled compound spiking solution (see Section 7.7.4) and 1.0 mL of the LCS spiking solution (see Section 7.8.4).

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

12.2.4 Calculation for LCS

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 analytes using the following equation:

EQ. 21 Percent Recovery of LCS Analytes

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

12.2.5 Technical Acceptance Criteria for LCS

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

12.2.5.2 The LCS must meet the technical acceptance criteria for sample analyses in Section 11.3.

12.2.5.3 The Percent Recovery of each of the compounds in the LCS must be within the acceptance limits in Table 6. Up to three target compounds may fail to meet the recovery limits listed in Table 6. However, frequent failures to meet the limits for recovery warrant investigation by the laboratory, and may result in questions from USEPA.

12.2.6 Corrective Action for LCS

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

13.0 METHOD PERFORMANCE

Not applicable.

14.0 POLLUTION PREVENTION

- 14.1 The solvents used in this method pose little threat to the environment when managed properly.
- 14.2 Standards should be prepared in volumes consistent with laboratory use to minimize the volume of expired standards to be disposed.

15.0 WASTE MANAGEMENT

- 15.1 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.
- 15.2 Samples containing HCl to pH < 2 are hazardous and must be neutralized before being poured down a drain or handled as hazardous waste.
- 15.3 The chlorinated dibenzo-p-dioxins/chlorinated dibenzofurans (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.
- 15.4 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.
- 15.5 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.

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Exhibit D CDDs/CDFs -- Section 16
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17.0 TABLES/DIAGRAMS/FLOWCHARTS

Table 1. Chlorinated Dibenzo-P-Dioxins/Chlorinated Dibenzofurans (CDDs/CDFs)
Determined by Isotope Dilution and Internal Standard High Resolution Gas
Chromatography /High Resolution Mass Spectrometry (HRGC/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 (Cleanup) ¹³ C ₁₂ -1,2,3,4-TCDD (IS)	76523-40-5 85508-50-5 114423-99-3
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 (IS)	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 (RT) References, Quantitation References, Relative Retention Times (RRTs), and Minimum Levels for CDDs/CDFs

CDD/CDF	Retention Time and Quantitation Reference	Relative Retention Time	CRQL		
			Water (pg/L; ppq)	Solid (ng/kg; ppt)	Extract (pg/μL; ppb)
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 ¹³C₁₂-1,2,3,6,7,8-HxCDD. 1,2,3,7,8,9-HxCDD is quantified using the averaged responses of ¹³C₁₂-1,2,3,4,7,8-HxCDD and ¹³C₁₂-1,2,3,6,7,8-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	0.2
2,3,7,8-TCDF	—	—	40	0.2
1,2,3,7,8-PeCDD	—	—	200	1
1,2,3,7,8-PeCDF	—	—	200	1
2,3,4,7,8-PeCDF	—	—	200	1
1,2,3,4,7,8-HxCDD	—	—	200	1
1,2,3,6,7,8-HxCDD	—	—	200	1
1,2,3,7,8,9-HxCDD	—	—	200	1
1,2,3,4,7,8-HxCDF	—	—	200	1
1,2,3,6,7,8-HxCDF	—	—	200	1
1,2,3,7,8,9-HxCDF	—	—	200	1
2,3,4,6,7,8-HxCDF	—	—	200	1
1,2,3,4,6,7,8-HpCDD	—	—	200	1
1,2,3,4,6,7,8-HpCDF	—	—	200	1
1,2,3,4,7,8,9-HpCDF	—	—	200	1
OCDD	—	—	400	2
OCDF	—	—	400	2
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	—	—
Cleanup Standard⁵				
³⁷ Cl ₄ -2,3,7,8-TCDD	0.8			
Internal Standards⁶				
¹³ C ₁₂ -1,2,3,4-TCDD	200			
¹³ C ₁₂ -1,2,3,7,8,9-HxCDD	200			

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

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

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

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

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

⁶Section 7.8.2, prepared in nonane and added to the concentrated extract immediately prior to injection into the GC (see 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 -2,3,4,6,7,8-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

¹See Section 7.8.3, calibration verification solution.

Table 5. Gas Chromatography (GC) RT Window Defining Mixture (WDM) and Isomer Specificity Check Standard (see Section 7.8.5)

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 Mass-to-Charge (m/z) Ratios, m/z Types, and Elemental Compositions of the CDDs/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	HxCDFE
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	HpCDFE
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
	437.8140	M+4	¹³ C ₁₂ H ³⁵ Cl ₅ ³⁷ Cl ₂ O ₂	HpCDD ³

Descriptor	Exact m/z ¹	m/z Type	Elemental Composition	Substance ²
	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 ¹³C = 13.003355 F = 18.9984
O = 15.994915 ³⁵Cl = 34.968853 ³⁷Cl = 36.965903

²TCDD = 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

³Labeled compound.

⁴There is only one m/z for ³⁷Cl₄-2,3,7,8,-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

¹QC limits represent $\pm 15\%$ windows around the theoretical ion abundance ratios.

²Does not apply to ³⁷Cl₄-2,3,7,8-TCDD (cleanup standard).

³Used for ¹³C₁₂-HxCDF only.

⁴Used for ¹³C₁₂-HpCDF only.

Table 10. Suggested Sample Quantities to be Extracted for Various Matrices¹

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				
<i>Liquid/Solid</i>				
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			
<i>Liquid/Liquid</i>				
Aqueous/organic	In-process effluent	<1	Organic	10 g
	Untreated effluent			
Aqueous/organic/solid	Untreated effluent	>1	Organic & solid	10 g

¹The quantity of sample to be extracted is adjusted to provide 10 g of solids (dry weight). 1 L 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.

²The sample matrix may be amorphous for some samples. In general, when the chlorinated dibenzo-p-dioxins/chlorinated dibenzofurans (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.

³Aqueous 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.

Table 11. Toxicity Equivalence Factors (TEF)

TARGET ANALYTE	TEF	TEF		
	Animals	Mammal	Fish	Bird
2378-TCDD	1	1	1	1
2378-TCDF	0.1	0.1	0.05	1
12378-PeCDF	0.05	0.03	0.05	0.1
12378-PeCDD	0.5	1	1	1
23478-PeCDF	0.5	0.3	0.5	1
123478-HxCDF	0.1	0.1	0.1	0.1
123678-HxCDD	0.1	0.1	0.1	0.1
123478-HxCDD	0.1	0.1	0.5	0.05
123678-HxCDD	0.1	0.1	0.01	0.01
123789-HxCDD	0.1	0.1	0.01	0.1
234678-HxCDF	0.1	0.1	0.1	0.1
123789-HxCDF	0.1	0.1	0.1	0.1
1234678-HpCDF	0.01	0.01	0.01	0.01
1234678-HpCDD	0.01	0.01	0.001	0.001
1234789-HpCDF	0.01	0.01	0.01	0.01
OCDD	0.001	0.0003	0.0001	0.0001
OCDF	0.001	0.0003	0.0001	0.0001
Source	EPA/625/3-89/016 March 1989	WHO* 2005	WHO* 1998	

*World Health Organization