EXHIBIT D

CHLORINATED DIBENZO-*p*-DIOXINS AND CHLORINATED DIBENZOFURANS ANALYSIS

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Exhibit D - Chlorinated Dibenzo-*p*-Dioxins and Chlorinated Dibenzofurans

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1.0 SCOPE AND APPLICATION

1.1 Method

The analytical method that follows is designed to analyze aqueous/water, soil/sediment, sludge, tissue (non-human), biosolids, ash, oil, and oily matrices by High Resolution Gas Chromatography/High Resolution Mass Spectrometry (HRGC/HRMS) to determine the presence and concentration of tetra through octa Chlorinated Dibenzo-*p*-Dioxins (CDDs) and Chlorinated Dibenzofurans (CDFs) contained in the Target Analyte List (TAL) for CDDs/CDFs in Exhibit C - Chlorinated Dibenzo-*p*-Dioxins and Chlorinated Dibenzofurans and Chlorinated Biphenyl Congeners Target Analyte List and Contract Required Quantitation Limits. The method is based on U.S. Environmental Protection Agency (EPA) Method 1613, Revision B (October 1994) and Method 8290A, Revision 1 (February 2007).

1.1.1 This method allows for the determination of the CDD/CDF Toxic Equivalents (TEQs) for mammal, fish, and bird tissues using Toxic Equivalency Factors (TEFs).

1.1.2 This method also allows for the estimation of homologue totals by level of chlorination (LOC) and estimation of total CDD/CDF in a sample by summation of the concentrations of the congeners.

1.2 Quantitation Levels

The levels listed in Exhibit C - Chlorinated Dibenzo-*p*-Dioxins and Chlorinated Dibenzofurans and Chlorinated Biphenyl Congeners Target Analyte List and Contract Required Quantitation Limits, Table 1 are the Contract Required Quantitation Limits (CRQLs). These limits are set based on the low calibration standard (CS1) analyzed for each 2,3,7,8-substituted congener.

The ability to achieve the CRQLs of this Statement of Work (SOW) is dependent on the level of interferences and laboratory background levels rather than instrumental limitations. Care shall be exercised to eliminate these background contaminants and interferences from the laboratory.

1.3 Qualitative Identification

The qualitative identification criteria (Section 11.1) include requirements for Retention Times (RTs) and limits on the ratio of the abundance of two exact m/z signals 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.

1.4 Caution

Because of the extreme toxicity of these compounds, the analyst must take necessary precautions to prevent the exposure of Contractor personnel or others to materials known or believed to contain CDDs or CDFs. Typical infectious waste incinerators are not satisfactory devices for disposal of materials highly contaminated with Polychlorinated Dibenzodioxins (PCDDs) or Polychlorinated Dibenzofurans (PCDFs). A Contractor planning to use these compounds should prepare a disposal plan. Additional safety instructions are outlined in Section 5.0.

2.0 SUMMARY OF METHOD

2.1 Extraction

2.1.1 Aqueous/Water Samples [samples containing less than one percent solids (%Solids)]

A mixture of ^{13}C-labeled analogs of the fifteen 2,3,7,8-substituted chlorinated CDDs/CDFs is spiked into a 1 liter (L) sample. The sample is extracted by one of the following two procedures:

2.1.1.1 Samples containing no visible particulates are extracted with methylene chloride in a separatory funnel, by continuous liquid-liquid extraction or the Solid Phase Extraction (SPE) technique. The extract is concentrated for cleanup.

2.1.1.2 Samples containing greater than or equal to 1% solids are vacuum filtered through a glass fiber filter. The filter containing the particulates is extracted as a soil sample and the filtrate is extracted as a water sample. The extract from the aqueous phase is concentrated and combined with the filter extract prior to cleanup.

2.1.2 Soil/Sediment, Multiphase, and Other Solid Samples

A mixture of ^{13}C-labeled analogs of the fifteen 2,3,7,8-substituted chlorinated CDDs/CDFs is spiked into a sample containing 10 grams (g) (dry weight) of solids and the sample is either extracted in an Soxhlet/Dean-Stark (SDS) extractor or mixed with sodium sulfate to a free-flowing consistency, allowed to equilibrate, and is extracted using the Soxhlet technique. The extract is concentrated for cleanup. Samples containing coarse solids are ground or homogenized prior to extraction.

2.1.3 Tissue (Non-Human) Samples

The sample is extracted by one of the following 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 anhydrous sodium sulfate, allowed to dry for 12-24 hours or overnight, and extracted for 18-24 hours using methylene chloride:hexane (1:1) in a Soxhlet extractor. The extract is evaporated to near dryness, and the lipid content is determined gravimetrically.

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

2.2 Cleanup

After extraction, the Cleanup Standard is added to each extract to measure the efficiency of the cleanup procedure. Sample cleanups may include back-extraction with acid and/or base, Gel Permeation Chromatography (GPC), alumina, silica gel, and Florisil. Activated carbon columns and High Performance Liquid Chromatography (HPLC) can be used for further isolation of specific isomers or congeners. Tissue extracts are cleaned up using an anthropogenic isolation column prior to the cleanup procedures listed above.

2.3 Analysis

2.3.1 After cleanup, the extract is concentrated to 10 microliters (µL). Immediately before injection, the two internal standards, $^{13}C_{12}$-1,2,3,4-TCDD, $^{13}C_{12}$-1,2,3,4-TCDF (optional) and $^{13}C_{12}$-1,2,3,7,8,9-HxCDD, are added to each extract. The $^{13}C_{12}$-1,2,3,4-TCDD is used to determine the Percent Recoveries (%R) of $^{13}C_{12}$-labeled tetrachlorinated and pentachlorinated CDD/CDF isotope dilution standard congeners, while the $^{13}C_{12}$-1,2,3,7,8,9-HxCDD is used to determine the recoveries of the $^{13}C_{12}$-labeled hexachlorinated, heptachlorinated, and octachlorinated CDD/CDF isotope dilution standard congeners. The final extract volume, including the 10 µL of internal standard, is 20 µL.

2.3.2 An aliquot of the extract is injected into the HRGC instrument. The analytes are separated by the HRGC system and detected by a High Resolution (≥10,000) Mass Spectrometer (HRMS). Two exact m/z signals are monitored for each analyte throughout a predetermined RT window.

2.3.3 An individual CDD/CDF is identified by comparing the HRGC RT and ion abundance ratio of two exact m/z with the corresponding RT of an authentic standard and the theoretical or acquired ion abundance ratio of the two exact m/z. Column performance in the HRGC system is monitored to ensure specificity in the identification of 2,3,7,8-TCDD and 2,3,7,8-TCDF.

2.4 Quantitation

Quantitative analysis is performed using Selected Ion Current Profile (SICP) areas in one of the following ways:

2.4.1 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 remaining analytes are quantitated by the internal standard technique.

2.4.2 For non-2,3,7,8-substituted isomers and all congeners in the tetra through octa levels of chlorination (e.g., Homologue totals), concentrations are estimated using Relative Response Factors (RRFs) from calibration of 2,3,7,8 analogs at the same level of chlorination.

2.4.3 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. The internal standards are labeled compounds spiked into the extract immediately prior to injection of an aliquot of the extract into the HRGC/HRMS system.

3.0 DEFINITIONS

See Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations for a complete list of definitions.

4.0 INTERFERENCES

4.1 Sources of Contamination

Solvents, reagents, glassware, and other sample processing hardware may yield artifacts, elevated baselines, and/or lock-mass suppression 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 after use. Sonication of glassware containing a detergent solution for approximately 30 seconds may aid in cleaning. Glassware with removable parts, particularly separatory funnels with polytetrafluoroethylene (PTFE) stopcocks, must be disassembled before detergent washing.

4.2.2 After detergent washing, glassware should be rinsed immediately, first with methanol, then with hot tap water. Another methanol rinse, then an acetone rinse, and then a methylene chloride rinse follow the tap water rinse.

4.2.3 Baking of glassware in a kiln or other high temperature furnace (450-500°C) may be warranted after particularly dirty samples are encountered. The kiln or furnace should be vented to an exhaust duct to prevent laboratory contamination by CDD/CDF vapors. Baking should be minimized, as repeated baking of glassware may cause active sites on the glass surface that may irreversibly adsorb CDDs/CDFs. Volumetric glassware and separatory funnels with ground glass sealing surface should not be baked at high temperatures that may cause the deformation of the volumetric glassware or the sealing surface of separatory funnels.

4.2.4 Immediately prior to use, the Soxhlet and SDS apparatus should be pre-extracted with toluene, and the continuous liquid-liquid extraction apparatus should be pre-extracted with methylene chloride 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.

4.2.5 A separate set of glassware may be necessary to effectively preclude contamination when low-level samples are analyzed.

4.3 Reagents and Materials

All materials used in the analysis shall be demonstrated to be free of interferences by analyzing reference matrix method blanks (Section 12.1.2) initially and with each sample batch (samples started through the extraction process in a given 12-hour period, to a maximum of 20 samples).

4.3.1 The reference matrix must simulate, as closely as possible, the sample matrix being extracted. The reference matrix should not contain the CDDs/CDFs in detectable amounts. The EPA 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 (Section 7.6.1) can be used to simulate aqueous/water samples; playground sand (Section 7.6.2) or white quartz sand (Section 7.3.2) can be used to simulate soils/sediments and other matrices such as biosolids, sludge, or ash; and corn oil (Section 7.6.3) can be used to simulate tissue samples.

4.4 Interfering Compounds

Interferences coextracted from samples will vary considerably from source to source, depending upon 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 can be used to reduce or eliminate these interferences and thereby permit reliable determination of the CDDs/CDFs at the levels in Exhibit C - Chlorinated Dibenzo-*p*-Dioxins and Chlorinated Dibenzofurans and Chlorinated Biphenyl Congeners Target Analyte List and Contract Required Quantitation Limits, Table 1.

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 Contractor 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 Contamination of Calibration Solutions

To prevent CDD/CDF contamination of the calibration solutions, the calibration solutions must be prepared in an area free of contamination using glassware free of CDD/CDF contamination. If these requirements cannot be met or are difficult to meet in the laboratory, the Contractor should have a vendor prepare the calibration standards and ensure a lack of contamination or should prepare the calibration solutions in a contamination-free facility.

4.7 Lipids

The natural lipid content of tissue can interfere in the analysis of tissue samples for the CDDs/CDFs. The lipid content 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, including anthropogenic isolation column and/or GPC, followed by Florisil and carbon, as minimum additional cleanup steps.

4.8 Polychlorinated Diphenyl Ethers

If polychlorinated diphenyl ethers are detected, as indicated by the presence of peaks at the exact m/z monitored for these interferents, alumina and/or Florisil cleanup must be employed to eliminate these interferences.

5.0 SAFETY

5.1 Toxicity

The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined; however, each 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 parts-per-trillion (ppt), and in organic solvents to 0.14%. Based on the available toxicological and physical properties of 2,3,7,8-TCDD, any material known to contain CDDs/CDFs should be handled only by highly trained personnel thoroughly familiar with their handling including cautionary procedures and the associated risks.

5.1.2 It is recommended that the Contractor purchase dilute standard solutions of the analytes in this method. However, if primary solutions are prepared, they shall be prepared in a hood or glove box.

5.2 Occupational Safety and Health Administration Requirements

The Contractor is responsible for maintaining a current awareness file of Occupational Safety and Health Administration (OSHA) regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDSs) should also be made available to all personnel involved in these analyses.

5.3 Sample Handling

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 and 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. 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 should not be allowed.

5.3.2 Protective Equipment - Disposable plastic gloves, apron or laboratory coat, safety glasses or mask, and a glove box or fume hood adequate for radioactive work should be used. During analytical operations that may give rise to aerosols or dusts, personnel should wear respirators equipped with activated carbon filters. Eye protection (preferably full-face shields) should 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 CDDs/CDFs, an additional set of gloves can also be worn beneath the latex gloves. A Tyvek® suit with foot covers and a hood is an option for protective clothing.

5.3.3 Training - Workers must be trained in the proper method of removing contaminated gloves and clothing without contacting the exterior surfaces. Workers should also be trained to recognize the symptoms of chloroacne.

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 from sample splitters on the GC and from roughing pumps on the MS should pass through either a column of activated charcoal or be bubbled through a trap containing oil or high-boiling alcohol to condense CDD/CDF vapors. Traps should be replaced at a minimum annually or more frequently as needed 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. All personnel should be advised of the hazard and 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 - 1,1,1-trichloroethane solvent can be effective in removing CDDs/CDFs. Satisfactory cleaning may be accomplished by rinsing with 1,1,1-trichloroethane, then washing with any detergent and water.

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 perform a wipe test of the surface suspected of being contaminated.

5.4.4.1 Using a piece of filter paper moistened with 1,1,1-trichloroethane or other solvent, wipe an area approximately 10 x 10 centimeters (cm).

5.4.4.2 Extract the wipe as specified in Section 10.2.4 and analyze by this analytical method or by GC with an Electron Capture Detector (ECD).

5.4.4.3 Using the area wiped [e.g., 10 x 10 cm = 0.01 square meter (m^2)], calculate the concentration in micrograms per square meter ($\mu g/m^2$). A concentration less than 1 $\mu g/m^2$ indicates acceptable cleanliness; anything higher warrants further cleaning. Concentrations more than 100 $\mu g/m^2$ constitute an acute hazard and requires prompt cleaning before further use of the equipment or workspace, and indicate that unacceptable work practices have been employed.

5.4.5 Biosolids samples may contain high concentrations of biohazards, and must be handled with gloves and opened in a hood or biological safety cabinet to prevent exposure. Contractor staff should know and observe the safety procedures required in a microbiology laboratory that handles pathogenic organisms when handling biosolids samples.

5.4.6 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

Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using equipment and supplies other than those specified here; however, a demonstration of equivalent performance meeting the requirements of this SOW is the responsibility of the Contractor. The Contractor shall document any use of alternate equipment or supplies in the Sample Delivery Group (SDG) Narrative.

6.1 Glassware Cleaning

6.1.1 Cleaning solvent – 1,1,1-trichloroethane.

6.1.2 Kiln – Properly vented to an exhaust duct to the outside of the laboratory and capable of reaching 450°C within 2 hours and maintaining 450-500°C within ±10°C, with a temperature controller and safety switch.

6.1.3 Laboratory sink with an overhead fume hood.

6.2 Equipment for Sample Preparation

6.2.1 Aluminum foil.

6.2.2 Balances

6.2.2.1 Analytical - Capable of weighing ±0.1 milligrams (mg).

6.2.2.2 Top loading - Capable of weighing ±10 mg.

6.2.2.3 A balance calibration must be checked with known masses once per each day of use. This verification consists of a check with two weights covering the range expected (approximately ±50% of the expected measured mass) for each type of balance and be accurate to ±0.1 mg and ±10 mg, respectively. The masses that are used to check the balances daily must be checked on a monthly basis using

National Institute of Standards and Technology (NIST)-traceable known reference masses (Class ‘0’, Class ‘1’, or Class ‘2’) as defined by ASTM E617-97 (2008), or equivalent (e.g., earlier Class ‘S’ defined masses). All balances must be checked at least once annually by a certified technician. The reference masses used by the Contractor must be recertified at least every five years, or sooner if there is reason to believe damage (corrosion, nicks) has occurred. The Contractor shall maintain documentation that demonstrates these criteria have been met.

6.2.3 Equipment for Determining Percent Solids

6.2.3.1 Oven – Properly vented to an exhaust duct to the outside of the laboratory and capable of maintaining a temperature of 110°C (±5°C).

6.2.3.2 Desiccator – Containing an expended desiccant indicator compound.

6.2.4 Glove box.

6.2.5 Laboratory fume hood of sufficient size to contain the sample preparation equipment.

6.2.6 Meat Grinder - With 3-5 millimeter (mm) holes in inner plate.

6.2.7 Sieve - #18 (1 mm nominal sieve opening).

6.2.8 Tissue Homogenizer - With stainless steel Macro-shaft and Turbo-shear blade.

6.3 Extraction Apparatus

6.3.1 Aqueous/Water Samples

6.3.1.1 pH Meter - With combination glass electrode. Calibrate according to manufacturer’s instructions. The pH meter shall be calibrated prior to each use, using reference standards bracketing the range expected in samples. The pH reference standards shall be replaced when their expiration dates have passed.

6.3.1.2 pH Paper - Wide range.

6.3.1.3 Graduated Cylinder Class A TC - 1 L capacity.

6.3.1.4 Separatory Funnels - 250 mL, 500 mL, and 2000 mL, with PTFE stopcocks.

6.3.1.5 Solid Phase Extraction

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 Glass-Fiber Filter - 1 micron nominal pore size, to fit filtration apparatus described in Section 6.3.1.5.1.

6.3.1.5.3 SPE Disk - Containing octadecyl (C_{18}) bonded silica uniformly enmeshed in an inert matrix, to fit filtration apparatus described in Section 6.3.1.5.1.

6.3.1.5.4 Vacuum Source - Capable of maintaining 25 in. Hg, equipped with shutoff valve and vacuum gauge.

6.3.1.6 Continuous Liquid-Liquid Extraction

PTFE or glass connecting joints and stopcocks without lubrication, 1.5-2 L capacity.

6.3.2 Solid Samples [Soil/Sediment, Sludge, Tissue (Non-Human), Biosolids, Ash, Oil, Filters]

6.3.2.1 Heating Mantle - Hemispherical, to fit 500 mL round-bottom flask.

6.3.2.2 SDS Extractor.

6.3.2.2.1 Moisture Trap - Dean-Stark or Barret with PTFE stopcock, to fit Soxhlet.

6.3.2.2.2 Soxhlet - 50 mm ID, 200 mL capacity with 500 mL flask.

6.3.2.2.3 Thimble - 43 mm x 123 mm or 33 mm x 94 mm cellulose thimble to fit Soxhlet.

6.3.2.3 Variable Transformer - Powerstat (or equivalent), 110 volt, 10 ampere (A).

6.3.3 Acid/Base Back-Extraction of Tissue Extracts

6.3.3.1 Bottle for Back-Extraction - 100-200 mL narrow-mouth clear glass with PTFE-lined cap.

6.3.3.2 Mechanical Shaker - Wrist-action or platform-type rotary shaker that produces vigorous agitation.

6.3.3.3 Rack - Attached to shaker table to permit agitation of 4-9 samples simultaneously.

6.3.4 Beakers - 400-500 mL.

6.3.5 Extraction Jars – Glass, 250 mL, equipped with PTFE-lined screw caps.

6.3.6 Spatulas - Stainless steel or glass rod.

6.4 Filtration Apparatus

6.4.1 Borosilicate Glass Wool - Solvent-extracted using a Soxhlet or SDS Extractor for 3 hours minimum. DO NOT BAKE.

6.4.2 Buchner Funnel - 15 cm.

6.4.2.1 Glass-Fiber Filter Paper - To fit Buchner funnel.

6.4.3 Drying Column - 15-20 mm ID borosilicate chromatographic column equipped with a coarse-glass frit or glass wool plug.

6.4.4 Filtration Flasks - 1.5-2.0 L, with a side arm.

6.4.5 Glass Funnel - 125-250 mL.

6.4.5.1 Glass-Fiber Filter Paper - To fit glass funnel.

6.4.6 Pressure Filtration Apparatus.

6.5 Centrifuge Apparatus

6.5.1 Centrifuge - Capable of rotating 500 mL centrifuge bottles or 15 mL centrifuge tubes at 5,000 revolutions per minute (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

6.6.1.1 Column - 600-700 mm long × 25 mm ID glass, packed with 70 g of 200-400 mesh SX-3 Bio Beads.

6.6.1.2 Syringe - 10 mL, with Luer-Lok fitting.

6.6.1.3 Syringe Filter Holder - Stainless steel, and glass-fiber or PTFE filters.

6.6.1.4 Ultraviolet (UV) Detector - 254 nanometers (nm), preparative or semi-preparative flow cell.

6.6.1.5 Temperature-controlled environment to ensure reproducible performance.

6.6.2 Reverse-Phase High-Performance Liquid Chromatograph

6.6.2.1 Column – Two 6.2 mm x 250 mm Zorbax-ODS columns in series, operated at 50°C with 2.0 mL/minute methanol isocratic eluent.

6.6.2.2 Detector - Operated at 0.02 AUFS at 235 nm.

6.6.2.3 Fraction Collector.

6.6.2.4 Injector – With 50 µL sample loop.

6.6.2.5 Pump.

6.6.2.6 Switching Valve.

6.6.3 Glass Chromatographic Columns

6.6.3.1 150 mm long x 8 mm ID, with coarse-glass frit or glass wool plug and 250 mL reservoir.

6.6.3.2 200 mm long x 15 mm ID, with coarse-glass frit or glass wool plug and 250 mL reservoir.

6.6.3.3 300 mm long x 25 mm ID, with coarse-glass frit or glass wool plug, 300 mL reservoir, and glass or PTFE stopcock.

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

6.6.5 Oven or Tube Furnace - For baking and activating adsorbents, capable of maintaining a constant temperature (±5°C) in the range of 400-700°C.

6.6.6 Pipettes

6.6.6.1 Disposable, Pasteur - 150 mm long x 5 mm ID.

6.6.6.2 Disposable, Serological - 10 mL (6 mm ID).

6.6.7 Stirring Apparatus - For batch silica cleanup of tissue extracts.

6.6.7.1 Mechanical Stirrer.

6.6.7.2 Bottle - 500-600 mL, wide-mouth clear glass.

6.7 Concentration Apparatus

6.7.1 Rotary Evaporator - Equipped with a variable temperature water bath.

6.7.1.1 A recirculating water pump and chiller are recommended. 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.

6.7.1.2 Boiling Flask – 125 mL pear-shaped borosilicate glass boiling flask for rotary evaporation of extracts.

6.7.1.3 Round-bottom Flasks - 100 mL and 500 mL or larger, with ground-glass fittings compatible with the rotary evaporator.

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

6.7.2 Kuderna-Danish Apparatus

6.7.2.1 Boiling Chips

6.7.2.1.1 Glass or Silicon Carbide - Approximately 10/40 mesh, solvent-rinse with methylene chloride and baked at 450°C for 1 hour minimum.

6.7.2.1.2 PTFE (optional) - Solvent-rinsed with methylene chloride.

6.7.2.2 Concentrator Tube - 10 mL, graduated with calibration verified. Ground-glass stopper (size 19/22 joint) is used to prevent evaporation of extracts.

6.7.2.3 Evaporation Flask - 500 mL, attached to concentrator tube with springs or Plastic Keck clips.

6.7.2.4 Snyder Column - Three-ball macro.

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

6.7.3 Nitrogen Evaporation Device - Equipped with water bath controlled in the range of 30-60°C, installed in a fume hood.

6.7.4 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 (LCS) spiking solution, labeled reference compounds solution, and internal standard solution].

6.7.5 Sample Vials

6.7.5.1 Amber Glass - 2-5 mL with PTFE-lined screw-cap.

6.7.5.2 Glass - 0.3 mL, conical, with PTFE-lined screw or crimp cap.

6.7.6 Volumetric Flasks 10 mL, Class A - For the preparation of stock standard solutions.

6.8 Gas Chromatograph

The GC shall have splitless or on-column injection port for a capillary column, a temperature program with isothermal hold, and shall meet all of the performance specifications listed in Section 9.0.

6.8.1 GC Column - Any GC column or column system (two or more columns) that provide unique resolution and identification of the 2,3,7,8 isomers for determination of a CDD’s/CDF’s TEQ using TEFs. Unique resolution means a valley height less than 25% of the most closely eluted isomers and the 2,3,7,8-substituted isomers when the isomer specificity check standard is analyzed. If any detected 2,3,7,8 isomers are not resolved on the primary column used, then confirmation is required.

6.8.1.1 If a DB-5 column is used, it must meet the specifications in Section 6.8.1 and the following additional specifications:

6.8.1.1.1 The column must uniquely resolve all target analytes listed in Exhibit C - Chlorinated Dibenzo-*p*-Dioxins and Chlorinated Dibenzofurans and Chlorinated Biphenyl Congeners Target Analyte List and Contract Required Quantitation Limits, Table 1. The percent valley between the GC peaks of the unlabeled TCDD isomer that elute most closely to the 2,3,7,8-TCDD and the 2,3,7,8-TCDD is less than 25% in the isomer specificity check standard (Section 9.4.5.4).

6.8.1.2 The column must be replaced when any of the criteria in Sections 6.8.1 – 6.8.1.1.1 are not met.

6.8.1.3 Suggested Column – 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 (DB-5 or equivalent). This column is capable of meeting the requirements in Sections 6.8.1 – 6.8.1.1.1.

6.8.1.4 Confirmation Column for isomer specificity for 2,3,7,8-TCDF – 30 ±5 m long x 0.32 ±0.02 mm ID; 0.25 µm bonded-phase fused-silica capillary column (J&W DB-225 or equivalent). The percent valley between the GC peaks of the unlabeled TCDF isomer that elute most closely to the 2,3,7,8-TCDF and the 2,3,7,8-TCDF is less than 25% in the isomer specificity check standard (Section 9.4.5.4).

NOTE: The criteria for minimum RT of the labeled standards and Relative Retention Time (RRT) of the CDDs and CDFs (Exhibit D – CCD/CDF, Table 2) were established using these columns. Whatever column or system of columns is used, the performance criteria specified in this section shall be met.

6.9 Mass Spectrometer

6.9.1 The GC/MS reference methods require the following conditions: 28- to 40-eV electron impact ionization that shall be capable of repetitively selectively monitoring 12 exact m/z minimum at high resolution (≥10,000) during a period of approximately one second, and meet all of the performance specifications in Section 9.0.

6.9.2 Alternate technologies may be utilized, only after approval in advance by EPA, if the following performance criteria can be met:

- Efficient ionization of all target analytes;
- Repetitive, selective monitoring of the exact target m/z at high resolution (≥10,000 with 10% valley definition, 20,000 at full width-half maximum definition) during a period of approximately one second; and
- Attainment of all of the performance specifications in Section 9.0.

6.10 Gas Chromatograph/Mass Spectrometer 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 Systems/Data Storage

Capable of collecting, recording, storing, and processing MS data.

6.11.1 Data Acquisition - The signal at each exact m/z must be collected repetitively throughout the monitoring period and stored on a mass storage device.

6.11.2 RFs and Multi-point Calibrations - The data system must record and maintain lists of RFs (response ratios for isotope dilution) and multi-point calibrations. Computations of Relative Standard Deviation (RSD) are to be used to test calibration linearity.

7.0 REAGENTS AND STANDARDS

The Contractor must provide all standards to be used with the contract. The Contractor must be able to verify that the standards are certified. Manufacturer's certificates of analysis must be retained by the Contractor and presented upon request.

Reagents shall be dated with the receipt date and used on a first-in, first-out basis. The purity of the reagents shall be verified before use.

7.1 pH Adjustment and Back-Extraction

7.1.1 Hydrochloric Acid, 6N (1:1) - Add 500 mL concentrated HCl, reagent grade to 400 mL reagent water and dilute to 1 L.

7.1.2 Potassium Hydroxide - Dissolve 20 g reagent grade KOH in 100 mL reagent water.

7.1.3 Sodium Chloride Solution - Reagent grade, prepare at 5% (w/v) solution in reagent water.

7.1.4 Sodium Hydroxide, 1N – Dissolve 40 g of NaOH in 500 mL reagent water and dilute to 1 L.

7.1.5 Sodium Thiosulfate.

7.1.6 Sulfuric Acid - Reagent grade (specific gravity 1.84).

7.1.7 Hydrochloric Acid, 1N.

7.2 Solution Drying and Evaporation

7.2.1 Sodium sulfate, reagent grade, granular, anhydrous, 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 Hydromatrix™ - Diatomaceous earth-based material rinsed with methylene chloride and dried at 400°C for 4 hours in a shallow tray, cooled in a desiccator, and stored in a glass bottle.

7.2.3 Pre-purified Nitrogen.

7.3 Extraction

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

7.3.2 White quartz sand, 60/70 mesh - For SDS extraction. Bake at 450°C for 4 hours minimum.

7.4 Gel Permeation Chromatography Calibration Solution

Prepare a GPC calibration solution in methylene chloride containing the following analytes at the minimum concentration listed (in elution order). The solution must be prepared every 6 months, or sooner if the solution has degraded or concentrated.

Compound	Concentration (mg/mL)
Corn oil (CAS #8001-30-7)	25.0
Bis(2-ethylhexyl)phthalate (CAS #117-81-7)	0.5
Methoxychlor (CAS #72-43-5)	0.1
Perylene (CAS #198-55-0)	0.02
Sulfur (CAS #7704-34-9)	0.08

NOTE: Sulfur is not very soluble in methylene chloride, but it is soluble in warm corn oil. Therefore, one approach is to weigh out the corn oil, warm it, and transfer the weighed amount of sulfur into the warm corn oil. Mix it and then transfer into a volumetric flask with methylene chloride, along with the other calibration compounds.

7.5 Adsorbents for Sample Cleanup

7.5.1 Silica Gel

7.5.1.1 Acid Silica Gel (30% w/w) - Thoroughly mix 44 g of concentrated sulfuric acid 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 PTFE-lined screw-cap.

7.5.1.2 Activated Silica Gel - 100-200 mesh, 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.

7.5.1.3 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 PTFE lined screw-cap.

7.5.1.4 Potassium Silicate.

7.5.1.4.1 Dissolve 56 g of high purity potassium hydroxide in 300 mL of methanol in a 750-1000 mL flat-bottom flask.

7.5.1.4.2 Add 100 g of activated silica gel and a stirring bar, and stir on an explosion-proof hot plate at 60-70°C for 1-2 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 2-4 hours in a hood.

7.5.1.4.5 Activate overnight at 200-250°C before use.

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 - Activate by heating to 130°C for a minimum of 12 hours.

7.5.2.2 Basic Alumina - 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 cubic centimeters (cc)/minute. Do not heat over 700°C, as this can lead to reduced capacity for retaining the analytes. Store at 130°C in a covered flask. Use within five days of baking.

7.5.3 Carbon

7.5.3.1 Carbopak C.

7.5.3.2 Celite 545.

7.5.3.3 Thoroughly mix 9.0 g Carbopak C and 41.0 g Celite 545. Activate the mixture at 130°C for a minimum of 6 hours. Store in a desiccator.

7.5.4 Anthropogenic Isolation Column - Pack the column in Section 6.6.3.3 from bottom to top with the following:

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

7.5.5 Florisil Column

7.5.5.1 Florisil - PR grade, activated magnesium silicate, 60-100 mesh. If necessary, Soxhlet extract in 500 g portions for 24 hours. Alternatively, pre-packaged Florisil columns may be used.

7.5.5.2 Insert a glass wool plug into the tapered end of a graduated serological pipette (Section 6.6.6.2). Pack with 1.5 g (approximately 2 mL) of Florisil topped with approximately 1 mL of anhydrous sodium sulfate (Section 7.2.1) and a glass wool plug.

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

7.6 Reference Matrices

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

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

7.6.2 Soil/Sediment Reference Matrix - Playground sand or similar material demonstrated to be free of the analytes of interest and potentially interfering substances. 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 demonstrated to be free of the analytes of interest and potentially interfering substances.

7.6.4 Other Matrices - This method may be verified on any reference matrix that is free of CDDs/CDFs. In no case shall the background level of CDDs/CDFs exceed 1/2 the CRQLs in Exhibit C – Chlorinated Dibenzo-*p*-Dioxins and Chlorinated Dibenzofurans and Chlorinated Biphenyl Congeners Target Analyte List and Contract Required Quantitation Limits, Table 1.

7.7 Perfluorokerosene (PFK)

Reference compound used for tuning the mass spectrometer. Exhibit D – CCD/CDF, Table 3, offers some suggestions for the lock-mass m/z.

7.8 Standard Solutions

Prepare standard solutions from materials of known purity and composition or purchase as solutions or mixtures with certification to their purity, concentration, and authenticity. 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 PTFE-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. All solution standards are to be clearly labeled with information or a unique standard ID identifier which allows for traceability to the identity of the analyte or analytes, concentration, date prepared, solvent, expiration date of the solution, special storage requirements (if any), and initials of the preparer.

7.8.1 Stock Solutions

7.8.1.1 Preparation — Prepare in nonane per the steps below or purchase as dilute solutions. Observe the safety precautions outlined in Section 5.0, paying close attention to the recommendation in Section 5.1.2.

7.8.1.1.1 For preparation of stock solutions from neat materials, dissolve an appropriate amount of assayed reference material in solvent. For example, weigh 1.0-2.0 mg of 2,3,7,8-TCDD, to two (2) significant figures, 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 PTFE-lined cap.

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

7.8.1.2 Native CDD/CDF Stock Solution – Prepare the native CDDs/CDFs stock solution at the concentrations in Exhibit D – CCD/CDF, Table 4, or purchase.

7.8.1.3 Labeled Compound Stock Solution - Prepare the labeled compounds for CDDs/CDFs, Cleanup Standard, and internal standards stock solutions in nonane at the concentrations in Exhibit D – CCD/CDF, Table 4, or purchase.

7.8.2 Working Standards

7.8.2.1 Labeled Compound Spiking Solution – This solution is spiked into each sample, blank, and Laboratory Control Sample/Laboratory Control Sample Duplicate (LCS/LCSD) to measure recovery. Prepare a sufficient volume of the labeled compound spiking solution at the concentrations shown in Exhibit D – CCD/CDF, Table 4, with acetone prior to use. Seal with PTFE tape and mark the meniscus of the solution to reduce and monitor evaporation of acetone. Each sample requires 1 mL of the diluted spiking solution, and this solution shall be prepared daily. When 1 mL of this solution is spiked into a sample, blank, and LCS/LCSD, and concentrated to a final extract volume of 20 µL, the concentration in the final extract volume will be as in Exhibit D – CCD/CDF, Table 4.

NOTE 1: Labeled compound 13C-OCDF may be included in the labeled compound spiking solution provided that all chromatographic performance criteria are met.

NOTE 2: The Contractor shall maintain a standard preparation log documenting the daily preparation of the labeled compound spiking solution.

7.8.2.2 LCS/LCSD Spiking Solution - This solution is spiked into each LCS/LCSD. Dilute the native CDD/CDF stock solution (Section 7.8.1.2) with acetone to produce the concentration as in Exhibit D – CCD/CDF, Table 4. When 1 mL of this solution is spiked into the LCS/LCSD, and concentrated to a final volume of 20 µL, the concentration in the final volume will be at the test concentration listed in Exhibit D – CCD/CDF, Table 5. Prepare only the amount necessary for each reference matrix with each sample batch.

7.8.2.3 Cleanup Standard - This solution is spiked into each sample, blank, and LCS/LCSD before cleanup to measure the efficiency of the cleanup process. Dilute $^{37}Cl_4$-2,3,7,8-TCDD from the stock solution in hexane to produce the concentration shown in Exhibit D – CCD/CDF, Table 4. Prepare only the amount necessary for each sample batch.

7.8.2.4 Internal Standards - This solution is added to each concentrated extract before injection into the HRGC/HRMS system. Dilute the internal standard stock solution to contain $^{13}C_{12}$-1,2,3,4-TCDD, $^{13}C_{12}$-1,2,3,7,8,9-HxCDD, and $^{13}C_{12}$-1,2,3,4-TCDF (optional) in nonane to produce a concentration shown in Exhibit D – CCD/CDF, Table 4.

7.8.3 Calibration Standards

Calibration Standard Solutions - Combine and dilute the solutions described in Sections 7.8.1.2 and 7.8.1.3 to produce the five calibration solutions (CS1 through CS5) in Exhibit D – CCD/CDF, Table 6, in nonane, or purchase. These solutions permit the Relative Response (RR) (labeled to native) and RRF to be measured as a function of concentration. The CS3 Standard is used for continuing calibration verification.

7.8.4 Window Defining Mixture 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 Exhibit D – CCD/CDF, Table 7, in nonane.

7.9 Stability of Solutions

Standard solutions used for quantitative purposes should be assayed periodically (e.g., every 6 months) against Standard Reference Materials (SRMs) from NIST (if available), or certified reference materials from a source that will attest to the authenticity and concentration, to assure that the composition and concentrations have not changed.

7.10 Storage of Standard Solutions

7.10.1 Store the working standards at ≤6°C, but not frozen, in PTFE-sealed containers. The solutions should be checked frequently for stability. These solutions must be replaced after 6 months, or sooner if comparison with Quality Control (QC) check samples indicates a problem.

7.10.2 Store the stock standard solutions at ≤6°C, but not frozen, in PTFE-lined screw-cap amber bottles.

7.10.3 Standard solutions purchased from a chemical supply company as ampulated extracts in glass vials may be retained and used until the expiration date provided by the manufacturer. If no manufacturer's expiration date is provided, the standard solutions as ampulated extracts may be retained and used for 2 years from the preparation date. Standard solutions prepared by the Contractor that are immediately ampulated in glass vials may be retained for 2 years from the preparation date. The expiration date of the ampulated standards, upon the breaking of the glass seal, is 6 months (or sooner if the standard has degraded or evaporated).

7.10.4 Refrigeration of the GPC calibration solution may cause the corn oil to precipitate. Before use, allow the solution to stand at room temperature until the corn oil dissolves. Replace this calibration solution every 6 months, or more frequently if necessary.

7.10.5 Protect all standards from light.

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

7.10.7 The Contractor is responsible for maintaining and verifying the integrity of standard solutions prior to use. This means, at a minimum, the standards must be brought to room temperature prior to use, checked for losses, and checked that all components have remained in solution.

7.11 Temperature Records for Storage of Standards

7.11.1 The temperature of all standard storage refrigerators/freezers shall be recorded daily.

7.11.2 Temperature excursions shall be noted and appropriate corrective actions shall be taken to correct problems.

7.11.3 Corrective action Standard Operating Procedures (SOPs) shall be posted on the refrigerators/freezers.

8.0 SAMPLE COLLECTION, PRESERVATION, STORAGE, AND HOLDING TIMES

8.1 Sample Collection and Preservation

8.1.1 Aqueous/water grab and composite samples must be collected in amber glass containers following conventional sampling practices. If residual chlorine is present, add 80 mg sodium thiosulfate per liter of water. EPA Methods 330.4 and 330.5 may be used to measure residual chlorine. If sample pH is greater than 9, adjust to pH 7-9 with sulfuric acid. All samples must be iced or refrigerated at ≤6°C and stored in the dark from the time of collection until sample receipt at the laboratory.

8.1.2 Soil/sediment samples are collected in amber glass jars. All samples must be iced or refrigerated at ≤6°C and stored in the dark from the time of collection until sample receipt at the laboratory.

8.1.3 Tissue (non-human) samples collected in the field must be wrapped in aluminum foil, and must be maintained at a temperature ≤6°C from the time of collection until receipt at the laboratory, to a maximum time of 24 hours. If a longer transport is necessary, freeze the sample. Ideally, tissues should be frozen upon collection and shipped to the laboratory under dry ice.

8.1.4 Refer to Section 10.1 for oily and multiphase samples.

8.2 Procedures for Sample and Sample Extract Storage

8.2.1 Maintain aqueous/water grab and composite samples in the dark at ≤6°C from time of receipt until extraction. If the sample will be frozen, allow room for expansion. It is recommended that sludge, biosolids, and oil/oily matrix samples be kept at their initial conditions. After extraction, the samples must be protected from light and refrigerated at ≤6°C, but not frozen, until 60 days after delivery of a complete, reconciled data package to the EPA. After 60 days, the samples may be disposed of in a manner that complies with all applicable regulations.

8.2.2 Store soil/sediment samples in the dark at ≤6°C. After extraction, the samples must be protected from light and refrigerated at ≤6°C, but not frozen, until 60 days after delivery of a complete, reconciled data package to the EPA. After 60 days, the samples may be disposed of in a manner that complies with all applicable regulations.

8.2.3 Tissue (non-human) samples must be frozen upon receipt at the laboratory and stored in the dark at less than -10°C until prepared. Unused sample portions and unused homogenized tissues must be stored in the dark at less than -10°C. Once thawed, tissue samples must be extracted within 24 hours. After extraction, the samples must be protected from light and stored at less than -10°C until 60 days after delivery of a complete, reconciled data package to the EPA. After 60 days, the samples may be disposed of in a manner that complies with all applicable regulations.

8.2.4 Sample extracts must be protected from light until 365 days after delivery of a complete, reconciled data package to the EPA.

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

8.3 Contract Required Holding Times

8.3.1 All samples should be extracted, analyzed, and completed within the time period specified during scheduling.

8.3.2 Analysis of sample extracts must be completed within one year following the start of extraction, when sample extracts are stored under the appropriate conditions.

9.0 CALIBRATION AND STANDARDIZATION

9.1 Initial Instrument Set-Up

9.1.1 High Resolution Gas Chromatograph Initial Set-up

9.1.1.1 Prior to analyzing the calibration solutions, blanks, samples, and LCS/LCSD samples, establish the HRGC system operating conditions necessary to meet the GC resolution requirements (Section 6.8.1) and the RRTs for CDD/CDF (Exhibit D – CCD/CDF, Table 2). Once optimized, the same conditions must be used for the analysis of all standards, samples, blanks, and LCS/LCSD samples.

NOTE: RTs, RRTs, and RRT limits may differ slightly from those in Exhibit D – CCD/CDF, Table 2.

9.1.1.1.1 The recommended GC operating conditions for DB-5 Column are provided in Exhibit D – CCD/CDF, Table 8.

9.1.1.2 Retention Time Calibrations for Chlorinated Dibenzo-*p*-Dioxins/Chlorinated Dibenzofurans

9.1.1.2.1 Separately inject each of the calibration standard solutions (Section 7.8.3). Establish the beginning and ending RTs for the scan descriptors in Exhibit D – CCD/CDF, Table 3. Scan descriptors other than those listed in Table 3 may be used, provided that the CRQLs in Exhibit C - Chlorinated Dibenzo-*p*-Dioxins and Chlorinated Dibenzofurans and Chlorinated Biphenyl Congeners Target Analyte List and Contract Required Quantitation Limits, Table 1 are met.

9.1.1.2.2 If an alternate column is used, adjust the conditions for that column. If column performance is unacceptable, optimize the analysis conditions or replace the column and repeat the performance tests. All alternate column performance criteria established by the laboratory must be thoroughly documented in the SDG Narrative.

9.1.1.2.3 After the column performance tests are passed, calculate and store the RT and RRT data for the target analytes. The windows in Exhibit D – CCD/CDF, Table 2, were developed based on the GC conditions given in Exhibit D – CCD/CDF, Table 8.

9.1.2 High Resolution Mass Spectrometer

9.1.2.1 Obtain an HRMS SICP of each analyte at the two exact m/z specified in Exhibit D – CCD/CDF, Table 3 at greater than or equal to 10,000 resolving power (with 10% valley definition) by injecting an authentic standard of CDDs/CDFs. The total cycle time for each Selected Ion Monitoring (SIM) descriptor must be less than or equal to 1 second, including the sum of all the ion dwell and voltage reset times.

9.1.2.2 The analysis time for CDDs/CDFs may exceed the long-term mass stability of the MS. Because the instrument is operated in the high resolution mode, mass drifts of a few parts-per-million (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 signal from PFK is used for drift correction. The lock-mass m/z signal is dependent on the exact m/z monitored within each descriptor, as in Exhibit D – CCD/CDF, Table 3. 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 these 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 reanalysis time. The GC run should be completed to allow elution of octachlorinated dibenzo-*p*-dioxin (OCDD) and octachlorinated dibenzofuran (OCDF).

9.2 Summary of High Resolution Gas Chromatography/High Resolution Mass Spectrometry System Performance Check

9.2.1 At the beginning and end of each 12-hour period and prior to analysis of any samples, blanks, LCS/LCSD, and calibration standards, the Contractor shall establish that the HRGC/HRMS system meets the system performance check criteria.

9.2.2 The HRGC/HRMS system performance check consists of three parts: 1) the HRMS system must be tuned to meet the minimum static resolving power, using a suitable calibrant such as PFK; 2) the performance of the HRGC system and the accuracy/appropriateness of the descriptor switching times must be verified by the analyses of the Window Defining Mixture (WDM); and 3) the resolution must be verified by the analysis of the Isomer Specificity Check Standard.

NOTE: The WDM and Isomer Specificity Check Standard 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 (Exhibit D – CCD/CDF, Table 7).

9.2.3 The HRMS System Tune, the WDM, and the Isomer Specificity Check Standard must also be completed at the end of each 12-hour period or analytical sequence. These analyses may also be used to establish the beginning of the next 12-hour period or analytical sequence.

9.3 High Resolution Mass Spectrometry System Tune

9.3.1 Frequency of HRMS System Tune

9.3.1.1 The HRMS System Tune (PFK tune) must be performed prior to the analysis of calibration standards, samples, LCS/LCSD, and blanks, and verified at the end of each 12-hour period or at the end of the sample analysis.

9.3.1.2 The 12-hour time period for the HRGC/HRMS system performance check does not begin until the HRMS resolution requirements have been met.

9.3.2 Procedure for HRMS System Tune

9.3.2.1 The mass spectrometer must be operated in the electron ionization mode. A static resolving power of at least 10,000 (10% valley definition) must be demonstrated at appropriate masses before any analysis is performed. Static resolving power checks must be performed at the beginning and at the end of each 12-hour period of operation. However, it is recommended that a check of the static resolution be made and documented before and after each analysis.

9.3.2.2 Documentation of the instrument resolving power shall be completed by recording the peak profiles of the reference peaks chosen for each descriptor. While generating the peak profiles, the detector zero shall be adjusted to allow presentation of the profile shoulders on-scale (Method 8290A, Figure 5 – Peak Profiles Representing Two PFK Reference Ions at m/z 305 and 381) so that the resolution can be manually evaluated. The format of the peak profiles shall show a horizontal axis calibrated in u or ppm, and a vertical scale in percent maximum signal. The result of the peak width measurement (perform at 5% of the maximum, which corresponds to the 10% valley definition) must appear on the profile, and must not exceed 100 ppm (i.e., 0.038 u for a peak at m/z 380.9760). Both the low and the high exact masses must be displayed to demonstrate the accuracy of the mass calibration. The mass resolution and the accelerating voltages of each mass profile must be present on the profile. This documentation shall be provided for a minimum of one descriptor during each check of the static resolving power of each instrument used, and shall contain identifying information, including instrument ID, time, and date.

9.3.3 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.

9.3.4 Corrective Action for HRMS System Tune

9.3.4.1 Technical acceptance criteria must be met before any standards, samples, LCS/LCSD, and required blanks are analyzed. Any analysis conducted when the technical acceptance criteria have not been met will require reanalysis at no additional cost to the EPA.

9.3.4.2 If the technical acceptance criteria at the end of the 12-hour period 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 the EPA.

9.3.4.3 If the technical acceptance criteria are not met, the instrument must be adjusted until the technical acceptance criteria are met. Refer to manufacturer’s instructions for trouble-shooting systems that will not meet tune criteria.

9.4 Window Defining Mixture

9.4.1 Frequency of Window Defining Mixture

9.4.1.1 The WDM must be analyzed as follows:

- After the HRMS System Tune and before any initial calibration on each instrument and HRGC column used for analysis;
- Once at the beginning and end 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.

9.4.1.2 The 12-hour time period for the HRGC/HRMS system performance check and calibration standards (initial or continuing calibration criteria) begins at the moment of injection of the WDM that the Contractor submits as documentation of a compliant instrument performance check. The time period ends after 12 hours have elapsed according to the system clock.

9.4.2 Procedure for Window Defining Mixture

9.4.2.1 Analyze a 1 or 2 µL aliquot of the WDM.

9.4.2.2 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. Exhibit D – CCD/CDF, Table 7, 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.

9.4.3 Technical Acceptance Criteria for Window Defining Mixture

The analysis of the WDM is acceptable if the beginning and ending CDDs/CDFs are eluting as given in Exhibit D – CCD/CDF, Table 7.

9.4.3.1 The RTs for the switching of m/z characteristic of one homologous series to the next higher homologous series must be indicated in the SICP.

9.4.3.2 All congeners included in the WDM standard must be present in the SICP.

9.4.3.3 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.

9.4.3.4 The presence in the appropriate descriptors and chromatographic separation of all 2,3,7,8-substituted congeners must be demonstrated.

9.4.4 Corrective Action for Window Defining Mixture

9.4.4.1 Technical acceptance criteria must be met before any standards, samples, LCS/LCSD, and required blanks are analyzed. Any analysis conducted when the technical acceptance criteria have not been met will require reanalysis at no additional cost to the EPA.

9.4.4.2 If the technical acceptance criteria of the WDM are not met, the analytical conditions must be adjusted and the test repeated or the HRGC column must be replaced.

9.4.5 Isomer Specificity Check Standard

9.4.5.1 Frequency of Isomer Specificity Check Standard

9.4.5.1.1 The Isomer Specificity Check Standard 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 and end 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.

9.4.5.2 Procedure for Isomer Specificity Check Standard

9.4.5.2.1 Analyze a 1 or 2 µL aliquot of the Isomer Specificity Check Standard. This volume should be consistent with the aliquot used for WDM, samples, and other standards throughout the analysis.

9.4.5.2.2 Compute the Percent Valley (%Valley) between the HRGC peaks that elute most closely to the 2,3,7,8-TCDD and TCDF isomers.

9.4.5.3 Calculations for Isomer Specificity Check Standard

Calculate the %Valley using the measurements made on the SICP for the appropriate m/z for each isomer using Equation 1 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

9.4.5.4 Technical Acceptance Criteria for Isomer Specificity Check Standard

The Isomer Specificity Check Standard is acceptable if the height of the valley between the least resolved adjacent isomer and the 2,3,7,8-substituted isomer is less than or equal to 25%.

9.4.5.5 Corrective Action for Isomer Specificity Check Standard

9.4.5.5.1 Technical acceptance criteria must be met before any standards, samples, LCS/LCSD, and required blanks are analyzed. Any analysis conducted when the technical acceptance criteria have not been met will require reanalysis at no additional cost to the EPA.

9.4.5.5.2 If the technical acceptance criteria at the end of the 12-hour period 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 the EPA.

9.4.5.5.3 If the technical acceptance criteria are not met, the analytical system parameters must be adjusted, the instrument recalibrated, and the WDM and isomer specificity tests repeated.

9.4.6 Ion Abundance Ratios and Signal-to-Noise Ratios

Choose an injection volume of either 1 or 2 µL, consistent with the capability of the HRGC/HRMS instrument. Inject a 1 or 2 µL aliquot of the calibration solution of lowest concentration (Exhibit D – CCD/CDF, Table 6), using the GC conditions in Section 9.1.1.

9.4.6.1 Measure the SICP areas for each CDDs/CDFs group, and compute the ion abundance ratios at the exact m/z specified in Exhibit D – CCD/CDF, Table 3. Compare the computed ratio to the theoretical ratio given in Exhibit D – CCD/CDF, Table 9.

9.4.6.2 The exact m/z to be monitored in each descriptor are in Exhibit D – CCD/CDF, Table 3. Each group or descriptor must be monitored in succession, as a function of GC RT, to ensure that the target analytes of interest are detected.

9.4.6.3 The MS must be operated in a mass-drift correction mode, using PFK to provide lock m/z ratios. The lock-mass for each group of m/z is shown in Exhibit D – CCD/CDF, Table 3. Each lock-mass must be monitored and must not vary by more than ±20% throughout its respective RT window. Variations of lock-mass by more than 20% indicate the presence of coeluting interferences that raise the source pressure and may significantly reduce the sensitivity of the MS.

9.4.6.4 All CDDs/CDFs and labeled compounds in the CS1 Standard must be within the QC limits (Exhibit D – CCD/CDF, Table 9), for their respective ion abundance ratios; otherwise, the MS must be adjusted and the test repeated until the m/z fall within the limits specified. If the adjustment alters the resolution of the MS, resolution must be verified (Section 9.3.2) before a repeat of the test.

9.4.6.5 The peaks representing the CDDs/CDFs and labeled compounds in the lowest concentration calibration standard must have Signal–to-Noise ratios (S/N) greater than or equal to 10; otherwise, the MS must be adjusted and the test repeated until this requirement is met.

9.5 Initial Calibration

9.5.1 Summary of Initial Calibration

Prior to the analysis of samples, LCS/LCSD, and blanks, and after the HRGC/HRMS system performance check criteria have been met, each HRGC/HRMS system must be calibrated with a minimum of five concentration levels to determine instrument sensitivity and linearity of the HRGC/HRMS response for CDDs/CDFs.

9.5.2 Calibration by 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. The reference compounds for each native compound and its labeled compound are listed in Exhibit D – CCD/CDF, Table 2.

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

9.5.2.2 The response of each CDD/CDF relative to its labeled compound is determined using the area responses of both the primary and secondary exact m/z specified in Exhibit D – CCD/CDF, Table 3, for each calibration standard, using Equation 2 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

9.5.3 Calibration by Internal Standard

The internal standard calibration method is applied to the determination of 1,2,3,7,8,9-HxCDD, OCDF if necessary, the non-2,3,7,8-substituted CDD/CDF, the labeled compounds, and the Cleanup Standard.

9.5.3.1 The RRF is determined using Equation 3 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

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

9.5.4 Frequency of Initial Calibration

9.5.4.1 Each HRGC/HRMS system must be calibrated prior to sample analyses, whenever the Contractor takes corrective action that may change or affect the initial calibration criteria (e.g., repair or replacement of any mass spectrometer components, etc.), or if the continuing calibration verification technical acceptance criteria are not met.

9.5.4.2 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 an opening Continuing Calibration Verification Standard within this 12-hour time period if the Initial Calibration Standard that is the same concentration as the Continuing Calibration Verification Standard meets the technical acceptance criteria. Quantitate all sample, LCS/LCSD, and blank results as necessary against the RR and the mean RRF from the initial calibration.

9.5.5 Procedure for Initial Calibration

9.5.5.1 Inject a volume identical to the volume chosen in Section 9.4.2 and the conditions in Section 9.1.1 of each of the Calibration Standards (CS1 through CS5). This volume must be identical to the volume and conditions chosen for the HRGC/HRMS system performance check.

9.5.5.2 Compute and store the RR and RRF for each native and labeled compound, respectively, at each concentration using Equations 2 and 3 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

9.5.5.3 Determine the RTs, S/N ratios, and ion abundance ratios for all Calibration Standards (Section 9.4.6).

9.5.5.4 Determine linearity of the calibration standards (CS1-CS5) by calculating the Percent Relative Standard Deviation (%RSD) over the five-point calibration range using Equation 7 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

9.5.6 Calculations for Initial Calibration

9.5.6.1 The response of each native CDD/CDF relative to its labeled compound is determined using the area responses of both the primary and secondary exact m/z (specified in Exhibit D – CCD/CDF, Table 3, for each calibration standard, using Equation 2 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

9.5.6.2 The RRF is determined for each labeled compound using Equation 3 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

9.5.6.3 The mean Relative Response is determined using Equation 4 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

9.5.6.4 The mean RRF is determined using Equation 5 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

9.5.6.5 The Standard Deviation (SD) for a statistically small set of values is determined using Equation 6 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

9.5.6.6 The %RSD is determined for both the native CDD/CDF and the labeled compounds using Equation 7 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

9.5.7 Technical Acceptance Criteria for Initial Calibration

9.5.7.1 All initial calibration standards must be analyzed at the concentration levels and frequency described, as a minimum requirement.

9.5.7.2 The m/z ratios must be within the limits specified in Exhibit D – CCD/CDF, Table 9.

9.5.7.3 The S/N ratios for the HRGC/HRMS signal in every SICP must be greater than or equal to 10.

9.5.7.4 The RTs of the isomers must fall within the appropriate RT windows established by analysis of the WDM. The Absolute RT of $^{13}C_{12}$-1,2,3,4-TCDD shall exceed 25 minutes on the DB-5 column and the retention time of $^{13}C_{12}$-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. The purpose of this requirement is to ensure that target analytes are adequately resolved and enhance the ability of the system to resolve target analytes from interferences.

9.5.7.5 The %RSD for the RR must be ≤20% and the %RSD for the RRF must be ≤35% over the five-point calibration range.

9.5.8 Corrective Action for Initial Calibration

9.5.8.1 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.

9.5.8.2 All initial calibration technical acceptance criteria must be met before any samples, LCS/LCSD, and required blanks are analyzed. Any analysis conducted when the technical acceptance criteria have not been met will require reanalysis at no additional cost to the EPA.

9.6 Continuing Calibration Verification

9.6.1 Summary of Continuing Calibration Verification

9.6.1.1 The analyses of a CS3 standard and an instrument blank or a method blank constitute the continuing calibration verification. All performance criteria must be verified through checking the RR, RRF, and RRT in the CS3 standard. Adjustment and/or recalibration (Section 9.5) must be performed until all performance criteria are met. Only after all performance criteria are met may samples and blanks be analyzed.

9.6.1.2 Mass Spectrometer Resolution

Static resolving power checks must be performed at the beginning and at the end of each shift. If the requirement in Section 9.0 cannot be met, the problem must be corrected before analyses can proceed. If any of the samples in the previous shift may be affected by poor resolution, those samples must be reanalyzed at no additional cost to the EPA.

9.6.2 Frequency of Continuing Calibration Verification

The CS3 CCV standard must bracket the 12-hour period during which sample data are collected. The CCV must be analyzed after the HRMS system tune, WDM and ICS, or CPS when the WDM and ICS are combined. An instrument blank must be analyzed after the CS3 opening CCV in the absence of a method blank during the 12-hour period. The CS3 closing CCV must be analyzed to bracket the end of the 12-hour period. This CS3 closing CCV may also be used as the beginning of the next 12-hour period. All acceptable samples must be analyzed within a valid analysis sequence as given below:

Time	Analysis
0 Hour	HRMS System Tune
First 12-Hour	WDM and ICS or CPS (when WDM and ICS are combined) Evaluate WDM and ISC or CPS (if meeting criteria) CS1 to CS5 Instrument Blank or Method Blank LCS, LCSD, and Samples (if time still remains on the 12-hour clock) Closing WDM and ICS or Closing CPS Closing CCV CS3 Ending HRMS System Tune
Next 12-Hour	HRMS System Tune WDM and ICS or CPS (when WDM and ICS are combined) Opening CCV CS3 Instrument Blank or Method Blank Samples Closing WDM and ICS or Closing CPS
Another 12-Hour	Closing CCV CS3 Instrument Blank or Method Blank Samples Closing WDM and ICS or Closing CPS Closing CCV CS3 Ending HRMS System Tune

9.6.3 Procedure for Continuing Calibration Verification

Inject the CS3 Continuing Calibration Verification Standard using the volume chosen in Section 9.4.2 and the conditions in Section 9.1.1 and measure the SICP areas for the analytes and compute the ion abundance ratios at the exact m/z. 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 continuing calibration verification RR/RRF using Equation 8 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

9.6.4 Calculations for Continuing Calibration Verification.

9.6.4.1 Calculate the RR and RRF according to Equations 2 and 3 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

9.6.4.2 The %D between the initial calibration and the continuing calibration verification response for each target and labeled analyte is determined using Equation 8 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

9.6.5 Technical Acceptance Criteria for Continuing Calibration Verification

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

9.6.5.2 The RRTs of the native and labeled CDDs/CDFs shall be within the limits defined in Exhibit D – CCD/CDF, Table 2, or, if an alternate column is used, within their respective RRT limits for the alternate column (Section 6.8).

9.6.5.3 The %D between the continuing calibration verification RR and the mean RR from the initial calibration must be within ±25%. The %D between the continuing calibration verification RRF and the mean RRF from the initial calibration must be within ±35%.

9.6.5.4 The peaks representing both native and labeled compounds in the CS3 standard must have a S/N ratio greater than or equal to 10.

9.6.5.5 The HRMS tune data must be checked and meet the resolution requirement at greater than or equal to 10,000.

9.6.6 Corrective Action for Continuing Calibration Verification

9.6.6.1 Continuing calibration verification technical acceptance criteria must be met before any samples, LCS/LCSD, or blanks are analyzed. Any analysis conducted when the technical acceptance criteria have not been met will require reanalysis at no additional cost to the EPA.

9.6.6.2 If the continuing calibration verification technical acceptance criteria are not met, inspect the system for problems. It may be necessary to change columns, adjust the system, and recalibrate. It may be necessary to segregate samples with particularly serious matrix issues for further cleanup or dilution and reanalysis at a later time in order to obtain compliant beginning and ending calibration checks.

9.6.6.3 If tune criteria were not met, refer to Section 9.3.4.

10.0 PROCEDURE

10.1 Sample Preparation

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

Blank and LCS/LCSD samples must be carried through the same preparation procedures as the field samples to check for losses and contamination in the preparation methods.

Extract pre-screening using a detector that is not the primary analytical unit, [i.e., electron capture GC (GC/ECD) or low resolution GC/MS (GC/LRMS)] is strongly recommended prior to HRGC/HRMS sample extraction as some congeners may be present in high concentration.

10.1.1 Multiphase/Insufficient Samples

10.1.1.1 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 contact the EPA Region. If all phases of the sample are amenable to analysis, the EPA 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 the 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 the EPA Sample Numbers for the additional phases, if required; or
- Not analyze the sample.

10.1.1.2 If an insufficient sample amount (less than the required amount) is received to perform the analyses, the Contractor shall contact SMO and proceed with the analysis of the sample at reduced volume. The Contractor shall document this action and the response from SMO in the SDG Narrative.

10.1.2 Aqueous/Water Samples

Because CDDs/CDFs may be bound to suspended particles, the preparation of aqueous/water samples is dependent on the apparent solids content of the sample.

10.1.2.1 Aqueous/water samples free from visible particulates are extracted directly using the separatory funnel extraction (Section 10.2.1), continuous liquid-liquid extraction (Section 10.2.2), or the SPE technique (Section 10.2.3).

10.1.2.1.1 The sample volume may be determined by: (1) marking the level of water in the sample container; (2) pouring the sample to an extractor; (3) rinsing the emptied bottle with a small amount of the extraction solvent, which shall be combined with the extraction solvent to be used for sample extraction; (4) refilling the emptied bottle with tap water to the marked

line; and (5) finally pouring the tap water to a graduated cylinder to measure the sample volume.

10.1.2.2 Aqueous/water samples containing visible particulates must be processed to determine the percent suspended solids, as in Section 10.1.4.1.1. If the sample is found to contain less than 1% suspended solids, it may be extracted as described above. Samples containing 1% or more of suspended solids must be prepared as indicated below.

10.1.2.3 Aqueous/water samples that contain 1% or more solids (sufficient to provide 10 g of dry solids) and solid samples that show an aqueous phase, must be filtered using the procedure in Section 10.1.3.6. The aqueous filtrate and the filtered solid are treated as two separate samples. The filtered solid is prepared following the procedure for soil/sediment samples (Section 10.2.4 or 10.2.5) and the filtrate is extracted using one of the three options outlined in Section 10.1.2.1. The extracts from these fractions may be combined after initial extraction.

10.1.3 Preparation of Aqueous/Water Samples

10.1.3.1 Mark the original level of the sample on the sample bottle for reference. Weigh the sample plus bottle to ±1 g. After taking the sample aliquot, reweigh the sample bottle and convert the weight to volume assuming a density of 1.0 g/mL.

10.1.3.2 Spike 1 mL of the labeled compound spiking solution (Section 7.8.2.1) into the sample bottle. Cap the bottle and mix the sample by careful shaking. Allow the sample to equilibrate for 1-2 hours, with occasional shaking.

10.1.3.3 For each sample or sample batch (to a maximum of 20 samples) to be extracted during the same 12-hour period, place three 1 L aliquots of reagent water in clean sample bottles or flasks.

10.1.3.4 Spike 1 mL of the labeled compound spiking solution (Section 7.8.2.1) into the three reagent water aliquots. One of these aliquots will serve as the blank and the others will serve as the LCS/LCSD. Spike each LCS/LCSD with 1 mL of the LCS/LCSD spiking solution (Section 7.8.2.2).

10.1.3.5 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 10.2.3 for extraction. If SPE is not to be used, and the sample has no visible particulates, proceed to Section 10.2 for extraction.

10.1.3.6 Filtration of Particulate Matter

10.1.3.6.1 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 particulates.

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

10.1.3.6.3 Rinse any particulates off the sides of the Buchner funnel with small quantities of reagent water.

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

10.1.3.6.5 Extract the filtrate using the separatory funnel (Section 10.2.1), continuous liquid-liquid extraction (Section 10.2.2), or SPE (Section 10.2.3) procedure.

10.1.3.6.6 Extract the filter containing the particulates using the appropriate soil/sediment procedure in Section 10.2.4 or 10.2.5.

10.1.4 Soil/Sediment Samples

Mix samples thoroughly, especially composite samples. Discard any foreign objects such as sticks, leaves, and rocks. If sample contains standing water, follow instructions in Section 10.1.1.

10.1.4.1 Determination of Percent Suspended Solids, Percent Solids, and Estimation of Particle Size

10.1.4.1.1 Determination of Percent Suspended Solids

For aqueous liquids and multiphase samples consisting of mainly an aqueous phase, desiccate and weigh a filter to three (3) significant figures.

10.1.4.1.1.1 Filter 1000 mL of well-mixed sample through the filter.

10.1.4.1.1.2 Dry the filter for a minimum of 12 hours at 110°C (±5°C) and cool in a desiccator. This aliquot is used for determining the Percent Suspended Solids (%Suspended Solids) of the sample, not for analysis of CDD/CDFs.

10.1.4.1.1.3 Calculate the %Suspended Solids using Equation 9 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

10.1.4.1.2 Determination of Percent Solids

For non-aqueous liquids, solids, semi-solid samples, and multiphase samples in which the main phase is not aqueous, but not tissues:

10.1.4.1.2.1 Weigh 5-10 g of sample to three (3) significant figures into a tared beaker.

10.1.4.1.2.2 Dry for a minimum of 12 hours at 110°C (±5°C) and cool in a desiccator. At the start and end of the drying procedure, record the oven temperature, the date, and the time. This aliquot is used for determining the %Solids of the sample, not for analysis of CDD/CDFs.

10.1.4.1.2.3 Calculate the %Solids using Equation 10 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

10.1.4.1.3 Estimation of Particle Size

10.1.4.1.3.1 Spread the dried sample from Section 10.1.4.1.2.2 on a piece of filter paper or aluminum foil in a fume hood or glove box.

10.1.4.1.3.2 Estimate the size of the particles in the sample. If the size of the largest particles is greater than 1 mm, the particle size must be reduced to 1 mm or less prior to extraction using the procedures in Section below.

10.1.4.2 Sample Sieving or Removal of Large Particles

Samples with particle sizes greater than 1 mm (as determined in Section 10.1.4.1.3) are subjected to sieving, or manual removal of just a few of the larger particles. Any sieving procedures must be carried out in a glove box or fume hood to prevent fugitive particles from contaminating the work environment.

10.1.4.3 Preparation of Soil/Sediment Samples

10.1.4.3.1 If instructed to remove water by the EPA Region, filter the sample through a glass-fiber filter. The water portion of the sample shall be handled based on EPA Regional direction.

10.1.4.3.2 Weigh a well-mixed aliquot of each solid sample sufficient to provide 10 g of dry soil/sediments (based on the percent solids determination in Section 10.1.4.1.2) into a clean beaker or glass jar. Record sample weight to the nearest 0.1 g.

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

10.1.4.3.4 Spike 1.0 mL of the labeled compound spiking solution (Section 7.8.2.1) into each sample and reference matrix aliquot. One aliquot of the reference matrix will serve as the blank and the others will serve as the LCS/LCSD. Spike each LCS/LCSD with 1.0 mL of the LCS/LCSD spiking solution (Section 7.8.2.2).

10.1.4.3.5 If particles greater than 1 mm are present in the sample, spread the sample on clean aluminum foil. After the sample is dry, reduce the particle size (Section 10.1.4.2). Keep the temperature cold throughout the process.

10.1.4.3.6 Extract the sample and reference matrix aliquots using the appropriate soil/sediment procedure in Section 10.2.4 or 10.2.5.

10.1.5 Tissue (Non-Human) Samples

Prior to processing tissue samples, the Contractor shall 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 Contractor), 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 Samples are homogenized while still frozen, where practical or partially frozen if not. If the Contractor shall dissect the whole fish to obtain the appropriate tissue for analysis, the unused tissues may be carefully and rapidly re-frozen using a flash freezer, liquid nitrogen, or dry carbon dioxide, and stored in a clean glass jar for subsequent use. The laboratory shall observe all applicable safety precautions when handling these materials.

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

10.1.5.1.3 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. Keep the temperature cold so the fatty tissue is not separated.

10.1.5.2 Preparation of Tissue (Non-Human) Samples

10.1.5.2.1 Transfer approximately 10 g (wet weight) of homogenized tissue to a clean, tared 400-500 mL beaker. For the alternate HCl digestion/extraction, transfer the tissue to a clean, tared 500-600 mL wide-mouth bottle. Record the weight to the nearest 0.1 g.

10.1.5.2.2 Prepare the blank, LCS, and LCSD by adding approximately 1-2 g of the tissue reference matrix (Section 7.6.3) to a 400-500 mL beaker. Record the weight to the nearest 0.1 g. For the alternate HCl digestion/extraction, add the reference matrix to a 500-600 mL wide-mouth bottle. Record the weight to the nearest 0.1 g.

10.1.5.2.3 Spike 1.0 mL of the labeled compound spiking solution (Section 7.8.2.1) into the samples, blank, LCS, and LCSD. Spike each LCS/LCSD with 1 mL of the LCS/LCSD spiking solution (Section 7.8.2.2).

10.1.5.2.4 Extract the sample and reference matrix aliquots using the Soxhlet extraction or HCl digestion/extraction and concentration procedures in Sections 10.2.5 and 10.2.6.

10.1.6 Oily Samples

Prior to processing oily samples, the Contractor 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 any standing water phase, and process as instructed in Section 10.1.1.

10.1.6.1 Preparation of Oily Samples

10.1.6.1.1 Weigh 2 grams of sample into a clean beaker or glass jar. Record the sample weight to the nearest 0.1 g.

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

10.1.6.1.3 Spike 1 mL of the labeled compound spiking solution (Section 7.8.2.1) into each sample and reference matrix aliquot. One aliquot of the reference matrix will serve as the blank and the others will serve as the LCS/LCSD. Spike each LCS/LCSD with 1 mL of the LCS/LCSD spiking solution (Section 7.8.2.2).

10.1.6.1.4 Extract the sample and reference matrix aliquots using the appropriate soil/sediment procedure in Section 10.2.4 or 10.2.5. If the sample aliquot is completely soluble in the extraction solvent, the extraction step may be skipped and the sample processed through cleanup.

10.1.7 Fly Ash Samples

All handling steps should be performed in a hood in order to minimize contamination because of the tendency of fly ash to "fly". The percent solids for the fly ash samples shall be determined following the procedures in Section 10.1.4.1.2.

10.1.7.1 Preparation of Fly Ash Samples

10.1.7.1.1 Weigh about 10 g of fly ash into a clean glass extraction jar. Record the sample weight to the nearest 0.1 g.

10.1.7.1.2 Spike 1.0 mL of the labeled compound spiking solution (Section 7.8.2.1) and allow the solution to dry. Add 150 mL of 1N HCl to the sample.

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

10.1.7.1.4 Spike 1.0 mL of the labeled compound spiking solution (Section 7.8.2.1) and add 150 mL of 1N HCl into each reference matrix aliquot. One aliquot will serve as the blank and the others will serve as the LCS/LCSD. Spike each LCS/LCSD with 1.0 mL of the LCS/LCSD spiking solution (Section 7.8.2.2).

10.1.7.1.5 Seal the jars with the PTFE-lined screw cap and shake for 3 hours at room temperature.

10.1.7.1.6 Rinse a glass fiber with toluene, and filter the sample through the filter paper, placed in a Buchner funnel, into a 1 L flask. Wash the fly ash cake with approximately 500 mL of organic-free reagent water and dry the filter cake overnight at room temperature in a desiccator. Follow the same procedures for each of the reference matrix aliquots.

10.1.7.1.7 Extract the sample, reference matrix aliquots, and filter paper using the appropriate soil/sediment procedure in Section 10.2.4 or 10.2.5.

10.2 Sample Extraction

Extraction procedures include separatory funnel (Section 10.2.1), continuous liquid-liquid extraction (Section 10.2.2), and SPE (Section 10.2.3) for aqueous/water samples; SDS extraction (Section 10.2.4) and Soxhlet extraction (Section 10.2.5) for soil/sediment or oily matrices; and Soxhlet extraction (Section 10.2.5) and HCl digestion (Section 10.2.6) for tissue (non-human) samples. Acid/base back-extraction (Section 10.2.7) is used for initial cleanup of extracts. Appropriate extraction methods shall be selected based on sample conditions. Soxhlet extraction alone is not appropriate for wet sediment samples.

10.2.1 Separatory Funnel Extraction

10.2.1.1 Pour the spiked sample (Section 10.1.3.4) or filtrate (Section 10.1.3.6.5) 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, 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 (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, discard the aqueous layer and rinse the separatory funnel with at least 20 mL of methylene chloride. 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 particulates. Set aside the concentration device for use after back-extraction or other cleanup.

10.2.1.4 Concentrate the extract using one of the macro-concentration procedures (Section 10.3). Set aside the concentration device for use after back-extraction or other cleanup.

10.2.1.4.1 If the extract is free from visible particulates, 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 10.2.7.

10.2.1.4.2 If the extract is from the aqueous filtrate (Section 10.1.3.6.5), set aside the Kuderna-Danish (K-D) apparatus for addition of the extract from the particulates (Section 10.2.4.9).

10.2.2 Continuous Liquid-Liquid Extraction

10.2.2.1 Place 100-150 mL methylene chloride in each continuous extractor and 200-300 mL in each distilling flask.

10.2.2.2 Pour the spiked sample (Section 10.1.3.4) into the extractor. Rinse the sample container with 50-100 mL of methylene chloride and add this rinse to the extractor.

10.2.2.3 Begin the extraction by heating the flask until the methylene chloride is boiling. When properly adjusted, 1-2 drops of methylene chloride per second will fall from the condenser tip into the water. Extract the sample for 16-24 hours. The accelerated continuous liquid-liquid extractor that uses the hydrophobic membrane to reduce the overall extraction time may be used, provided that all performance criteria are met and the prior Method Detection Limit (MDL) study was performed using the same procedure.

10.2.2.4 Remove the distilling flask, estimate and record the volume of extract (to the nearest 100 mL), and pour the contents through a drying column containing 7-10 cm of granular anhydrous sodium sulfate into a 500 mL K-D evaporator flask equipped with a 10 mL concentrator tube. Rinse the distilling flask with 30-50 mL of methylene chloride and pour through the drying column.

10.2.2.5 Concentrate and exchange to hexane per Section 10.3 and back-extract per Section 10.2.7.

10.2.3 Solid Phase Extraction

10.2.3.1 Disk Preparation

10.2.3.1.1 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.

10.2.3.1.2 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.

10.2.3.1.3 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 filter/disk to go dry from this point until the extraction is completed.

10.2.3.1.4 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. Do not allow the filter/disk to go dry from this point until the extraction is completed.

10.2.3.2 Sample Extraction

10.2.3.2.1 Allow the samples (Section 10.1.3.5) to stand for 1-2 hours, if necessary, to settle the suspended particulates. Decant the clear layer of the sample, blank, LCS, and LCSD into its respective 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 a high amount of particulates (suspended solids), the extraction time may be an hour or longer.

10.2.3.2.2 If the filter clogs with particulates and more rapid extraction is desired, replace the filter during the extraction by pouring 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, cover and label with the Sample ID, for later extraction. Reassemble the apparatus with a clean filter and proceed with the extraction. Pull the sample through the filter/disk, leaving a layer of water on the surface of the filter.

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

10.2.3.2.4 Before the sample and rinses have been pulled through the filter/disk, rinse the sides of the reservoir with small portions of reagent water. Allow the filter/disk to partially dry under the vacuum for approximately 3 minutes, then elute the analytes using the procedure in Section 10.2.3.3. Alternatively, extract all filter(s) and disk used per the SDS or Soxhlet procedure (Sections 10.2.4 or 10.2.5).

10.2.3.3 Elution of the Filter/Disk

10.2.3.3.1 Release the vacuum, remove the entire filter/disk/reservoir assembly from the vacuum flask, and empty the flask. Insert a test tube for eluant collection into the flask. The test tube should have sufficient capacity to contain the total volume of the elution solvent (approximately 50 mL) and should fit around the drip tip. The drip tip should protrude into the test tube to preclude loss of a sample from spattering when the vacuum is applied. Reassemble the filter/disk/reservoir assembly on the vacuum flask.

10.2.3.3.2 Wet the filter/disk with 4-5 mL of acetone. Allow the acetone to spread evenly across the disk and soak for 15-20 seconds. Pull the acetone through the disk, releasing the vacuum when approximately 1 mm thickness remains on the filter.

10.2.3.3.3 Rinse the sample bottle with approximately 20 mL of methylene chloride and transfer to the reservoir. Pull approximately half of the solvent through the filter/disk and release the vacuum. Allow the filter/disk to soak for approximately 1 minute. Pull all of the solvent through the disk. Repeat the bottle rinsing and elution step with another 20 mL of methylene chloride. Pull all of the solvent through the disk.

10.2.3.3.4 Remove the filter/disk/reservoir assembly, and remove the test tube containing the sample solution. Quantitatively transfer the solution to a 250 mL separatory funnel and proceed to Section 10.2.7 for back-extraction.

10.2.4 Soxhlet/Dean-Stark Extraction

10.2.4.1 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.

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

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

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

10.2.4.5 Load the sample from Sections 10.1.3.6.6, 10.1.4.3.6, 10.1.6.1.4, and 10.1.7.1.7 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.

10.2.4.6 Reassemble 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.

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

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

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

10.2.4.9.1 For the extracts from the particulate portion of a water sample containing less than 1% solids:

10.2.4.9.1.1 Concentrate the extract to approximately 5 mL using the rotary evaporation or the heating mantle procedures (Section 10.3.1 or 10.3.2).

10.2.4.9.1.2 Quantitatively transfer the extract through the sodium sulfate (Section 10.2.1.3) into the apparatus that was set aside (Section 10.2.1.4) and reconcentrate to the level of the toluene.

10.2.4.9.1.3 Adjust to approximately 10 mL with hexane, transfer to a 250 mL separatory funnel, and proceed with back-extraction (Section 10.2.7).

10.2.4.9.2 For the extracts from soil/sediments or from the SPE filter(s) and disk:

10.2.4.9.2.1 Concentrate to approximately 10 mL using the rotary evaporator or heating mantle (Section 10.3.1 or 10.3.2), transfer to a 250 mL separatory funnel, and proceed with back-extraction (Section 10.2.7).

10.2.5 Soxhlet Extraction

This procedure includes determination of the lipid content of tissue samples (Section 10.2.5.8), using the same sample extract that is analyzed by high resolution GC/MS. Alternatively, a separate sample aliquot may be used for the lipid determination.

10.2.5.1 Add 30-40 g of powdered anhydrous sodium sulfate to each of the beakers and mix thoroughly. Cover the beakers with aluminum foil and dry until the mixture becomes a free-flowing powder (30 minutes minimum). Remix prior to extraction to prevent clumping.

10.2.5.2 Assemble and pre-extract the Soxhlet apparatus per Sections 10.2.4.1 – 10.2.4.4, however, use the methylene chloride:hexane (1:1) mixture for the pre-extraction and rinsing and omit the quartz sand.

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

10.2.5.4 Transfer the prepared sample to the Soxhlet thimble and install the thimble in the Soxhlet apparatus.

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

10.2.5.6 After extraction, cool and quantitatively transfer the extract to a macro-concentration device and concentrate to near dryness. Disassemble the apparatus and set aside for re-use.

10.2.5.7 Complete the removal of the solvent using the nitrogen evaporation procedure (Section 10.4) and a water bath temperature at 60°C. Weigh the receiver, record the weight, and return the receiver to the nitrogen evaporation device, concentrating the residue until a constant weight is obtained.

NOTE: Close attention is required when performing the extract concentration.

10.2.5.8 Percent Lipids Determination

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

10.2.5.8.1 Redissolve the residue in the concentration device in hexane and spike 1.0 mL of the Cleanup Standard into the extract prior to cleanup in Section 10.5.6 for tissue samples.

10.2.5.8.2 Transfer the residue/hexane to the anthropogenic isolation column (Section 10.5.6.1) or the narrow-mouth 100-200 mL bottle for acidified silica gel cleanup (Section 10.5.6.2), 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 redissolved. Allow the receiver to dry. Weigh the receiver and boiling chips.

10.2.5.8.3 Calculate the lipid content to the nearest three (3) significant figures using Equation 11 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

10.2.6 HCl Digestion/Extraction

10.2.6.1 Add 200 mL of 6-N HCl and 200 mL of methylene chloride:hexane (1:1) to the sample, blank, and LCS/LCSD aliquots (Section 10.1.5.2.4).

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

10.2.6.3 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 12-24 hours.

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

10.2.6.5 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 (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.

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

10.2.6.7 Complete the removal of the solvent using the nitrogen evaporation device (Section 10.4) and a water bath temperature at 60°C. Weigh the receiver, record the weight, and return the receiver to the nitrogen evaporation device, concentrating the residue until a constant weight is obtained.

10.2.6.8 Determine the lipid content per the procedure described in Section 10.2.5.8.

10.2.6.9 Clean up the extract per Section 10.5, beginning with the sulfuric acid and base back-extraction cleanup procedure in Section 10.5.6.3.

10.2.7 Back-Extraction with Base and Acid

10.2.7.1 Spike 1 mL of the Cleanup Standard (Section 7.8.2.3) into each extract of samples, blanks, and LCS/LCSD, or verify that this has been done. Back-extraction may not be necessary for some samples. For some samples, the presence of color in the extract may indicate that back-extraction is necessary. If back-extraction is not to be performed, proceed to micro-concentration (Section 10.4), or to extract cleanup (Section 10.5). If back-extraction is necessary, proceed to Section 10.2.7.2.

10.2.7.2 Partition the extract against 50 mL of potassium hydroxide solution (Section 7.1.2). Shake not more than 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 with minimum contact time, provided that the Contractor meets the specifications for labeled compound recovery.

10.2.7.3 Partition the extract against 50 mL of sodium chloride solution (Section 7.1.3) in the same way as with base. Discard the water layer.

10.2.7.4 Partition the extract against 50 mL of sulfuric acid (Section 7.1.6) 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.

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

10.2.7.6 Pour each extract through a drying column containing 7-10 cm of granular anhydrous sodium sulfate into a macro-concentration device (Section 10.3). If a concentration device was set aside from extraction, that concentration device may be re-used. Rinse the separatory funnel with 30-50 mL of solvent, and pour through the drying column. Collect each extract in a round-bottom flask. Reconcentrate the sample per Sections 10.3 through 10.4, and clean up the samples 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 2-3 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 15-20 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 Proceed to Section 10.2.7 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 1-2 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 15-20 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 10.2.7 for back-extraction with base and acid or to Section 10.4 for micro-concentration and solvent exchange.

10.3.3 Kuderna-Danish Apparatus

Concentrate the extracts in separate 500 mL K-D flasks equipped with 10 mL concentrator tubes. The K-D technique is used for solvents such as methylene chloride and hexane.

10.3.3.1 Add 1-2 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 15-20 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 5-10 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 10.2.7 for back-extraction with base and acid or to Section 10.4 for micro-concentration and solvent exchange.

10.3.4 Preparation for Back-Extraction or Micro-Concentration and Solvent Exchange

10.3.4.1 For back-extraction, transfer the extract to a 250-mL separatory funnel. Rinse the concentration vessel with small portions of hexane, adjust the hexane volume in the separatory funnel to 10-20 mL, and proceed to back-extraction (Section 10.2.7).

10.3.4.2 For cleanup procedures other than back-extraction, transfer the extract to a nitrogen evaporation vial using 2-3 rinses of solvent. Proceed with micro-concentration and solvent exchange (Section 10.4).

10.4 Micro-Concentration and Solvent Exchange

10.4.1 Extracts to be subjected to GPC or HPLC cleanup are exchanged into methylene chloride. Extracts that are to be cleaned up using silica gel, alumina, carbon (Carbopak/Celite), 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 2-3 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 10.0 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 (Section 10.5.1).

10.4.6 If the extract is to be cleaned up by column chromatography (alumina, silica gel, carbon, or Florisil), bring the final volume to 1 mL with hexane. Proceed with column cleanups (Sections 10.5.2, 10.5.3, 10.5.4, and 10.5.7).

10.4.7 For extracts to be concentrated for injection into the HRGC/HRMS system, 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 percent lipids determination, evaporate until a constant weight is obtained.

10.5 Cleanup Procedures

Cleanup may not be necessary for relatively clean samples (e.g., groundwater and drinking water). However, the Contractor is required to perform all clean-up procedures necessary to achieve the CRQLs, or to fully document the reasons why they could not be achieved. If particular circumstances require the use of cleanup procedures, the Contractor may use any or all of the procedures in this method or any other appropriate procedure. Any additional cleanup procedure step shall be documented in the SDG Narrative and must also be carried out on the associated method blanks and LCS/LCSD.

The following cleanup procedures are available in this method:

- GPC - Removes many high molecular weight interferences that cause GC column performance to degrade. It should be used for all tissue, 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.
- Silica Gel, Alumina, and Florisil - Used to remove non-polar and polar interferences. Alumina and Florisil are used to remove polychlorinated diphenyl ethers.
- Carbon (Carbopak/Celite) - Used to remove non-polar interferences.
- HPLC - Used to provide specificity for the 2,3,7,8-substituted and other CDD and CDF congeners.
- Anthropogenic Isolation Column - Used for removal of lipids from tissue samples.

10.5.1 Gel Permeation Chromatography Cleanup

10.5.1.1 Introduction

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

the molecular size of the molecules to be separated. The Contractor may prepare columns by the procedure described below or purchase commercially available columns.

10.5.1.2 Column Packing

10.5.1.2.1 Place 200-400 mesh, 70-75 g of SX-3 Bio Beads in a 400-500 mL beaker.

10.5.1.2.2 Cover the beads with methylene chloride and swirl the container to ensure the wetting of all beads. Allow the beads to swell overnight (a minimum of 12 hours).

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

10.5.1.2.4 After purging the column with solvent for 1-2 hours, adjust the column head pressure to 7-10 pounds per square inch gauge (psig), and purge for 4-5 hours to remove air. Maintain a head pressure of 7-10 psig. Connect the column to the UV detector.

10.5.1.3 Column Calibration

10.5.1.3.1 Summary of Calibration

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

10.5.1.3.2 Frequency of GPC Calibration

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

10.5.1.3.3 Procedure for GPC Calibration

10.5.1.3.3.1 Follow the manufacturer’s instructions for operating the GPC system. Using a 10 mL syringe, load the calibration solution onto the GPC. 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.

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

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

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

10.5.1.3.4 Technical Acceptance Criteria for GPC Calibration

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

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

10.5.1.3.4.3 The recovery of the methoxychlor shall be greater than 85%.

10.5.1.3.5 Corrective Action for GPC Calibration

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

10.5.1.4 Sample Extract Cleanup by GPC

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

10.5.1.4.1 Frequency of Sample Extract Cleanup by GPC

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

10.5.1.4.2 Procedure for Sample Extract Cleanup by GPC

10.5.1.4.2.1 Filter the extract or load through the filter holder to remove any particulates. Follow the manufacurer’s instructions for operation of the system being utilized. A 2 mL injection loop may be used in place of a 5 mL injection loop. If a 2 mL injection loop is used, concentrate the sample extract to 4 mL instead of 10 mL and then inject 4 mL instead of 10 mL. Load the concentrated sample extract into the sample injection loop and inject it onto the GPC column.

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

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

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

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

10.5.2 Silica Gel Cleanup

10.5.2.1 Procedure for Silica Gel Cleanup

10.5.2.1.1 Place a glass wool plug in a 15 mm ID chromatography column. Pack the column bottom to top with: 1 g silica gel (Section 7.5.1.2), 4 g basic silica gel (Section 7.5.1.3), 1 g silica gel, 8 g acid silica gel (Section 7.5.1.1), 2 g silica gel, and 4 g granular anhydrous sodium sulfate (Section 7.2.1). Tap the column to settle the adsorbents. Column packing material may be slurried in hexane to aid settling.

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

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

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

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

10.5.2.1.6 For extracts of samples known to contain large quantities of other organic compounds, 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 1N 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 from the column.

10.5.3 Alumina Cleanup

10.5.3.1 Procedure for Alumina Cleanup

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

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

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

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

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

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

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

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

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

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

10.5.4 Carbon Column Cleanup

10.5.4.1 Procedure for Carbon Column Cleanup

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

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

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

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

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

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

10.5.5 High Performance Liquid Chromatography Cleanup

10.5.5.1 Procedure for HPLC Calibration

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

10.5.5.1.2 Inject 30 µL of the calibration solution into the HPLC and record the signal from the detector. Collect the eluant for reuse. The elution order will be the tetrachlorinated through octachlorinated isomers.

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

10.5.5.2 Technical Acceptance Criteria for HPLC Calibration

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

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

10.5.5.3 Corrective Action for HPLC Calibration

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

10.5.5.4 Sample Extract Cleanup by HPLC

HPLC requires that the column not be overloaded. The column specified in this method is designed to handle a maximum of 5-50 µg depending on the congener. If the amount of material in the extract will overload the column, split the extract into fractions and combine the fractions after elution from the column.

10.5.5.4.1 Procedure for Sample Extract Cleanup by HPLC

10.5.5.4.1.1 Spike 1 mL of the Cleanup Standard (Section 7.8.2.3) into each extract of samples, blanks, and LCS/LCSD, or verify that this has been done. Rinse the sides of the vial twice with 30 µL of methylene chloride and reduce to 30 µL with the evaporation apparatus.

10.5.5.4.1.2 Inject the 30 µL extract into the HPLC.

10.5.5.4.1.3 Elute the extract using the calibration data determined in Section 10.5.5.1. Collect the fraction(s) in clean 20 mL concentrator tubes containing 5 mL of hexane:acetone (1:1 v/v).

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

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

10.5.6 Anthropogenic Isolation Column, Acidified Silica Gel, and Sulfuric Acid and Base Back-Extraction Cleanup

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

10.5.6.1 Anthropogenic Isolation Column Cleanup

10.5.6.1.1 Procedure for Anthropogenic Isolation Column Cleanup

10.5.6.1.1.1 Prepare the column as given in Section 7.5.4.

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

10.5.6.1.1.3 Load the sample and rinses (Section 10.2.5.8.2) 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 (Section 10.3) using 200 mL of hexane.

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

10.5.6.1.1.5 If necessary, exchange the extract to a solvent suitable for the additional cleanups to be used (Sections 10.5.1 - 10.5.5, and Section 10.5.7).

10.5.6.1.1.6 Spike 1.0 mL of the Cleanup Standard into the residue/solvent.

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

10.5.6.1.1.8 Following cleanup, concentrate the extract to 10 µL as described in Section 10.4 and proceed with the analysis in Section 10.6.

10.5.6.2 Acidified Silica Gel Cleanup

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

10.5.6.2.1 Procedure for Acidified Silica Gel Cleanup

10.5.6.2.1.1 Adjust the volume of hexane in the bottle (Section 10.2.5.8.2) to approximately 200 mL.

10.5.6.2.1.2 Spike 1.0 mL of the Cleanup Standard into the residue/solvent.

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

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

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

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

10.5.6.2.1.6 Concentrate the extract to a volume suitable for the cleanups given in Section 10.5.

10.5.6.3 Sulfuric Acid and Base Back-Extraction Cleanup

This procedure is used with HCl digested extracts (Section 10.2.6).

10.5.6.3.1 Procedure for Sulfuric Acid and Base Back-Extraction Cleanup

10.5.6.3.1.1 Spike 1.0 mL of the Cleanup Standard into the residue/solvent, or verify that this has been done.

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

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

10.5.6.3.1.4 Back-extract the solvent/residue with 50 mL of potassium hydroxide solution per Section 10.2.7.2, followed by two reagent water rinses.

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

10.5.6.3.1.6 Concentrate the cleaned up extract to a volume suitable for the additional cleanups given in Sections 10.5.1 - 10.5.6. GPC, alumina, and carbon (Carbopak/Celite) are recommended as minimum additional cleanup steps.

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

10.5.7 Florisil Cleanup

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

10.5.7.2 Spike 1 mL of the Cleanup Standard (Section 7.8.2.3) into each extract of samples, blanks, and LCS/LCSD, or verify that this has been done. When the solvent is within 1 mm of the packing, apply the sample extract (in hexane) to the column. Rinse the sample container twice with 1 mL portions of hexane and apply to the column.

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

10.5.7.4 Elute the CDDs/CDFs with 35 mL of methylene chloride and collect the eluate. Concentrate the eluate(s) per Sections 10.3 through 10.4 for further cleanup or for injection into the HPLC or HRGC/HRMS instrument.

10.6 High Resolution Gas Chromatography/High Resolution Mass Spectrometry Analysis

10.6.1 Introduction

10.6.1.1 Establish the operating conditions given in Section 9.1.1.

10.6.1.2 Sample extracts shall be analyzed only after the HRGC/HRMS system has met the resolution, RT, RRT, and ion abundance ratio requirements in Section 9.0 and has met the requirements for initial calibration and continuing calibration verification. The same instrument conditions must be employed for the analysis of samples as were used for calibration.

10.6.2 Procedure for Sample Analysis by HRGC/HRMS

10.6.2.1 Add 10 µL of the appropriate internal standard (Section 7.8.2.4) 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 reanalyzed and evaporation has occurred, do not add more internal standard. 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).

10.6.2.2 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 (Section 9.0). Start the GC column initial isothermal hold upon injection. Start HRMS data collection after the solvent peak elutes. Stop data collection after the OCDD and OCDF have eluted. Return the column to the initial temperature for analysis of the next extract or standard.

10.6.2.3 Analysis of Complex Samples

Some samples may contain high levels [>10 ng/L; >1,000 nanograms/kilogram (ng/kg)] of the compounds of interest, interfering compounds, and/or polymeric materials. Some extracts may not concentrate to 10 µL (Section 10.4.7); others may overload the HRGC column and/or MS. Analyze a smaller aliquot of the sample (Section 10.6.3.2) when the extract will not concentrate to 20 µL after all cleanup procedures have been exhausted. If a smaller aliquot of soils or mixed-phase samples is analyzed, assure that the aliquot is representative of the sample as a whole.

10.6.3 Sample Dilutions

If the concentration of any target analyte given in Exhibit C - Chlorinated Dibenzo-*p*-Dioxins and Chlorinated Dibenzofurans and Chlorinated Biphenyl Congeners Target Analyte List and Contract Required Quantitation Limits, Table 1 (except for OCDD or OCDF), exceeds the calibration range of the system (as defined by the five-point initial calibration performed as specified in Section 9.5), the Contractor shall do one or a combination of the following. Any and all steps taken to obtain analyte results from within the calibration range of the analytical system must be thoroughly documented.

10.6.3.1 Dilute the extract or a portion thereof. A dilution sufficient to bring the target analytes within calibration range is allowed as long as the labeled extraction standard in the extract meets the criteria in Section 11.1.1. This reanalysis is not billable.

10.6.3.2 Re-extract a smaller aliquot of the original sample. Care must be taken to preserve sample representativeness. This re-extraction and reanalysis is not billable.

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

10.6.3.2.2 For samples containing greater than 1% solids, extract a fraction of the solid and/or liquid phase portions of the sample. This aliquot should not be less than 1/20 of the original amount without the permission of the client, contacted through SMO. Re-prepare, extract, clean up, and analyze.

10.6.3.3 In the event that it is determined a cut or dilution of more than 1/10 would be necessary to achieve responses within the range of the calibration curve, the Contractor shall perform one or both of the following:

10.6.3.3.1 Perform a dilution or take a subsample of the extract produced in Section 10.2 prior to adding the Cleanup Standard. This procedure must not prevent the labeled extraction standards from passing the criteria in Section 11.1.1.

10.6.3.3.2 Add a higher calibration point to the initial calibration to expand the calibration range of the system.

10.6.3.4 If the target compounds cannot be measured reliably by isotope dilution, prepare a reduced volume or mass of sample, dilute the extract prior to cleanup, adding a larger aliquot of Cleanup Standard, and further dilute the final extract by adding additional labeled internal standard. This dilution and subsequent reanalysis are not billable. The exact procedure followed in this step must be documented in the SDG Narrative.

11.0 DATA ANALYSIS AND CALCULATIONS

11.1 Qualitative Identification

11.1.1 Identification of Target Analytes

A CDD or CDF (native or labeled) is identified when all of the criteria in Section 11.1 are met.

11.1.1.1 The signals for the two exact m/z in Exhibit D – CCD/CDF, Table 3, must be present and must maximize within the same 2 seconds.

11.1.1.2 The S/N for each exact m/z for native analytes in sample extracts must be greater than or equal to 3. The S/N for native analytes in a calibration standard, and for all labeled compounds in sample extracts as well as standards, must be greater than or equal to 10.

11.1.1.3 The ratio of the integrated areas of the two exact m/z specified in Exhibit D – CCD/CDF, Table 3, must be within the limits in Exhibit D – CCD/CDF, Table 9, or within ±10% of the ratio in the most recent mid-point calibration standard (CS3).

11.1.1.4 The RRT of the peak representing a native 2,3,7,8-substituted CDD or CDF must be within the limits specified in Exhibit D – CCD/CDF, Table 2 , or, if an alternate column or column system is employed, within its respective RRT QC limits for the alternate column or column system (Section 6.8). The RT of peaks representing non-2,3,7,8-substituted CDDs/CDFs must be within the RTs established during the analysis of the WDM for the first and last eluting compounds.

11.1.1.5 Confirmatory Analysis of 2,3,7,8-TCDF

11.1.1.5.1 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 below or above the CRQL by analysis on a DB-5 column (or equivalent) must have a confirmatory analysis performed on a DB-225 or equivalent HRGC column. The operating conditions in Section 9.1.1 may be adjusted for analysis on the second HRGC column, but the HRGC/HRMS must meet the mass resolution and calibration specifications in Section 9.0. 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 9.0.

11.1.2 Technical Acceptance Criteria for Qualitative Identification

11.1.2.1 If the criteria for identification listed above are not met, check calculations, internal standards, and system performance. If significant sample matrix–induced background is present, reanalysis of the extract may either resolve the issue or prove matrix effects.

11.1.2.2 The relative ion abundance ratio criteria for target analytes and labeled compounds must be met using peak areas to calculate ratios.

11.1.3 Corrective Action for Qualitative Identification

11.1.3.1 If the ion abundance ratios are not met by using peak areas, but all other criteria are met, the Contractor can use peak heights to evaluate the m/z ratio.

11.1.3.2 If positive identification is not possible due to non-compliant m/z ratio, report the target analyte as an EMPC. Document in the SDG Narrative as necessary.

11.1.3.3 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 1A-HR).

11.2 Quantitative Analysis

11.2.1 Isotope Dilution Quantitation

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 compound exhibit similar behavior upon extraction, concentration, and GC.

11.2.1.2 The labeled compound for the OCDF isomer may be added if GC parameters eliminate any potential interference, otherwise the labeled compound of the isomer OCDF is not added to the sample. Therefore, native OCDF is quantitated against either labeled OCDF or against labeled OCDD. If labeled OCDD is used, 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 compounds 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 RR values from the initial calibration data described in Section 9.5.6 are used to determine concentrations directly, so long as labeled compound spiking levels are constant, using Equation 12 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

11.2.2 Internal Standard Quantitation and Labeled Compound Recovery

11.2.2.1 Compute the concentrations in the extract of the 1,2,3,7,8,9-HxCDD, OCDF (if necessary), ^{13}C-labeled compounds, and the ^{37}Cl-labeled Cleanup Standard in the extract using the RRFs determined from the initial calibration data (Section 9.5.6) and Equation 13 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

11.2.2.1.1 The $^{13}C_{12}$-1,2,3,4-TCDD ($^{13}C_{12}$-1,2,3,4-TCDF, optional) internal standard is used to quantitate the tetrachlorinated and pentachlorinated labeled compounds and the Cleanup Standard. The $^{13}C_{12}$-1,2,3,7,8,9-HxCDD internal standard is used to quantitate the hexachlorinated, heptachlorinated, and octachlorinated labeled compounds.

11.2.2.1.2 There is only one m/z for the $^{37}Cl_4$ labeled Cleanup Standard.

11.2.2.2 Using the concentration in the extract determined above, compute the %R of the labeled compounds and Cleanup Standard using Equation 14 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

11.2.3 Sample Concentration

11.2.3.1 The concentration of a native compound in a solid sample is computed using the concentration of the compound in the extract and the weight of the sample (Section 10.1.4.3), using Equation 15 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

11.2.3.2 The concentration of a native compound in the tissue sample is computed using the concentration of the compound in the extract and the weight of the tissues, using Equation 16 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

11.2.3.3 The concentration of a native compound in aqueous phase of the sample is computed using the concentration of the compound in the extract and the volume of water extracted (Section 10.1.2) using Equation 17 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

11.2.3.4 Results are reported to two (2) significant figures, as appropriate for the CDDs/CDFs and labeled compounds found in all standards, blanks, LCS/LCSD, and samples.

11.2.3.5 For water samples, report results in pg/L (parts-per-quadrillion).

11.2.3.6 For samples containing greater than 1% solids (soils, sediments, filter cake, compost), report results in ng/kg (parts-per-trillion) based on the dry weight of the sample.

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

11.2.3.8 For all samples, report results for all peaks with S/N ratios greater than or equal to 3, even if below the CRQL (Exhibit C - Chlorinated Dibenzo-*p*-Dioxins and Chlorinated Dibenzofurans and Chlorinated Biphenyl Congeners Target Analyte List and Contract Required Quantitation Limits, Table 1).

11.2.4 Sample-Specific Estimated Detection Limit

The Estimated Detection Limit (EDL) is the concentration of a given analyte required to produce a signal with a peak height of at least 3 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 Contractor may note, for completeness of documentation in the data package, that no correction was performed. This is applicable where this practice differs from the Contractor's Standard Operating Procedures (SOPs).

11.2.4.1 If the calculated EDL is less than the adjusted analyte/matrix/instrument-specific MDL, then report the MDL.

11.2.4.2 Use the expression for EDL below to calculate an EDL for each absent 2,3,7,8-substituted isomer CDD/CDF (i.e., S/N<3). The background level is determined by measuring the range of the noise (peak-to-peak) for the two quantitation m/z (see Exhibit D – CCD/CDF, Table 9) of a particular CDD/CDF, in the region of the SICP trace corresponding to the elution of the labeled compound or in the region of the SICP where the congener is expected to elute by comparison with the routine calibration data (for those congeners that do not have a ^{13}C-labeled compound), multiplying that noise height by 3, and relating the product to an estimated concentration that would produce that peak height.

11.2.4.3 Use Equation 18 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations for aqueous/water samples.

11.2.4.4 Use Equation 19 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations for solid samples.

NOTE: If interferences can be shown to be present (e.g., PCBs), then descriptor ions other than those listed in Exhibit D – CCD/CDF, Table 3, may be used for quantitation. This deviation shall be adequately documented in the SDG Narrative.

11.2.5 Estimated Maximum Possible Concentration

An EMPC is calculated for 2,3,7,8-substituted isomers that are characterized by a response with an S/N of at least 3 for both quantitation m/z, but that do not meet all the identification criteria described in Section 11.1.

The EMPC is not adjusted with the theoretical ion balance. However, the Contractor may note, for comp leteness of documentation in the data package, that no correction was performed. This is applicable where this practice differs from the Contractor's SOPs.

11.2.5.1 The EMPC of a target analyte in all matrices other than aqueous/water is computed using the concentration of the compound in the extract and the dilution factor, if any (Section 10.6.3), using Equation 20 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

11.2.5.2 The EMPC of a target analyte in an aqueous/water matrix is computed using the concentration of the compound in the extract and the dilution factor, if any (Section 10.6.3), using Equation 21 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

11.2.6 Contract Required Quantitation Limit Calculations

11.2.6.1 Solid

Calculate the adjusted CRQL for solid samples using Equation 22 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

11.2.6.2 Tissue

Calculate the adjusted CRQL for tissue samples using Equation 23 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

11.2.6.3 Aqueous/Water

Calculate the adjusted CRQL for aqueous/water samples using Equation 24 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

11.2.7 Toxic Equivalency Factor and Toxic Equivalent Summary

11.2.7.1 The concentration of each of the 2,3,7,8-substituted CDD and CDF congeners is multiplied by a TEF to arrive at an equivalent toxicity concentration. Calculate the adjusted TEF concentration (using Equation 25 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations) 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 (Exhibit D – CCD/CDF, Table 11).

11.2.7.2 Add all 17 TEF-adjusted concentrations to calculate the Total TEQ using Equation 26 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations. The Total TEQ calculations of all three species (mammal, fish, and bird) are required.

11.2.8 Total Homologue Concentration

The total concentration of all isomers at a given level of chlorination (homologue; i.e., Total TCDD, Total PeCDD) is calculated by summing the concentrations of all identified 2,3,7,8-substituted isomers and the estimated concentrations of all other possible non-2,3,7,8 isomers at that LOC.

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, HRMS System Tune, resolution check, initial calibration, continuing 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 target and spiked target analytes must meet the qualitative identification criteria in Exhibit D – CCD/CDF, Table 2 and Table 9.

11.3.5 The labeled compounds of the sample must meet the acceptance criteria in Exhibit D – CCD/CDF, Table 5.

11.3.6 If any target analyte concentration exceeds the upper limit of the initial calibration, see Section 10.6.3.

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 (Section 12.1.2.5) will require re-extraction and reanalysis at no additional cost to the EPA. Any samples analyzed that do not meet the technical acceptance criteria will require re-extraction and/or reanalysis at no additional cost to the EPA.

11.4.2 If the sample analysis technical acceptance criteria are not met, check calculations, internal standards, and system performance. If significant sample matrix–induced background is present, reanalysis of the extract may either resolve the issue or prove matrix effects, in which instance the finding should be explained in the SDG Narrative and all chromatographic evidence provided. 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 reanalyzed at no additional cost to the EPA after the corrective action.

11.4.3 System performance checks, initial calibration, and continuing calibration verification must be acceptable before the reanalysis of samples.

12.0 QUALITY CONTROL

12.1 Blank Analyses

12.1.1 Summary

There are two types of blanks required by this method: the method blank and the instrument blank.

12.1.2 Method Blank

12.1.2.1 Summary of Method Blank

A method blank is a volume or weight of a clean reference matrix (reagent water for aqueous/water samples, sand for soil/sediment samples, or corn oil for tissue samples) that is carried through the entire analytical procedure. The volume or weight 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 levels of contamination associated with the processing and analysis of samples. When sample extracts are subjected to cleanup procedures, the associated method blank must also be subjected to the same cleanup procedures. In cases when there is no more method blank volume available, the Contractor should use the instrument blank for analysis (Section 12.1.3).

12.1.2.2 Frequency of Method Blank

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 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 HRGC/HRMS system used to analyze associated samples. At least one method blank or one instrument blank (Section 12.1.3) must be analyzed as part of each analytical sequence that includes samples from an SDG.

12.1.2.3 Procedure for Method Blank

A method blank for aqueous/water samples consists of 1 L of reagent water spiked with 1 mL of the Labeled Compound Spiking Solution (Section 7.8.2.1). For soil/sediment samples, a method blank consists of 10 g of sand spiked with 1 mL of the Labeled Compound Spiking Solution (Section 7.8.2.1). For tissue samples, a method blank consists of 1.0 g of corn oil spiked with 1 mL of the Labeled Compound Spiking Solution (Section 7.8.2.1). The Cleanup Standard is added to all method blanks prior to cleanup.

12.1.2.4 Calculations for Method Blank

Perform data analysis and calculations according to Section 11.0.

12.1.2.5 Technical Acceptance Criteria for Method Blank

12.1.2.5.1 All blanks must be extracted and analyzed at the frequency described in Section 12.1.2.2 on an HRGC/HRMS system meeting all the technical acceptance criteria in Section 11.0.

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

12.1.2.5.3 For all CDDs/CDFs except OCDD/OCDF, the concentration of the target analyte in the method blank must be less than 1/2 the CRQL. The concentration of OCDD/OCDF in the method blank must be less than three times the CRQL, if detected.

12.1.2.5.4 All method blanks must be analyzed undiluted.

12.1.2.6 Corrective Action for Method Blank

12.1.2.6.1 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 (except for OCDD/OCDF) and associated with a contaminated blank must be re-extracted and reanalyzed at no additional cost to the EPA.

12.1.2.6.2 If contamination is the problem, then 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 solvents, reagents, glassware, and sample storage and processing hardware that lead to discrete artifacts and/or elevated baselines in the HRGC/HRMS system are eliminated. All samples associated with a contaminated blank must be re-extracted and reanalyzed at no additional cost to the EPA.

12.1.2.6.3 If the method blank fails to meet the technical acceptance criteria due to instrument problem, correct the instrument problem, recalibrate the instrument (if necessary), and reanalyze the method blank.

12.1.3 Instrument Blank

12.1.3.1 Summary of Instrument Blank

An instrument blank is a volume of clean solvent spiked with labeled compounds and analyzed on the instrument used for sample analysis when there is not enough method blank volume available for analysis. The purpose of the instrument blank is to determine the levels of contamination associated with the instrumental analysis, particularly with regard to carryover of analytes from standards or highly contaminated samples into other analyses.

12.1.3.2 Frequency of Instrument Blank

At least one instrument blank shall be analyzed as part of each analytical sequence that includes samples from an SDG and when there is no method blank analysis in the analytical sequence. The injection of the instrument blank shall be performed after the continuing calibration verification to initiate a new analytical sequence.

12.1.3.3 Procedure for Instrument Blank

12.1.3.3.1 Prepare the instrument blank by spiking the Labeled Compound Spiking Solution (Section 7.8.2.1) into nonane for a final concentration of 100 ng/mL.

12.1.3.3.2 Analyze the instrument blank according to the frequency listed in Section 12.1.3.2.

12.1.3.4 Calculations for Instrument Blank

Assuming that the material in the instrument blank resulted from the extraction of a 1 L water sample, calculate the concentration of each analyte using the equations in Section 11.2. Compare the results to the CRQL values for water samples in Exhibit C - Chlorinated Dibenzo-*p*-Dioxins and Chlorinated Dibenzofurans and Chlorinated Biphenyl Congeners Target Analyte List and Contract Required Quantitation Limits, Table 1.

12.1.3.5 Technical Acceptance Criteria for Instrument Blank

12.1.3.5.1 All blanks must be analyzed at the frequency described in Section 12.1.3.2 on an HRGC/HRMS system meeting all the technical acceptance criteria in Section 11.0.

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

12.1.3.5.3 For all CDDs/CDFs, the concentration of any target analyte in the instrument blank must be less than 1/2 the CRQL.

12.1.3.5.4 All instrument blanks must be analyzed undiluted.

12.1.3.6 Corrective Action for Instrument Blank

12.1.3.6.1 If an instrument blank does not meet the technical acceptance criteria for blank analysis, the Contractor shall consider the analytical system to be out of control.

12.1.3.6.2 If contamination is the problem, then the source of the contamination must be investigated and appropriate corrective measures taken and documented before further sample analysis proceeds. All samples associated with a contaminated instrument blank must be reanalyzed at no additional cost to the EPA.

12.1.3.6.3 If the instrument blank fails to meet the technical acceptance criteria due to instrument problem, correct the instrument problem, recalibrate the instrument (if necessary), and reanalyze the instrument blank. An acceptable instrument blank must be analyzed before additional data are collected.

12.2 Laboratory Control Sample and Laboratory Control Sample Duplicate

12.2.1 Summary of Laboratory Control Sample and Laboratory Control Sample Duplicate

The LCS/LCSD is a volume or weight of clean reference matrix that is spiked and carried through the entire analytical procedure. To evaluate the accuracy and precision of the method used for CDD/CDF analyses, a mixture of CDD/CDF target analytes is spiked into an aliquot of reference matrix and analyzed in accordance with the appropriate method.

12.2.2 Frequency of Laboratory Control Sample and Laboratory Control Sample Duplicate

The LCS/LCSD shall be extracted with each SDG. The number of samples extracted with each LCS/LCSD should not exceed 20 field samples (excluding PE samples). The LCS/LCSD must be extracted and analyzed concurrently with the samples in the SDG using the same extraction protocol, cleanup procedure, and instrumentation as the samples in the SDG.

12.2.3 Procedure for Laboratory Control Sample and Laboratory Control Sample Duplicate

For water samples, prepare 1 L aliquots of reagent water. For soil/sediment/other samples, prepare aliquots or weigh the appropriate reference matrix (Section 7.6). For tissue samples, use 1 g of corn oil. All LCS/LCSD are spiked with 1 mL of the LCS/LCSD Spiking Solution (Section 7.8.2.2), the Labeled Compound Spiking Solution (Section 7.8.2.1), and the Cleanup Standard (Section 7.8.2.3) according to the procedures in Section 10.0.

12.2.4 Calculations for Laboratory Control Sample and Laboratory Control Sample Duplicate

12.2.4.1 Calculate the concentration of each analyte according to Section 11.0.

12.2.4.2 Compute the %R and RPD of the LCS/LCSD analytes using Equations 27 and 28 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

12.2.5 Technical Acceptance Criteria for Laboratory Control Sample and Laboratory Control Sample Duplicate

12.2.5.1 All LCS/LCSD must be prepared and analyzed at the frequency described in Section 12.2.2.

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

12.2.5.3 The %R of each of the spiked analytes in the LCS/LCSD must be within the acceptance limits in Exhibit D – CCD/CDF, Table 5.

12.2.5.4 The RPD between LCS/LCSD for the target analytes should be within 30%. This limit is only advisory; no further action by the Contractor is required.

12.2.6 Corrective Action for Laboratory Control Sample and Laboratory Control Sample Duplicate

12.2.7 If both LCS and LCSD do not meet the technical acceptance criteria for LCS %R, the Contractor must consider the analytical system to be out of control. All samples associated with a non-compliant LCS/LCSD must be re-extracted and reanalyzed at no additional cost to the EPA.

12.3 Method Detection Limit Determination

12.3.1 Before any field samples are analyzed under the contract, the MDL for each CDD/CDF target analyte shall be determined on each instrument under the same conditions used for analysis (i.e., analytical system configuration) as well as type and dimensions of GC column, prior to the start of contract analyses and verified annually thereafter. MDL determination is matrix-specific (i.e., the MDL shall be determined for aqueous/water, soil/sediment, and tissue samples). The MDL determined for soil/sediment shall be used for sludge, ash, and oil samples. An MDL study shall be performed after major instrument maintenance, or changes in instrumentation or instrumental conditions, to verify the current sensitivity of the analysis. Major instrument maintenance includes, but is not limited to repair or replacement of any mass spectrometer components. A new MDL study will not be required after changing the GC column, as long as the replacement has the same length, inner diameter, and stationary phase.

12.3.2 To determine the MDLs, the Contractor shall perform MDL studies following the procedures specified in Title 40 of the Code of Federal Regulations (CFR), Part 136, Appendix B, Revision 2.

12.3.3 The determined concentration of the MDL must be less than 1/2 the CRQL listed in Exhibit C – Chlorinated Dibenzo-*p*-Dioxins and Chlorinated Dibenzofurans and Chlorinated Biphenyl Congeners Target Analyte List and Contract Required Quantitation Limits, Table 1.

12.3.4 The delivery requirements for the MDL values are specified in Exhibit B – Reporting and Deliverables Requirements, Table 1.

13.0 METHOD PERFORMANCE

Not applicable.

14.0 POLLUTION PREVENTION

Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operations. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. When feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the EPA recommends recycling as the next best option.

15.0 WASTE MANAGEMENT

15.1 The EPA requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The EPA urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with applicable environmental rules 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 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.

16.0 REFERENCES

16.1 U.S. Environmental Protection Agency, Tetra-Through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, Method 1613, Revision B, October 1994.

16.2 U.S. Environmental Protection Agency, Polychlorinated Dibenzo-*p*-Dioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) by High-Resolution Gas Chromatography/High-Resolution Mass Spectrometry (HRGC/HRMS), Method 8290A, Revision 1, February 2007.

16.3 U.S. Government Printing Office, Title 40 of the Code of Federal Regulations, Chapter 1, Subchapter D, Part 136, Appendix B Definition and Procedure for the Determination of the Method Detection Limit, Revision 2.

17.0 TABLES/DIAGRAMS/FLOWCHARTS

TABLE 1. ANALYTE NAMES AND CHEMICAL ABSTRACTS SERVICE (CAS) REGISTRY NUMBERS FOR CHLORINATED DIBENZO-*p*-DIOXINS/CHLORINATED DIBENZOFURANS

Analyte Name[1]	CAS Registry Number	Labeled Compound	CAS Registry Number
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)[2]	76523-40-5 85508-50-5 114423-99-3
2,3,7,8-TCDF	51207-31-9	$^{13}C_{12}$-2,3,7,8-TCDF $^{13}C_{12}$-1,2,3,4-TCDF (IS)[2]	89059-46-1 89059-36-9
1,2,3,7,8-PeCDD	40321-76-4	$^{13}C_{12}$-1,2,3,7,8-PeCDD	109719-79-1
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
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)[2]	109719-82-6
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
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
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
OCDD	3268-87-9	$^{13}C_{12}$-OCDD	114423-97-1
OCDF	39001-02-0	$^{13}C_{12}$-OCDF*	—109719-78-0
Total TCDD	41903-57-5	—	—
Total TCDF	55722-27-5	—	—
Total PeCDD	36088-22-9	—	—
Total PeCDF	30402-15-4	—	—
Total HxCDD	34465-46-8	—	—
Total HxCDF	55684-94-1	—	—
Total HpCDD	37871-00-4	—	—
Total HpCDF	38998-75-3	—	—

[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

[2]IS = Internal Standard.

*Optional Labeled Compound.

TABLE 2. RETENTION TIME REFERENCES, QUANTITATION REFERENCES, AND RELATIVE RETENTION TIMES FOR CHLORINATED DIBENZO-*p*-DIOXINS/ CHLORINATED DIBENZOFURANS

Analyte Name	RT and Quantitation Reference	RRT
Compounds using $^{13}C_{12}$-1,2,3,4-TCDD as the internal standard		
2,3,7,8-TCDF	$^{13}C_{12}$-2,3,7,8-TCDF	0.999-1.003
2,3,7,8-TCDD	$^{13}C_{12}$-2,3,7,8-TCDD	0.999-1.002
1,2,3,7,8-PeCDF	$^{13}C_{12}$-1,2,3,7,8-PeCDF	0.999-1.002
2,3,4,7,8-PeCDF	$^{13}C_{12}$-2,3,4,7,8-PeCDF	0.999-1.002
1,2,3,7,8-PeCDD	$^{13}C_{12}$-1,2,3,7,8-PeCDD	0.999-1.002
$^{13}C_{12}$-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 $^{13}C_{12}$-1,2,3,4-TCDF as the internal standard*		
2,3,7,8-TCDF	$^{13}C_{12}$-2,3,7,8-TCDF	0.999-1.003
1,2,3,7,8-PeCDF	$^{13}C_{12}$-1,2,3,7,8-PeCDF	0.999-1.002
2,3,4,7,8-PeCDF	$^{13}C_{12}$-2,3,4,7,8-PeCDF	0.999-1.002
$^{13}C_{12}$-2,3,7,8-TCDF	$^{13}C_{12}$-2,3,7,8-TCDF	0.923-1.103
$^{13}C_{12}$-1,2,3,7,8-PeCDF	$^{13}C_{12}$-2,3,7,8-TCDF	1.000-1.425
$^{13}C_{12}$-2,3,4,7,8-PeCDF	$^{13}C_{12}$-2,3,7,8-TCDF	1.011-1.526
Compounds using $^{13}C_{12}$-1,2,3,7,8,9-HxCDD as the internal standard		
1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	0.999-1.001
1,2,3,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	0.997–1.005
1,2,3,7,8,9-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	0.999–1.001
2,3,4,6,7,8-HxCDF	$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	0.999–1.001
1,2,3,4,7,8-HxCDD	$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	0.999–1.001
1,2,3,6,7,8-HxCDD	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	0.998–1.004
1,2,3,7,8,9-HxCDD[1]		1.000–1.019
1,2,3,4,6,7,8-HpCDF	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	0.999–1.001
1,2,3,4,7,8,9-HpCDF	$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	0.999–1.001
1,2,3,4,6,7,8-HpCDD	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	0.999–1.001
OCDF	$^{13}C_{12}$-OCDD (or $^{13}C_{12}$-OCDF*)	0.999–1.008
OCDD	$^{13}C_{12}$-OCDD	0.999–1.001
$^{13}C_{12}$-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

TABLE 2. RETENTION TIME REFERENCES, QUANTITATION REFERENCES, AND RELATIVE RETENTION TIMES FOR CHLORINATED DIBENZO-*p*-DIOXINS/ CHLORINATED DIBENZOFURANS (CON'T)

Analyte Name	RT and Quantitation Reference	RRT
$^{13}C_{12}$-OCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	1.032–1.311
$^{13}C_{12}$-OCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	1.000–1.311

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

*Optional Internal Standard that can be used provided that the column performance criteria are still met.

TABLE 3. DESCRIPTORS, m/z INFORMATION, AND SUBSTANCES

Descriptor	Exact m/z[1]	m/z Type	m/z Formula	Substance[2]
1	292.9825	Lock	$C_7 F_{11}$	PFK
	303.9016	M	$C_{12} H_4 {}^{35}Cl_4 O$	TCDF
	305.8987	M+2	$C_{12} H_4 {}^{35}Cl_3{}^{37}Cl O$	TCDF
	315.9419	M	$^{13}C_{12} H_4 {}^{35}Cl_4 O$	TCDF[3]
	317.9389	M+2	$^{13}C_{12} H_4 {}^{35}Cl_3 {}^{37}Cl O$	TCDF[3]
	319.8965	M	$C_{12} H_4 {}^{35}Cl_4 O_2$	TCDD
	321.8936	M+2	$C_{12} H_4 {}^{35}Cl_3 {}^{37}Cl O_2$	TCDD
	327.8847	M	$C_{12} H_4 {}^{37}Cl_4 O_2$	TCDD[4]
	330.9792	QC	$C_7 F_{13}$	PFK
	331.9368	M	$^{13}C_{12} H_4 {}^{35}Cl_4 O_2$	TCDD[3]
	333.9339	M+2	$^{13}C_{12} H_4 {}^{35}Cl_3 {}^{37}Cl O_2$	TCDD[3]
	375.8364	M+2	$C_{12} H_4 {}^{35}Cl_5 {}^{37}Cl O$	HxCDPE
2	339.8597	M+2	$C_{12} H_3 {}^{35}Cl_4 {}^{37}Cl O$	PeCDF
	341.8567	M+4	$C_{12} H_3 {}^{35}Cl_3 {}^{37}Cl_2 O$	PeCDF
	351.9000	M+2	$^{13}C_{12} H_3 {}^{35}Cl_4 {}^{37}Cl O$	PeCDF
	353.8970	M+4	$^{13}C_{12} H_3 {}^{35}Cl_3 {}^{37}Cl_2 O$	PeCDF[3]
	354.9792	Lock	$C_9 F_{13}$	PFK
	355.8546	M+2	$C_{12} H_3 {}^{35}Cl_4 {}^{37}Cl O_2$	PeCDD
	357.8516	M+4	$C_{12} H_3 {}^{35}Cl_3 {}^{37}Cl_2 O_2$	PeCDD
	367.8949	M+2	$^{13}C_{12} H_3 {}^{35}Cl_4 {}^{37}Cl O_2$	PeCDD[3]
	369.8919	M+4	$^{13}C_{12} H_3 {}^{35}Cl_3 {}^{37}Cl_2 O_2$	PeCDD[3]
	409.7974	M+2	$C_{12} H_3 {}^{35}Cl_6 {}^{37}Cl O$	HpCDPE
3	373.8208	M+2	$C_{12} H_2 {}^{35}Cl_5 {}^{37}Cl O$	HxCDF
	375.8178	M+4	$C_{12} H_2 {}^{35}Cl_4 {}^{37}Cl_2 O$	HxCDF
	383.8639	M	$^{13}C_{12} H_2 {}^{35}Cl_6 O$	HxCDF[3]
	385.8610	M+2	$^{13}C_{12} H_2 {}^{35}Cl_5 {}^{37}Cl O$	HxCDF[3]
	389.8157	M+2	$C_{12} H_2 {}^{35}Cl_5 {}^{37}Cl O_2$	HxCDD
	391.8127	M+4	$C_{12} H_2 {}^{35}Cl_4 {}^{37}Cl_2 O_2$	HxCDD
	392.9760	Lock	$C_9 F_{15}$	PFK
	401.8559	M+2	$^{13}C_{12} H_2 {}^{35}Cl_5 {}^{37}Cl O_2$	HxCDD[3]
	403.8529	M+4	$^{13}C_{12} H_2 {}^{35}Cl_4 {}^{37}Cl_2 O_2$	HxCDD[3]
	430.9729	QC	$C_9 F_{17}$	PFK
	445.7555	M+4	$C_{12} H_2 {}^{35}Cl_6 {}^{37}Cl_2 O$	OCDPE
4	407.7818	M+2	$C_{12} H {}^{35}Cl_6 {}^{37}Cl O$	HpCDF
	409.7789	M+4	$C_{12} H {}^{35}Cl_5 {}^{37}Cl_2 O$	HpCDF
	417.8253	M	$^{13}C_{12} H {}^{35}Cl_7 O$	HpCDF[3]
	419.8220	M+2	$^{13}C_{12} H {}^{35}Cl_6 {}^{37}Cl O$	HpCDF[3]
	423.7766	M+2	$C_{12} H {}^{35}Cl_6 {}^{37}Cl O_2$	HpCDD
	425.7737	M+4	$C_{12} H {}^{35}Cl_5 {}^{37}Cl_2 O_2$	HpCDD
	430.9729	Lock	$C_9 F_{17}$	PFK
	435.8169	M+2	$^{13}C_{12} H {}^{35}Cl_6 {}^{37}Cl O_2$	HpCDD[3]
	437.8140	M+4	$^{13}C_{12} H {}^{35}Cl_5 {}^{37}Cl_2 O_2$	HpCDD[3]

TABLE 3. DESCRIPTORS, m/z INFORMATION, AND SUBSTANCES (CON'T)

Descriptor	Exact m/z[1]	m/z Type	m/z Formula	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 ^{13}C = 13.003355 F = 18.9984
O = 15.994915 ^{35}Cl = 34.968853 ^{37}Cl = 36.965903

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

[3]Labeled compound.

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

TABLE 4. CONCENTRATIONS OF NATIVE AND LABELED CHLORINATED DIBENZO-*p*-DIOXINS/CHLORINATED DIBENZOFURANS IN STOCK AND SPIKING SOLUTIONS

Analyte Name	**Stock Solution[1] (ng/mL)**	**Spiking Solution[2] (ng/mL)**
2,3,7,8-TCDD	40	0.20
2,3,7,8-TCDF	40	0.20
1,2,3,7,8-PeCDD	200	1.0
1,2,3,7,8-PeCDF	200	1.0
2,3,4,7,8-PeCDF	200	1.0
1,2,3,4,7,8-HxCDD	200	1.0
1,2,3,6,7,8-HxCDD	200	1.0
1,2,3,7,8,9-HxCDD	200	1.0
1,2,3,4,7,8-HxCDF	200	1.0
1,2,3,6,7,8-HxCDF	200	1.0
1,2,3,7,8,9-HxCDF	200	1.0
2,3,4,6,7,8-HxCDF	200	1.0
1,2,3,4,6,7,8-HpCDD	200	1.0
1,2,3,4,6,7,8-HpCDF	200	1.0
1,2,3,4,7,8,9-HpCDF	200	1.0
OCDD	400	2.0
OCDF	400	2.0
$^{13}C_{12}$-2,3,7,8-TCDD	100	2.0
$^{13}C_{12}$-2,3,7,8-TCDF	100	2.0
$^{13}C_{12}$-1,2,3,7,8-PeCDD	100	2.0
$^{13}C_{12}$-1,2,3,7,8-PeCDF	100	2.0
$^{13}C_{12}$-2,3,4,7,8-PeCDF	100	2.0
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	100	2.0
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	100	2.0
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	100	2.0
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	100	2.0
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	100	2.0
$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	100	2.0
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	100	2.0
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	100	2.0
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	100	2.0
$^{13}C_{12}$-OCDD	200	4.0
$^{13}C_{12}$-OCDF*	200	4.0
Cleanup Standard[3]		
$^{37}Cl_4$-2,3,7,8-TCDD	0.80	

TABLE 4. CONCENTRATIONS OF NATIVE AND LABELED CHLORINATED DIBENZO-*p*-DIOXINS/CHLORINATED DIBENZOFURANS IN STOCK AND SPIKING SOLUTIONS (CON'T)

Analyte Name	Stock Solution[1] (ng/mL)	Spiking Solution[2] (ng/mL)
Internal Standards[4]		
$^{13}C_{12}$-1,2,3,4-TCDD	200	
$^{13}C_{12}$-1,2,3,4-TCDF*	200	
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	200	

[1]See Section 7.8.1.3, prepared in nonane and diluted to prepare spiking solution.

[2]See Section 7.8.2.1, prepared in acetone from stock solution daily.

[3]See Section 7.8.2.3, prepared in hexane and added to extract prior to cleanup.

[4]See Section 7.8.2.4, prepared in nonane and added to the concentrated extract immediately prior to injection into the GC (Section 10.6.2.1).

*Optional Labeled Compound and Internal Standard.

TABLE 5. QUALITY CONTROL ACCEPTANCE CRITERIA FOR CHLORINATED DIBENZO-*p*-DIOXINS AND CHLORINATED DIBENZOFURANS IN LABORATORY CONTROL SAMPLE/LABORATORY CONTROL SAMPLE DUPLICATE AND SAMPLES

CDD/CDF	Test Conc (ng/mL)	LCS/LCSD %R	Labeled Compound %R in Sample
2,3,7,8-TCDD	10	67-158	N/A
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	
Labeled Compound			
$^{13}C_{12}$-2,3,7,8-TCDD	100	20-175	25-164
$^{13}C_{12}$-2,3,7,8-TCDF	100	22-152	24-169
$^{13}C_{12}$-1,2,3,7,8-PeCDD	100	21-227	25-181
$^{13}C_{12}$-1,2,3,7,8-PeCDF	100	21-192	24-185
$^{13}C_{12}$-2,3,4,7,8-PeCDF	100	13-328	21-178
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	100	21-193	32-141
$^{13}C_{12}$-1,2,3,6,7,8,-HxCDD	100	25-163	28-130
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	100	19-202	26-152
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	100	21-159	26-123
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	100	17-205	29-147
$^{13}C_{12}$-2,3,4,6,7,8,-HxCDF	100	22-176	28-136
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	100	26-166	23-140
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	100	21-158	28-143
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	100	20-186	26-138
$^{13}C_{12}$-OCDD	200	13-198	17-157
$^{13}C_{12}$-OCDF*	200	17-160	17-160
Cleanup Standard			
$^{37}Cl_4$-2,3,7,8-TCDD	10	31-191	35-197

*Optional Labeled Compound.

TABLE 6. CONCENTRATIONS OF CHLORINATED DIBENZO-p-DIOXINS/CHLORINATED DIBENZOFURANS IN CALIBRATION AND VERIFICATION SOLUTIONS

Analyte Name	Solution Concentration (ng/mL)				
	CS1	CS2	CS3[1]	CS4	CS5
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
$^{13}C_{12}$-OCDF*	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
$^{13}C_{12}$-1,2,3,4-TCDF*	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	100	100	100	100	100

[1]See Section 7.8.3, continuing calibration verification solution.

*Optional Labeled Compound and Internal Standard.

TABLE 7. GAS CHROMATOGRAPHY RETENTION TIME WINDOW DEFINING MIXTURE AND ISOMER SPECIFICITY CHECK STANDARD

Analyte Name	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 8. GAS CHROMATOGRAPH ANALYTICAL CONDITIONS

Injector Temperature	270°C
Interface Temperature	290°C
Initial Temperature	200°C
Initial Time	2 min.
Temperature Program	200-220°C at 5°C/min. 220°C for 16 min. 220-235°C at 5°C/min 235°C at 7 min. 235-330°C at 5°C/min.
Final Time	1 min.

TABLE 9. THEORETICAL ION ABUNDANCE RATIOS AND QUALITY CONTROL LIMITS

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

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

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

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

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

TABLE 10. SUGGESTED SAMPLE QUANTITIES TO BE EXTRACTED FOR VARIOUS MATRICES[1]

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

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

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

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

TABLE 11. TOXIC EQUIVALENCY FACTORS

Analyte Name	TEF		
	Mammal	Fish	Bird
2,3,7,8-TCDD	1	1	1
2,3,7,8-TCDF	0.1	0.05	1
1,2,3,7,8-PeCDF	0.03	0.05	0.1
1,2,3,7,8-PeCDD	1	1	1
2,3,4,7,8-PeCDF	0.3	0.5	1
1,2,3,4,7,8-HxCDF	0.1	0.1	0.1
1,2,3,6,7,8-HxCDF	0.1	0.1	0.1
1,2,3,4,7,8-HxCDD	0.1	0.5	0.05
1,2,3,6,7,8-HxCDD	0.1	0.01	0.01
1,2,3,7,8,9-HxCDD	0.1	0.01	0.1
2,3,4,6,7,8-HxCDF	0.1	0.1	0.1
1,2,3,7,8,9-HxCDF	0.1	0.1	0.1
1,2,3,4,6,7,8-HpCDF	0.01	0.01	0.01
1,2,3,4,6,7,8-HpCDD	0.01	0.001	0.001
1,2,3,4,7,8,9-HpCDF	0.01	0.01	0.01
OCDD	0.0003	0.0001	0.0001
OCDF	0.0003	0.0001	0.0001
Source	WHO* 2005	WHO* 1998	

*World Health Organization

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EXHIBIT D

CHLORINATED BIPHENYL CONGENERS ANALYSIS

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Exhibit D – Chlorinated Biphenyl Congeners

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Exhibit D – Chlorinated Biphenyl Congeners

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1.0 SCOPE AND APPLICATION

1.1 Method

The analytical method that follows is designed to analyze aqueous/water, soil/sediment, sludge, tissue (non-human), biosolids, ash, oil, and oily matrices by High Resolution Gas Chromatography/High Resolution Mass Spectrometry (HRGC/HRMS) to determine the presence and concentration of Chlorinated Biphenyl Congeners (CBCs) contained in the Target Analyte List (TAL) for CBCs in Exhibit C – Chlorinated Dibenzo-*p*-Dioxins and Chlorinated Dibenzofurans and Chlorinated Biphenyl Congeners Target Analyte List and Contract Required Quantitation Limits. The method is based on U.S. Environmental Protection Agency (EPA) Method 1668, Revision C (April 2010).

1.1.1 This method allows for the determination of the Polychlorinated Biphenyl (PCB) Toxic Equivalents (TEQs) for mammal tissue using Toxic Equivalency Factors (TEFs). A second column analysis is an option for resolution of the two toxic CB Congeners (PCB-156 and PCB-157) that are not resolved in the primary column.

1.1.2 This method also allows for the estimation of homologue totals by level of chlorination (LOC) and estimation of total CBCs in a sample by summation of the concentrations of the CBCs.

1.1.3 The CBCs that can be determined by this method are the 12 PCBs designated as toxic by the World Health Organization (WHO), plus the remaining 197 CBCs, approximately 125 of which are resolved adequately on a SPB-Octyl Gas Chromatographic (GC) column to be determined as individual congeners. The approximately 70 remaining congeners are determined as mixtures of isomers (coelutions).

1.1.4 The list of 209 congener target analytes is given in Exhibit D – CBC, Table 1.

1.2 Quantitation Levels

The levels listed in Exhibit C - Chlorinated Dibenzo-*p*-Dioxins and Chlorinated Dibenzofurans and Chlorinated Biphenyl Congeners Target Analyte List and Contract Required Quantitation Limits, Table 3, are the Contract Required Quantitation Limits (CRQLs). These limits are set based on the low calibration standard (CS1) analyzed for each WHO Toxic Congener and LOC.

The ability to achieve the CRQLs of this Statement of Work (SOW) is dependent on the level of interferences and laboratory background levels rather than instrumental limitations. Care shall be exercised to eliminate these background contaminants and interferences from the laboratory.

1.3 Qualitative Identification

The qualitative identification criteria (Section 11.1) include requirements for Retention Times (RTs) and limits on the ratio of the abundance of two exact m/z signals produced by each compound. In the instance where, for a WHO Toxic Congener, 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.

2.0 SUMMARY OF METHOD

2.1 Extraction

2.1.1 Aqueous/Water Samples [samples containing less than one percent solids (%Solids)]

A mixture of ^{13}C-labeled WHO Toxic Congeners and LOC definition CBCs is spiked into a 1 liter (L) sample. The sample is extracted by one of the following two procedures:

2.1.1.1 Samples containing no visible particulates are extracted with methylene chloride in a separatory funnel, by continuous liquid/liquid extraction or the Solid Phase Extraction (SPE) technique. The extract is concentrated for cleanup.

2.1.1.2 Samples containing greater than or equal to 1% solids are vacuum filtered through a glass fiber filter. The filter containing the particulates is extracted as a soil sample and the filtrate is extracted as a water sample. The extract from the aqueous phase is concentrated and combined with the filter extract prior to cleanup.

2.1.2 Soil/Sediment, Multiphase, and Other Solid Samples

A mixture of ^{13}C-labeled WHO Toxic Congeners and LOC definition CBCs is spiked into a sample containing 10 grams (g) (dry weight) of solids and the sample is either extracted in an Soxhlet/Dean-Stark (SDS) extractor or mixed with sodium sulfate to a free-flowing consistency, allowed to equilibrate, and is extracted using the Soxhlet technique. The extract is concentrated for cleanup. Samples containing coarse solids are ground or homogenized prior to extraction.

2.1.3 Tissue (Non-Human) Samples

The sample is extracted by the following procedure:

A 20 g aliquot of frozen sample is homogenized and a 10 g aliquot is spiked with the labeled compounds of WHO Toxic Congeners and LOC. The sample is mixed with anhydrous sodium sulfate, allowed to dry for 12-24 hours or overnight, and extracted for 18-24 hours using methylene chloride in a Soxhlet extractor. The extract is evaporated to near dryness, and the lipid content is determined gravimetrically.

2.2 Cleanup

After extraction, the Cleanup Standard is added to each extract to measure the efficiency of the cleanup procedure. Sample cleanups may include back-extraction with acid and/or base, Gel Permeation Chromatography (GPC), silica gel, and Florisil. Activated carbon columns and High Performance Liquid Chromatography (HPLC) can be used for further isolation of specific isomers or congeners. Tissue extracts are cleaned up using an anthropogenic isolation column prior to the cleanup procedures listed above.

2.3 Analysis

2.3.1 After cleanup, the extract is concentrated to 10 microliters (µL). Immediately before injection, the labeled internal standards are added to each extract. The final extract volume, including the 10 µL of internal standard, is 20 µL.

2.3.2 An aliquot of the extract is injected into the HRGC instrument. The analytes are separated by the HRGC system and detected by a High Resolution (≥10,000) Mass Spectrometer (HRMS). Two exact m/z signals are monitored for each analyte throughout a predetermined RT window.

2.3.3 An individual congener is identified by comparing the HRGC RT and ion abundance ratio of two exact m/z with the corresponding RT of an authentic standard and the theoretical or acquired ion abundance ratio of the two exact m/z. Column performance in the HRGC system is monitored to ensure specificity in the identification of the WHO Toxic Congeners.

2.4 Quantitation

Quantitative analysis is performed using Selected Ion Current Profile (SICP) areas in one of the following ways:

2.4.1 For the 12 WHO Toxic Congeners and the LOC, the HRGC/HRMS system is calibrated using multiple initial calibration points and the concentration is determined using the isotope dilution technique.

2.4.2 For all congeners other than the WHO Toxic Congeners and LOC, the HRGC/HRMS system is calibrated at a single concentration and the concentrations are estimated using the internal standard quantitation technique.

2.4.3 For the labeled WHO Toxic Congeners, labeled LOC, and Cleanup Standard, the Gas Chromatograph/Mass Spectrometer (GC/MS) system is calibrated using replicates at a single concentration and the concentrations of these labeled compounds in samples are determined using the internal standard technique. The internal standards are labeled compounds spiked into the extract immediately prior to injection of an aliquot of the extract into the HRGC/HRMS system.

3.0 DEFINITIONS

See Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations for a complete list of definitions.

4.0 INTERFERENCES

4.1 Sources of Contamination

Solvents, reagents, glassware, and other sample processing hardware may yield artifacts, elevated baselines, and/or lock-mass suppression 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 after use. Sonication of glassware containing a detergent solution for approximately 30 seconds may aid in cleaning. Glassware with removable parts, particularly separatory funnels with polytetrafluoroethylene (PTFE) stopcocks, must be disassembled before detergent washing.

4.2.2 After detergent washing, glassware should be rinsed immediately, first with methanol, then with hot tap water. Another methanol rinse, then an acetone rinse, and then a methylene chloride rinse follow the tap water rinse.

4.2.3 Baking of glassware in a kiln or other high temperature furnace (450-500°C) may be warranted after particularly dirty samples are encountered. The kiln or furnace should be vented to an exhaust duct to prevent laboratory contamination by CBC vapors. Baking should be minimized, as repeated baking of glassware may cause active sites on the glass surface that may irreversibly adsorb CBCs. Volumetric glassware and separatory funnels with ground glass sealing surface should not be baked at high temperatures that may cause the deformation of the volumetric glassware or the sealing surface of separatory funnels.

4.2.4 Immediately prior to use, the Soxhlet and SDS apparatus should be pre-extracted with toluene, and the continuous liquid-liquid extraction apparatus should be pre-extracted with methylene chloride 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.

4.2.5 A separate set of glassware may be necessary to effectively preclude contamination when low-level samples are analyzed.

4.3 Reagents and Materials

All materials used in the analysis shall be demonstrated to be free of interferences by analyzing reference matrix method blanks (Section 12.1.2) initially and with each sample batch (samples started through the extraction process in a given 12-hour period, to a maximum of 20 samples).

4.3.1 The reference matrix must simulate, as closely as possible, the sample matrix being extracted. The reference matrix should not contain the CBCs in detectable amounts. The EPA 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 (Section 7.6.1) can be used to simulate aqueous/water samples; playground sand (Section 7.6.2) or white quartz sand (Section 7.3.2) can be used to simulate soils/sediments and other matrices such as biosolids, sludge, or ash; and corn oil (Section 7.6.3) can be used to simulate tissue samples.

4.4 Interfering Compounds

Interferences coextracted from samples will vary considerably from source to source, depending upon the diversity of the site being sampled. Interfering compounds may be present at concentrations several orders of magnitude higher than the CBC. The most frequently encountered interferences are chlorinated dioxins and dibenzofurans, methoxy biphenyls, hydroxydiphenyl ethers, benzylphenyl ethers, brominated diphenyl ethers, polynuclear aromatics, polychlorinated naphthalenes, polychlorinated diphenylethers, and pesticides. Because very low levels of CBC are measured by this method, the elimination of interferences is essential. The cleanup steps given can be used to reduce or eliminate these interferences and thereby permit reliable determination of the CBC at the levels in Exhibit C - Chlorinated Dibenzo-*p*-Dioxins and Chlorinated Dibenzofurans and Chlorinated Biphenyl Congeners Target Analyte List and Contract Required Quantitation Limits, Table 3.

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 Contractor 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 Contamination of Calibration Solutions

To prevent CBC contamination of the calibration solutions, the calibration solutions must be prepared in an area free of contamination using glassware free of CBC contamination. If these requirements cannot be met or are difficult to meet in the laboratory, the Contractor should have a vendor prepare the calibration standards and ensure a lack of contamination or should prepare the calibration solutions in a contamination-free facility.

4.7 Lipids

The natural lipid content of tissue can interfere in the analysis of tissue samples for the CBCs. The lipid content 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, including anthropogenic isolation column and/or GPC, followed by Florisil and carbon, as minimum additional cleanup steps.

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 compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level.

5.1.1 CBCs have been tentatively classified as known or suspected human or mammalian carcinogens. Based on the available toxicological and physical properties of CBCs, any material known to contain CBCs should be handled only by highly trained personnel thoroughly familiar with their handling including cautionary procedures and the associated risks.

5.1.2 It is recommended that the Contractor purchase dilute standard solutions of the analytes in this method. However, if primary solutions are prepared, they shall be prepared in a hood or glove box.

5.2 Occupational Safety and Health Administration Requirements

The Contractor is responsible for maintaining a current awareness file of Occupational Safety and Health Administration (OSHA) regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDSs) should also be made available to all personnel involved in these analyses.

5.3 Sample Handling

CBCs and samples suspected to contain these compounds are handled using essentially the same techniques employed in handling radioactive or infectious materials. Well-ventilated and 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 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 should not be allowed.

5.3.2 Protective Equipment - Disposable plastic gloves, apron or laboratory coat, safety glasses or mask, and a glove box or fume hood adequate for radioactive work should be used. During analytical operations that may give rise to aerosols or dusts, personnel should wear respirators equipped with activated carbon filters. Eye protection (preferably full-face shields) should 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 CBCs, an additional set of gloves can also be worn beneath the latex gloves. A Tyvek® suit with foot covers and a hood is an option for protective clothing.

5.3.3 Training - Workers must be trained in the proper method of removing contaminated gloves and clothing without contacting the exterior surfaces. Workers should also be trained to recognize the symptoms of chloroacne.

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 from sample splitters on the GC and from roughing pumps on the MS should pass through either a column of activated charcoal or be bubbled through a trap containing oil or high-boiling alcohol to condense CBC vapors. Traps should be replaced a minimum of annually or more frequently as needed 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. All personnel should be advised of the hazard and 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 - 1,1,1-trichloroethane solvent can be effective in removing CBCs. Satisfactory cleaning may be accomplished by rinsing with 1,1,1-trichloroethane, then washing with any detergent and water.

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 perform a wipe test of the surface suspected of being contaminated.

5.4.4.1 Using a piece of filter paper moistened with 1,1,1-trichloroethane or other solvent, wipe an area approximately 10 x 10 centimeters (cm).

5.4.4.2 Extract the wipe as specified in Section 10.2.4 and analyze by this analytical method, or by GC with an Electron Capture Detector (ECD) using, for example, EPA Method 8082A, Revision 1 (February 2007).

5.4.4.3 Using the area wiped [e.g., 10 x 10 cm = 0.01 square meter (m^2)], calculate the concentration in micrograms per square meter ($\mu g/m^2$). A concentration less than 1 $\mu g/m^2$ indicates acceptable cleanliness; anything higher warrants further cleaning. Concentrations more than 100 $\mu g/m^2$ constitute an acute hazard and requires prompt cleaning before further use of the equipment or workspace, and indicate that unacceptable work practices have been employed.

5.4.5 Biosolids samples may contain high concentrations of biohazards, and must be handled with gloves and opened in a hood or biological safety cabinet to prevent exposure. Contractor staff should know and observe the safety procedures required in a microbiology laboratory that handles pathogenic organisms when handling biosolids samples.

5.4.6 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

Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using equipment and supplies other than those specified here; however, a demonstration of equivalent performance meeting the requirements of this SOW is the responsibility of the Contractor. The Contractor shall document any use of alternate equipment or supplies in the Sample Delivery Group (SDG) Narrative.

6.1 Glassware Cleaning

6.1.1 Cleaning solvent – 1,1,1-trichloroethane.

6.1.2 Kiln - Properly vented to an exhaust duct to the outside of the laboratory and capable of reaching 450°C within 2 hours and maintaining 450-500°C within ±10°C, with a temperature controller and safety switch.

6.1.3 Laboratory sink with an overhead fume hood.

6.2 Equipment for Sample Preparation

6.2.1 Aluminum foil.

6.2.2 Balances

6.2.2.1 Analytical - Capable of weighing ±0.1 milligrams (mg).

6.2.2.2 Top loading - Capable of weighing ±10 mg.

6.2.2.3 A balance calibration must be checked with known masses once per each day of use. This verification consists of a check with two weights covering the range expected (approximately ±50% of the expected measured mass) for each type of balance and be accurate to ±0.1 mg and ±10 mg, respectively. The masses that are used to check the balances daily must be checked on a monthly basis using National Institute of Standards and Technology (NIST)-traceable known reference masses (Class ‘0’, Class ‘1’, or Class ‘2’) as defined by ASTM E617-97 (2008), or equivalent (e.g., earlier Class ‘S’ defined masses). All balances must be checked at least once annually by a certified technician. The reference masses used by the Contractor must be recertified at least every five years, or sooner if there is reason to believe damage (corrosion, nicks) has occurred. The Contractor shall maintain documentation that demonstrates these criteria have been met.

6.2.3 Equipment for Determining Percent Solids

6.2.3.1 Oven - Properly vented to an exhaust vent to the outside of the laboratory and capable of maintaining a temperature of 110°C (±5°C).

6.2.3.2 Desiccator - Containing an expended desiccant indicator compound.

6.2.4 Glove box.

6.2.5 Laboratory fume hood of sufficient size to contain the sample preparation equipment.

6.2.6 Meat Grinder - With 3-5 millimeter (mm) holes in inner plate.

6.2.7 Sieve - #18 (1 mm nominal sieve opening).

6.2.8 Tissue Homogenizer - With stainless steel Macro-shaft and Turbo-shear blade.

6.3 Extraction Apparatus

6.3.1 Aqueous/Water Samples

6.3.1.1 Graduated Cylinder Class A TC - 1 L capacity.

6.3.1.2 pH Meter - With a combination glass electrode. Calibrate according to manufacturer’s instructions. The pH meter shall be calibrated prior to each use using reference standards bracketing the range expected in samples. The pH reference standards shall be replaced when their expiration dates have passed.

6.3.1.3 pH Paper - Wide range.

6.3.1.4 Separatory Funnels - 250 milliliters (mL), 500 mL, and 2000 mL, with PTFE stopcocks.

6.3.1.5 Solid Phase Extraction

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 Glass-Fiber Filter - 1 micron nominal pore size, to fit filtration apparatus described in Section 6.3.1.5.1.

6.3.1.5.3 SPE Disk - Containing octadecyl (C_{18}) bonded silica uniformly enmeshed in an inert matrix, to fit filtration apparatus described in Section 6.3.1.5.1.

6.3.1.5.4 Vacuum Source - Capable of maintaining 25 in. Hg, equipped with shutoff valve and vacuum gauge.

6.3.1.6 Continuous Liquid-Liquid Extraction

PTFE or glass connecting joints and stopcocks without lubrication, 1.5-2 L capacity.

6.3.2 Solid Samples [Soil/Sediment, Sludge, Tissue (Non-Human), Biosolids, Ash, Oil, Filters]

6.3.2.1 Heating Mantle - Hemispherical, to fit 500 mL round-bottom flask.

6.3.2.2 SDS Extractor.

6.3.2.2.1 Moisture Trap - Dean-Stark or Barret with PTFE stopcock, to fit Soxhlet.

6.3.2.2.2 Soxhlet - 50 mm ID, 200 mL capacity with 500 mL flask.

6.3.2.2.3 Thimble - 43 mm x 123 mm or 33 mm x 94 mm cellulose thimble to fit Soxhlet.

6.3.2.3 Variable Transformer - Powerstat (or equivalent), 110 volt, 10 ampere (A).

6.3.3 Acid/Base Back-Extraction of Tissue Extracts

6.3.3.1 Bottle for Back-Extraction - 100-200 mL narrow-mouth clear glass with PTFE-lined cap.

6.3.3.2 Mechanical Shaker - Wrist-action or platform-type rotary shaker that produces vigorous agitation.

6.3.3.3 Rack - Attached to shaker table to permit agitation of 4-9 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 using a Soxhlet or SDS Extractor for 3 hours minimum. DO NOT BAKE.

6.4.2 Buchner Funnel - 15 cm.

6.4.2.1 Glass-Fiber Filter Paper - To fit Buchner funnel.

6.4.3 Drying Column - 15-20 mm ID borosilicate chromatographic column equipped with a coarse-glass frit or glass wool plug.

6.4.4 Filtration Flasks - 1.5-2.0 L, with a side arm.

6.4.5 Glass Funnel - 125-250 mL.

6.4.5.1 Glass-Fiber Filter Paper - To fit glass funnel.

6.4.6 Pressure Filtration Apparatus.

6.5 Centrifuge Apparatus

6.5.1 Centrifuge - Capable of rotating 500 mL centrifuge bottles or 15 mL centrifuge tubes at 5,000 revolutions per minute (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

6.6.1.1 Column - 600-700 mm long × 25 mm ID glass, packed with 70 g of 200-400 mesh SX-3 Bio Beads.

6.6.1.2 Syringe - 10 mL, with Luer-Lok fitting.

6.6.1.3 Syringe Filter Holder - Stainless steel, and glass-fiber or PTFE filters.

6.6.1.4 Ultraviolet (UV) Detector - 254 nanometers (nm), preparative or semi-preparative flow cell.

6.6.1.5 Temperature-controlled environment to ensure reproducible performance.

6.6.2 Reverse-Phase High-Performance Liquid Chromatograph

6.6.2.1 Column - Hypercarb, 100 x 4.6 mm, 5 µm particle size, Keystone Scientific, or equivalent.

6.6.2.2 Detector - Operated at 0.02 AUFS at 235 nm.

6.6.2.3 Fraction Collector.

6.6.2.4 Injector – With 50 µL sample loop.

6.6.2.5 Pump.

6.6.2.6 Switching Valve.

6.6.3 Glass Chromatographic Columns

6.6.3.1 150 mm long x 8 mm ID, with coarse-glass frit or glass wool plug and 250 mL reservoir.

6.6.3.2 200 mm long x 15 mm ID, with coarse-glass frit or glass wool plug and 250 mL reservoir.

6.6.3.3 300 mm long x 22 mm ID, with coarse-glass frit or glass wool plug, 300 mL reservoir, and glass or PTFE stopcock.

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

6.6.5 Oven or Tube Furnace - For baking and activating adsorbents, capable of maintaining a constant temperature (±5°C) in the range of 400-700°C.

6.6.6 Pipettes

6.6.6.1 Disposable, Pasteur - 150 mm long x 5 mm ID.

6.6.6.2 Disposable, Serological - 50 mL (8-10 mm ID).

6.6.7 Stirring Apparatus - For batch silica cleanup of tissue extracts.

6.6.7.1 Mechanical Stirrer.

6.6.7.2 Bottle - 500-600 mL, wide-mouth clear glass.

6.7 Concentration Apparatus

6.7.1 Rotary Evaporator - Equipped with a variable temperature water bath.

6.7.1.1 A recirculating water pump and chiller are recommended. 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.

6.7.1.2 Boiling Flask – 125 mL pear-shaped borosilicate glass boiling flask for rotary evaporation of extracts.

6.7.1.3 Round-bottom Flasks - 100 mL and 500 mL or larger, with ground-glass fittings compatible with the rotary evaporator.

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

6.7.2 Kuderna-Danish Apparatus

6.7.2.1 Boiling Chips

6.7.2.1.1 Glass or Silicon Carbide - Approximately 10/40 mesh, solvent-rinsed with methylene chloride and baked at 450°C for 1 hour minimum.

6.7.2.1.2 PTFE (optional) – Solvent-rinsed with methylene chloride.

6.7.2.2 Concentrator Tube - 10 mL, graduated with calibration verified. Ground-glass stopper (size 19/22 joint) is used to prevent evaporation of extracts.

6.7.2.3 Evaporation Flask - 500 mL, attached to concentrator tube with springs or Plastic Keck clips.

6.7.2.4 Snyder Column - Three-ball macro.

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

6.7.3 Nitrogen Evaporation Device - Equipped with water bath controlled in the range of 30-60°C, installed in a fume hood.

6.7.4 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 (LCS) spiking solution, labeled reference compounds solution, and internal standards].

6.7.5 Sample Vials

6.7.5.1 Amber Glass - 2-5 mL with PTFE-lined screw-cap.

6.7.5.2 Glass - 0.3 mL, conical, with PTFE-lined screw or crimp cap.

6.7.6 Volumetric Flasks 10 mL, Class A - For the preparation of stock standard solutions.

6.8 Gas Chromatograph

The GC shall have splitless or on-column injection port for a capillary column, a temperature program with isothermal hold, and shall meet all of the performance specifications listed in Section 9.0.

6.8.1 GC Column - Any GC column or column system (two or more columns) that provide unique resolution and identification of the WHO Toxic Congeners for determination of a PCB TEQ using TEFs. Isomers may be unresolved as long as they have the same TEF and Response Factor (RF) and these unresolved isomers are uniquely resolved from all other congeners. For example, the SPB-Octyl column (Section 6.8.1.1.3) achieves unique GC resolution of all WHO Toxic Congeners except PCB-156 and PCB-157. This isomeric pair is uniquely resolved from all other congeners, and these congeners have the same TEF and RF.

6.8.1.1 If an SPB-Octyl column is used, it must meet the specifications in Section 6.8.1 and the following additional specifications:

6.8.1.1.1 The column must uniquely resolve Congeners 34 from 23 and 187 from 182. Congeners 156 and 157 must coelute within 2 seconds at the peak maximum. Unique resolution means a valley height less than 40% of the shorter of the two peaks that result when the Diluted Combined 209-Congener Standard Solution (Section 7.8.3.3) is analyzed.

6.8.1.1.2 The column must be replaced when any of the criteria in Sections 6.8.1 - 6.8.1.1.1 are not met.

6.8.1.1.3 Suggested Column - 30(±5) m long x 0.25 (±0.02) mm ID; 0.25 µm film SPB-Octyl. This column is capable of meeting the requirements in Sections 6.8.1 - 6.8.1.1.1.

NOTE: The SPB-Octyl column is subject to rapid degradation when exposed to oxygen. The analyst should exclude oxygen from the carrier gas, eliminate air leaks, and cool the injector, column, and transfer line before opening the column to the atmosphere. For further information on precluding oxidation, contact the column manufacturer.

6.8.1.2 An additional column may be used for resolution of additional congeners. A DB-1 column may be used for this purpose. See specifications in Appendix A - Preliminary Information for Determination of 209 CBCs on the DB-1 Column.

6.9 Mass Spectrometer

6.9.1 The GC/MS reference methods require the following conditions: 28- to 40-eV electron impact ionization that shall be capable of selectively monitoring a minimum of 22 exact m/z minimum at high resolution (≥10,000) during a period of less than 1.5 seconds, and meet all of the performance specifications in Section 9.0.

6.9.2 Alternate technologies may be utilized, only after approval in advance by EPA, if the following performance criteria can be met:

- Efficient ionization of all target analytes;
- Repetitive, selective monitoring of the exact target m/z at high resolution (≥10,000 with 10% valley definition, 20,000 at full width-half maximum definition) during a period of approximately one second; and
- Attainment of all of the performance specifications in Section 9.0.

6.10 Gas Chromatograph/Mass Spectrometer 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 Systems/Data Storage

Capable of collecting, recording, storing, and processing MS data.

6.11.1 Data Acquisition - The signal at each exact m/z must be collected repetitively throughout the monitoring period and stored on a mass storage device.

6.11.2 RFs and Multi-point Calibrations - The data system must record and maintain lists of RFs (response ratios for isotope dilution) and multi-point calibrations. Computations of Relative Standard Deviation (RSD) are to be used to test calibration linearity.

7.0 REAGENTS AND STANDARDS

The Contractor must provide all standards to be used with the contract. The Contractor must be able to verify that the standards are certified. Manufacturer's certificates of analysis must be retained by the Contractor and presented upon request.

Reagents shall be dated with the receipt date and used on a first-in, first-out basis. The purity of the reagents shall be verified before use.

7.1 pH Adjustment and Back-Extraction

7.1.1 Hydrochloric Acid, 6N (1:1) - Add 500 mL concentrated HCl, reagent grade to 400 mL reagent water and dilute to 1 L.

7.1.2 Potassium Hydroxide - Dissolve 20 g reagent grade KOH in 100 mL reagent water.

7.1.3 Sodium Chloride Solution - Reagent grade, prepare at 5% (w/v) solution in reagent water.

7.1.4 Sodium Hydroxide, 1N – Dissolve 40 g of NaOH in 500 mL reagent water and dilute to 1 L.

7.1.5 Sodium Thiosulfate.

7.1.6 Sulfuric Acid - Reagent grade (specific gravity 1.84).

7.2 Solution Drying and Evaporation

7.2.1 Sodium sulfate, reagent grade, granular, anhydrous, 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 the 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 Hydromatrix™ - Diatomaceous earth-based material rinsed with methylene chloride and dried at 400°C for 4 hours in a shallow tray, cooled in a desiccator, and stored in a glass bottle.

7.2.3 Pre-purified Nitrogen.

7.3 Extraction

7.3.1 Solvents - Acetone, toluene, hexane, ether, methanol, methylene chloride, iso-octane, nonane, decane, or tetradecane; distilled in glass, pesticide quality, lot-certified to be free of interferences.

NOTE: Some solvents (e.g., ether, iso-octane and nonane) may need to be redistilled to eliminate CBC background contamination.

7.3.2 White quartz sand, 60/70 mesh - For SDS extraction. Bake at 450°C for 4 hours minimum.

7.4 Gel Permeation Chromatography Calibration Solution

Prepare a GPC calibration solution in methylene chloride containing the following analytes at the minimum concentration listed (in elution order). The solution must be prepared every 6 months, or sooner if the solution has degraded or concentrated.

Compound	Concentration (mg/mL)
Corn oil (CAS #8001-30-7)	25.0
Bis(2-ethylhexyl)phthalate (CAS #117-81-7)	0.5
Methoxychlor (CAS #72-43-5)	0.1
Perylene (CAS #198-55-0)	0.02
Sulfur (CAS #7704-34-9)	0.08

NOTE: Sulfur is not very soluble in methylene chloride, but it is soluble in warm corn oil. Therefore, one approach is to weigh out the corn oil, warm it, and transfer the weighed amount of sulfur into the warm corn oil. Mix it and then transfer into a volumetric flask with methylene chloride, along with the other calibration compounds.

7.5 Adsorbents for Sample Cleanup

7.5.1 Silica Gel

7.5.1.1 Acid Silica Gel (30% w/w) - Thoroughly mix 44 g of concentrated sulfuric acid 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 PTFE-lined screw-cap.

7.5.1.2 Activated Silica Gel - 100-200 mesh, 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.

7.5.1.3 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 PTFE-lined screw-cap.

7.5.1.4 Potassium Silicate.

7.5.1.4.1 Dissolve 56 g of high purity potassium hydroxide in 300 mL of methanol in a 750-1000 mL flat-bottom flask.

7.5.1.4.2 Add 100 g of activated silica gel and a stirring bar, and stir on an explosion-proof hot plate at 60-70°C for 1-2 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 2-4 hours in a hood.

7.5.1.4.5 Activate overnight at 200-250°C before use.

7.5.2 Carbon

7.5.2.1 Carbopak C.

7.5.2.2 Celite 545.

7.5.2.3 Thoroughly mix 18 g Carbopak C and 18 g Celite 545 to produce a 50% w/w mixture. Activate the mixture at 130°C for a minimum of 6 hours. Store in a desiccator.

NOTE: The carbon column has been included in this method to allow separation of coplanar Congeners 77, 126, and 169 from other congeners and interferences, should such separation be desired.

7.5.3 Anthropogenic Isolation Column - Pack the column in Section 6.6.3.3 from bottom to top with the following:

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

7.5.4 Florisil Column

7.5.4.1 Florisil - PR grade, activated magnesium silicate, 60-100 mesh. Alternatively, pre-packaged Florisil columns may be used. If necessary, Soxhlet extract in 500 g portions for 24 hours. Use the following procedure for Florisil activation and column packing:

7.5.4.1.1 Fill a clean 1-2 L bottle 1/2 to 2/3 full with Florisil and place in an oven at 130-150°C for a minimum of 3 days to activate the Florisil.

7.5.4.1.2 Immediately before use, dry pack a 300 mm x 22 mm ID glass column (Section 6.6.3.3) bottom to top with 0.5-1.0 cm of warm to hot anhydrous sodium sulfate (Section 7.2.1), 10-10.5 cm of warm to hot activated Florisil (Section 7.5.4.1.1), and 1-2 cm of warm to hot anhydrous sodium sulfate. Allow the column to cool and wet immediately with 100 mL of n-hexane to prevent moisture from entering.

7.5.4.2 Using the procedure in Section 10.5.6.4, establish the elution pattern for each carton of Florisil or each lot of Florisil columns received.

7.6 Reference Matrices

Matrices in which the CBCs and interfering compounds are not detected by this method.

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

7.6.2 Soil/Sediment Reference Matrix - Playground sand or similar material demonstrated to be free of the analytes of interest and potentially interfering substances. 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 demonstrated to be free of the analytes of interest and potentially interfering substances.

7.6.4 Other Matrices - This method may be verified on any reference matrix that is free of CBCs. In no case shall the background level of CBCs exceed 1/2 the CRQLs in Exhibit C - Chlorinated Dibenzo-*p*-Dioxins and Chlorinated Dibenzofurans and Chlorinated Biphenyl Congeners Target Analyte List and Contract Required Quantitation Limits, Table 3.

7.7 Perfluorokerosene (PFK)

Reference compound used for tuning the mass spectrometer. Exhibit D – CBC, Table 2, and Appendix A - Preliminary Information for Determination of 209 CBCs on the DB-1 Column, Table A-2, offers some suggestions for the lock-mass m/z.

7.8 Standard Solutions

Prepare standard solutions from materials of known purity and composition or purchase as solutions or mixtures with certification to their purity, concentration, and authenticity. 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 PTFE-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. All solution standards are to be clearly labeled with information or a unique standard ID identifier which allows for traceability to the identity of the analyte or analytes, concentration, date prepared, solvent, expiration date of the solution, special storage requirements (if any), and initials of the preparer.

7.8.1 Stock Solutions

7.8.1.1 Preparation – Prepare in nonane per the steps below or purchase as dilute solutions. Observe the safety precautions outlined in Section 5.0, paying close attention to the recommendation in Section 5.1.2.

7.8.1.1.1 For preparation of stock solutions from neat materials, dissolve an appropriate amount of assayed reference material in solvent. For example, weigh 10.0-20.0 mg of PCB-126, to two (2) significant figures, in a 10 mL ground-glass stoppered volumetric flask and fill to the mark with nonane. After the compound is completely dissolved, transfer the solution to a clean 15 mL vial with a PTFE-lined cap.

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

7.8.1.2 Native CBC Stock Solutions

7.8.1.2.1 Native WHO Toxic Congeners/LOC Stock Solution — Prepare the native WHO Toxic Congeners and LOC at the concentrations in Exhibit D – CBC, Table 3, or purchase.

7.8.1.2.2 Native 209 Congener Stock Solutions - Solutions containing all 209 congener target analytes to calibrate the SPB-Octyl column.

NOTE: If a column other than the SPB-Octyl column is used, solutions that will allow separation of all 209 congener target analytes on that column must be used. The contents of all five solutions must be documented in a table similar to Exhibit D – CBC, Table 4, and included with the SDG Narrative.

7.8.1.2.2.1 Native 209 Congener Stock Solution for separation of individual congeners on the SPB-Octyl column – Prepare the five solutions with the congeners listed in Exhibit D – CBC, Table 4, at the concentration in Exhibit D – CBC, Table 3, or purchase.

7.8.1.2.2.2 Combined 209 Congener Stock Solution – Combine equal volumes of the standards in Section 7.8.1.2.2.1 to form a stock solution containing all 209 congener target analytes. This solution will be at 1/5 the concentration of the five individual solutions.

7.8.1.3 Labeled Compound Stock Solutions

7.8.1.3.1 Labeled Toxic/LOC/Window-Defining Congeners Stock Solution - Prepare in isooctane or nonane at the concentrations in Exhibit D – CBC, Table 3, or purchase.

7.8.1.3.2 Labeled Cleanup Standard Stock Solution - Prepare labeled PCB-28, PCB-111, and PCB-178 in iso-octane or nonane at the concentrations in Exhibit D – CBC, Table 3, or purchase.

7.8.1.3.3 Labeled Internal Standard Stock Solution - Prepare labeled PCB-9, PCB-52, PCB-101, PCB-138, and PCB-194 in nonane or iso-octane at the concentrations in Exhibit D – CBC, Table 3, or purchase.

7.8.2 Working Standards

7.8.2.1 Labeled Toxic/LOC/Window-Defining Congeners Spiking Solution - This solution is spiked into each sample, blank, and Laboratory Control Sample/Laboratory Control Sample Duplicate (LCS/LCSD) to measure recovery. Prepare a sufficient volume of the labeled compound spiking solution at the concentrations in Exhibit D – CBC, Table 3, with acetone prior to use. Seal with PTFE tape and mark the meniscus of the solution to reduce and monitor evaporation of acetone. Each sample requires 1 mL of the diluted spiking solution, and this solution shall be prepared daily. When 1 mL of this solution is spiked into a sample, blank, and LCS/LCSD, and concentrated to a final extract volume of 20 µL, the concentration in the final extract volume will be as in Table 3.

NOTE: The Contractor shall maintain a standard preparation log documenting the daily preparation of the labeled compound spiking solution.

7.8.2.2 LCS/LCSD Spiking Solution – This solution is spiked into each LCS/LCSD. Dilute the native WHO Toxic Congeners/LOC stock solution (Section 7.8.1.2.1) with acetone to produce the concentrations shown in Exhibit D – CBC, Table 3. When 1 mL of this solution is spiked into the LCS/LCSD, and concentrated to a final volume of 20 µL, the concentration in the final volume will be at the test concentrations or as in Exhibit D – CBC, Table 5. Prepare only the amount necessary for each reference matrix with each sample batch.

7.8.2.3 Cleanup Standard - This solution is spiked into each sample, blank, and LCS/LCSD before cleanup to measure the efficiency of the cleanup process. Dilute the Labeled Cleanup Standard Stock Solution (Section 7.8.1.3.2) in methylene chloride to produce the concentration of the Cleanup Standards shown in Exhibit D – CBC, Table 3. Prepare only the amount necessary for each sample batch.

7.8.2.4 Internal Standards - This solution is added to each concentrated extract before injection into the HRGC/HRMS system. Dilute the Labeled Internal Standard Stock Solution (Section 7.8.1.3.3) in nonane to produce the concentration of the Internal Standards shown in Exhibit D – CBC, Table 3.

7.8.3 Calibration Standards

7.8.3.1 Calibration Standard Solutions

Combine and dilute the solutions described in Sections 7.8.1.2.1 and 7.8.1.3 to produce the five calibration solutions (CS1 through CS5) in Exhibit D – CBC, Table 6, or purchase. These solutions permit the Relative Response (RR) (labeled to native) and a Relative Response Factor (RRF) to be measured as a function of concentration. The CS3 Standard is used for continuing calibration verification.

7.8.3.2 Diluted Individual Mix Solutions

7.8.3.2.1 The five individual solutions, when analyzed, allow resolution of all 209 congener target analytes on the SPB-Octyl column, and are used for establishing RT and other data for each congener. For the SPB-Octyl column, the elution order of the congeners present in each of the five solutions is given in Exhibit D – CBC, Table 4.

7.8.3.2.2 Combine an aliquot of each individual mix stock solution (Section 7.8.1.2.2.1) with an aliquot of the Labeled Toxic/LOC/Window-Defining Congeners Stock Solution (Section 7.8.1.3.1), the Labeled Cleanup Standard Stock Solution (Section 7.8.1.3.2), and the Labeled Internal Standard Stock Solution (Section 7.8.1.3.3) to produce concentrations of 100 ng/mL for the labeled compounds, and 25, 50, and 75 for the MoCB - TrCB, TeCB - HpCB, and OcCB - DeCB, respectively, as in Exhibit D – CBC, Table 3.

7.8.3.3 Diluted Combined 209-Congener Standard Solution

7.8.3.3.1 This solution combines the five individual mixes with the labeled compounds to allow a single-point calibration of the congeners not included in the multi-point calibration, and establishes an average RF for the coeluting isomeric congeners.

7.8.3.3.2 Combine an aliquot of the Combined 209 Congener Stock Solution with an aliquot of the Labeled Toxic/LOC/Window-Defining Congeners Stock Solution, the Labeled Cleanup Standard Stock Solution, and the Labeled Internal Standard Stock Solution to produce the same concentrations as in the diluted individual mix solutions (Section 7.8.1.2.2.2 and Exhibit D – CBC, Table 3. This final mix standard is referred to as CS209.

7.8.4 Window Defining Mixture

Used to define the beginning and ending RTs for congeners at each LOC. The mixture must contain an appropriate amount of Labeled Toxic/LOC/Window-Defining Congeners Spiking Solution. The standard must contain the compounds listed in Exhibit D – CBC, Table 3. The CS3 or CS209 calibration standards may be used for this purpose, and therefore there is no need for a separate injection.

7.9 Stability of Solutions

Standard solutions used for quantitative purposes should be assayed periodically (e.g., every 6 months) against Standard Reference Materials (SRMs) from NIST (if available), or certified reference materials from a source that will attest to the authenticity and concentration, to assure that the composition and concentrations have not changed.

7.10 Storage of Standard Solutions

7.10.1 Store the working standards at ≤6°C, but not frozen, in PTFE-sealed containers. The solutions should be checked frequently for stability. These solutions must be replaced after 6 months, or sooner if comparison with Quality Control (QC) check samples indicates a problem.

7.10.2 Store the stock standard solutions at ≤6°C, but not frozen, in PTFE-lined screw-cap amber bottles.

7.10.3 Standard solutions purchased from a chemical supply company as ampulated extracts in glass vials may be retained and used until the expiration date provided by the manufacturer. If no manufacturer's expiration date is provided, the standard solutions as ampulated extracts may be retained and used for 2 years from the preparation date. Standard solutions prepared by the Contractor that are immediately ampulated in glass vials may be retained for 2 years from the preparation date. The expiration date of the ampulated standards, upon the breaking of the glass seal, is 6 months (or sooner if the standard has degraded or evaporated).

7.10.4 Refrigeration of the GPC calibration solution may cause the corn oil to precipitate. Before use, allow the solution to stand at room temperature until the corn oil dissolves. Replace this calibration solution every 6 months, or more frequently if necessary.

7.10.5 Protect all standards from light.

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

7.10.7 The Contractor is responsible for maintaining and verifying the integrity of standard solutions prior to use. This means, at a minimum, the standards must be brought to room temperature prior to use, checked for losses, and checked that all components have remained in solution.

7.11 Temperature Records for Storage of Standards

7.11.1 The temperature of all standard storage refrigerators/freezers shall be recorded daily.

7.11.2 Temperature excursions shall be noted and appropriate corrective actions shall be taken to correct problems.

7.11.3 Corrective action Standard Operating Procedures (SOPs) shall be posted on the refrigerators/freezers.

8.0 SAMPLE COLLECTION, PRESERVATION, STORAGE, AND HOLDING TIMES

8.1 Sample Collection and Preservation

8.1.1 Aqueous/water grab and composite samples must be collected in amber glass containers following conventional sampling practices. If residual chlorine is present, add 80 mg sodium thiosulfate per liter of water. EPA Methods 330.4 and 330.5 may be used to measure residual chlorine. All samples must be iced or refrigerated at ≤6°C and stored in the dark from the time of collection until sample receipt at the laboratory.

8.1.2 Soil/sediment samples are collected in amber glass jars. All samples must be iced or refrigerated at ≤6°C and stored in the dark from the time of collection until sample receipt at the laboratory.

8.1.3 Tissue (non-human) samples collected in the field must be wrapped in aluminum foil, and must be maintained at a temperature ≤6°C from the time of collection until receipt at the laboratory, to a maximum time of 24 hours. If a longer transport is necessary, freeze the sample. Ideally, tissues should be frozen upon collection and shipped to the laboratory under dry ice.

8.1.4 Refer to Section 10.1 for oily and multiphase samples.

8.2 Procedures for Sample and Sample Extract Storage

8.2.1 Maintain aqueous/water grab and composite samples in the dark at ≤6°C from time of receipt until extraction. If the sample will be frozen, allow room for expansion. It is recommended that sludge, biosolids, and oil/oily matrix samples be kept at their initial conditions. After extraction, the samples must be protected from light and refrigerated at ≤6°C, but not frozen, until 60 days after delivery of a complete, reconciled data package to the EPA. After 60 days, the samples may be disposed of in a manner that complies with all applicable regulations.

8.2.2 Store soil/sediment samples in the dark at ≤6°C. After extraction, the samples must be protected from light and refrigerated at ≤6°C, but not frozen, until 60 days after delivery of a complete, reconciled data package to the EPA. After 60 days, the samples may be disposed of in a manner that complies with all applicable regulations.

8.2.3 Tissue (non-human) samples must be frozen upon receipt at the laboratory and stored in the dark at less than -10°C until prepared. Unused sample portions and unused homogenized tissues must be stored in the dark at less than -10°C. Once thawed, tissue samples must be extracted within 24 hours. After extraction, the samples must be protected from light and stored at less than -10°C until 60 days after delivery of a complete, reconciled data package to the EPA. After 60 days, the samples may be disposed of in a manner that complies with all applicable regulations.

8.2.4 Sample extracts must be protected from light until 365 days after delivery of a complete, reconciled data package to the EPA.

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

8.3 Contract Required Holding Times

8.3.1 All samples should be extracted, analyzed, and completed within the time period specified during scheduling.

8.3.2 Analysis of sample extracts must be completed within one year following the start of extraction, when sample extracts are stored under the appropriate conditions.

9.0 CALIBRATION AND STANDARDIZATION

9.1 Initial Instrument Set-Up

9.1.1 High Resolution Gas Chromatograph Initial Set-up

9.1.1.1 Prior to analyzing the calibration solutions, blanks, samples, and LCS/LCSD samples, establish the HRGC system operating conditions necessary to meet the GC resolution requirements (Section 6.8.1), to properly define the descriptor groups, and to establish the Relative Retention Times (RRTs) for CBCs (Exhibit D – CBC, Table 7). Once optimized, the same conditions must be used for the analysis of all standards, samples, blanks, and LCS/LCSD samples.

NOTE: RTs, RRTs, and RRT limits may differ slightly from those in Exhibit D – CBC, Table 7.

9.1.1.1.1 The recommended GC Operating Conditions for SPB-Octyl Column are provided in Exhibit D – CBC, Table 8.

9.1.1.2 Retention Time Calibrations for Chlorinated Biphenyl Congeners

9.1.1.2.1 Separately inject each of the diluted individual mix solutions (Section 7.8.3.2). Establish the beginning and ending RTs for the scan descriptors in Exhibit D – CBC, Table 2. Scan descriptors other than those listed in Table 2 may be used, provided that the CRQLs in Exhibit C - Chlorinated Dibenzo-*p*-Dioxins and Chlorinated Dibenzofurans and Chlorinated Biphenyl Congeners Target Analyte List and Contract Required Quantitation Limits, Table 3 are met.

9.1.1.2.2 Inject the diluted combined 209-congener standard solution (Section 7.8.3.3 and Exhibit D – CBC, Table 6. Adjust the chromatographic conditions and scan descriptors until the RT and RRT for all congeners are approximately within the windows in Exhibit D – CBC, Table 7, and the column performance specifications in Section 6.8 are met.

9.1.1.2.3 If an alternate column is used, adjust the conditions for that column. If column performance is unacceptable, optimize the analysis conditions or replace the column and repeat the performance tests. All alternate column performance criteria established by the laboratory must be thoroughly documented in the SDG Narrative.

9.1.1.2.4 After the column performance tests are passed, calculate and store the RT and RRT data for the resolved congeners and the RT and RRT for the isomeric congeners that coelute. The windows in Exhibit D – CBC, Table 7, were developed based on the GC conditions given in Exhibit D – CBC, Table 8.

9.1.2 High Resolution Mass Spectrometer

9.1.2.1 Obtain an HRMS SICP of each analyte at the two exact m/z specified in Exhibit D – CBC, Table 2, and at greater than or equal to 10,000 resolving power (with 10% valley definition) at

each LOC for the native congeners and congener groups and for the labeled congeners. Due to the extensive mass range covered in each function, it may not be possible to maintain 10,000 resolution throughout the mass range during the function. Therefore, resolution must be greater than or equal to 8,000 throughout the mass range and must be greater than or equal to 10,000 in the center of the mass range for each function.

9.1.2.2 The analysis time for CBCs may exceed the long-term mass stability of the MS. Because the instrument is operated in the high-resolution mode, mass drifts of a few parts-per-million (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 signal from PFK is used for drift correction. The lock-mass m/z signal is dependent on the exact m/z monitored within each descriptor, as in Exhibit D – CBC, Table 2. The deviation between the exact m/z and the theoretical m/z (Table 2) for each exact m/z monitored must be less than 5 ppm. 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 these 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 8,000 resolving power to save reanalysis time. The GC run should be completed to allow elution of PCB-209.

9.2 Summary of High Resolution Gas Chromatography/High Resolution Mass Spectrometry System Performance Check

9.2.1 At the beginning and end of each 12-hour period and prior to analysis of any samples, blanks, LCS/LCSD, and calibration standards, the Contractor shall establish that the HRGC/HRMS system meets the system performance check criteria.

9.2.2 The HRGC/HRMS system performance check consists of three parts: 1) the HRMS system must be tuned to meet the minimum static resolving power, using a suitable calibrant such as PFK; 2) the performance of the HRGC system and the accuracy/appropriateness of the descriptor switching times must be verified by the analyses of the Window Defining Mixture (WDM); and 3) the resolution must be verified by the analysis of the Isomer Specificity Check Standard (CS1 or CS3).

9.2.3 The HRMS System Tune, the WDM, and the Isomer Specificity Check Standard must also be completed at the end of each 12-hour period or analytical sequence. These analyses may also be used to establish the beginning of the next 12-hour period or analytical sequence.

9.3 High Resolution Mass Spectrometry System Tune

9.3.1 Frequency of HRMS System Tune

9.3.1.1 The HRMS System Tune (PFK tune) must be performed prior to the analysis of calibration standards, samples, LCS/LCSD, and blanks, and verified at the end of each 12-hour period or at the end of the sample analysis.

9.3.1.2 The 12-hour time period for the HRGC/HRMS system performance check does not begin until the HRMS resolution requirements have been met.

9.3.2 Procedure for HRMS System Tune

9.3.2.1 The mass spectrometer must be operated in the electron ionization mode. A static resolving power of at least 10,000 (10% valley definition) must be demonstrated at appropriate masses before any analysis is performed. Static resolving power checks must be performed at the beginning and at the end of each 12-hour period of operation. However, it is recommended that a check of the static resolution be made and documented before and after each analysis.

9.3.2.2 Documentation of the instrument resolving power shall be completed by recording the peak profiles of the reference peaks chosen for each descriptor. While generating the peak profiles, the detector zero shall be adjusted to allow presentation of the profile shoulders on-scale (Method 8290A, Figure 5 – Peak Profiles Representing Two PFK Reference Ions at m/z 305 and 381) so the resolution can be manually evaluated. The format of the peak profiles shall show a horizontal axis calibrated in u or ppm, and a vertical scale in percent maximum signal. The result of the peak width measurement (perform at 5% of the maximum, which corresponds to the 10% valley definition) must appear on the profile, and must not exceed 100 ppm (i.e., 0.038 u for a peak at m/z 380.9760). Both the low and the high exact masses must be displayed to demonstrate the accuracy of the mass calibration. The mass resolution and the accelerating voltages of each mass profile must be present on the profile. This documentation shall be provided for a minimum of one descriptor during each check of the static resolving power of each instrument used, and shall contain identifying information, including instrument ID, time, and date.

9.3.3 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.

9.3.4 Corrective Action for HRMS System Tune

9.3.4.1 Technical acceptance criteria must be met before any standards, samples, LCS/LCSD, and required blanks are analyzed. Any analysis conducted when the technical acceptance criteria have not been met will require reanalysis at no additional cost to the EPA.

9.3.4.2 If the technical acceptance criteria at the end of the 12-hour period 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 the EPA.

9.3.4.3 If the technical acceptance criteria are not met, the instrument must be adjusted until the technical acceptance criteria are met. Refer to manufacturer’s instructions for trouble-shooting systems that will not meet tune criteria.

9.4 Window Defining Mixture

9.4.1 Frequency of Window Defining Mixture

9.4.1.1 The WDM must be analyzed as follows:

- After the HRMS System Tune and before any initial calibration on each instrument and HRGC column used for analysis;
- Once at the beginning and end 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.

9.4.1.2 The 12-hour time period for the HRGC/HRMS system performance check and calibration standards (initial or continuing calibration criteria) begins at the moment of injection of the WDM that the Contractor submits as documentation of a compliant instrument performance check. The time period ends after 12 hours have elapsed according to the system clock.

9.4.2 Procedure for Window Defining Mixture

9.4.2.1 Analyze a 1 or 2 µL aliquot of the WHO Toxic Congeners/LOC/Window-Defining Congeners Spiking Solution (CS1 or CS3 may be used).

9.4.2.2 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. Exhibit D – CBC, Table 7, gives the elution order of all congeners on the SPB-Octyl column.

9.4.3 Technical Acceptance Criteria for Window Defining Mixture

The analysis of the WDM is acceptable if the beginning and ending congeners are eluting as given in Exhibit D – CBC, Table 7.

9.4.3.1 The RTs for the switching of m/z characteristic of one homologous series to the next higher homologous series must be indicated in the SICP.

9.4.3.2 All congeners included in the WDM standard must be present in the SICP.

9.4.3.3 If the GC/MS system is not capable of detecting all congeners within one analysis, corrective action must be taken.

9.4.4 Corrective Action for Window Defining Mixture

9.4.4.1 Technical acceptance criteria must be met before any standards, samples, LCS/LCSD, and required blanks are analyzed. Any analysis conducted when the technical acceptance criteria have not been met will require reanalysis at no additional cost to the EPA.

9.4.4.2 If the technical acceptance criteria of the WDM are not met, the analytical conditions must be adjusted and the test repeated or the HRGC column must be replaced.

9.4.5 Isomer Specificity Check Standard

9.4.5.1 Frequency of Isomer Specificity Check Standard

9.4.5.1.1 The Isomer Specificity Check Standard 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 and end 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.

9.4.5.2 Procedure for Isomer Specificity Check Standard

9.4.5.2.1 Analyze a 1 or 2 µL aliquot of the CS209 (Isomer Specificity Check). This volume should be consistent with the aliquot used for WDM, samples, and other standards throughout the analysis.

9.4.5.2.2 Compute the Percent Valley (%Valley) between the HRGC peaks for PCB-34 and PCB-23, as well as PCB-187 and PCB-182.

9.4.5.2.3 Examine the peak representing PCB-156 and PCB-157 on the SPB-Octyl column.

9.4.5.3 Calculations for Isomer Specificity Check Standard

Calculate the %Valley using the measurements made on the SICP for the appropriate m/z for each isomer using Equation 1 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

9.4.5.4 Technical Acceptance Criteria for Isomer Specificity Check Standard

The Isomer Specificity Check Standard is acceptable if the height of the valley between the least resolved adjacent isomer is less than 40% of the height of the shorter peak and if PCB-156 and PCB-157 elute within 2 seconds of each other.

9.4.5.5 Corrective Action for Isomer Specificity Check Standard

9.4.5.5.1 Technical acceptance criteria must be met before any standards, samples, LCS/LCSD, and required blanks are analyzed. Any analysis conducted when the technical acceptance criteria have not been met will require reanalysis at no additional cost to the EPA.

9.4.5.5.2 If the technical acceptance criteria at the end of the 12-hour period 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 the EPA.

9.4.5.5.3 If the technical acceptance criteria are not met, the analytical system parameters must be adjusted, the instrument recalibrated, and the WDM and isomer specificity tests repeated.

9.4.6 Ion Abundance Ratios and Signal-to-Noise Ratios

Choose an injection volume of either 1 or 2 µL, consistent with the capability of the HRGC/HRMS instrument. Inject a 1 or 2 µL aliquot of the calibration solution of lowest concentration (Exhibit D – CBC, Table 6), using the GC conditions in Section 9.1.1.

9.4.6.1 Measure the SICP areas for each congener or congener group, and compute the ion abundance ratios at the exact m/z specified in Exhibit D – CBC, Table 2. Compare the computed ratio to the theoretical ratio given in Exhibit D – CBC, Table 9.

9.4.6.2 The exact m/z to be monitored in each descriptor are in Exhibit D – CBC, Table 2. Each group or descriptor must be monitored in succession, as a function of GC RT, to ensure that the CBCs of interest are detected. Additional m/z may be monitored in each descriptor, and the m/z may be divided among more than the descriptors listed in Table 2, provided that the Contractor is able to monitor the m/z of all CBCs that may be eluted from the GC in a given LOC window. In particular, the Contractor may monitor the M+4 and M+6 m/z for the nona-substituted congeners. The Contractor shall also monitor exact m/z for congeners at higher LOC to determine if fragments will compromise measurement of congeners at lower LOC. Any additional m/z monitored shall be documented in the SDG Narrative.

9.4.6.3 The MS must be operated in a mass-drift correction mode, using PFK to provide lock m/z ratios. The lock-mass for each group of m/z is shown in Exhibit D – CBC, Table 2. Each lock-mass must be monitored and must not vary by more than ±20% throughout its respective RT window. Variations of lock-mass by more than 20% indicate the presence of coeluting interferences that raise the source pressure and may significantly reduce the sensitivity of the MS.

9.4.6.4 All WHO Toxic Congener target analytes and labeled compounds in the CS1 Standard must be within the QC limits (Exhibit D – CBC, Table 9) for their respective ion abundance ratios; otherwise, the MS must be adjusted and the test repeated until the m/z fall within the limits specified. If the adjustment alters the resolution of the MS, resolution must be verified (Section 9.3.2) before a repeat of the test.

9.4.6.5 The peaks representing the CBCs and labeled compounds in the lowest concentration calibration standard must have Signal-to-Noise ratios (S/N) greater than or equal to 10; otherwise, the MS must be adjusted and the test repeated until this requirement is met.

9.5 Initial Calibration

9.5.1 Summary of Initial Calibration

Prior to the analysis of samples, LCS/LCSD, and blanks, and after the HRGC/HRMS system performance check criteria have been met, each HRGC/HRMS system must be calibrated with a minimum of five concentration levels to determine instrument sensitivity and linearity of the HRGC/HRMS response for the WHO Toxic Congeners/LOC.

9.5.2 Calibration by Isotope Dilution

Isotope dilution calibration is used for the WHO Toxic Congeners/LOC. The reference compounds for each native compound and its labeled compound are listed in Exhibit D – CBC, Table 7.

9.5.2.1 A calibration curve encompassing the concentration range is prepared for the WHO Toxic Congeners/LOC.

9.5.2.2 For the WHO Toxic Congeners/LOC determined by isotope dilution, the RR (labeled to native) vs. concentration in the calibration solutions (Exhibit D – CBC, Table 6) is computed over the calibration range according to the procedures described in Section 9.6.6. Five calibration points are employed for less-sensitive HRMS instruments; five or six-points may be employed for more sensitive instruments.

9.5.2.3 The response of each WHO Toxic Congener/LOC relative to its labeled compound is determined using the area responses of both the primary and secondary exact m/z specified in Exhibit D – CBC, Table 2, for each calibration standard using Equation 2 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

9.5.3 Calibration by Internal Standard

The internal standard calibration method is applied to the determination of the native CBCs for which a labeled compound is not available, Labeled Toxic/LOC/Window-Defining Congeners and Cleanup Standards, and Internal Standards (except for PCB-138L). For the native congeners (other than the native WHO Toxic Congeners/LOC), calibration is performed at a single point using CS209. For the labeled compounds, a calibration is performed using data from the five points in the calibration for the native WHO Toxic Congeners/LOC (Section 9.5.2).

9.5.3.1 The RRF is determined using Equation 3 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

9.5.4 Frequency of Initial Calibration

9.5.4.1 Each HRGC/HRMS system must be calibrated prior to sample analyses, whenever the Contractor takes corrective action that may change or affect the initial calibration criteria (e.g., repair or replacement of any mass spectrometer components, etc.), or if the continuing calibration verification technical acceptance criteria are not met.

9.5.4.2 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 an opening Continuing Calibration Verification Standard within this 12-hour time period if the Initial Calibration Standard that is the same concentration as the Continuing Calibration Verification Standard meets the technical acceptance criteria. Quantitate all sample, LCS/LCSD, and blank results as necessary against the **RR** and the mean RRF from the initial calibration.

NOTE: Initial calibration for the native congener target analytes other than the native WHO Toxic Congeners/LOCs/Labeled compounds and Cleanup Standards is performed each 12-hour period of sample analysis using the single concentration of CS209.

9.5.5 Procedure for Initial Calibration

9.5.5.1 Inject a volume identical to the volume chosen in Section 9.4.2 and the conditions in Section 9.1.1 of each of the Calibration Standards (CS1 through CS5). This volume must be identical to the volume and conditions chosen for the HRGC/HRMS system performance check. An injection of the CS209 standard with the identical volume selected in Section 9.4.2 and the conditions in Section 9.1.1 is also required as part of the initial calibration procedure.

9.5.5.2 Compute and store the RR for each native WHO Toxic Congener/LOC at each concentration using Equation 2 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

9.5.5.3 Determine the RTs, S/N ratios, and ion abundance ratios for all Calibration Standards (Section 9.4.6).

9.5.5.4 Determine linearity of the calibration standards (CS1-CS5) by calculating the Percent Relative Standard Deviation (%RSD) over the five-point calibration range using Equation 7 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

9.5.5.5 Compute the mean RR of the five RRs using Equation 4 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

9.5.5.6 To calibrate the analytical system for native CBCs other than the native WHO Toxic Congeners/LOC by Internal Standard, inject the single-concentration of CS 209 (Section 7.8.3.3 and Exhibit D – CBC, Table 3). Use a volume identical to the volume chosen in Section 9.4.2 and the conditions in Section 9.1.1.

9.5.5.6.1 Compute and store the RRF for all native congener target analytes except the native WHO Toxic Congeners/LOC using Equation 3 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations. Use the average response of the labeled compounds at each LOC as the quantitation reference, to a maximum of 5 labeled congeners, as in Exhibit D – CBC, Table 7. For the combination of isomeric congeners that coelute, compute a combined RRF for the coeluted group. For example, for PCB-122, the areas at the two exact m/z for PCB-104L, PCB-105L, PCB-114L, PCB-118L, and PCB-123L are summed and the total area is divided by 5 (because there are 5 congeners in the quantitation reference).

NOTE: All labeled congeners at each LOC are used as reference to reduce the effect of interference if a single congener is used as reference. Other quantitation references and procedures may be used if the results produced are as accurate as results produced by the quantitation references and procedures described in this Section.

9.5.5.6.2 Compute and store the RRF for the labeled compounds, except PCB-138L. For the Labeled Toxic/LOC/Window-Defining Congeners and the Cleanup Standards, use the nearest eluted Labeled Internal Standard as the quantitation reference, as listed in Exhibit D – CBC, Table 7. The Labeled Internal Standards are referenced to PCB-138L, as in Table 7.

9.5.6 Calculations for Initial Calibration

9.5.6.1 The response of each native congener target analyte relative to its labeled compound is determined using the area responses of both the primary and secondary exact m/z (specified in Exhibit D – CBC, Table 9), for each calibration standard, using Equation 2 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

9.5.6.2 The RRF is determined for each labeled compound using Equation 3 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

9.5.6.3 The mean Relative Response is determined using Equation 4 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

9.5.6.4 The mean RRF is determined using Equation 5 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

9.5.6.5 The Standard Deviation (SD) for a statistically small set of values is determined using Equation 6 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

9.5.6.6 The %RSD is determined for both the native CBCs and the labeled compounds using Equation 7 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

9.5.7 Technical Acceptance Criteria for Initial Calibration

9.5.7.1 All initial calibration standards must be analyzed at the concentration levels and frequency described, as a minimum requirement.

9.5.7.2 The peaks shall be resolved with a valley of ≤40% for PCB-34 from PCB-23, and PCB-187 from PCB-182.

9.5.7.3 The m/z ratios must be within the limits specified in Exhibit D – CBC, Table 9.

9.5.7.4 The S/N ratios for the HRGC/HRMS signal in every SICP must be greater than or equal to 10.

9.5.7.5 The RTs must fall within the appropriate RT windows established by analyses of CS1 and CS209.

9.5.7.6 The %RSD for the RR must be ≤20% and the %RSD for the RRF must be ≤35% over the five-point calibration range.

9.5.8 Corrective Action for Initial Calibration

9.5.8.1 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.

9.5.8.2 All initial calibration technical acceptance criteria must be met before any samples, LCS/LCSD, and required blanks are analyzed. Any analysis conducted when the technical acceptance criteria have not been met will require reanalysis at no additional cost to the EPA.

9.6 Continuing Calibration Verification

9.6.1 Summary of Continuing Calibration Verification

9.6.1.1 The analyses of a CS3 or CS209 standard and an instrument blank or a method blank constitute the continuing calibration verification. CS3 standards must be analyzed as the opening/closing CCVs with sample analyses requested for the WHO Toxic Congeners only, while CS209 standards must be analyzed as the opening/closing CCVs with sample analyses involving 209 congener target analytes. All performance criteria must be verified through checking the RR, RRF, and RRT in the CS3 or CS209 standard. Adjustment and/or recalibration (Section 9.5) must be performed until all performance criteria are met. Only after all performance criteria are met may samples and blanks be analyzed.

9.6.1.2 Mass Spectrometer Resolution

Static resolving power checks must be performed at the beginning and at the end of each shift. If the requirement in Section 9.0 cannot be met, the problem must be corrected before analyses can proceed. If any of the samples in the previous shift may be affected by poor resolution, those samples must be reanalyzed at no additional cost to the EPA.

9.6.2 Frequency of Continuing Calibration Verification

The CS3 or CS209 CCV standards must bracket the 12-hour period during which sample data are collected. The CCV must be analyzed after the HRMS system tune. An instrument blank must be analyzed after the CS3 or CS209 opening CCV in the absence of a method blank during the 12-hour period. The CS3 or CS209 closing CCV must be analyzed to bracket the end of the 12-hour period. This CS3 or CS209 closing CCV may also be used as the beginning of the next 12-hour period. All acceptable samples must be analyzed within a valid analysis sequence as given below:

Time	Analysis
0 Hour	HRMS System Tune
First 12-Hour	CS1 to CS5
	Evaluate CS1 or CS3 against resolution check criteria
	Opening CCV CS209 with 209 congener target analyte analysis
	Instrument Blank or Method Blank
	LCS, LCSD, and Samples (if time still remains on the 12-hour clock)
	Closing CCV CS209 with 209 congener target analyte analysis or
	Closing CCV CS3 with WHO Toxic Congener analysis
	Ending HRMS System Tune
Next 12-Hour	HRMS System Tune
	Opening CCV CS209 or CS3
	Instrument Blank or Method Blank
	Samples
Another 12-Hour	Closing CCV CS209 or CS3
	Instrument Blank or Method Blank
	Samples
	Closing CCV CS209 or CS3
	Ending HRMS System Tune

9.6.3 Procedure for Continuing Calibration Verification

Inject the CS3 or CS209 CCV standard using the volume chosen in Section 9.4.2 and the conditions in Section 9.1.1 and measure the SICP areas for the analytes and compute the ion abundance ratios at the exact m/z. 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 concentrations of the WHO Toxic Congeners/LOC/Labeled compounds and Cleanup Standards based on the initial calibration. Determine the Percent Difference (%D) between the mean RR/RRF from the initial calibration and the continuing calibration verification RR/RRF using Equation 8 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

9.6.4 Calculations for Continuing Calibration Verification

9.6.4.1 Calculate the RR and RRF according to Equations 2 and 3 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

9.6.4.2 The %D between the initial calibration and the continuing calibration verification response in the CS3 or CS209 standard for each WHO Toxic Congener target analyte and applicable labeled compound is determined using Equation 8 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

9.6.5 Technical Acceptance Criteria for Continuing Calibration Verification

9.6.5.1 All 209 congener target analytes in the calibration standard (both native and labeled) must be within their respective ion abundance ratios.

9.6.5.2 The RRTs of all 209 congener target analytes and labeled compounds shall be within the limits defined in Exhibit D – CBC, Table 7, or, if an alternate column is used, within their respective RRT limits for the alternate column (Section 6.8).

9.6.5.3 The %D between the continuing calibration verification RR/RRF and the mean RR/RRF from the initial calibration must be within ±25% for the WHO Toxic Congeners/LOC and refer to Exhibit D – CBC, Table 5, for the labeled compounds and Cleanup Standards.

9.6.5.4 The peaks representing both native and labeled compounds in the CS3 or CS209 standard must have a S/N ratio greater than or equal to 10.

9.6.5.5 The HRMS tune data must be checked and meet the resolution requirement at greater than or equal to 10,000.

9.6.5.6 The absolute RTs of the Labeled Toxic/LOC/Window-Defining Congeners Standard Spiking Solution in the verification test must be within ±15 seconds of the respective RTs in the calibration. If an alternate column is employed, which requires different criteria for peak identification, the laboratory must report the alternate RTs and RRT windows which are used for peak acceptance in the SDG Narrative.

9.6.6 Corrective Action for Continuing Calibration Verification

9.6.6.1 Continuing calibration verification technical acceptance criteria must be met before any samples, LCS/LCSD, or blanks are analyzed. Any analysis conducted when the technical acceptance criteria have not been met will require reanalysis at no additional cost to the EPA.

9.6.6.2 If the continuing calibration verification technical acceptance criteria are not met, inspect the system for problems. It may be necessary to change columns, adjust the system, and recalibrate. If recalibration is required, recalibration for the 209 congeners must also be performed. It may be necessary to segregate samples with particularly serious matrix issues for further cleanup or dilution and reanalysis at a later time in order to obtain compliant beginning and ending calibration checks.

9.6.6.3 If the absolute or RRT of any compound is not within the limits specified, the GC is not performing properly. In this event, adjust the GC and repeat the verification test (Section 9.6.2) or recalibrate (Section 9.5). If the GC column requires replacement, adjust and recalibrate until all system performance criteria are met.

9.6.6.4 Update the RTs, RRTs, and response factors for all congeners except the WHO Toxic Congeners and LOC. For the WHO Toxic Congeners and LOC, the multi-point calibration data must be used.

9.6.6.5 If tune criteria were not met, refer to Section 9.3.4.

10.0 PROCEDURE

10.1 Sample Preparation

Sample preparation involves modifying the physical form of the sample so that the CBCs 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. Exhibit D – CBC, Table 10, lists the phases and suggested quantities for extraction of various sample matrices.

Blank and LCS/LCSD samples must be carried through the same preparation procedures as the field samples to check for losses and contamination in the preparation methods.

Extract pre-screening using a detector that is not the primary analytical unit, [i.e., electron capture GC (GC/ECD) or low resolution GC/MS (GC/LRMS)] is strongly recommended prior to HRGC/HRMS sample extraction as some congeners may be present in high concentration.

10.1.1 Multiphase/Insufficient Samples

10.1.1.1 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 contact the EPA Region. If all phases of the sample are amenable to analysis, the EPA 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 the 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 the EPA Sample Numbers for the additional phases, if required; or
- Not analyze the sample.

10.1.1.2 If an insufficient sample amount (less than the required amount) is received to perform the analyses, the Contractor shall contact SMO and proceed with the analysis of the sample at reduced volume. The Contractor shall document this action and the response from SMO in the SDG Narrative.

10.1.2 Aqueous/Water Samples

Because CBCs may be bound to suspended particles, the preparation of aqueous/water samples is dependent on the apparent solids content of the sample.

10.1.2.1 Aqueous/water samples free from visible particulates are extracted directly using the separatory funnel extraction (Section 10.2.1), continuous liquid-liquid extraction (Section 10.2.2), or the SPE technique (Section 10.2.3).

10.1.2.1.1 The sample volume may be determined by: (1) marking the level of water in the sample container; (2) pouring the sample to an extractor; (3) rinsing the emptied bottle with a small amount of the extraction solvent, which shall be combined with the extraction solvent to be used for sample extraction; (4) refilling the emptied bottle with tap water to the marked line; and (5) finally pouring the tap water to a graduated cylinder to measure the sample volume.

10.1.2.2 Aqueous/water samples containing visible particulates must be processed to determine the percent suspended solids, as in Section 10.1.4.1.1. If the sample is found to contain less than 1% suspended solids, it may be extracted as described above. Samples containing 1% or more of suspended solids must be prepared as indicated below.

10.1.2.3 Aqueous/water samples that contain 1% or more solids (sufficient to provide 10 g of dry solids) and solid samples that show an aqueous phase, must be filtered using the procedure in Section 10.1.3.6. The aqueous filtrate and the filtered solid are treated as two separate samples. The filtered solid is prepared following the procedure for soil/sediment samples (Section 10.2.4 or 10.2.5) and the filtrate is extracted using one of the three options outlined in Section 10.1.2.1. The extracts from these fractions may be combined after initial extraction.

10.1.3 Preparation of Aqueous/Water Samples

10.1.3.1 Mark the original level of the sample on the sample bottle for reference. Weigh the sample plus bottle to ±1 g. After taking the sample aliquot, reweigh the sample bottle and convert the weight to volume assuming a density of 1.00 g/mL.

10.1.3.2 Spike 1 mL of the labeled compound spiking solution (Section 7.8.2.1) into the sample bottle. Cap the bottle and mix the sample by careful shaking. Allow the sample to equilibrate for 1-2 hours, with occasional shaking.

10.1.3.3 For each sample or sample batch (to a maximum of 20 samples) to be extracted during the same 12-hour period, place three 1 L aliquots of reagent water in clean sample bottles or flasks.

10.1.3.4 Spike 1 mL of the labeled compound spiking solution (Section 7.8.2.1) into the three reagent water aliquots. One of these aliquots will serve as the blank and the others will serve as the LCS/LCSD. Spike each LCS/LCSD with 1 mL of the LCS/LCSD spiking solution (Section 7.8.2.2).

10.1.3.5 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 10.2.3 for extraction. If SPE is not to be used, and the sample has no visible particulates, proceed to Section 10.2 for extraction.

10.1.3.6 Filtration of Particulate Matter

10.1.3.6.1 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 particulates.

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

10.1.3.6.3 Rinse any particulates off the sides of the Buchner funnel with small quantities of reagent water.

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

10.1.3.6.5 Extract the filtrate using the separatory funnel (Section 10.2.1), continuous liquid-liquid extraction, (Section 10.2.2), or SPE (Section 10.2.3) procedure.

10.1.3.6.6 Extract the filter containing the particulates using the appropriate soil/sediment procedure in Section 10.2.4 or 10.2.5.

10.1.4 Soil/Sediment Samples

Mix samples thoroughly, especially composite samples. Discard any foreign objects such as sticks, leaves, and rocks. If sample contains standing water, follow instructions in Section 10.1.1.

10.1.4.1 Determination of Percent Suspended Solids, Percent Solids, and Estimation of Particle Size

10.1.4.1.1 Determination of Percent Suspended Solids

For aqueous liquids and multiphase samples consisting of mainly an aqueous phase, desiccate and weigh a filter to three (3) significant figures.

10.1.4.1.1.1 Filter 1000 mL of well-mixed sample through the filter.

10.1.4.1.1.2 Dry the filter for a minimum of 12 hours at 110 ±5°C and cool in a desiccator. This aliquot is used for determining the Percent Suspended Solids (%Suspended Solids) of the sample, not for analysis of CBCs.

10.1.4.1.1.3 Calculate the %Suspended Solids using Equation 9 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

10.1.4.1.2 Determination of Percent Solids

For non-aqueous liquids, solids, semi-solid samples, and multiphase samples in which the main phase is not aqueous, but not tissues.

10.1.4.1.2.1 Weigh 5-10 g of sample to three (3) significant figures into a tared beaker.

10.1.4.1.2.2 Dry for a minimum of 12 hours at 110(±5)°C and cool in a desiccator. At the start and end of the drying procedure, record the oven temperature, the date, and the time. This aliquot is used for determining the %Solids of the sample, not for analysis of CBCs.

10.1.4.1.2.3 Calculate the %Solids using Equation 10 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

10.1.4.1.3 Estimation of Particle Size

10.1.4.1.3.1 Spread the dried sample from Section 10.1.4.1.2.2 on a piece of filter paper or aluminum foil in a fume hood or glove box.

10.1.4.1.3.2 Estimate the size of the particles in the sample. If the size of the largest particles is greater than 1 mm, the particle size must be reduced to 1 mm or less prior to extraction using the procedures in Section below.

10.1.4.2 Sample Sieving or Removal of Larger Particles

Samples with particle sizes greater than 1 mm (as determined in Section 10.1.4.1.3) are subjected to sieving, or manual removal of just a few of the larger particles. Any sieving procedures must be carried out in a glove box or fume hood to prevent fugitive particles from contaminating the work environment.

10.1.4.3 Preparation of Soil/Sediment Samples

10.1.4.3.1 If instructed to remove water by the EPA Region, filter the sample through a glass-fiber filter. The water portion of the sample shall be handled based on EPA Regional direction.

10.1.4.3.2 Weigh a well-mixed aliquot of each solid sample sufficient to provide 10 g of dry soil/sediments (based on the percent solids determination in Section 10.1.4.1.2) into a clean beaker or glass jar. Record sample weight to the nearest 0.1 g.

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

10.1.4.3.4 Spike 1.0 mL of the labeled compound spiking solution (Section 7.8.2.1) into each sample and reference matrix aliquot. One aliquot of the reference matrix will serve as the blank and the others will serve as the LCS/LCSD. Spike each LCS/LCSD with 1.0 mL of the LCS/LCSD spiking solution (Section 7.8.2.2).

10.1.4.3.5 Extract the sample and reference matrix aliquots using the appropriate soil/sediment procedure in Section 10.2.4 or 10.2.5.

10.1.5 Tissue (Non-Human) Samples

Prior to processing tissue samples, the Contractor shall 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 Contractor), 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 Samples are homogenized while still frozen, where practical or partially frozen if not. If the Contractor shall dissect the whole fish to obtain the appropriate tissue for analysis, the unused tissues may be carefully and rapidly re-frozen using a flash freezer, liquid nitrogen, or dry carbon dioxide, and stored in a clean glass jar for subsequent use. The laboratory shall observe all applicable safety precautions when handling these materials.

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

10.1.5.1.3 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. Keep the temperature cold so the fatty tissue is not separated.

10.1.5.2 Preparation of Tissue (Non-Human) Samples

10.1.5.2.1 Transfer approximately 10 g (wet weight) of homogenized tissue to a clean, tared 400-500 mL beaker. Record the weight to the nearest 0.1 g.

10.1.5.2.2 Prepare the blank, LCS, and LCSD by adding approximately 1-2 g of the tissue reference matrix (Section 7.6.3) to a 400-500 mL beaker. Record the weight to the nearest 0.1 g.

10.1.5.2.3 Spike 1.0 mL of the labeled compound spiking solution (Section 7.8.2.1) into the samples, blank, LCS, and LCSD. Spike each LCS/LCSD with 1 mL of the LCS/LCSD spiking solution (Section 7.8.2.2).

10.1.5.2.4 Extract the sample and reference matrix aliquots using the Soxhlet extraction and concentration procedures in Section 10.2.5.

10.1.6 Oily Samples

Prior to processing oily samples, the Contractor 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 any standing water phase, and process as instructed in Section 10.1.1.

10.1.6.1 Preparation of Oily Samples

10.1.6.1.1 Weigh 2 grams of sample into a clean beaker or glass jar. Record the sample weight to the nearest 0.1 g.

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

10.1.6.1.3 Spike 1 mL of the labeled compound spiking solution (Section 7.8.2.1) into each sample and reference matrix aliquot. One aliquot of the reference matrix will serve as the blank and the others will serve as the LCS/LCSD. Spike each LCS/LCSD with 1 mL of the LCS/LCSD spiking solution (Section 7.8.2.2).

10.1.6.1.4 Extract the sample and reference matrix aliquots using the appropriate soil/sediment procedure in Section 10.2.4 or 10.2.5. If the sample aliquot is completely soluble in the extraction solvent, the extraction step may be skipped and the sample processed through cleanup.

10.2 Sample Extraction

Extraction procedures include separatory funnel (Section 10.2.1), continuous liquid-liquid extraction (Section 10.2.2), and SPE (Section 10.2.3) for aqueous/water samples; SDS extraction (Section 10.2.4) and Soxhlet extraction (Section 10.2.5) for soil/sediment or oily matrices; and Soxhlet extraction (Section 10.2.5) for tissue (non-human) samples. Acid/base back-extraction (Section 10.2.6) is used for initial cleanup of extracts. Appropriate extraction methods shall be selected based on sample conditions. Soxhlet extraction alone is not appropriate for wet sediment samples.

10.2.1 Separatory Funnel Extraction

10.2.1.1 Pour the spiked sample (Section 10.1.3.4) or filtrate (Section 10.1.3.6.5) 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, 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 (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, discard the aqueous layer and rinse the separatory funnel with at least 20 mL of methylene chloride. 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 particulates. Set aside the concentration device for use after back-extraction or other cleanup.

10.2.1.4 Concentrate the extract using one of the macro-concentration procedures (Section 10.3). Set aside the concentration device for use after back extraction or other cleanup.

10.2.1.4.1 If the extract is free from visible particulates, 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 aqueous filtrate (Section 10.1.3.6.5), set aside the Kuderna-Danish (K-D) apparatus for addition of the extract from the particulates (Section 10.2.4.9).

10.2.2 Continuous Liquid-Liquid Extraction

10.2.2.1 Place 100-150 mL methylene chloride in each continuous extractor and 200-300 mL in each distilling flask.

10.2.2.2 Pour the spiked sample (Section 10.1.3.4) into the extractor. Rinse the sample container with 50-100 mL of methylene chloride and add this rinse to the extractor.

10.2.2.3 Begin the extraction by heating the flask until the methylene chloride is boiling. When properly adjusted, 1-2 drops of methylene chloride per second will fall from the condenser tip into the water. Extract the sample for 16-24 hours. The accelerated continuous liquid-liquid extractor that uses the hydrophobic membrane to reduce the overall extraction time may be used, provided that all performance criteria are met and the prior Method Detection Limit (MDL) study was performed using the same procedure.

10.2.2.4 Remove the distilling flask, estimate and record the volume of extract (to the nearest 100 mL), and pour the contents through a drying column containing 7-10 cm of granular anhydrous sodium sulfate into a 500 mL K-D evaporator flask equipped with a 10 mL concentrator tube. Rinse the distilling flask with 30-50 mL of methylene chloride and pour through the drying column.

10.2.2.5 Concentrate and exchange to hexane per Section 10.3 and back-extract per Section 10.2.6.

10.2.3 Solid Phase Extraction

10.2.3.1 Disk Preparation

10.2.3.1.1 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 methylene chloride. Clamp the filter and SPE disk between a 1 L glass reservoir and a 2 L vacuum filtration flask.

10.2.3.1.2 Rinse the sides of the filtration flask with approximately 15 mL of methylene chloride 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 methylene chloride through the filter/disk. Repeat the wash step with approximately 15 mL of acetone and allow the filter/disk to air dry.

10.2.3.1.3 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 filter/disk to go dry from this point until the extraction is completed.

10.2.3.1.4 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. Do not allow the filter/disk to go dry from this point until the extraction is completed.

10.2.3.2 Sample Extraction

10.2.3.2.1 Allow the samples (Section 10.1.3.5) to stand for 1-2 hours, if necessary, to settle the suspended particulates. Decant the clear layer of the sample, blank, LCS, and LCSD into its respective 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 a high amount of particulates (suspended solids), the extraction time may be an hour or longer.

10.2.3.2.2 If the filter clogs with particulates and more rapid extraction is desired, replace the filter during the extraction by pouring 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, cover and label with the Sample ID, for later extraction. Reassemble the apparatus with a clean filter and proceed with the extraction. Pull the sample through the filter/disk, leaving a layer of water on the surface of the filter.

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

10.2.3.2.4 Before the sample and rinses have been pulled through the filter/disk, rinse the sides of the reservoir with small portions of reagent water. Allow the filter/disk to partially dry under the vacuum for approximately 3 minutes, then elute the analytes using the procedure in Section 10.2.3.3. Alternatively, extract all filter(s) and disk used per the SDS or Soxhlet procedure (Sections 10.2.4 or 10.2.5).

10.2.3.3 Elution of the Filter/Disk

10.2.3.3.1 Release the vacuum, remove the entire filter/disk/reservoir assembly from the vacuum flask, and empty the flask. Insert a test tube for eluant collection into the flask. The test tube should have sufficient capacity to contain the total volume of the elution solvent (approximately 50 mL) and should fit around the drip tip. The drip tip should protrude into the test tube to preclude loss of a sample from spattering when the vacuum is applied. Reassemble the filter/disk/reservoir assembly on the vacuum flask.

10.2.3.3.2 Wet the filter/disk with 4-5 mL of acetone. Allow the acetone to spread evenly across the disk and soak for 15-20 seconds Pull the acetone through the disk, releasing the vacuum when approximately 1 mm thickness remains on the filter.

10.2.3.3.3 Rinse the sample bottle with approximately 20 mL of methylene chloride and transfer to the reservoir. Pull approximately half of the solvent through the filter/disk and release the vacuum. Allow the filter/disk to soak for approximately 1 minute. Pull all of the solvent through the disk. Repeat the bottle rinsing and elution step with another 20 mL of methylene chloride. Pull all of the solvent through the disk.

10.2.3.3.4 Remove the filter/disk/reservoir assembly and remove the test tube containing the sample solution. Quantitatively transfer the solution to a 250 mL separatory funnel and proceed to Section 10.2.6 for back-extraction.

10.2.4 Soxhlet/Dean-Stark Extraction

SDS extraction with toluene may cause loss of some of the mono- through tri-CBCs. If this loss is excessive, use Soxhlet extraction with methylene chloride (Section 10.2.5) and increase the amount of powdered, anhydrous sodium sulfate as necessary to provide a free-flowing mixture.

10.2.4.1 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.

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

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

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

10.2.4.5 Load the wet sample from Sections 10.1.3.6.6, 10.1.4.3.6, and 10.1.6.1.4 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.

10.2.4.6 Reassemble 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.

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

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

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

10.2.4.9.1 For the extracts from the particulate portion of a water sample containing less than 1% solids:

10.2.4.9.1.1 Concentrate the extract to approximately 5 mL using the rotary evaporation or the heating mantle procedures (Section 10.3.1 or 10.3.2).

10.2.4.9.1.2 Quantitatively transfer the extract through the sodium sulfate (Section 10.2.1.3) into the apparatus that was set aside (Section 10.2.1.4) and reconcentrate to the level of the methylene chloride.

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

10.2.4.9.2 For the extracts from soil/sediments (Section 10.1.3) or from the SPE filter(s) and disk:

10.2.4.9.2.1 Concentrate to approximately 10 mL using the rotary evaporator or heating mantle (Section 10.3.1 or 10.3.2), transfer to a 250 mL separatory funnel, and proceed with back-extraction (Section 10.2.6).

10.2.5 Soxhlet Extraction

This procedure includes determination of the lipid content of tissue samples (Section 10.2.5.8), using the same sample extract that is analyzed by high resolution GC/MS. Alternatively, a separate sample aliquot may be used for the lipid determination. If a separate aliquot is used, use nitrogen to evaporate the main portion of the sample extract only to the extent necessary to affect the solvent exchange to n-hexane, so that loss of low molecular weight congener target analytes is avoided. Thus, it is not necessary to dry the main portion of the sample to constant weight (Section 10.2.5.7).

10.2.5.1 Add 30-40 g of powdered anhydrous sodium sulfate to each of the beakers and mix thoroughly. Cover the beakers with aluminum foil and dry until the mixture becomes a free-flowing powder (30 minutes minimum). Remix prior to extraction to prevent clumping.

10.2.5.2 Assemble and pre-extract the Soxhlet apparatus per Sections 10.2.4.1 - 10.2.4.4, however, use the methylene chloride for the pre-extraction and rinsing and omit the quartz sand.

10.2.5.3 Reassemble the pre-extracted Soxhlet apparatus and add a fresh charge of methylene chloride to the reflux flask.

10.2.5.4 Transfer the prepared sample to the Soxhlet thimble and install the thimble in the Soxhlet apparatus.

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

10.2.5.6 After extraction, cool and quantitatively transfer the extract to a macro-concentration device and concentrate to near dryness. Disassemble the apparatus and set aside for re-use.

10.2.5.7 Complete the removal of the solvent using the nitrogen evaporation procedure (Section 10.4) and a water bath temperature at 60°C. Weigh the receiver, record the weight, and return the receiver to the nitrogen evaporation device, concentrating the residue until a constant weight is obtained.

NOTE: Close attention is required when performing the extract concentration.

10.2.5.8 Percent Lipids Determination

10.2.5.8.1 Redissolve the residue in the concentration device in hexane and spike 1.0 mL of the Cleanup Standard into the extract prior to cleanup in Section 10.5.5.1 for tissue samples.

10.2.5.8.2 Transfer the residue/hexane to the anthropogenic isolation column (Section 10.5.5), 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 redissolved. Allow the receiver to dry. Weigh the receiver and boiling chips.

10.2.5.8.3 Calculate the lipid content to the nearest three (3) significant figures using Equation 11 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

10.2.6 Back-Extraction with Base and Acid

10.2.6.1 Spike 1 mL of the Cleanup Standard (Section 7.8.2.3) into each extract of samples, blanks, and LCS/LCSD, or verify that this has been done. Back-extraction may not be necessary for some samples. For some samples, the presence of color in the extract may indicate that back-extraction is necessary. If back-extraction is not to be performed, proceed to micro-concentration (Section 10.4), or to extract cleanup (Section 10.5). If back-extraction is necessary, proceed to Section 10.2.6.2).

10.2.6.2 Partition the extract against 50 mL of potassium hydroxide solution (Section 7.1.2). Shake not more than 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 CBCs. Stronger potassium hydroxide solutions may be employed for back-extraction with minimum contact time, provided that the Contractor meets the specifications for labeled compound recovery.

10.2.6.3 Partition the extract against 50 mL of sodium chloride solution (Section 7.1.3) in the same way as with base. Discard the water layer.

10.2.6.4 Partition the extract against 50 mL of sulfuric acid (Section 7.1.6) 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 7-10 cm of granular anhydrous sodium sulfate into a macro-concentration device (Section 10.3). If a concentration device was set aside from extraction, that concentration device may be re-used. Rinse the separatory funnel with 30-50 mL of solvent, and pour through the drying column. Collect each extract in a round-bottom flask. Reconcentrate the sample per Sections 10.3 through 10.4, and clean up the samples 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.

NOTE: In the concentration procedures below, the extract must not be allowed to concentrate to dryness because the mono- through tri-chlorobiphenyls may be totally or partially lost.

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 2-3 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 15-20 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 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 1-2 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 15-20 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 Apparatus

Concentrate the extracts in separate 500 mL K-D flasks equipped with 10 mL concentrator tubes. The K-D technique is used for solvents such as methylene chloride and hexane.

10.3.3.1 Add 1-2 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 15-20 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 5-10 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.

10.3.4 Preparation for Back-Extraction or Micro-Concentration and Solvent Exchange

10.3.4.1 For back-extraction, transfer the extract to a 250-mL separatory funnel. Rinse the concentration vessel with small portions of hexane, adjust the hexane volume in the separatory funnel to 10-20 mL, and proceed to back-extraction (Section 10.2.6).

10.3.4.2 For cleanup procedures other than back-extraction, transfer the extract to a nitrogen evaporation vial using 2-3 rinses of solvent. Proceed with micro-concentration and solvent exchange (Section 10.4).

10.4 Micro-Concentration and Solvent Exchange

10.4.1 Extracts to be subjected to GPC cleanup are exchanged into methylene chloride. Extracts that are to be cleaned up using silica gel, carbon (Carbopak/Celite), HPLC, 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 2-3 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 10.0 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 (Section 10.5.1).

10.4.6 If the extract is to be cleaned up by column chromatography (silica gel, carbon, or Florisil), bring the final volume to 1 mL with hexane. Proceed with column cleanups (Sections 10.5.2, 10.5.3, and 10.5.6).

10.4.7 For extracts to be concentrated for injection into the HRGC/HRMS system, 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 percent lipids determination, evaporate until a constant weight is obtained.

10.5 Cleanup Procedures

Cleanup may not be necessary for relatively clean samples (e.g., groundwater and drinking water). However, the Contractor is required to perform all clean-up procedures necessary to achieve the CRQLs, or to fully document the reasons why they could not be achieved. If particular circumstances require the use of cleanup procedures, the Contractor may use any or all of the procedures in this method or any other appropriate procedure. Any additional cleanup procedure step shall be documented in the SDG Narrative and must also be carried out on the associated method blanks and LCS/LCSD.

The following cleanup procedures are available in this method:

- GPC - Removes many high molecular weight interferences that cause GC column performance to degrade. It should be used for all tissue, 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.
- Silica Gel and Florisil - Used to remove non-polar and polar interferences.
- Carbon (Carbopak/Celite) - Used to separate PCB-77, PCB-126, and PCB-169 from the mono- and di- ortho-substituted CBCs.

- HPLC - Used to provide specificity for certain congeners and congeners groups.
- Anthropogenic Isolation Column - Used for removal of lipids from tissue samples.

10.5.1 Gel Permeation Chromatography Cleanup

10.5.1.1 Introduction

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

10.5.1.2 Column Packing

10.5.1.2.1 Place 200-400 mesh, 70- 75 g of SX-3 Bio Beads in a 400-500 mL beaker.

10.5.1.2.2 Cover the beads with methylene chloride and swirl the container to ensure the wetting of all beads. Allow the beads to swell overnight (a minimum of 12 hours).

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

10.5.1.2.4 After purging the column with solvent for 1-2 hours, adjust the column head pressure to 7-10 pounds per square inch gauge (psig), and purge for 4-5 hours to remove air. Maintain a head pressure of 7-10 psig. Connect the column to the UV detector.

10.5.1.3 Column Calibration

10.5.1.3.1 Summary of Calibration

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

10.5.1.3.2 Frequency of GPC Calibration

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

10.5.1.3.3 Procedure for GPC Calibration

10.5.1.3.3.1 Follow the manufacturer’s instructions for operating the GPC system. Using a 10 mL syringe, load the GPC calibration solution onto the GPC. 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.

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

10.5.1.3.3.3 Set the "DUMP" time to allow greater than 85% removal of bis(2-ethyl hexyl)phthalate and greater than 85% collection of methoxychlor.

10.5.1.3.3.4 Set the "COLLECT" time to the time of the sulfur peak maximum.

10.5.1.3.4 Technical Acceptance Criteria for GPC Calibration

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

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

10.5.1.3.4.3 The recovery of the methoxychlor shall be greater than 85%.

10.5.1.3.5 Corrective Action for GPC Calibration

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

10.5.1.4 Sample Extract Cleanup by GPC

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

10.5.1.4.1 Frequency of Sample Extract Cleanup by GPC

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

10.5.1.4.2 Procedure for Sample Extract Cleanup by GPC

10.5.1.4.2.1 Filter the extract or load through the filter holder to remove any particulates. Follow the manufacturer’s instructions for operation of the GPC system being utilized. A 2 mL injection loop may be used in place of a 5 mL injection loop. If a 2 mL injection loop is used, concentrate the sample extract to 4 mL instead of 10 mL, and then inject 4 mL instead of 10 mL. Load the concentrated sample extract into the sample injection loop and inject it onto the GPC column.

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

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

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

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

10.5.2 Silica Gel Cleanup

10.5.2.1 Procedure for Silica Gel Cleanup

10.5.2.1.1 Place a glass wool plug in a 15 mm ID chromatography column. Pack the column bottom to top with: 1 g silica gel (Section 7.5.1.2), 4 g basic silica gel (Section 7.5.1.3), 1 g silica gel, 8 g acid silica gel (Section 7.5.1.1), 2 g silica gel, and 4 g granular anhydrous sodium sulfate (Section 7.2.1). Tap the column to settle the adsorbents. Column packing material may be slurried in hexane to aid settling.

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

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

10.5.2.1.4 Rinse the receiver twice with 1 mL portions of hexane and apply separately to the column. Elute the CBCs with 25 mL of hexane and collect the eluate.

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

10.5.2.1.6 For extracts of samples known to contain large quantities of other organic compounds, 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 40% w/w (6.7 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 1N 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 CBCs. 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 from the column.

10.5.3 Carbon Column Cleanup

10.5.3.1 Procedure for Carbon Column Cleanup

10.5.3.1.1 The carbon column cleanup can be used to separate PCB-77, PCB-126, and PCB-169 from the mono- and di-ortho-substituted CBCs.

NOTE: If PCB-77, PCB-126, and PCB-169 were detected in the sample, then column cleanup should be performed and the sample should be reanalyzed.

10.5.3.1.2 Cut both ends from a 50 mL disposable serological pipette to produce a 20 cm column. Fire-polish both ends and flare both ends if desired. Insert a glass wool plug at one end, and pack the column with 3.6 g of Carbopak/Celite to form an adsorbent bed 20 cm long. Insert a glass wool plug on top of the bed to hold the adsorbent in place.

10.5.3.1.3 Pre-rinse the column with 20 mL each in succession of toluene, methylene chloride, and hexane.

10.5.3.1.4 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.

10.5.3.1.5 Elute the column with 25 mL of n-hexane and collect the eluate. This fraction will contain the mono- and di-ortho CBCs. If carbon particles are present in the eluate, filter through glass-fiber filter paper.

10.5.3.1.6 Elute the column with 15 mL of methanol and discard the eluate. The fraction discarded will contain residual lipids and other potential interferents, if present.

10.5.3.1.7 Elute the column with 15 mL of toluene and collect the eluate. This fraction will contain Congeners 77, 126, and 169. If carbon particles are present in the eluate, filter through glass-fiber filter paper.

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

10.5.4 High Performance Liquid Chromatography Cleanup

10.5.4.1 Procedure for HPLC Calibration

10.5.4.1.1 Prepare a calibration standard containing the WHO Toxic Congeners and other congeners of interest in methylene chloride at the concentrations of the stock solution in Exhibit D – CBC, Table 3, or at a concentration appropriate to the response (not exceeding the linearity) of the detector.

10.5.4.1.2 Inject 30 µL of the calibration standard into the HPLC and record the signal from the detector. Collect the eluant for reuse. Elution will be in the order of the di-ortho, mono-ortho, and non-ortho congeners.

10.5.4.1.3 Establish the collection time for the congeners of interest. Following calibration, flush the injection system with solvent to ensure that residual CBCs are removed from the system.

10.5.4.2 Technical Acceptance Criteria for HPLC Calibration

10.5.4.2.1 Verify the calibration with the calibration standard after every 20 extracts.

10.5.4.2.2 Calibration is verified if the recovery of the CBCs from the calibration standard (Section 10.5.4.1.1) is within 75-125%.

10.5.4.3 Corrective Action for HPLC Calibration

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

10.5.4.4 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 5-50 µg of a given CBC, depending on the congener. If the amount of material in the extract will overload the column, split the extract into fractions and combine the fractions after elution from the column.

10.5.4.4.1 Procedure for Sample Extract Cleanup by HPLC

10.5.4.4.1.1 Spike 1 mL of the Cleanup Standard (Section 7.8.2.3) into each extract of samples, blanks, and LCS/LCSD, or verify that this has been done. Rinse the sides of the vial containing the sample and adjust to the volume required for the sample loop for injection.

10.5.4.4.1.2 Inject the sample extract into the HPLC.

10.5.4.4.1.3 Elute the extract using the calibration data determined in Section 10.5.4.1. Collect the fraction(s) in clean 20 mL concentrator tubes.

10.5.4.4.1.4 If an extract containing greater than 500 µg of total CBCs is encountered, an instrument blank must be run through the system to check for carry-over.

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

10.5.5 Anthropogenic Isolation Column Cleanup

Anthropogenic isolation column cleanup is used for removal of lipids from tissue extracts.

NOTE: Anthropogenic isolation column cleanup is mandatory for all tissue samples.

10.5.5.1 Procedure for Anthropogenic Isolation Column Cleanup

10.5.5.1.1 Prepare the column as given in Section 7.5.3.

10.5.5.1.2 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.

10.5.5.1.3 Load the sample and rinses (Section 10.2.5.8.2) onto the column by draining each portion to the top of the bed. Elute the CBCs from the column into the apparatus used for concentration (Section 10.3) using 200 mL of hexane.

10.5.5.1.4 Remove a small portion (e.g., 50 µL) of the extract for percent lipids determination of residue content. Estimate the percent of the total that this portion represents. Concentrate the small portion to constant weight per Section 10.4.8. Calculate the total amount of residue in the extract. If more than 500 mg of material remains, repeat the cleanup using a fresh anthropogenic isolation column.

10.5.5.1.5 If necessary, exchange the extract to a solvent suitable for the additional cleanups to be used. GPC (Section 10.5.1) and Florisil (Section 10.5.6) are recommended as minimum additional cleanup steps.

10.5.5.1.6 Following cleanup, concentrate the extract to 20 µL as described in Section 10.4 and proceed with the analysis in Section 10.6.

10.5.6 Florisil Cleanup

10.5.6.1 Pre-elute the column with 20 mL of hexane.

10.5.6.2 Begin draining the n-hexane from the column (Section 7.5.4). Adjust the flow rate of eluant to 4.5-5.0 mL/minute.

10.5.6.3 Spike 1 mL of the Cleanup Standard (Section 7.8.2.3) into each extract of samples, blanks, and LCS/LCSD, or verify that this has been done. When the n-hexane is within 1 mm of the sodium sulfate, apply the sample extract (in hexane) to the column as close to the packing material as possible. Rinse the sample container twice with 1 mL portions of hexane and apply to the column.

10.5.6.4 Elute the mono-ortho and di-ortho CBCs with approximately 165 mL of n-hexane and collect the eluate. Elute the non-ortho co-planar CBCs with approximately 100 mL of 6% ether/hexane and collect the eluate. The exact volumes of solvents will need to be determined for each batch of Florisil. If the mono/di-ortho CBCs are not to be separated from the non-ortho co-planar CBCs, elute all CBCs with 6% ether/hexane.

10.5.6.5 Concentrate the eluate(s) per Sections 10.3 through 10.4 for further cleanup or for injection into the HPLC or HRGC/HRMS instrument.

10.6 High Resolution Gas Chromatography/High Resolution Mass Spectrometry Analysis

10.6.1 Introduction

10.6.1.1 Establish the operating conditions given in Section 9.1.1.

10.6.1.2 Sample extracts shall be analyzed only after the HRGC/HRMS system has met the resolution, RT, RRT, and ion abundance ratio requirements in Section 9.0 and has met the requirements for initial calibration and continuing calibration verification. The same instrument conditions must be employed for the analysis of samples as were used for calibration.

10.6.2 Procedure for Sample Analysis by HRGC/HRMS

10.6.2.1 Add 1 µL of the appropriate internal standard (Section 7.8.2.4) 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 reanalyzed and evaporation has occurred, do not add more Labeled Internal Standard Spiking Solution. Rather, bring the extract back to its previous volume (e.g., 19 µL) with pure nonane (18 µL if 2 µL injections are used).

10.6.2.2 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 (Section 9.0). Start the GC column initial isothermal hold upon injection. Start MS data collection after the solvent peak elutes. Monitor the exact m/z at each LOC throughout the LOC RT window. If necessary, monitor m/z associated with congeners at a higher LOC to assure that fragments are not interfering with the m/z for congeners at a lower LOC. Also where warranted, monitor m/z associated with interferents expected to be present. Stop data collection after $^{13}C_{12}$-DeCB has eluted. Return the column to the initial temperature for analysis of the next extract or standard.

10.6.2.3 Analysis of Complex Samples

10.6.2.3.1 Some samples may contain high levels [>10 ng/L; >1,000 nanograms/kilogram (ng/kg)] of the compounds of interest, interfering compounds, and/or polymeric materials. Some extracts may not concentrate to 20 µL (Section 10.4.7); others may overload the HRGC column and/or MS. Fragment ions from congeners at higher LOC may interfere with determination of congeners at a lower LOC. Analyze a smaller aliquot of the sample (Section 10.6.3.2) when the extract will not concentrate to 20 µL after all cleanup procedures have been exhausted. If a smaller aliquot of soils or mixed-phase samples is analyzed, assure that the aliquot is representative of the sample as a whole.

10.6.2.3.2 Interferences may pose a problem in the determination of congeners 81, 123, 126, and 169 in some environmental samples. Loss of one or more chlorines from a highly chlorinated congener may inflate or produce a false concentration from a less-chlorinated congener that elutes at the same retention time. If, upon inspection of the chromatogram, the possibility of interferences is evident (e.g., high concentrations of fragments from the loss of one or two chlorines from higher chlorinated, closely eluting congeners), carbon column cleanup (Section 10.5.3) and reanalysis is recommended.

10.6.3 Sample Dilutions

If the concentration of any target analyte given in Exhibit C - Chlorinated Dibenzo-*p*-Dioxins and Chlorinated Dibenzofurans and Chlorinated Biphenyl Congeners Target Analyte List and Contract Required Quantitation Limits, Table 3, exceeds the calibration range of the system (as defined by the five-point initial calibration performed as specified in Section 9.5), the Contractor shall do one or a combination of the following. Any and all steps taken to obtain analyte results from within the calibration range of the analytical system must be thoroughly documented.

10.6.3.1 Dilute the extract or a portion thereof. A dilution sufficient to bring the target analytes within calibration range is allowed as long as the labeled extraction standard in the extract meets the criteria in Section 11.1.1. This reanalysis is not billable.

10.6.3.2 Re-extract a smaller aliquot of the original sample. Care must be taken to preserve sample representativeness. This re-extraction and reanalysis is not billable.

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

10.6.3.2.2 For samples containing greater than 1% solids, extract a fraction of the solid and/or liquid phase portions of the sample. This aliquot should not be less than 1/20 of the original amount without the permission of the client, contacted through SMO. Re-prepare, extract, clean up, and analyze.

10.6.3.3 In the event that it is determined a cut or dilution of more than 1/10 would be necessary to achieve responses within the range of the calibration curve, the Contractor shall perform one or both of the following:

10.6.3.3.1 Perform a dilution or take a subsample of the extract produced in Section 10.1 prior to adding the Cleanup Standard. This procedure must not prevent the labeled extraction standards from passing the criteria in Section 11.1.1.

10.6.3.3.2 Add a higher calibration point to the initial calibration to expand the calibration range of the system.

10.6.3.4 If the target compounds cannot be measured reliably by isotope dilution, prepare a reduced volume or mass of sample, dilute the extract prior to cleanup, adding a larger aliquot of Cleanup Standard, and further dilute the final extract by adding additional labeled internal standard. This dilution and subsequent reanalysis are not billable. The exact procedure followed in this step must be documented in the SDG Narrative.

11.0 DATA ANALYSIS AND CALCULATIONS

11.1 Qualitative Identification

11.1.1 Identification of Target Analytes

A CBC (native or labeled) is identified when all of the criteria in Section 11.1 are met.

11.1.1.1 The signals for the two exact m/z in Exhibit D – CBC, Table 2, must be present and must maximize within the same two seconds.

11.1.1.2 The S/N for each exact m/z for native analytes in sample extracts must be greater than or equal to 3. The S/N for native analytes in a calibration standard, and for all labeled compounds in sample extracts as well as standards, must be greater than or equal to 10.

11.1.1.3 The ratio of the integrated areas of the two exact m/z specified in Exhibit D – CBC, Table 2, must be within the limits in Exhibit D – CBC, Table 9, or within ±15% of the ratio in the most recent mid-point calibration standard (CS3).

11.1.1.4 The RRT of the peak for a CBC must be within the limits specified in Exhibit D – CBC, Table 7, or if an alternate column or column system is employed, within its respective RRT QC limits for the alternate column or column system (Section 6.8).

NOTE: For native CBC determined by internal standard quantitation, a given CBC may fall within more than one RT window and be misidentified unless the RRT windows are made very narrow, as in Exhibit D – CBC, Table 7. Therefore, consistency of the RT and RRT with other congeners and the labeled compounds may be required for rigorous congener identification. RT regression analysis may aid in this identification.

11.1.1.5 It is possible that not all of the identification criteria may be met because of congener overlap and the potential for interfering substances. It is also possible that loss of one or more chlorines from a highly chlorinated congener may inflate or produce a false concentration for a less-chlorinated congener that elutes at the same RT. If identification is ambiguous, an experienced spectrometrist shall determine the presence or absence of the congener.

11.1.2 Technical Acceptance Criteria for Qualitative Identification

11.1.2.1 If the criteria for identification listed above are not met, check calculations, internal standards, and system performance. If significant sample matrix-induced background is present, reanalysis of the extract may either resolve the issue or prove matrix effects.

11.1.2.2 The relative ion abundance ratio criteria for target analytes and labeled compounds must be met using peak areas to calculate ratios.

11.1.3 Corrective Action for Qualitative Identification

11.1.3.1 If the ion abundance ratios are not met by using peak areas, but all other criteria are met, the Contractor can use peak heights to evaluate the m/z ratio.

11.1.3.2 If positive identification is not possible due to non-compliant m/z ratio, report the target analyte as an EMPC for WHO Toxic Congeners and as a non-detect for non-WHO Toxic Congeners. Document in the SDG Narrative as necessary.

11.2 Quantitative Analysis

11.2.1 Isotope Dilution Quantitation

11.2.1.1 By adding a known amount of the Labeled Toxic/LOC/Window-Defining Congeners to every sample before extraction, correction for recovery of the CBC can be made because the native compound and its labeled compound exhibit similar behavior upon extraction, concentration, and GC. RRs are used in conjunction with the calibration data in Section 9.5.6 to determine concentrations in the final extract, so long as labeled compound spiking levels are constant.

11.2.1.2 Compute the concentrations in the extract of the native WHO Toxic Congeners/LOC using the RRs from the calibration data (Section 9.5) and Equation 12 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

11.2.2 Internal Standard Quantitation and Labeled Compound Recovery

11.2.2.1 Compute the concentrations in the extract of the native compounds other than those in the native WHO Toxic Congeners/LOC Standard, in the Cleanup Standard, and in the Labeled Internal Standard (except for PCB-138L) using the RRF determined from the calibration data (Section 9.5.6) and Equation 13 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

11.2.2.2 Using the concentration in the extract determined above, compute the %R of the Labeled Toxic/LOC/Window-Defining CBC and Cleanup Standard using Equation 14 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

11.2.3 Sample Concentration

11.2.3.1 The concentration of a native CBC in a solid sample is computed using the concentration of the compound in the extract and the weight of the sample (Section 10.1.3), using Equation 15 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

11.2.3.2 The concentration of a native CBC in the tissue sample is computed using the concentration of the compound in the extract and the weight of the tissues, using Equation 16 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

NOTE: Tissue samples are reported in wet weight basis unless otherwise justified. Data should not be blank-corrected.

11.2.3.3 The concentration of a native CBC in the aqueous phase of the sample is computed using the concentration of the compound in the extract and the volume of water extracted (Section 10.1.2), using Equation 17 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

11.2.3.4 Results are reported to two (2) significant figures, as appropriate for the CBCs and labeled compounds found in all standards, blanks, LCS/LCSD, and samples.

11.2.3.5 For water samples, report results in pg/L (parts-per-quadrillion).

11.2.3.6 For samples containing greater than 1% solids (soils, sediments, filter cake, compost), report results in ng/kg (parts-per-trillion) based on the dry weight of the sample.

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

11.2.3.8 For all samples, report results for all peaks with S/N ratios greater than or equal to 3, even if below the CRQL (Exhibit C - Chlorinated Dibenzo-*p*-Dioxins and Chlorinated Dibenzofurans and Chlorinated Biphenyl Congeners Target Analyte List and Contract Required Quantitation Limits, Table 3).

11.2.4 Sample-Specific Estimated Detection Limit

The Estimated Detection Limit (EDL) is the concentration of a given analyte required to produce a signal with a peak height of at least 3 times the background signal level. An EDL is calculated for each WHO Toxic Congener that is not identified.

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

11.2.4.1 If the calculated EDL is less than the adjusted analyte/matrix/instrument-specific MDL, then report the MDL.

11.2.4.2 Use the expression for EDL below to calculate an EDL for each absent WHO Toxic Congener (i.e., S/N<3). The background level is determined by measuring the range of the noise (peak-to-peak) for the two quantitation m/z (Exhibit D – CBC, Table 2) of a particular congener, in the region of the SICP trace corresponding to the elution of the labeled compound or in the region of the SICP where the congener is expected to elute by comparison with the routine calibration data (for those congeners that do not have a ^{13}C-labeled compound), multiplying that noise height by 3, and relating the product to an estimated concentration that would produce that peak height.

11.2.4.3 Use Equation 18 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations for aqueous/water samples.

11.2.4.4 Use Equation 19 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations for solid samples.

NOTE: If interferences can be shown to be present (e.g., CDD/CDF), then descriptor ions other than those listed in Exhibit D – CBC, Table 2, may be used for quantitation. This deviation shall be adequately documented in the SDG Narrative.

11.2.5 Estimated Maximum Possible Concentration

An EMPC is calculated for the WHO Toxic Congeners that are characterized by a response with an S/N of at least 3 for both quantitation m/z, but that do not meet all the identification criteria described in Section 11.1.

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

11.2.5.1 The EMPC of a target analyte in all matrices other than aqueous/water is computed using the concentration of the compound in the extract and the dilution factor, if any (Section 10.6.3), using Equation 20 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

11.2.5.2 The EMPC of a target analyte in an aqueous/water matrix is computed using the concentration of the compound in the extract and the dilution factor, if any (Section 10.6.3), using Equation 21 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

11.2.6 Contract Required Quantitation Limit Calculations

11.2.6.1 Solid

Calculate the adjusted CRQL for solid samples using Equation 22 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

11.2.6.2 Tissue

Calculate the adjusted CRQL for tissue samples using Equation 23 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

NOTE: Tissue samples are reported on a wet weight basis unless otherwise justified. Data should not be blank-corrected.

11.2.6.3 Aqueous/Water

Calculate the adjusted CRQL for aqueous/water samples using Equation 24 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

11.2.7 Toxic Equivalency Factor and Toxic Equivalent Summary

11.2.7.1 The concentration of each of the WHO Toxic Congeners is multiplied by a TEF to arrive at an equivalent toxicity concentration. Calculate the adjusted TEF concentration (using Equation 25 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations) for each species using the TEF associated with the species. The 2005 WHO TEFs for mammal and the 1998 WHO TEFs for fish and bird are used (Exhibit D – CBC, Table 11).

11.2.7.2 Add all TEF-adjusted concentrations to calculate the Total TEQ using Equation 26 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations. The Total TEQ calculations of all three species (mammal, fish, and bird) are required.

11.2.8 Total Homologue Concentration

The total concentration of all isomers at a given level of chlorination (homologue; i.e., Total TrCB, Total PeCB) is calculated by summing the concentrations of all isomers identified at that LOC, including both the Toxics and other isomers. Also, the Total PCBs is calculated by summing all congeners identified at all LOCs.

11.3 Technical Acceptance Criteria for Sample Analysis

11.3.1 The samples must be analyzed on a HRGC/HRMS system meeting the isomer specificity check, HRMS System Tune, resolution check, initial calibration, continuing 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 target and spiked target analytes must meet the qualitative identification criteria in Exhibit D – CBC, Table 7 and Table 9.

11.3.5 The labeled compounds of the sample must meet the acceptance criteria in Exhibit D – CBC, Table 5.

11.3.6 If any target analyte concentration exceeds the upper limit of the initial calibration, see Section 10.6.3.

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 (Section 12.1.2.5) will require re-extraction and reanalysis at no additional cost to the EPA. Any samples analyzed that do not meet the technical acceptance criteria will require re-extraction and/or reanalysis at no additional cost to the EPA.

11.4.2 If the sample analysis technical acceptance criteria are not met, check calculations, internal standards, and system performance. If significant sample matrix-induced background is present, reanalysis of the extract may either resolve the issue or prove matrix effects, in which instance the finding should be explained in the SDG Narrative and all chromatographic evidence provided. 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 reanalyzed at no additional cost to the EPA after the corrective action.

11.4.3 System performance checks, initial calibration, and continuing calibration verification must be acceptable before the reanalysis of samples.

12.0 QUALITY CONTROL

12.1 Blank Analyses

12.1.1 Summary

There are two types of blanks required by this method: the method blank and the instrument blank.

12.1.2 Method Blank

12.1.2.1 Summary of Method Blank

A method blank is a volume or weight of a clean reference matrix (reagent water for aqueous/water samples, sand for soil/sediment samples, or corn oil for tissue samples) that is carried through the entire analytical procedure. The volume or weight 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 levels of contamination associated with the processing and analysis of the samples. When sample extracts are subjected to cleanup procedures, the associated method blank must also be subjected to the same cleanup procedures. In cases when there is no more method blank volume available, the Contractor should use the instrument blank for analysis (Section 12.1.3).

12.1.2.2 Frequency of Method Blank

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 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 HRGC/HRMS system used to analyze associated samples. At least one method blank or one instrument blank (Section 12.1.3) must be analyzed as part of each analytical sequence that includes samples from an SDG.

12.1.2.3 Procedure for Method Blank

A method blank for aqueous/water samples consists of 1 L of reagent water spiked with 1 mL of the Labeled Toxic/LOC/Window-Defining Congeners Spiking Solution (Section 7.8.2.1). For soil/sediment samples, a method blank consists of 10 g of sand spiked with 1 mL of the Labeled Toxic/LOC/Window-Defining Congeners Spiking Solution (Section 7.8.2.1). For tissue samples, a method blank consists of 1.0 g of corn oil spiked with 1 mL of the Labeled Toxic/LOC/Window-Defining Congeners Spiking

Solution (Section 7.8.2.1). The Cleanup Standards are added to all method blanks prior to cleanup.

12.1.2.4 Calculations for Method Blank

Perform data analysis and calculations according to Section 11.0.

12.1.2.5 Technical Acceptance Criteria for Method Blank

12.1.2.5.1 All blanks must be extracted and analyzed at the frequency described in Section 12.1.2.2 on an HRGC/HRMS system meeting all the technical acceptance criteria in Section 11.0.

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

12.1.2.5.3 For the WHO Toxic Congeners, the concentration in the method blank must contain less than 1/2 the CRQL.

12.1.2.5.4 All method blanks must be analyzed undiluted.

12.1.2.6 Corrective Action for Method Blank

12.1.2.6.1 If a method blank does not meet the technical acceptance criteria for method blank analysis, the Contractor shall consider the analytical system to be out of control.

12.1.2.6.2 If contamination is the problem, then 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 solvents, reagents, glassware, and sample storage and processing hardware that lead to discrete artifacts and/or elevated baselines in the HRGC/HRMS system are eliminated. All samples associated with a contaminated blank must be re-extracted and reanalyzed at no additional cost to the EPA.

12.1.2.6.3 If the method blank fails to meet the technical acceptance criteria due to instrument problem, correct the instrument problem, recalibrate the instrument (if necessary), and reanalyze the method blank.

12.1.3 Instrument Blank

12.1.3.1 Summary of Instrument Blank

An instrument blank is a volume of clean solvent spiked with labeled compounds and analyzed on the instrument used for sample analysis when there is not enough method blank volume available for analysis. The purpose of the instrument blank is to determine the levels of contamination associated with the instrumental analysis, particularly with regard to carryover of analytes from standards or highly contaminated samples into other analyses.

12.1.3.2 Frequency of Instrument Blank

At least one instrument blank shall be analyzed as part of each analytical sequence that includes samples from an SDG and when there is no method blank analysis in the analytical sequence. The injection of the instrument blank shall be performed after the continuing calibration verification to initiate a new analytical sequence.

12.1.3.3 Procedure for Instrument Blank

12.1.3.3.1 Prepare the instrument blank by spiking the Labeled Toxic/LOC/Window-Defining Congeners Spiking Solution (Section 7.8.2.1) into nonane for a final concentration of 100 ng/mL.

12.1.3.3.2 Analyze the instrument blank according to the frequency listed in Section 12.1.3.2.

12.1.3.4 Calculations for Instrument Blank

Assuming that the material in the instrument blank resulted from the extraction of a 1 L water sample, calculate the concentration of each analyte using the equations in Section 11.2. Compare the results to the CRQL values for water samples in Exhibit C - Chlorinated Dibenzo-*p*-Dioxins and Chlorinated Dibenzofurans and Chlorinated Biphenyl Congeners Target Analyte List and Contract Required Quantitation Limits, Table 3.

12.1.3.5 Technical Acceptance Criteria for Instrument Blank

12.1.3.5.1 All blanks must be analyzed at the frequency described in Section 12.1.3.2 on an HRGC/HRMS system meeting all the technical acceptance criteria in Section 11.0.

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

12.1.3.5.3 For the WHO Toxic Congeners, the concentration in the instrument blank must be less than 1/2 the CRQL.

12.1.3.5.4 All instrument blanks must be analyzed undiluted.

12.1.3.6 Corrective Action for Instrument Blank

12.1.3.6.1 If an instrument blank does not meet the technical acceptance criteria for blank analysis, the Contractor shall consider the analytical system to be out of control.

12.1.3.6.2 If contamination is the problem, then the source of the contamination must be investigated and appropriate corrective measures taken and documented before further sample analysis proceeds. All samples associated with a contaminated instrument blank must be reanalyzed at no additional cost to the EPA.

12.1.3.6.3 If the instrument blank fails to meet the technical acceptance criteria due to instrument problem, correct the instrument problem, recalibrate the instrument (if necessary), and reanalyze the instrument blank. An acceptable instrument blank must be analyzed before additional data are collected.

12.2 Laboratory Control Sample and Laboratory Control Sample Duplicate

12.2.1 Summary of Laboratory Control Sample and Laboratory Control Sample Duplicate

The LCS/LCSD is a volume or weight of clean reference matrix that is spiked and carried through the entire analytical procedure. To evaluate the accuracy and precision of the method used for CBC analysis, a mixture of the Native Toxic/LOC target analytes is spiked into an aliquot of reference matrix and analyzed in accordance with the appropriate method.

12.2.2 Frequency of Laboratory Control Sample and Laboratory Control Sample Duplicate

The LCS/LCSD shall be extracted with each SDG. The number of samples extracted with each LCS/LCSD should not exceed 20 field samples (excluding PE samples). The LCS/LCSD must be extracted and analyzed concurrently with the samples in the SDG using the same extraction protocol, cleanup procedure, and instrumentation as the samples in the SDG.

12.2.3 Procedure for Laboratory Control Sample and Laboratory Control Sample Duplicate

For water samples, prepare 1 L aliquots of reagent water. For soil/sediment/other samples, prepare aliquots or weigh the appropriate reference matrix (Section 7.6). For tissue samples, use 1 g of corn oil. All LCS/LCSD are spiked with 1 mL of the LCS/LCSD Spiking Solution (Section 7.8.2.2), the Labeled Toxic/LOC/Window-Defining Congeners Spiking Solution (Section 7.8.2.1), and the Cleanup Standard (Section 7.2.3) according to the procedures in Section 10.0.

12.2.4 Calculations for Laboratory Control Sample and Laboratory Control Sample Duplicate

12.2.4.1 Calculate the concentration of each analyte according to Section 11.0.

12.2.4.2 Compute the %R and RPD of the LCS/LCSD analytes using Equations 27 and 28 in Exhibit G – List of Abbreviations & Acronyms, Glossary of Terms, and Equations.

12.2.5 Technical Acceptance Criteria for Laboratory Control Sample and Laboratory Control Sample Duplicate

12.2.5.1 All LCS/LCSD must be prepared and analyzed at the frequency described in Section 12.2.2.

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

12.2.5.3 The %R of each of the spiked analytes in the LCS/LCSD must be within the acceptance limits in Exhibit D – CBC, Table 5.

12.2.5.4 The RPD between LCS/LCSD for the target analytes should be within 30%. This limit is only advisory; no further action by the Contractor is required.

12.2.6 Corrective Action for Laboratory Control Sample and Laboratory Control Sample Duplicate

If both LCS and LCSD do not meet the technical acceptance criteria for LCS %R, the Contractor must consider the analytical system to be out of control. All samples associated with a non-compliant LCS/LCSD must be re-extracted and reanalyzed at no additional cost to the EPA.

12.3 Method Detection Limit Determination

12.3.1 Before any field samples are analyzed under the contract, the MDL for each WHO Toxic Congener shall be determined on each instrument under the same conditions used for analysis (i.e., analytical system configuration) as well as type and dimensions of GC column, prior to the start of contract analyses and verified annually thereafter. MDL determination is matrix-specific (i.e., the MDL shall be determined for aqueous/water, soil/sediment, and tissue samples).

The MDLs determined for soil/sediment shall be used for sludge, ash, and oil samples. An MDL study shall also be performed after major instrument maintenance or changes in instrumentation, or instrumental conditions, to verify the current sensitivity of the analysis. Major instrument maintenance includes, but is not limited to repair or replacement of any mass spectrometer components. A new MDL study will not be required after changing the GC column, as long as the replacement column has the same length, inner diameter, and stationary phase.

12.3.2 To determine the MDLs, the Contractor shall perform MDL studies following the procedures specified in Title 40 of the Code of Federal Regulations (CFR), Part 136, Appendix B, Revision 2.

12.3.3 The determined concentration of the MDL must be less than 1/2 the CRQL listed in Exhibit C – Chlorinated Dibenzo-*p*-Dioxins and Chlorinated Dibenzofurans and Chlorinated Biphenyl Congeners Target Analyte List and Contract Required Quantitation Limits, Table 5.

12.3.4 The delivery requirements for the MDL values are specified in Exhibit B – Reporting and Deliverables Requirements, Table 1.

13.0 METHOD PERFORMANCE

Not applicable.

14.0 POLLUTION PREVENTION

Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operations. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. When feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the EPA recommends recycling as the next best option.

15.0 WASTE MANAGEMENT

The EPA requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The EPA urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with applicable environmental rules and regulations.

16.0 REFERENCES

16.1 U.S. Environmental Protection Agency, Chlorinated Biphenyl Congeners in Water, Soil, Sediment, Biosolids, and Tissue by HRGC/HRMS, Method 1668, Revision C, April 2010.

16.2 U.S. Environmental Protection Agency, Polychlorinated Biphenyls (PCBs) by Gas Chromatography, Method 8082A, Revision 1, February 2007.

16.3 U.S. Environmental Protection Agency, Polychlorinated Dibenzo-*p*-Dioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) by High-Resolution Gas Chromatography/High-Resolution Mass Spectrometry (HRGC/HRMS), Method 8290A, Revision 1, February 2007.

16.4 U.S. Government Printing Office, Title 40 of the Code of Federal Regulations, Chapter 1, Subchapter D, Part 136, Appendix B, Definition and Procedure for the Determination of the Method Detection Limit, Revision 2.

17.0 TABLES/DIAGRAMS/FLOWCHARTS

TABLE 1. ANALYTE NAMES AND CHEMICAL ABSTRACTS SERVICE (CAS) REGISTRY NUMBERS FOR NATIVE AND LABELED CHLORINATED BIPHENYL CONGENERS

CB Congener[1]	Analyte Name	CAS Registry Number	Labeled Compound	Labeled Analyte Name	CAS Registry Number
2-MoCB	PCB-1	2051-60-7	$^{13}C_{12}$-2-MoCB[2]	PCB-1L	234432-85-0
3-MoCB	PCB-2	2051-61-8			
4-MoCB	PCB-3	2051-62-9	$^{13}C_{12}$-4-MoCB[2]	PCB-3L	208263-77-8
2,2'-DiCB	PCB-4	13029-08-8	$^{13}C_{12}$-2,2'-DiCB[2]	PCB-4L	234432-86-1
2,3-DiCB	PCB-5	16605-91-7			
2,3'-DiCB	PCB-6	25569-80-6			
2,4-DiCB	PCB-7	33284-50-3			
2,4'-DiCB[3]	PCB-8	34883-43-7			
2,5-DiCB	PCB-9	34883-39-1	$^{13}C_{12}$-2,5-DiCB[4]	PCB-9L	250694-89-4
2,6-DiCB	PCB-10	33146-45-1			
3,3'-DiCB	PCB-11	2050-67-1			
3,4-DiCB	PCB-12	2974-92-7			
3,4'-DiCB	PCB-13	2974-90-5			
3,5-DiCB	PCB-14	34883-41-5			
4,4'-DiCB	PCB-15	2050-68-2	$^{13}C_{12}$-4,4'-DiCB[2]	PCB-15L	208263-67-6
2,2',3-TrCB	PCB-16	38444-78-9			
2,2',4-TrCB	PCB-17	37680-66-3			
2,2',5-TrCB[3]	PCB-18	37680-65-2			
2,2',6-TrCB	PCB-19	38444-73-4	$^{13}C_{12}$-2,2',6-TrCB[2]	PCB-19L	234432-87-2
2,3,3'-TrCB	PCB-20	38444-84-7			
2,3,4-TrCB	PCB-21	55702-46-0			
2,3,4'-TrCB	PCB-22	38444-85-8			
2,3,5-TrCB	PCB-23	55720-44-0			
2,3,6-TrCB	PCB-24	55702-45-9			
2,3',4-TrCB	PCB-25	55712-37-3			
2,3',5-TrCB	PCB-26	38444-81-4			
2,3',6-TrCB	PCB-27	38444-76-7			

TABLE 1. ANALYTE NAMES AND CHEMICAL ABSTRACTS SERVICE (CAS) REGISTRY NUMBERS FOR NATIVE AND LABELED CHLORINATED BIPHENYL CONGENERS (CON'T)

CB Congener[1]	Analyte Name	CAS Registry Number	Labeled Compound	Labeled Analyte Name	CAS Registry Number
2,4,4'-TrCB[3]	PCB-28	7012-37-5	$^{13}C_{12}$-2,4,4'-TriCB[5]	PCB-28L	208263-76-7
2,4,5-TrCB	PCB-29	15862-07-4			
2,4,6-TrCB	PCB-30	35693-92-6			
2,4',5-TrCB	PCB-31	16606-02-3			
2,4',6-TrCB	PCB-32	38444-77-8			
2',3,4-TrCB	PCB-33	38444-86-9			
2',3,5-TrCB	PCB-34	37680-68-5			
3,3',4-TrCB	PCB-35	37680-69-6			
3,3',5-TrCB	PCB-36	38444-87-0			
3,4,4'-TrCB	PCB-37	38444-90-5	$^{13}C_{12}$-3,4,4'-TrCB[2]	PCB-37L	208263-79-0
3,4,5-TrCB	PCB-38	53555-66-1			
3,4',5-TrCB	PCB-39	38444-88-1			
2,2',3,3'-TeCB	PCB-40	38444-93-8			
2,2',3,4-TeCB	PCB-41	52663-59-9			
2,2',3,4'-TeCB	PCB-42	36559-22-5			
2,2',3,5-TeCB	PCB-43	70362-46-8			
2,2',3,5'-TeCB[3]	PCB-44	41464-39-5			
2,2',3,6-TeCB	PCB-45	70362-45-7			
2,2',3,6'-TeCB	PCB-46	41464-47-5			
2,2',4,4'-TeCB	PCB-47	2437-79-8			
2,2',4,5-TeCB	PCB-48	70362-47-9			
2,2',4,5'-TeCB	PCB-49	41464-40-8			
2,2',4,6-TeCB	PCB-50	62796-65-0			
2,2',4,6'-TeCB	PCB-51	68194-04-7			
2,2',5,5'-TeCB[3]	PCB-52	35693-99-3	$^{13}C_{12}$-2,2',5,5'-TeCB[4]	PCB-52L	208263-80-3
2,2',5,6'-TeCB	PCB-53	41464-41-9			
2,2',6,6'-TeCB	PCB-54	15968-05-5	$^{13}C_{12}$-2,2',6,6'-TeCB[2]	PCB-54L	234432-88-3
2,3,3',4-TeCB	PCB-55	74338-24-2			
2,3,3',4'-TeCB	PCB-56	41464-43-1			

TABLE 1. ANALYTE NAMES AND CHEMICAL ABSTRACTS SERVICE (CAS) REGISTRY NUMBERS FOR NATIVE AND LABELED CHLORINATED BIPHENYL CONGENERS (CON'T)

CB Congener[1]	Analyte Name	CAS Registry Number	Labeled Compound	Labeled Analyte Name	CAS Registry Number
2,3,3',5-TeCB	PCB-57	70424-67-8			
2,3,3',5'-TeCB	PCB-58	41464-49-7			
2,3,3',6-TeCB	PCB-59	74472-33-6			
2,3,4,4'-TeCB	PCB-60	33025-41-1			
2,3,4,5-TeCB	PCB-61	33284-53-6			
2,3,4,6-TeCB	PCB-62	54230-22-7			
2,3,4',5-TeCB	PCB-63	74472-34-7			
2,3,4',6-TeCB	PCB-64	52663-58-8			
2,3,5,6-TeCB	PCB-65	33284-54-7			
2,3',4,4'-TeCB[3]	PCB-66	32598-10-0			
2,3',4,5-TeCB	PCB-67	73575-53-8			
2,3',4,5'-TeCB	PCB-68	73575-52-7			
2,3',4,6-TeCB	PCB-69	60233-24-1			
2,3',4',5-TeCB	PCB-70	32598-11-1			
2,3',4',6-TeCB	PCB-71	41464-46-4			
2,3',5,5'-TeCB	PCB-72	41464-42-0			
2,3',5',6-TeCB	PCB-73	74338-23-1			
2,4,4',5-TeCB	PCB-74	32690-93-0			
2,4,4',6-TeCB	PCB-75	32598-12-2			
2',3,4,5-TeCB	PCB-76	70362-48-0			
3,3',4,4'-TeCB[3,6]	PCB-77	32598-13-3	$^{13}C_{12}$-3,3',4,4'-TeCB[2,7]	PCB-77L	105600-23-5
3,3',4,5-TeCB	PCB-78	70362-49-1			
3,3',4,5'-TeCB	PCB-79	41464-48-6			
3,3',5,5'-TeCB	PCB-80	33284-52-5			
3,4,4',5-TeCB[6]	PCB-81	70362-50-4	$^{13}C_{12}$-3,4,4',5-TeCB[7]	PCB-81L	208461-24-9
2,2',3,3',4-PeCB	PCB-82	52663-62-4			
2,2',3,3',5-PeCB	PCB-83	60145-20-2			
2,2',3,3',6-PeCB	PCB-84	52663-60-2			
2,2',3,4,4'-PeCB	PCB-85	65510-45-4			

TABLE 1. ANALYTE NAMES AND CHEMICAL ABSTRACTS SERVICE (CAS) REGISTRY NUMBERS
FOR NATIVE AND LABELED CHLORINATED BIPHENYL CONGENERS (CON'T)

CB Congener[1]	Analyte Name	CAS Registry Number	Labeled Compound	Labeled Analyte Name	CAS Registry Number
2,2',3,4,5-PeCB	PCB-86	55312-69-1			
2,2',3,4,5'-PeCB	PCB-87	38380-02-8			
2,2',3,4,6-PeCB	PCB-88	55215-17-3			
2,2',3,4,6'-PeCB	PCB-89	73575-57-2			
2,2',3,4',5-PeCB	PCB-90	68194-07-0			
2,2',3,4',6-PeCB	PCB-91	68194-05-8			
2,2',3,5,5'-PeCB	PCB-92	52663-61-3			
2,2',3,5,6-PeCB	PCB-93	73575-56-1			
2,2',3,5,6'-PeCB	PCB-94	73575-55-0			
2,2',3,5',6-PeCB	PCB-95	38379-99-6			
2,2',3,6,6'-PeCB	PCB-96	73575-54-9			
2,2',3',4,5-PeCB	PCB-97	41464-51-1			
2,2',3',4,6-PeCB	PCB-98	60233-25-2			
2,2',4,4',5-PeCB	PCB-99	38380-01-7			
2,2',4,4',6-PeCB	PCB-100	39485-83-1			
2,2',4,5,5'-PeCB[3]	PCB-101	37680-73-2	$^{13}C_{12}$-2,2',4,5,5'-PeCB[4]	PCB-101L	104130-39-4
2,2',4,5,6'-PeCB	PCB-102	68194-06-9			
2,2',4,5',6-PeCB	PCB-103	60145-21-3			
2,2',4,6,6'-PeCB	PCB-104	56558-16-8	$^{13}C_{12}$-2,2',4,6,6'-PeCB[2]	PCB-104L	234432-89-4
2,3,3',4,4'-PeCB[3,6]	PCB-105	32598-14-4	$^{13}C_{12}$-2,3,3',4,4'-PeCB[7]	PCB-105L	208263-62-1
2,3,3',4,5-PeCB	PCB-106	70424-69-0			
2,3,3',4',5-PeCB	PCB-107	70424-68-9			
2,3,3',4,5'-PeCB	PCB-108	70362-41-3			
2,3,3',4,6-PeCB	PCB-109	74472-35-8			
2,3,3',4',6-PeCB	PCB-110	38380-03-9			
2,3,3',5,5'-PeCB	PCB-111	39635-32-0	$^{13}C_{12}$-2,3,3',5,5'-PeCB[5]	PCB-111L	235416-29-2
2,3,3',5,6-PeCB	PCB-112	74472-36-9			
2,3,3',5',6-PeCB	PCB-113	68194-10-5			
2,3,4,4',5-PeCB[6]	PCB-114	74472-37-0	$^{13}C_{12}$-2,3,4,4',5-PeCB[7]	PCB-114L	208263-63-2

TABLE 1. ANALYTE NAMES AND CHEMICAL ABSTRACTS SERVICE (CAS) REGISTRY NUMBERS
FOR NATIVE AND LABELED CHLORINATED BIPHENYL CONGENERS (CON'T)

CB Congener[1]	Analyte Name	CAS Registry Number	Labeled Compound	Labeled Analyte Name	CAS Registry Number
2,3,4,4',6-PeCB	PCB-115	74472-38-1			
2,3,4,5,6-PeCB	PCB-116	18259-05-7			
2,3,4',5,6-PeCB	PCB-117	68194-11-6			
2,3',4,4',5-PeCB[3,6]	PCB-118	31508-00-6	$^{13}C_{12}$-2,3',4,4',5-PeCB[7]	PCB-118L	104130-40-7
2,3',4,4',6-PeCB	PCB-119	56558-17-9			
2,3',4,5,5'-PeCB	PCB-120	68194-12-7			
2,3',4,5',6-PeCB	PCB-121	56558-18-0			
2',3,3',4,5-PeCB	PCB-122	76842-07-4			
2',3,4,4',5-PeCB[6]	PCB-123	65510-44-3	$^{13}C_{12}$-2',3,4,4',5-PeCB[7]	PCB-123L	208263-64-3
2',3,4,5,5'-PeCB	PCB-124	70424-70-3			
2',3,4,5,6'-PeCB	PCB-125	74472-39-2			
3,3',4,4',5-PeCB[3,6]	PCB-126	57465-28-8	$^{13}C_{12}$-3,3',4,4',5-PeCB[2,7]	PCB-126L	208263-65-4
3,3',4,5,5'-PeCB	PCB-127	39635-33-1			
2,2',3,3',4,4'-HxCB[3]	PCB-128	38380-07-3			
2,2',3,3',4,5-HxCB	PCB-129	55215-18-4			
2,2',3,3',4,5'-HxCB	PCB-130	52663-66-8			
2,2',3,3',4,6-HxCB	PCB-131	61798-70-7			
2,2',3,3',4,6'-HxCB	PCB-132	38380-05-1			
2,2',3,3',5,5'-HxCB	PCB-133	35694-04-3			
2,2',3,3',5,6-HxCB	PCB-134	52704-70-8			
2,2',3,3',5,6'-HxCB	PCB-135	52744-13-5			
2,2',3,3',6,6'-HxCB	PCB-136	38411-22-2			
2,2',3,4,4',5-HxCB	PCB-137	35694-06-5			
2,2',3,4,4',5'-HxCB[3]	PCB-138	35065-28-2	$^{13}C_{12}$-2,2',3,4,4',5'-HxCB[4]	PCB-138L	208263-66-5
2,2',3,4,4',6-HxCB	PCB-139	56030-56-9			
2,2',3,4,4',6'-HxCB	PCB-140	59291-64-4			
2,2',3,4,5,5'-HxCB	PCB-141	52712-04-6			
2,2',3,4,5,6-HxCB	PCB-142	41411-61-4			

TABLE 1. ANALYTE NAMES AND CHEMICAL ABSTRACTS SERVICE (CAS) REGISTRY NUMBERS FOR NATIVE AND LABELED CHLORINATED BIPHENYL CONGENERS (CON'T)

CB Congener[1]	Analyte Name	CAS Registry Number	Labeled Compound	Labeled Analyte Name	CAS Registry Number
2,2',3,4,5,6'-HxCB	PCB-143	68194-15-0			
2,2',3,4,5',6-HxCB	PCB-144	68194-14-9			
2,2',3,4,6,6'-HxCB	PCB-145	74472-40-5			
2,2',3,4',5,5'-HxCB	PCB-146	51908-16-8			
2,2',3,4',5,6-HxCB	PCB-147	68194-13-8			
2,2',3,4',5,6'-HxCB	PCB-148	74472-41-6			
2,2',3,4',5',6-HxCB	PCB-149	38380-04-0			
2,2',3,4',6,6'-HxCB	PCB-150	68194-08-1			
2,2',3,5,5',6-HxCB	PCB-151	52663-63-5			
2,2',3,5,6,6'-HxCB	PCB-152	68194-09-2			
2,2',4,4',5,5'-HxCB[3]	PCB-153	35065-27-1			
2,2',4,4',5,6'-HxCB	PCB-154	60145-22-4			
2,2',4,4',6,6'-HxCB	PCB-155	33979-03-2	$^{13}C_{12}$-2,2',4,4',6,6'-HxCB[2]	PCB-155L	234432-90-7
2,3,3',4,4',5-HxCB[6]	PCB-156	38380-08-4	$^{13}C_{12}$-2,3,3',4,4',5-HxCB[7]	PCB-156L	208263-68-7
2,3,3',4,4',5'-HxCB[6]	PCB-157	69782-90-7	$^{13}C_{12}$-2,3,3',4,4',5'-HxCB[7]	PCB-157L	235416-30-5
2,3,3',4,4',6-HxCB	PCB-158	74472-42-7			
2,3,3',4,5,5'-HxCB	PCB-159	39635-35-3			
2,3,3',4,5,6-HxCB	PCB-160	41411-62-5			
2,3,3',4,5',6-HxCB	PCB-161	74472-43-8			
2,3,3',4',5,5'-HxCB	PCB-162	39635-34-2			
2,3,3',4',5,6-HxCB	PCB-163	74472-44-9			
2,3,3',4',5',6-HxCB	PCB-164	74472-45-0			
2,3,3',5,5',6-HxCB	PCB-165	74472-46-1			
2,3,4,4',5,6-HxCB	PCB-166	41411-63-6			
2,3',4,4',5,5'-HxCB[6]	PCB-167	52663-72-6	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[7]	PCB-167L	208263-69-8
2,3',4,4',5',6-HxCB	PCB-168	59291-65-5			
3,3',4,4',5,5'-HxCB[3,6]	PCB-169	32774-16-6	$^{13}C_{12}$-3,3',4,4',5,5'-HxCB[2,7]	PCB-169L	208263-70-1
2,2',3,3',4,4',5-HpCB[3]	PCB-170	35065-30-6	$^{13}C_{12}$-2,2',3,3',4,4',5-HpCB	PCB-170L	160901-80-4
2,2',3,3',4,4',6-HpCB	PCB-171	52663-71-5			

TABLE 1. ANALYTE NAMES AND CHEMICAL ABSTRACTS SERVICE (CAS) REGISTRY NUMBERS FOR NATIVE AND LABELED CHLORINATED BIPHENYL CONGENERS (CON'T)

CB Congener[1]	Analyte Name	CAS Registry Number	Labeled Compound	Labeled Analyte Name	CAS Registry Number
2,2',3,3',4,5,5'-HpCB	PCB-172	52663-74-8			
2,2',3,3',4,5,6-HpCB	PCB-173	68194-16-1			
2,2',3,3',4,5,6'-HpCB	PCB-174	38411-25-5			
2,2',3,3',4,5',6-HpCB	PCB-175	40186-70-7			
2,2',3,3',4,6,6'-HpCB	PCB-176	52663-65-7			
2,2',3,3',4',5,6-HpCB	PCB-177	52663-70-4			
2,2',3,3',5,5',6-HpCB	PCB-178	52663-67-9	$^{13}C_{12}$-2,2',3,3',5,5',6-HpCB[5]	PCB-178L	232919-67-4
2,2',3,3',5,6,6'-HpCB	PCB-179	52663-64-6			
2,2',3,4,4',5,5'-HpCB[3]	PCB-180	35065-29-3	$^{13}C_{12}$-2,2',3,4,4',5,5'-HpCB[3]	PCB-180L	160901-82-6
2,2',3,4,4',5,6-HpCB	PCB-181	74472-47-2			
2,2',3,4,4',5,6'-HpCB	PCB-182	60145-23-5			
2,2',3,4,4',5',6-HpCB	PCB-183	52663-69-1			
2,2',3,4,4',6,6'-HpCB	PCB-184	74472-48-3			
2,2',3,4,5,5',6-HpCB	PCB-185	52712-05-7			
2,2',3,4,5,6,6'-HpCB	PCB-186	74472-49-4			
2,2',3,4',5,5',6-HpCB[3]	PCB-187	52663-68-0			
2,2',3,4',5,6,6'-HpCB	PCB-188	74487-85-7	$^{13}C_{12}$-2,2',3,4',5,6,6'-HpCB[2]	PCB-188L	234432-91-8
2,3,3',4,4',5,5'-HpCB[6]	PCB-189	39635-31-9	$^{13}C_{12}$-2,3,3',4,4',5,5'-HpCB[2,7]	PCB-189L	208263-73-4
2,3,3',4,4',5,6-HpCB	PCB-190	41411-64-7			
2,3,3',4,4',5',6-HpCB	PCB-191	74472-50-7			
2,3,3',4,5,5',6-HpCB	PCB-192	74472-51-8			
2,3,3',4',5,5',6-HpCB	PCB-193	69782-91-8			
2,2',3,3',4,4',5,5'-OcCB	PCB-194	35694-08-7	$^{13}C_{12}$-2,2',3,3',4,4',5,5'-OcCB[4]	PCB-194L	208263-74-5
2,2',3,3',4,4',5,6-OcCB[3]	PCB-195	52663-78-2			
2,2',3,3',4,4',5,6'-OcCB	PCB-196	42740-50-1			
2,2',3,3',4,4',6,6'-OcCB	PCB-197	33091-17-7			
2,2',3,3',4,5,5',6-OcCB	PCB-198	68194-17-2			
2,2',3,3',4,5,5',6'-OcCB	PCB-199	52663-75-9			
2,2',3,3',4,5,6,6'-OcCB	PCB-200	52663-73-7			

TABLE 1. ANALYTE NAMES AND CHEMICAL ABSTRACTS SERVICE (CAS) REGISTRY NUMBERS FOR NATIVE AND LABELED CHLORINATED BIPHENYL CONGENERS (CON'T)

CB Congener[1]	Analyte Name	CAS Registry Number	Labeled Compound	Labeled Analyte Name	CAS Registry Number
2,2',3,3',4,5',6,6'-OcCB	PCB-201	40186-71-8			
2,2',3,3',5,5',6,6'-OcCB	PCB-202	2136-99-4	$^{13}C_{12}$-2,2',3,3',5,5',6,6'-OcCB[2]	PCB-202L	105600-26-8
2,2',3,4,4',5,5',6-OcCB	PCB-203	52663-76-0			
2,2',3,4,4',5,6,6'-OcCB	PCB-204	74472-52-9			
2,3,3',4,4',5,5',6-OcCB	PCB-205	74472-53-0	$^{13}C_{12}$-2,3,3',4,4',5,5',6-OcCB[2]	PCB-205L	234446-64-1
2,2',3,3',4,4',5,5',6-NoCB[3]	PCB-206	40186-72-9	$^{13}C_{12}$-2,2',3,3',4,4',5,5',6-NoCB[2]	PCB-206L	208263-75-6
2,2',3,3',4,4',5,6,6'-NoCB	PCB-207	52663-79-3			
2,2',3,3',4,5,5',6,6'-NoCB	PCB-208	52663-77-1	$^{13}C_{12}$-2,2',3,3',4,5,5',6,6'-NoCB[2]	PCB-208L	234432-92-9
DeCB[3]	PCB-209	2051-24-3	$^{13}C_{12}$-DeCB[2]	PCB-209L	105600-27-9

[1]Abbreviations for chlorination levels:
MoCB = monochlorobiphenyl
DiCB = dichlorobiphenyl
TrCB = trichlorobiphenyl
TeCB = tetrachlorobiphenyl
PeCB = pentachlorobiphenyl
HxCB = hexachlorobiphenyl
HpCB = heptachlorobiphenyl
OcCB = octachlorobiphenyl
NoCB = nonachlorobiphenyl
DeCB = decachlorobiphenyl

[2]Labeled LOC Window-Defining Congener.

[3]National Oceanic and Atmospheric Administration (NOAA) Congener of Interest.

[4]Internal Standard.

[5]Cleanup Standard.

[6]WHO Toxic Congener.

[7]Labeled compound of WHO Toxic Congener.

TABLE 2. DESCRIPTORS, LEVELS OF CHLORINATION, m/z INFORMATION, AND SUBSTANCES

Function and Chlorine Level	Exact m/z[1]	m/z Type	m/z Formula	Substance
Fn-1; Cl-1	188.0393	M	$^{12}C_{12}$ H_9 ^{35}Cl	Cl-1 CB
	190.0363	M+2	$^{12}C_{12}$ H_9 ^{37}Cl	Cl-1 CB
	200.0795	M	$^{13}C_{12}$ H_9 ^{35}Cl	$^{13}C_{12}$ Cl-1 CB
	202.0766	M+2	$^{13}C_{12}$ H_9 ^{37}Cl	$^{13}C_{12}$ Cl-1 CB
	218.9856	lock	C_4 F_9	PFK
Fn-2; Cl-2,3	222.0003	M	$^{12}C_{12}$ H_8 $^{35}Cl_2$	Cl-2 PCB
	223.9974[2]	M+2	$^{12}C_{12}$ H_8 ^{35}Cl ^{37} Cl	Cl-2 PCB
	225.9944	M+4	$^{12}C_{12}$ H_8 $^{37}Cl_2$	Cl-2 PCB
	234.0406	M	$^{13}C_{12}$ H_8 $^{35}Cl_2$	$^{13}C_{12}$ Cl-2 PCB
	236.0376	M+2	$^{13}C_{12}$ H_8 ^{35}Cl ^{37} Cl	$^{13}C_{12}$ Cl-2 PCB
	242.9856	lock	C_6 F_9	PFK
	255.9613	M	$^{12}C_{12}$ H_7 $^{35}Cl_3$	Cl-3 PCB
	257.9584	M+2	$^{12}C_{12}$ H_7 $^{35}Cl_2$ ^{37}Cl	Cl-3 PCB
	268.0016	M	$^{13}C_{12}$ H_7 $^{35}Cl_3$	$^{13}C_{12}$ Cl-3 PCB
	269.9986	M+2	$^{13}C_{12}$ H_7 $^{35}Cl_2$ ^{37}Cl	$^{13}C_{12}$ Cl-3 PCB
Fn-3; Cl-3,4,5	255.9613	M	$^{12}C_{12}$ H_7 $^{35}Cl_3$	Cl-3 PCB
	257.9584	M+2	$^{12}C_{12}$ H_7 $^{35}Cl_2$ ^{37}Cl	Cl-3 PCB
	259.9554	M+4	$^{12}C_{12}$ H_7 ^{35}Cl $^{37}Cl_2$	Cl-3 PCB
	268.0016	M	$^{13}C_{12}$ H_7 $^{35}Cl_3$	$^{13}C_{12}$ Cl-3 PCB
	269.9986	M+2	$^{13}C_{12}$ H_7 $^{35}Cl_2$ ^{37}Cl	$^{13}C_{12}$ Cl-3 PCB
	280.9825	lock	C_6 F_{11}	PFK
	289.9224	M	$^{12}C_{12}$ H_6 $^{35}Cl_4$	Cl-4 PCB
	291.9194	M+2	$^{12}C_{12}$ H_6 $^{35}Cl_3$ ^{37}Cl	Cl-4 PCB
	293.9165	M+4	$^{12}C_{12}$ H_6 $^{35}Cl_2$ $^{37}Cl_2$	Cl-4 PCB
	301.9626	M	$^{13}C_{12}$ H_6 $^{35}Cl_4$	$^{13}C_{12}$ Cl-4 PCB
	303.9597	M+2	$^{13}C_{12}$ H_6 $^{35}Cl_3$ ^{37}Cl	$^{13}C_{12}$ Cl-4 PCB
	323.8834	M	$^{12}C_{12}$ H^5 $^{35}Cl_5$	Cl-5 PCB
	325.8804	M+2	$^{12}C_{12}$ H^5 $^{35}Cl_4$ ^{37}Cl	Cl-5 PCB
	327.8775	M+4	$^{12}C_{12}$ H^5 $^{35}Cl_3$ $^{37}Cl_2$	Cl-5 PCB
	337.9207	M+2	$^{13}C_{12}$ H^5 $^{35}Cl_4$ ^{37}Cl	$^{13}C_{12}$ Cl-5 PCB
	339.9178	M+4	$^{13}C_{12}$ H^5 $^{35}Cl_3$ $^{37}Cl_2$	$^{13}C_{12}$ Cl-5 PCB
Fn-4; Cl-4,5,6	289.9224	M	$^{12}C_{12}$ H_6 $^{35}Cl_4$	Cl-4 PCB
	291.9194	M+2	$^{12}C_{12}$ H_6 $^{35}Cl_3$ ^{37}Cl	Cl-4 PCB
	293.9165	M+4	$^{12}C_{12}$ H_6 $^{35}Cl_2$ $^{37}Cl_2$	Cl-4 PCB
	301.9626	M+2	$^{13}C_{12}$ H_6 $^{35}Cl_3$ ^{37}Cl	$^{13}C_{12}$ Cl-4 PCB
	303.9597	M+4	$^{13}C_{12}$ H_6 $^{35}Cl_2$ $^{37}Cl_2$	$^{13}C_{12}$ Cl-4 PCB
	323.8834	M	$^{12}C_{12}$ H_5 $^{35}Cl_5$	Cl-5 PCB
	325.8804	M+2	$^{12}C_{12}$ H_5 $^{35}Cl_4$ ^{37}Cl	Cl-5 PCB
	327.8775	M+4	$^{12}C_{12}$ H_5 $^{35}Cl_3$ $^{37}Cl_2$	Cl-5 PCB
	330.9792	lock	C_7 F_{15}	PFK
	337.9207	M+2	$^{13}C_{12}$ H_5 $^{35}Cl_4$ ^{37}Cl	$^{13}C_{12}$ Cl-5 PCB
	339.9178	M+4	$^{13}C_{12}$ H_5 $^{35}Cl_3$ $^{37}Cl_2$	$^{13}C_{12}$ Cl-5 PCB
	359.8415	M+2	$^{12}C_{12}$ H_4 $^{35}Cl_5$ ^{37}Cl	Cl-6 PCB
	361.8385	M+4	$^{12}C_{12}$ H_4 $^{35}Cl_4$ $^{37}Cl_2$	Cl-6 PCB

TABLE 2. DESCRIPTORS, LEVELS OF CHLORINATION, m/z INFORMATION, AND SUBSTANCES (CON'T)

Function and Chlorine Level	Exact m/z[1]	m/z Type	m/z Formula	Substance
Fn-4; Cl-4,5,6	363.8356	M+6	$^{12}C_{12}$ H_4 $^{35}Cl_3$ $^{37}Cl_2$	Cl-6 PCB
	371.8817	M+2	$^{13}C_{12}$ H_4 $^{35}Cl_5$ ^{37}Cl	$^{13}C_{12}$ Cl-6 PCB
	373.8788	M+4	$^{13}C_{12}$ H_4 $^{35}Cl_4$ $^{37}Cl_2$	$^{13}C_{12}$ Cl-6 PCB
Fn-5; Cl-5,6,7	323.8834	M	$^{12}C_{12}$ H_5 $^{35}Cl_5$	Cl-5 PCB
	325.8804	M+2	$^{12}C_{12}$ H_5 $^{35}Cl_4$ ^{37}Cl	Cl-5 PCB
	327.8775	M+4	$^{12}C_{12}$ H_5 $^{35}Cl_3$ $^{37}Cl_2$	Cl-5 PCB
	337.9207	M+2	$^{13}C_{12}$ H_5 $^{35}Cl_4$ ^{37}Cl	$^{13}C_{12}$ Cl-5 PCB
	339.9178	M+4	$^{13}C_{12}$ H_5 $^{35}Cl_3$ $^{37}Cl_2$	$^{13}C_{12}$ Cl-5 PCB
	354.9792	lock	C_9 F_{13}	PFK
	359.8415	M+2	$^{12}C_{12}$ H_4 $^{35}Cl_5$ ^{37}Cl	Cl-6 PCB
	361.8385	M+4	$^{12}C_{12}$ H_4 $^{35}Cl_4$ $^{37}Cl_2$	Cl-6 PCB
	363.8356	M+6	$^{12}C_{12}$ H_4 $^{35}Cl_3$ $^{37}Cl_3$	Cl-6 PCB
	371.8817	M+2	$^{13}C_{12}$ H_4 $^{35}Cl_5$ ^{37}Cl	$^{13}C_{12}$ Cl-6 PCB
	373.8788	M+4	$^{13}C_{12}$ H_4 $^{35}Cl_4$ $^{37}Cl_2$	$^{13}C_{12}$ Cl-6 PCB
	393.8025	M+2	$^{12}C_{12}$ H_3 $^{35}Cl_6$ ^{37}Cl	Cl-7 PCB
	395.7995	M+4	$^{12}C_{12}$ H_3 $^{35}Cl_5$ $^{37}Cl_2$	Cl-7 PCB
	397.7966	M+6	$^{12}C_{12}$ H_3 $^{35}Cl_4$ $^{37}Cl_3$	Cl-7 PCB
	405.8428	M+2	$^{13}C_{12}$ H_3 $^{35}Cl_6$ ^{37}Cl	$^{13}C_{12}$ Cl-7 PCB
	407.8398	M+4	$^{13}C_{12}$ H_3 $^{35}Cl_5$ $^{37}Cl_2$	$^{13}C_{12}$ Cl-7 PCB
	454.9728	QC	C_{11} F_{17}	PFK
Fn-6; Cl-7,8,9,10	393.8025	M+2	$^{12}C_{12}$ H_3 $^{35}Cl_6$ ^{37}Cl	Cl-7 PCB
	395.7995	M+4	$^{12}C_{12}$ H_3 $^{35}Cl_5$ $^{37}Cl_2$	Cl-7 PCB
	397.7966	M+6	$^{12}C_{12}$ H_3 $^{35}Cl_4$ $^{37}Cl_3$	Cl-7 PCB
	405.8428	M+2	$^{13}C_{12}$ H_3 $^{35}Cl_6$ ^{37}Cl	$^{13}C_{12}$ Cl-7 PCB
	407.8398	M+4	$^{13}C_{12}$ H_3 $^{35}Cl_5$ $^{37}Cl_2$	$^{13}C_{12}$ Cl-7 PCB
	427.7635	M+2	$^{12}C_{12}$ H_2 $^{35}Cl_7$ ^{37}Cl	Cl-8 PCB
	429.7606	M+4	$^{12}C_{12}$ H_2 $^{35}Cl_6$ $^{37}Cl_2$	Cl-8 PCB
	431.7576	M+6	$^{12}C_{12}$ H_2 $^{35}Cl_5$ $^{37}Cl_3$	Cl-8 PCB
	439.8038	M+2	$^{13}C_{12}$ H_2 $^{35}Cl_7$ ^{37}Cl	$^{13}C_{12}$ Cl-8 PCB
	441.8008	M+4	$^{13}C_{12}$ H_2 $^{35}Cl_6$ $^{37}Cl_2$	$^{13}C_{12}$ Cl-8 PCB
	442.9728	QC	C_{10} F_{13}	PFK
	454.9728	lock	C_{11} F_{13}	PFK
	461.7246	M+2	$^{12}C_{12}$ H_1 $^{35}Cl_8$ ^{37}Cl	Cl-9 PCB
	463.7216	M+4	$^{12}C_{12}$ H_1 $^{35}Cl_7$ $^{37}Cl_2$	Cl-9 PCB
	465.7187	M+6	$^{12}C_{12}$ H_1 $^{35}Cl_6$ $^{37}Cl_3$	Cl-9 PCB
	473.7648	M+2	$^{13}C_{12}$ H_1 $^{35}Cl_8$ ^{37}Cl	$^{13}C_{12}$ Cl-9 PCB
	475.7619	M+4	$^{13}C_{12}$ H_1 $^{35}Cl_7$ $^{37}Cl_2$	$^{13}C_{12}$ Cl-9 PCB
	495.6856	M+2	$^{12}C_{12}$ H_4 $^{35}Cl_9$ ^{37}Cl	Cl-10 PCB
	497.6826	M+4	$^{12}C_{12}$ $^{35}Cl_8$ $^{37}Cl_2$	Cl-10 PCB
	499.6797	M+6	$^{12}C_{12}$ $^{35}Cl_7$ $^{37}Cl_3$	Cl-10 PCB
	507.7258	M+2	$^{13}C_{12}$ $^{35}Cl_9$ ^{37}Cl	$^{13}C_{12}$ Cl-10 PCB
	509.7229	M+4	$^{13}C_{12}$ $^{35}Cl_8$ $^{37}Cl_2$	$^{13}C_{12}$ Cl-10 PCB
	511.7199	M+6	$^{13}C_{12}$ $^{35}Cl_7$ $^{37}Cl_3$	$^{13}C_{12}$ Cl-10 PCB

[1]Isotopic masses used for accurate mass calculation:

^{1}H 1.0078

^{12}C 12.0000

^{13}C 13.0034

^{35}Cl 34.9689

^{37}Cl 36.9659

^{19}F 18.9984

[2]An interference with PFK m/z 223.9872 may preclude meeting 10:1 S/N for the DiCB at the CS-1 calibration level (Exhibit D – CBC, Section 9.4.3 and Table 6). If this interference occurs, 10:1 S/N must be met at the CS-2 level.

TABLE 3. CONCENTRATIONS OF NATIVE AND LABELED CHLORINATED BIPHENYLS IN STOCK SOLUTIONS, SPIKING SOLUTIONS, AND FINAL EXTRACTS

Analyte Name	Solution Concentrations		
	Stock (µg/mL)	Spiking (ng/mL)	Extract (ng/mL)
Native Toxic/LOC[1]			
PCB-1	20	1.0	50
PCB-3	20	1.0	50
PCB-4	20	1.0	50
PCB-15	20	1.0	50
PCB-19	20	1.0	50
PCB-37	20	1.0	50
PCB-54	20	1.0	50
PCB-77	20	1.0	50
PCB-81	20	1.0	50
PCB-104	20	1.0	50
PCB-105	20	1.0	50
PCB-114	20	1.0	50
PCB-118	20	1.0	50
PCB-123	20	1.0	50
PCB-126	20	1.0	50
PCB-155	20	1.0	50
PCB-156	20	1.0	50
PCB-157	20	1.0	50
PCB-167	20	1.0	50
PCB-169	20	1.0	50
PCB-188	20	1.0	50
PCB-189	20	1.0	50
PCB-202	20	1.0	50
PCB-205	20	1.0	50
PCB-206	20	1.0	50
PCB-208	20	1.0	50
PCB-209	20	1.0	50
Native 209 Congener Mix Stock Solutions[2]			
MoCB thru TrCB	2.5		
TeCB thru HpCB	5.0		
OcCB thru DeCB	7.5		
Labeled Toxic/LOC/Window-Defining[3]			
PCB-1L	1.0	2.0	100
PCB-3L	1.0	2.0	100
PCB-4L	1.0	2.0	100
PCB-15L	1.0	2.0	100
PCB-19L	1.0	2.0	100
PCB-37L	1.0	2.0	100
PCB-54L	1.0	2.0	100
PCB-77L	1.0	2.0	100
PCB-81L	1.0	2.0	100
PCB-104L	1.0	2.0	100
PCB-105L	1.0	2.0	100
PCB-114L	1.0	2.0	100
PCB-118L	1.0	2.0	100
PCB-123L	1.0	2.0	100
PCB-126L	1.0	2.0	100
PCB-155L	1.0	2.0	100
PCB-156L	1.0	2.0	100

TABLE 3. CONCENTRATIONS OF NATIVE AND LABELED CHLORINATED BIPHENYLS IN STOCK SOLUTIONS, SPIKING SOLUTIONS, AND FINAL EXTRACTS (CON'T)

Analyte Name	Solution Concentrations		
	Stock (µg/mL)	Spiking (ng/mL)	Extract (ng/mL)
PCB-157L	1.0	2.0	100
PCB-167L	1.0	2.0	100
PCB-169L	1.0	2.0	100
PCB-188L	1.0	2.0	100
PCB-189L	1.0	2.0	100
PCB-202L	1.0	2.0	100
PCB-205L	1.0	2.0	100
PCB-206L	1.0	2.0	100
PCB-208L	1.0	2.0	100
PCB-209L	1.0	2.0	100
Labeled Cleanup Standard[4]			
PCB-28L	1.0	2.0	100
PCB-111L	1.0	2.0	100
PCB-178L	1.0	2.0	100
Labeled Internal Standard[5]			
PCB-9L	5	200	100
PCB-52L	5	200	100
PCB-101L	5	200	100
PCB-138L	5	200	100
PCB-194L	5	200	100

Diluted Combined 209-Congener[6]		
	Solution Concentration (ng/mL)	
Standard	**Native**	**Labeled**
Native congeners		
MoCB thru TrCB	25	
TeCB thru HpCB	50	
OcCB thru DeCB	75	
Labeled Toxic/LOC/Window-Defining		100
Cleanup Standard		100
Internal Standard		100

[1]Stock solution: Section 7.8.1.2.1; Spiking solution: Section 7.8.2.2.

[2]See Section 7.8.1.2.2.1.

[3]Stock Solution: Section 7.8.1.3.1; Spiking solution: Section 7.8.2.1.

[4]Stock Solution: Section 7.8.1.3.2; Spiking solution: Section 7.8.2.3.

[5]Stock Solution: Section 7.8.1.3.3; Spiking solution: Section 7.8.2.4.

[6]See Section 7.8.3.3.

TABLE 4. SUGGESTED COMPOSITION OF INDIVIDUAL NATIVE CHLORINATED BIPHENYL CONGENER SOLUTIONS[1]

Solution Identifier				
A2	**B2**	**C2**	**D2**	**E2**
2	7	13	25	1
10	5	17	21	3
9	12	29	69	4
6	18	20	47	15
8	24	46	42	19
14	23	65	64	16
11	28	59	70	37
30	22	40	102	54
27	39	67	97	43
32	53	76	115	44
34	51	80	123	74
26	73	93	134	56
31	48	84	131	77
33	62	101	163	104
36	71	112	180	98
38	68	86		125
35	58	116		110
50	61	107		126
45	55	154		155
52	60	147		138
49	94	140		169
75	100	146		188
41	91	141		189
72	121	164		202
57	90	158		205
63	99	182		208
66	109	174		206
79	117	173		209
78	111	193		
81	108			
96	118			
103	114			
95	150			
88	145			
89	135			
92	149			
113	139			
83	132			
119	165			
87	168			
85	137			
82	160			
120	128			
124	162			
106	157			
122	184			
105	186			

TABLE 4. SUGGESTED COMPOSITION OF INDIVIDUAL NATIVE CHLORINATED BIPHENYL CONGENER SOLUTIONS[1] (CON'T)

Solution Identifier				
A2	**B2**	**C2**	**D2**	**E2**
127	187			
152	185			
136	181			
148	192			
151	197			
144	199/201			
143	203			
142				
133				
161				
153				
130				
129				
166				
159				
167				
156				
179				
176				
178				
175				
183				
177				
171				
172				
191				
170				
190				
201/200				
204				
200/199				
198				
196				
195				
194				
207				
Total Number of Congeners				
83	**54**	**29**	**15**	**28**

[1]Congeners present in each standard listed in elution order for each level of chlorination. Congener Number listed first; Ballschmiter (BZ) Number listed second where ambiguous. See Exhibit D – CBC, Table 3 for concentrations of congeners in stock solutions, and Table 6 for concentrations in the Calibration Standard. The individual native congener (other than the native WHO Toxic Congeners/LOC) listed in Table 4 is calibrated at a single point.

TABLE 5. QUALITY CONTROL ACCEPTANCE CRITERIA FOR CHLORINATED BIPHENYLS IN CALIBRATION VERIFICATION, LABORATORY CONTROL SAMPLE/LABORATORY CONTROL SAMPLE DUPLICATE, AND SAMPLES[1]

Congener Name	Analyte Name[2]	Test Conc (ng/mL)[3]	VER(%)[4]	LCS/LCSD Recovery (%)	Labeled Compound Recovery in Samples (%)
2-MoCB	PCB-1	50	75 – 125	60 – 135	N/A
4-MoCB	PCB-3	50	75 – 125	60 – 135	
2,2'-DiCB	PCB-4	50	75 – 125	60 – 135	
4,4'-DiCB	PCB-15	50	75 – 125	60 – 135	
2,2'6-TrCB	PCB-19	50	75 – 125	60 – 135	
3,4,4'-TrCB	PCB-37	50	75 – 125	60 – 135	
2,2'6,6'-TeCB	PCB-54	50	75 – 125	60 – 135	
3,3',4,4'-TeCB	PCB-77	50	75 – 125	60 – 135	
3,4,4',5-TeCB	PCB-81	50	75 – 125	60 – 135	
2,2',4,6,6'-PeCB	PCB-104	50	75 – 125	60 – 135	
2,3,3',4,4'-PeCB	PCB-105	50	75 – 125	60 – 135	
2,3,4,4',5-PeCB	PCB-114	50	75 – 125	60 – 135	
2,3',4,4',5-PeCB	PCB-118	50	75 – 125	60 – 135	
2',3,4,4',5-PeCB	PCB-123	50	75 – 125	60 – 135	
3,3',4,4',5-PeCB	PCB-126	50	75 – 125	60 – 135	
2,2',4,4',6,6'-HxCB	PCB-155	50	75 – 125	60 – 135	
2,3,3',4,4',5-HxCB[5]	PCB-156	50	75 – 125	60 – 135	
2,3,3',4,4',5'-HxCB[5]	PCB-157	50	75 – 125	60 – 135	
2,3',4,4',5,5'-HxCB	PCB-167	50	75 – 125	60 – 135	
3,3',4,4',5,5'-HxCB	PCB-169	50	75 – 125	60 – 135	
2,2',3,4',5,6,6'-HpCB	PCB-188	50	75 – 125	60 – 135	
2,3,3',4,4',5,5'-HpCB	PCB-189	50	75 – 125	60 – 135	
2,2',3,3',5,5',6,6'-OcCB	PCB-202	50	75 – 125	60 – 135	
2,3,3',4,4',5,5',6-OcCB	PCB-205	50	75 – 125	60 – 135	
2,2',3,3',4,4',5,5',6-NoCB	PCB-206	50	75 – 125	60 – 135	
2,2',3,3',4,5,5',6,6'-NoCB	PCB-208	50	75 – 125	60 – 135	
DeCB	PCB-209	50	75 – 125	60 – 135	
$^{13}C_{12}$-2-MoCB	PCB-1L	100	50 – 145	15 – 145	5 – 145
$^{13}C_{12}$-4-MoCB	PCB-3L	100	50 – 145	15 – 145	5 – 145
$^{13}C_{12}$-2,2'-DiCB	PCB-4L	100	50 – 145	15 – 145	5 – 145
$^{13}C_{12}$-4,4'-DiCB	PCB-15L	100	50 – 145	15 – 145	5 – 145
$^{13}C_{12}$-2,2',6-TrCB	PCB-19L	100	50 – 145	15 – 145	5 – 145
$^{13}C_{12}$-3,4,4'-TrCB	PCB-37L	100	50 – 145	15 – 145	5 – 145
$^{13}C_{12}$-2,2',6,6'-TeCB	PCB-54L	100	50 – 145	15 – 145	5 – 145

TABLE 5. QUALITY CONTROL ACCEPTANCE CRITERIA FOR CHLORINATED BIPHENYLS IN CALIBRATION VERIFICATION, LABORATORY CONTROL SAMPLE/LABORATORY CONTROL SAMPLE DUPLICATE, AND SAMPLES[1] (CON'T)

Congener Name	Analyte Name[2]	Test Conc (ng/mL)[3]	VER(%)[4]	LCS/LCSD Recovery (%)	Labeled Compound Recovery in Samples (%)
$^{13}C_{12}$-3,3',4,4'-TeCB	PCB-77L	100	50 – 145	40 – 145	10 – 145
$^{13}C_{12}$-3,4,4',5-TeCB	PCB-81L	100	50 – 145	40 – 145	10 – 145
$^{13}C_{12}$-2,2',4,6,6'-PeCB	PCB-104L	100	50 – 145	40 – 145	10 – 145
$^{13}C_{12}$-2,3,3',4,4'-PeCB	PCB-105L	100	50 – 145	40 – 145	10 – 145
$^{13}C_{12}$-2,3,4,4',5-PeCB	PCB-114L	100	50 – 145	40 – 145	10 – 145
$^{13}C_{12}$-2,3',4,4',5-PeCB	PCB-118L	100	50 – 145	40 – 145	10 – 145
$^{13}C_{12}$-2',3,4,4',5-PeCB	PCB-123L	100	50 – 145	40 – 145	10 – 145
$^{13}C_{12}$-3,3',4,4',5-PeCB	PCB-126L	100	50 – 145	40 – 145	10 – 145
$^{13}C_{12}$-2,2',4,4',6,6'-HxCB	PCB-155L	100	50 – 145	40 – 145	10 – 145
$^{13}C_{12}$-2,3,3',4,4',5–HxCB[5]	PCB-156L	100	50 – 145	40 – 145	10 – 145
$^{13}C_{12}$-2,3,3',4,4',5'-HxCB[5]	PCB-157L	100	50 – 145	40 – 145	10 – 145
$^{13}C_{12}$-2,3',4,4',5,5'-HxCB	PCB-167L	100	50 – 145	40 – 145	10 – 145
$^{13}C_{12}$-3,3',4,4',5,5'-HxCB	PCB-169L	100	50 – 145	40 – 145	10 – 145
$^{13}C_{12}$-2,2',3,4',5,6,6'-HpCB	PCB-188L	100	50 – 145	40 – 145	10 – 145
$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB	PCB-189L	100	50 – 145	40 – 145	10 – 145
$^{13}C_{12}$-2,2',3,3',5,5',6,6'-OcCB	PCB-202L	100	50 – 145	40 – 145	10 – 145
$^{13}C_{12}$-2,3,3',4,4',5,5',6-OcCB	PCB-205L	100	50 – 145	40 – 145	10 – 145
$^{13}C_{12}$-2,2',3,3',4,4',5,5',6-NoCB	PCB-206L	100	50 – 145	40 – 145	10 – 145
$^{13}C_{12}$-2,2',3,3',4,5,5',6,6'-NoCB	PCB-208L	100	50 – 145	40 – 145	10 – 145
$^{13}C_{12}$-2,2',3,3',4,4',5,5',6,6'-DeCB	PCB-209L	100	50 – 145	40 – 145	10 – 145
Cleanup Standards					
$^{13}C_{12}$-2,4,4'-TrCB	PCB-28L	100	65 – 135	15 – 145	5 - 145
$^{13}C_{12}$-2,3,3',5,5'-PeCB	PCB-111L	100	75 – 125	40 – 145	10 – 145
$^{13}C_{12}$-2,2',3,3',5,5',6-HpCB	PCB-178L	100	75 – 125	40 – 145	10 – 145

[1]QC acceptance criteria for LCS, LCSD, and samples based on a 20 µL extract final volume.

[2]Suffix "L" indicates labeled compound.

[3]See Exhibit D – CBC, Table 6.

[4]See Section 9.6.4.

[5]Congeners 156 and 157 are tested as the sum of two concentrations.

TABLE 6. CONCENTRATIONS OF CHLORINATED BIPHENYL CONGENERS IN CALIBRATION AND VERIFICATION SOLUTIONS

CB Congener	Analyte Name[1]	Solution Concentration (ng/mL)				
		CS1	CS2	CS3 (CCV)	CS4	CS5
2-MoCB	PCB-1	1.0	5.0	50	400	2000
4-MoCB	PCB-3	1.0	5.0	50	400	2000
2,2'-DiCB	PCB-4	1.0	5.0	50	400	2000
4,4'-DiCB	PCB-15	1.0	5.0	50	400	2000
2,2',6-TrCB	PCB-19	1.0	5.0	50	400	2000
3,4,4'-TrCB	PCB-37	1.0	5.0	50	400	2000
2,2',6,6'-TeCB	PCB-54	1.0	5.0	50	400	2000
3,3',4,4'-TeCB	PCB-77	1.0	5.0	50	400	2000
3,4,4',5-TeCB	PCB-81	1.0	5.0	50	400	2000
2,2',4,6,6'-PeCB	PCB-104	1.0	5.0	50	400	2000
2,3,3',4,4'-PeCB	PCB-105	1.0	5.0	50	400	2000
2,3,4,4',5-PeCB	PCB-114	1.0	5.0	50	400	2000
2,3',4,4',5-PeCB	PCB-118	1.0	5.0	50	400	2000
2',3,4,4',5-PeCB	PCB-123	1.0	5.0	50	400	2000
3,3',4,4',5-PeCB	PCB-126	1.0	5.0	50	400	2000
2,2',4,4',6,6'-HxCB	PCB-155	1.0	5.0	50	400	2000
2,3,3',4,4',5-HxCB	PCB-156	1.0	5.0	50	400	2000
2,3,3',4,4',5'-HxCB	PCB-157	1.0	5.0	50	400	2000
2,3',4,4',5,5'-HxCB	PCB-167	1.0	5.0	50	400	2000
3,3',4,4',5,5'-HxCB	PCB-169	1.0	5.0	50	400	2000
2,2',3,4',5,6,6'-HpCB	PCB-188	1.0	5.0	50	400	2000
2,3,3',4,4',5,5'-HpCB	PCB-189	1.0	5.0	50	400	2000
2,2',3,3',5,5',6,6'-OcCB	PCB-202	1.0	5.0	50	400	2000
2,3,3',4,4',5,5',6-OcCB	PCB-205	1.0	5.0	50	400	2000
2,2',3,3',4,4',5,5',6-NoCB	PCB-206	1.0	5.0	50	400	2000
2,2',3,3',4,5,5',6,6'-NoCB	PCB-208	1.0	5.0	50	400	2000
DeCB	PCB-209	1.0	5.0	50	400	2000
$^{13}C_{12}$-2-MoCB	PCB-1L	100	100	100	100	100
$^{13}C_{12}$-4-MoCB	PCB-3L	100	100	100	100	100
$^{13}C_{12}$-2,2'-DiCB	PCB-4L	100	100	100	100	100
$^{13}C_{12}$-4,4'-DiCB	PCB-15L	100	100	100	100	100
$^{13}C_{12}$-2,2',6-TrCB	PCB-19L	100	100	100	100	100
$^{13}C_{12}$-3,4,4'-TrCB	PCB-37L	100	100	100	100	100
$^{13}C_{12}$-2,2',6,6'-TeCB	PCB-54L	100	100	100	100	100
$^{13}C_{12}$-3,3',4,4'-TeCB	PCB-77L	100	100	100	100	100
$^{13}C_{12}$-3,4,4',5-TeCB	PCB-81L	100	100	100	100	100
$^{13}C_{12}$-2,2',4,6,6'-PeCB	PCB-104L	100	100	100	100	100
$^{13}C_{12}$-2,3,3',4,4'-PeCB	PCB-105L	100	100	100	100	100
$^{13}C_{12}$-2,3,4,4',5-PeCB	PCB-114L	100	100	100	100	100
$^{13}C_{12}$-2,3',4,4',5-PeCB	PCB-118L	100	100	100	100	100
$^{13}C_{12}$-2',3,4,4',5-PeCB	PCB-123L	100	100	100	100	100
$^{13}C_{12}$-3,3',4,4',5-PeCB	PCB-126L	100	100	100	100	100

TABLE 6. CONCENTRATIONS OF CHLORINATED BIPHENYL CONGENERS IN CALIBRATION AND VERIFICATION SOLUTIONS (CON'T)

CB Congener	Analyte Name[1]	Solution Concentration (ng/mL)				
		CS1	CS2	CS3 (CCV)	CS4	CS5
$^{13}C_{12}$-2,2',4,4',6,6'-HxCB	PCB-155L	100	100	100	100	100
$^{13}C_{12}$-2,3,3',4,4',5-HxCB	PCB-156L	100	100	100	100	100
$^{13}C_{12}$-2,3,3',4,4',5'-HxCB	PCB-157L	100	100	100	100	100
$^{13}C_{12}$-2,3',4,4',5,5'-HxCB	PCB-167L	100	100	100	100	100
$^{13}C_{12}$-3,3',4,4',5,5'-HxCB	PCB-169L	100	100	100	100	100
$^{13}C_{12}$-2,2',3,4',5,6,6'-HpCB	PCB-188L	100	100	100	100	100
$^{13}C_{12}$-2,3,3',4,4',5,5'-HpCB	PCB-189L	100	100	100	100	100
$^{13}C_{12}$-2,2',3,3',5,5',6,6'-OcCB	PCB-202L	100	100	100	100	100
$^{13}C_{12}$-2,3,3',4,4',5,5',6-OcCB	PCB-205L	100	100	100	100	100
$^{13}C_{12}$-2,2',3,3',4,4',5,5',6-NoCB	PCB-206L	100	100	100	100	100
$^{13}C_{12}$-2,2',3,3',4,5,5',6,6'-NoCB	PCB-208L	100	100	100	100	100
$^{13}C_{12}$-DeCB	PCB-209L	100	100	100	100	100
Cleanup Standard						
$^{13}C_{12}$-2,4,4'-TrCB	PCB-28L	100	100	100	100	100
$^{13}C_{12}$-2,3,3',5,5'-PeCB	PCB-111L	100	100	100	100	100
$^{13}C_{12}$-2,2',3,3',5,5',6-HpCB	PCB-178L	100	100	100	100	100
Internal Standard						
$^{13}C_{12}$-2,5-DiCB	PCB-9L	100	100	100	100	100
$^{13}C_{12}$-2,2',5,5'-TeCB	PCB-52L	100	100	100	100	100
$^{13}C_{12}$-2,2',4',5,5'-PeCB	PCB-101L	100	100	100	100	100
$^{13}C_{12}$-2,2',3',4,4',5'-HxCB	PCB-138L	100	100	100	100	100
$^{13}C_{12}$-2,2',3,3',4,4',5,5'-OcCB	PCB-194L	100	100	100	100	100

[1]Suffix "L" indicates labeled compound.

TABLE 7. RETENTION TIME REFERENCES, QUANTITATION REFERENCES, AND RELATIVE RETENTION TIMES FOR CHLORINATED BIPHENYL CONGENERS ON SPB-OCTYL

Cl No.[1]	Congener No.[2,3]	RT Ref[4]	RT[5]	RRT[6]	RRT Limits[7]	Window (sec)[8]	Quantitation Reference[9]
Compounds using 9L ($^{13}C_{12}$-2,5-DiCB) as internal standard							
CB Congener							
	Monochlorobiphenyls						
1	1	1L	13:44	1.0012	0.9988-1.0036	-1+3	1L
1	2	3L	16:08	0.9878	0.9847-0.9908	6	1L/3L
1	3	3L	16:21	1.0010	0.9990-1.0031	-1+3	3L
	Dichlorobiphenyls						
2	4	4L	16:40	1.0010	0.9990-1.0030	-1+3	4L
2	10	4L	16:53	1.0140	1.0110-1.0170	6	4L/15L
2	9	4L	18:55	1.1361	1.1331-1.1391	6	4L/15L
2	7	4L	19:07	1.1481	1.1451-1.1512	6	4L/15L
2	6	4L	19:26	1.1672	1.1642-1.1702	6	4L/15L
2	5	4L	19:48	1.1892	1.1862-1.1922	6	4L/15L
2	8	4L	19:56	1.1972	1.1942-1.2002	6	4L/15L
2	14	15L	21:42	0.9267	0.9246-0.9288	6	4L/15L
2	11	15L	22:42	0.9694	0.9673-0.9715	6	4L/15L
2	13	15L	23:03	0.9843	0.9822-0.9865	6	4L/15L
2	12	15L	23:06	0.9865	0.9843-0.9886	6	4L/15L
2	13/12	15L	23:04	0.9851	0.9829-0.9872	6	4L/15L
2	15	15L	23:26	1.0007	0.9993-1.0021	-1+3	15L
	Trichlorobiphenyls						
3	19	19L	20:19	1.0008	0.9992-1.0025	-1+3	19L
3	30	19L	22:15	1.0961	1.0936-1.0985	6	19L/37L
3	18	19L	22:23	1.1026	1.1002-1.1051	6	19L/37L
3	30/18	19L	22:19	1.0993	1.0969-1.1018	6	19L/37L
3	17	19L	22:49	1.1240	1.1215-1.1264	6	19L/37L
3	27	19L	23:06	1.1379	1.1355-1.1404	6	19L/37L
3	24	19L	23:14	1.1445	1.1420-1.1470	6	19L/37L
3	16	19L	23:25	1.1535	1.1511-1.1560	6	19L/37L
3	32	19L	24:57	1.2291	1.2266-1.2315	6	19L/37L
3	34	19L	25:17	1.2455	1.2430-1.2479	6	19L/37L
3	23	19L	25:26	1.2529	1.2504-1.2553	6	19L/37L
3	29	19L	25:47	1.2701	1.2660-1.2742	10	19L/37L

TABLE 7. RETENTION TIME REFERENCES, QUANTITATION REFERENCES, AND RELATIVE RETENTION TIMES FOR CHLORINATED BIPHENYL CONGENERS ON SPB-OCTYL (CON'T)

Cl No.[1]	Congener No.[2,3]	RT Ref[4]	RT[5]	RRT[6]	RRT Limits[7]	Window (sec)[8]	Quantitation Reference[9]
3	26	19L	25:48	1.2709	1.2668-1.2750	10	19L/37L
3	26/29	19L	25:48	1.2709	1.2668-1.2750	10	19L/37L
3	25	37L	26:04	0.8364	0.8348-0.8380	6	19L/37L
3	31	37L	26:25	0.8476	0.8460-0.8492	6	19L/37L
3	28	37L	26:44	0.8578	0.8551-0.8604	10	19L/37L
3	20	37L	26:49	0.8604	0.8578-0.8631	10	19L/37L
3	28/20	37L	26:47	0.8594	0.8567-0.8620	10	19L/37L
3	21	37L	26:58	0.8652	0.8626-0.8679	10	19L/37L
3	33	37L	27:01	0.8668	0.8642-0.8695	10	19L/37L
3	21/33	37L	26:59	0.8658	0.8631-0.8684	10	19L/37L
3	22	37L	27:29	0.8818	0.8802-0.8834	6	19L/37L
3	36	37L	29:05	0.9332	0.9316-0.9348	6	19L/37L
3	39	37L	29:30	0.9465	0.9449-0.9481	6	19L/37L
3	38	37L	30:10	0.9679	0.9663-0.9695	6	19L/37L
3	35	37L	30:42	0.9850	0.9834-0.9866	6	19L/37L
3	37	37L	31:11	1.0005	0.9995-1.0011	-1+3	37L
Labeled Compounds							
1	1L	9L	13:43	0.7257	0.7125-0.7390	30	9L
1	3L	9L	16:20	0.8642	0.8510-0.8774	30	9L
2	4L	9L	16:39	0.8810	0.8677-0.8942	30	9L
2	15L	9L	23:25	1.2390	1.2302-1.2478	20	9L
3	19L	9L	20:18	1.0741	1.0608-1.0873	30	9L
3	37L	52L	31:10	1.0841	1.0754-1.0928	30	52L
Compounds using 52L ($^{13}C_{12}$-2,2',5,5'-TeCB) as internal standard							
CB Congener							
Tetrachlorobiphenyls							
4	54	54L	23:51	1.0007	0.9993-1.0021	-1+3	54L
4	50	54L	26:07	1.0958	1.0923-1.0993	10	54L/81L/77L
4	53	54L	26:09	1.0972	1.0937-1.1007	10	54L/81L/77L
4	50/53	54L	26:08	1.0965	1.0930-1.1000	10	54L/81L/77L
4	45	54L	26:55	1.1294	1.1259-1.1329	10	54L/81L/77L
4	51	54L	26:58	1.1315	1.1280-1.1350	10	54L/81L/77L
4	45/51	54L	26:57	1.1308	1.1273-1.1343	10	54L/81L/77L

TABLE 7. RETENTION TIME REFERENCES, QUANTITATION REFERENCES, AND RELATIVE RETENTION TIMES FOR CHLORINATED BIPHENYL CONGENERS ON SPB-OCTYL (CON'T)

Cl No.[1]	Congener No.[2,3]	RT Ref[4]	RT[5]	RRT[6]	RRT Limits[7]	Window (sec)[8]	Quantitation Reference[9]
4	46	54L	27:18	1.1455	1.1434-1.1476	6	54L/81L/77L
4	52	54L	28:45	1.2063	1.2042-1.2084	6	54L/81L/77L
4	73	54L	28:52	1.2112	1.2091-1.2133	6	54L/81L/77L
4	43	54L	28:58	1.2154	1.2133-1.2175	6	54L/81L/77L
4	69	54L	29:08	1.2224	1.2189-1.2259	10	54L/81L/77L
4	49	54L	29:16	1.2280	1.2245-1.2315	10	54L/81L/77L
4	69/49	54L	29:12	1.2252	1.2217-1.2287	10	54L/81L/77L
4	48	54L	29:33	1.2399	1.2378-1.2420	6	54L/81L/77L
4	65	54L	29:49	1.2510	1.2476-1.2545	10	54L/81L/77L
4	47	54L	29:50	1.2517	1.2483-1.2552	10	54L/81L/77L
4	44	54L	29:53	1.2538	1.2503-1.2573	10	54L/81L/77L
4	44/47/65	54L	29:50	1.2517	1.2483-1.2552	10	54L/81L/77L
4	62	54L	30:06	1.2629	1.2594-1.2664	10	54L/81L/77L
4	75	54L	30:08	1.2643	1.2608-1.2678	10	54L/81L/77L
4	59	54L	30:12	1.2671	1.2636-1.2706	10	54L/81L/77L
4	59/62/75	54L	30:09	1.2650	1.2615-1.2685	10	54L/81L/77L
4	42	54L	30:26	1.2769	1.2748-1.2790	6	54L/81L/77L
4	41	54L	30:52	1.2951	1.2916-1.2986	10	54L/81L/77L
4	71	54L	30:58	1.2993	1.2958-1.3028	10	54L/81L/77L
4	40	54L	31:01	1.3014	1.2979-1.3049	10	54L/81L/77L
4	41/40/71	54L	30:58	1.2993	1.2958-1.3028	10	54L/81L/77L
4	64	54L	31:12	1.3091	1.3070-1.3112	6	54L/81L/77L
4	72	81L	31:59	0.8336	0.8323-0.8349	6	54L/81L/77L
4	68	81L	32:18	0.8419	0.8406-0.8432	6	54L/81L/77L
4	57	81L	32:46	0.8540	0.8527-0.8553	6	54L/81L/77L
4	58	81L	33:05	0.8623	0.8610-0.8636	6	54L/81L/77L
4	67	81L	33:13	0.8658	0.8645-0.8671	6	54L/81L/77L
4	63	81L	33:30	0.8732	0.8719-0.8745	6	54L/81L/77L
4	61	81L	33:46	0.8801	0.8775-0.8827	12	54L/81L/77L
4	70	81L	33:53	0.8831	0.8805-0.8858	12	54L/81L/77L
4	76	81L	33:55	0.8840	0.8814-0.8866	12	54L/81L/77L
4	74	81L	33:57	0.8849	0.8827-0.8871	10	54L/81L/77L
4	61/70/74/76	81L	33:55	0.8840	0.8814-0.8866	12	54L/81L/77L

TABLE 7. RETENTION TIME REFERENCES, QUANTITATION REFERENCES, AND RELATIVE RETENTION TIMES FOR CHLORINATED BIPHENYL CONGENERS ON SPB-OCTYL (CON'T)

Cl No.[1]	Congener No.[2,3]	RT Ref[4]	RT[5]	RRT[6]	RRT Limits[7]	Window (sec)[8]	Quantitation Reference[9]
4	66	81L	34:15	0.8927	0.8914-0.8940	6	54L/81L/77L
4	55	81L	34:28	0.8983	0.8970-0.8997	6	54L/81L/77L
4	56	81L	35:03	0.9136	0.9123-0.9149	6	54L/81L/77L
4	60	81L	35:16	0.9192	0.9179-0.9205	6	54L/81L/77L
4	80	81L	35:32	0.9262	0.9248-0.9275	6	54L/81L/77L
4	79	81L	37:16	0.9713	0.9700-0.9726	6	54L/81L/77L
4	78	81L	37:52	0.9870	0.9857-0.9883	6	54L/81L/77L
4	81	81L	38:23	1.0004	0.9996-1.0013	-1+3	81L
4	77	77L	39:02	1.0004	0.9996-1.0013	-1+3	77L
Labeled Compounds							
4	54L	52L	23:50	0.8290	0.8232-0.8348	20	52L
4	81L	52L	38:22	1.3345	1.3287-1.3403	20	52L
4	77L	52L	39:01	1.3571	1.3513-1.3629	20	52L
Compounds using 101L ($^{13}C_{12}$-2,2',4,5,5'-PeCB) as internal standard							
CB Congener							
Pentachlorobiphenyls							
5	104	104L	29:46	1.0000	0.9994-1.0017	-1+3	104L
5	96	104L	30:17	1.0174	1.0146-1.0202	10	104L/123L/114L/118L/105L
5	103	104L	32:11	1.0812	1.0795-1.0829	6	104L/123L/114L/118L/105L
5	94	104L	32:29	1.0913	1.0896-1.0929	6	104L/123L/114L/118L/105L
5	95	104L	33:00	1.1086	1.1058-1.1114	10	104L/123L/114L/118L/105L
5	100	104L	33:06	1.1120	1.1092-1.1148	10	104L/123L/114L/118L/105L
5	93	104L	33:14	1.1165	1.1137-1.1193	10	104L/123L/114L/118L/105L
5	102	104L	33:21	1.1204	1.1176-1.1232	10	104L/123L/114L/118L/105L
5	98	104L	33:26	1.1232	1.1204-1.1260	10	104L/123L/114L/118L/105L
5	95/100/93/102/98	104L	33:13	1.1159	1.1131-1.1187	15	104L/123L/114L/118L/105L
5	88	104L	33:48	1.1355	1.1321-1.1389	12	104L/123L/114L/118L/105L
5	91	104L	33:55	1.1394	1.1366-1.1422	10	104L/123L/114L/118L/105L
5	88/91	104L	33:52	1.1377	1.1344-1.1411	12	104L/123L/114L/118L/105L
5	84	104L	34:14	1.1501	1.1484-1.1517	6	104L/123L/114L/118L/105L
5	89	104L	34:44	1.1669	1.1652-1.1685	6	104L/123L/114L/118L/105L
5	121	104L	34:57	1.1741	1.1725-1.1758	6	104L/123L/114L/118L/105L
5	92	123L	35:26	0.8639	0.8627-0.8651	6	104L/123L/114L/118L/105L
5	113	123L	36:01	0.8781	0.8761-0.8801	10	104L/123L/114L/118L/105L

TABLE 7. RETENTION TIME REFERENCES, QUANTITATION REFERENCES, AND RELATIVE RETENTION TIMES FOR CHLORINATED BIPHENYL CONGENERS ON SPB-OCTYL (CON'T)

Cl No.[1]	Congener No.[2,3]	RT Ref[4]	RT[5]	RRT[6]	RRT Limits[7]	Window (sec)[8]	Quantitation Reference[9]
5	90	123L	36:03	0.8789	0.8769-0.8809	10	104L/123L/114L/118L/105L
5	101	123L	36:04	0.8793	0.8773-0.8813	10	104L/123L/114L/118L/105L
5	113/90/101	123L	36:03	0.8789	0.8769-0.8809	10	104L/123L/114L/118L/105L
5	83	123L	36:39	0.8935	0.8911-0.8960	12	104L/123L/114L/118L/105L
5	99	123L	36:41	0.8944	0.8923-0.8964	10	104L/123L/114L/118L/105L
5	83/99	123L	36:40	0.8939	0.8915-0.8964	12	104L/123L/114L/118L/105L
5	112	123L	36:51	0.8984	0.8972-0.8996	6	104L/123L/114L/118L/105L
5	119	123L	37:12	0.9069	0.9037-0.9102	16	104L/123L/114L/118L/105L
5	108	123L	37:12	0.9069	0.9037-0.9102	16	104L/123L/114L/118L/105L
5	86	123L	37:17	0.9090	0.9057-0.9122	16	104L/123L/114L/118L/105L
5	97	123L	37:17	0.9090	0.9057-0.9122	16	104L/123L/114L/118L/105L
5	125	123L	37:21	0.9106	0.9074-0.9139	16	104L/123L/114L/118L/105L
5	87	123L	37:25	0.9122	0.9102-0.9143	10	104L/123L/114L/118L/105L
5	108/119/86/97/125/87	123L	37:19	0.9098	0.9065-0.9130	16	104L/123L/114L/118L/105L
5	117	123L	37:57	0.9252	0.9228-0.9277	12	104L/123L/114L/118L/105L
5	116	123L	38:02	0.9273	0.9248-0.9297	12	104L/123L/114L/118L/105L
5	85	123L	38:05	0.9285	0.9265-0.9305	10	104L/123L/114L/118L/105L
5	117/116/85	123L	38:00	0.9265	0.9240-0.9289	12	104L/123L/114L/118L/105L
5	110	123L	38:16	0.9330	0.9309-0.9350	10	104L/123L/114L/118L/105L
5	115	123L	38:18	0.9338	0.9317-0.9358	10	104L/123L/114L/118L/105L
5	110/115	123L	38:17	0.9334	0.9313-0.9354	10	104L/123L/114L/118L/105L
5	82	123L	38:40	0.9427	0.9415-0.9439	6	104L/123L/114L/118L/105L
5	111	123L	38:52	0.9476	0.9464-0.9488	6	104L/123L/114L/118L/105L
5	120	123L	39:21	0.9594	0.9581-0.9606	6	104L/123L/114L/118L/105L
5	107	123L	40:39	0.9911	0.9890-0.9931	10	104L/123L/114L/118L/105L
5	124	123L	40:40	0.9915	0.9894-0.9935	10	104L/123L/114L/118L/105L
5	107/124	123L	40:39	0.9911	0.9890-0.9931	10	104L/123L/114L/118L/105L
5	109	123L	40:54	0.9972	0.9959-0.9984	6	104L/123L/114L/118L/105L
5	123	123L	41:02	1.0004	0.9996-1.0012	-1+3	123L
5	106	123L	41:10	1.0037	1.0024-1.0049	6	104L/123L/114L/118L/105L
5	118	118L	41:22	1.0004	0.9996-1.0012	-1+3	118L
5	122	118L	41:49	1.0113	1.0101-1.0125	6	104L/123L/114L/118L/105L
5	114	114L	41:58	1.0004	0.9999-1.0012	-1+3	114L
5	105	105L	42:43	0.9996	0.9992-1.0012	-1+3	105L
5	127	105L	44:09	1.0332	1.0320-1.0343	6	104L/123L/114L/118L/105L
5	126	126L	45:58	1.0004	0.9996-1.0011	-1+3	126L

TABLE 7. RETENTION TIME REFERENCES, QUANTITATION REFERENCES, AND RELATIVE RETENTION TIMES FOR CHLORINATED BIPHENYL CONGENERS ON SPB-OCTYL (CON'T)

Cl No.[1]	Congener No.[2,3]	RT Ref[4]	RT[5]	RRT[6]	RRT Limits[7]	Window (sec)[8]	Quantitation Reference[9]
Labeled Compounds							
5	104L	101L	29:46	0.8257	0.8211-0.8303	20	101L
5	123L	101L	41:01	1.1378	1.1331-1.1424	20	101L
5	118L	101L	41:21	1.1470	1.1424-1.1516	20	101L
5	114L	101L	41:57	1.1637	1.1590-1.1683	20	101L
5	105L	101L	42:44	1.1854	1.1808-1.1900	20	101L
5	126L	101L	45:57	1.2746	1.2700-1.2792	20	101L
Compounds using 138L ($^{13}C_{12}$-2,2',3,4,4',5'-HxCB) as internal standard							
CB Congener							
Hexachlorobiphenyls							
6	155	155L	35:44	1.0000	0.9995-1.0014	-1+3	155L
6	152	155L	36:07	1.0107	1.0093-1.0121	6	155L/156L/157L/167L/169L
6	150	155L	36:15	1.0145	1.0131-1.0159	6	155L/156L/157L/167L/169L
6	136	155L	36:44	1.0280	1.0266-1.0294	6	155L/156L/157L/167L/169L
6	145	155L	37:00	1.0354	1.0340-1.0368	6	155L/156L/157L/167L/169L
6	148	155L	34:26	1.0756	1.0742-1.0770	6	155L/156L/157L/167L/169L
6	151	155L	39:10	1.0961	1.0938-1.0984	10	155L/156L/157L/167L/169L
6	135	155L	39:17	1.0993	1.0970-1.1017	10	155L/156L/157L/167L/169L
6	154	155L	39:21	1.1012	1.0989-1.1035	10	155L/156L/157L/167L/169L
6	151/135/154	155L	39:15	1.0984	1.0961-1.1007	10	155L/156L/157L/167L/169L
6	144	155L	39:47	1.1133	1.1119-1.1147	6	155L/156L/157L/167L/169L
6	147	155L	40:09	1.1236	1.1213-1.1259	10	155L/156L/157L/167L/169L
6	149	155L	40:12	1.1250	1.1227-1.1273	10	155L/156L/157L/167L/169L
6	147/149	155L	40:10	1.1241	1.1217-1.1264	10	155L/156L/157L/167L/169L
6	134	155L	40:27	1.1320	1.1297-1.1343	10	155L/156L/157L/167L/169L
6	143	155L	40:30	1.1334	1.1311-1.1357	10	155L/156L/157L/167L/169L
6	134/143	155L	40:29	1.1329	1.1306-1.1353	10	155L/156L/157L/167L/169L
6	139	155L	40:47	1.1413	1.1390-1.1437	10	155L/156L/157L/167L/169L
6	140	155L	40:48	1.1418	1.1395-1.1441	10	155L/156L/157L/167L/169L
6	139/140	155L	40:47	1.1413	1.1390-1.1437	10	155L/156L/157L/167L/169L
6	131	155L	41:03	1.1488	1.1474-1.1502	6	155L/156L/157L/167L/169L
6	142	155L	41:13	1.1535	1.1521-1.1549	6	155L/156L/157L/167L/169L
6	132	155L	41:36	1.1642	1.1618-1.1665	10	155L/156L/157L/167L/169L

TABLE 7. RETENTION TIME REFERENCES, QUANTITATION REFERENCES, AND RELATIVE RETENTION TIMES FOR CHLORINATED BIPHENYL CONGENERS ON SPB-OCTYL (CON'T)

Cl No.[1]	Congener No.[2,3]	RT Ref[4]	RT[5]	RRT[6]	RRT Limits[7]	Window (sec)[8]	Quantitation Reference[9]
6	133	155L	41:57	1.1740	1.1726-1.1754	6	155L/156L/157L/167L/169L
6	165	167L	42:23	0.8864	0.8853-0.8874	6	155L/156L/157L/167L/169L
6	146	167L	42:38	0.8916	0.8906-0.8926	6	155L/156L/157L/167L/169L
6	161	167L	42:47	0.8947	0.8937-0.8958	6	155L/156L/157L/167L/169L
6	153	167L	43:17	0.9052	0.9035-0.9069	10	155L/156L/157L/167L/169L
6	168	167L	43:21	0.9066	0.9048-0.9083	10	155L/156L/157L/167L/169L
6	153/168	167L	43:19	0.9059	0.9041-0.9076	10	155L/156L/157L/167L/169L
6	141	167L	43:34	0.9111	0.9101-0.9122	6	155L/156L/157L/167L/169L
6	130	167L	44:01	0.9205	0.9195-0.9216	6	155L/156L/157L/167L/169L
6	137	167L	44:14	0.9251	0.9240-0.9261	6	155L/156L/157L/167L/169L
6	164	167L	44:22	0.9278	0.9268-0.9289	6	155L/156L/157L/167L/169L
6	138	167L	44:42	0.9348	0.9324-0.9373	14	155L/156L/157L/167L/169L
6	163	167L	44:42	0.9348	0.9324-0.9373	14	155L/156L/157L/167L/169L
6	129	167L	44:47	0.9366	0.9341-0.9390	14	155L/156L/157L/167L/169L
6	160	167L	44:53	0.9387	0.9369-0.9404	10	155L/156L/157L/167L/169L
6	138/163/129/160	167L	44:47	0.9366	0.9341-0.9390	14	155L/156L/157L/167L/169L
6	158	167L	45:05	0.9428	0.9418-0.9439	6	155L/156L/157L/167L/169L
6	166	167L	45:59	0.9617	0.9599-0.9634	10	155L/156L/157L/167L/169L
6	128	167L	46:09	0.9651	0.9634-0.9669	10	155L/156L/157L/167L/169L
6	128/166	167L	46:04	0.9634	0.9617-0.9651	10	155L/156L/157L/167L/169L
6	159	167L	46:59	0.9826	0.9815-0.9836	6	155L/156L/157L/167L/169L
6	162	167L	47:18	0.9892	0.9881-0.9902	6	155L/156L/157L/167L/169L
6	167	167L	47:49	1.0000	0.9997-1.0010	-1+3	167L
6	156	156L/157L	49:05	0.9993	0.9983-1.0003	6	156L/157L
6	157	156L/157L	49:09	1.0007	0.9990-1.0024	10	156L/157L
6	156/157	156L/157L	45:07	1.0000	0.9990-1.0010	6	156L/157L
6	169	169L	52:31	1.0003	0.9997-1.0010	-1+3	169L
Labeled Compounds							
6	155L	138L	35:44	0.7997	0.7960-0.8034	20	138L
6	167L	138L	47:49	1.0701	1.0664-1.0739	20	138L
6	156L	138L	49:05	1.0985	1.0974-1.0996	20	138L
6	157L	138L	49:08	1.0996	1.0959-1.1033	20	138L
6	169L	138L	52:30	1.1749	1.1738-1.1761	20	138L
6	156L/157L	138L	49:07	1.0992	1.0981-1.1003	20	138L

TABLE 7. RETENTION TIME REFERENCES, QUANTITATION REFERENCES, AND RELATIVE RETENTION TIMES FOR CHLORINATED BIPHENYL CONGENERS ON SPB-OCTYL (CON'T)

Cl No.[1]	Congener No.[2,3]	RT Ref[4]	RT[5]	RRT[6]	RRT Limits[7]	Window (sec)[8]	Quantitation Reference[9]
Compounds using 194L($^{13}C_{12}$-2,2',3,3',4,4',5,5'-OcCB) as internal standard							
CB Congener							
Heptachlorobiphenyls							
7	188	188L	41:51	1.0000	0.9996-1.0012	-1+3	188L
7	179	188L	42:19	1.0112	1.0100-1.0123	6	188L/189L
7	184	188L	42:45	1.0215	1.0203-1.0227	6	188L/189L
7	176	188L	43:15	1.0335	1.0323-1.0346	6	188L/189L
7	186	188L	43:45	1.0454	1.0442-1.0466	6	188L/189L
7	178	188L	45:06	1.0777	1.0765-1.0789	6	188L/189L
7	175	188L	45:46	1.0936	1.0924-1.0948	6	188L/189L
7	187	188L	46:02	1.1000	1.0988-1.1012	6	188L/189L
7	182	188L	46:14	1.1047	1.1035-1.1059	6	188L/189L
7	183	188L	46:42	1.1159	1.1147-1.1171	6	188L/189L
7	185	188L	46:53	1.1203	1.1191-1.1215	6	188L/189L
7	183/185	188L	46:47	1.1179	1.1167-1.1191	6	188L/189L
7	174	188L	47:02	1.1239	1.1227-1.1251	6	188L/189L
7	177	188L	47:30	1.1350	1.1338-1.1362	6	188L/189L
7	181	188L	47:52	1.1438	1.1426-1.1450	6	188L/189L
7	171	188L	48:10	1.1509	1.1489-1.1529	10	188L/189L
7	173	188L	48:11	1.1513	1.1501-1.1525	6	188L/189L
7	171/173	188L	48:10	1.1509	1.1489-1.1529	10	188L/189L
7	172	189L	49:47	0.9035	0.9026-0.9044	6	188L/189L
7	192	189L	50:06	0.9093	0.9083-0.9102	6	188L/189L
7	193	189L	50:26	0.9153	0.9144-0.9162	6	188L/189L
7	180	189L	50:27	0.9156	0.9147-0.9165	6	188L/189L
7	180/193	189L	50:26	0.9153	0.9144-0.9162	6	188L/189L
7	191	189L	50:51	0.9229	0.9220-0.9238	6	188L/189L
7	170	189L	51:54	0.9419	0.9410-0.9428	6	188L/189L
7	190	189L	52:26	0.9516	0.9507-0.9525	6	188L/189L
7	189	189L	55:07	1.0003	0.9997-1.0009	-1+3	189L

TABLE 7. RETENTION TIME REFERENCES, QUANTITATION REFERENCES, AND RELATIVE RETENTION TIMES FOR CHLORINATED BIPHENYL CONGENERS ON SPB-OCTYL (CON'T)

Cl No.[1]	Congener No.[2,3]	RT Ref[4]	RT[5]	RRT[6]	RRT Limits[7]	Window (sec)[8]	Quantitation Reference[9]
Octachlorobiphenyls							
8	202	202L	47:32	1.0004	0.9996-1.0011	-1+3	202L
8	201	202L	48:31	1.0210	1.0193-1.0228	10	202L/205L
8	204	202L	49:11	1.0351	1.0340-1.0361	6	202L/205L
8	197	202L	49:27	1.0407	1.0396-1.0417	6	202L/205L
8	200	202L	49:40	1.0452	1.0442-1.0463	6	202L/205L
8	197/200	202L	49:33	1.0428	1.0417-1.0438	6	202L/205L
8	198	202L	52:30	1.1049	1.1031-1.1066	10	202L/205L
8	199	202L	52:32	1.1056	1.1045-1.1066	6	202L/205L
8	198/199	202L	52:31	1.1052	1.1035-1.1070	10	202L/205L
8	196	205L	53:13	0.9207	0.9198-0.9216	6	202L/205L
8	203	205L	53:26	0.9245	0.9236-0.9253	6	202L/205L
8	195	205L	54:55	0.9501	0.9493-0.9510	6	202L/205L
8	194	205L	57:19	0.9916	0.9908-0.9925	6	202L/205L
8	205	205L	57:49	1.0003	0.9997-1.0009	-1+3	205L
Nonachlorobiphenyls							
9	208	208L	54:33	1.0003	0.9997-1.0009	-1+3	208L
9	207	208L	55:32	1.0183	1.0174-1.0193	6	208L/206L
9	206	206L	59:37	1.0003	0.9997-1.0008	-1+3	206L
Decachlorobiphenyl							
10	209	209L	61:15	1.0003	0.9997-1.0008	-1+3	209L
Labeled Compounds							
7	188L	194L	41:51	0.7304	0.7275-0.7333	20	194L
7	180L	194L	50:27	0.8805	0.8775-0.8834	20	194L
7	170L	194L	51:53	0.9055	0.9026-0.9084	20	194L
7	189L	194L	55:06	0.9616	0.9587-0.9645	20	194L
8	202L	194L	47:31	0.8293	0.8264-0.8322	20	194L
8	205L	194L	57:48	1.0087	1.0044-1.0131	30	194L
9	208L	194L	54:32	0.9517	0.9488-0.9546	20	194L
9	206L	194L	59:36	1.0401	1.0358-1.0445	30	194L
10	209L	194L	61:14	1.0686	1.0643-1.0730	30	194L
Cleanup Standards							
3	28L	52L	26:44	0.9266	0.9209-0.9324	20	52L
5	111L	101L	38:51	1.0777	1.0730-1.0823	20	101L
7	178L	138L	45:05	1.0090	1.0052-1.0127	20	138L

TABLE 7. RETENTION TIME REFERENCES, QUANTITATION REFERENCES, AND RELATIVE RETENTION TIMES FOR CHLORINATED BIPHENYL CONGENERS ON SPB-OCTYL (CON'T)

Cl No.[1]	Congener No.[2,3]	RT Ref[4]	RT[5]	RRT[6]	RRT Limits[7]	Window (sec)[8]	Quantitation Reference[9]
Internal Standards							
2	9L	138L	18:54	0.4230	0.4183-0.4276	25	138L
4	52L	138L	28:45	0.6434	0.6388-0.6481	25	138L
5	101L	138L	36:03	0.8068	0.8021-0.8115	25	138L
6	138L	138L	44:41	1.0000	0.9996-1.0011	100	138L
8	194L	138L	57:18	1.2824	1.2777-1.2870	25	138L

[1]Number of Chlorines on congener.

[2]Suffix "L" indicates labeled compound.

[3]Multiple congeners in a box indicate a group of congeners that coelute or may not be adequately resolved on a 30-m SPB-Octyl column. Congeners included in the group are listed as the last entry in the box.

[4]RT reference that is used to locate target congener.

[5]RT of target congener.

[6]RRT between the RT for the congener and RT for the reference.

[7]RRT limits based on RT window. RTs, RRTs, and RRT limits may differ slightly from those in this table.

[8]RT window width for congener or group of two or more congeners.

[9]Labeled congeners that form the quantitation reference. Areas from the exact m/z of the congeners listed in the quantitation reference are summed, and divided by the number of congeners in the quantitation reference. For example, for Chlorinated Biphenyl (CB) 10, the areas at the exact m/z for 4L and 15L are summed and the sum is divided by 2 (because there are two congeners in the quantitation reference).

TABLE 8. GAS CHROMATOGRAPH ANALYTICAL CONDITIONS

Injector Temperature	270°C
Interface Temperature	290°C
Initial Temperature	75°C
Initial Time	2 min.
Temperature Program	75-150°C at 15°C/min. 150-290°C at 2.5°C/min.
Final Time	1 min.

TABLE 9. THEORETICAL ION ABUNDANCE RATIOS AND QUALITY CONTROL LIMITS

Chlorine Atoms	m/z Forming Ratio	Theoretical Ratio	QC Limit	
			Lower	Upper
1	m/m+2	3.13	2.66	3.60
2	m/(m+2)	1.56	1.33	1.79
3	m/(m+2)	1.04	0.88	1.20
4	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
7	(m+2)/(m+4)	1.05	0.89	1.21
8	(m+2)/(m+4)	0.89	0.76	1.02
9	(m+2)/(m+4)	0.77	0.65	0.89
10	(m+4)/(m+6)	1.16	0.99	1.33

TABLE 10. SUGGESTED SAMPLE QUANTITIES TO BE EXTRACTED FOR VARIOUS MATRICES[1]

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

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

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

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

TABLE 11. TOXIC EQUIVALENCY FACTORS

Analyte Name	TEF		
	Mammal	Fish	Bird
PCB-77	0.0001	0.0001	0.05
PCB-81	0.0003	0.0005	0.1
PCB-105	0.00003	0.000005	0.0001
PCB-114	0.00003	0.000005	0.0001
PCB-118	0.00003	0.000005	0.00001
PCB-123	0.00003	0.000005	0.00001
PCB-126	0.1	0.005	0.1
PCB-156	0.00003	0.000005	0.0001
PCB-157	0.00003	0.000005	0.0001
PCB-167	0.00003	0.000005	0.00001
PCB-169	0.03	0.00005	0.001
PCB-189	0.00003	0.000005	0.00001
Source	WHO* 2005	WHO* 1998	

*World Health Organization

APPENDIX A - PRELIMINARY INFORMATION FOR DETERMINATION OF 209 CBCs ON THE DB-1 COLUMN

A second-column option is provided for resolution of the two toxic PCB congeners (PCB156/157) that are not resolved on the SPB-Octyl column and for resolution of other Chlorinated Biphenyl Congeners (CBCs), if needed. An alternative column may be used as long as unique resolution of the WHO-Toxic congeners is achieved and all IPC criteria are met. For alternative columns, the Contractor shall provide an updated Table 7. Any alternate performance criteria established must first be approved by the EPA as a variance to the SOW.

1.0 COLUMN AND CONDITIONS

1.1 Column - 30 ± 5-m long x 0.25 ± 0.02-mm ID; 0.25 µm film DB-1 (J&W, or equivalent).

1.2 Suggested Gas Chromatograph (GC) Operating Conditions:

Injector temperature:	270°C
Interface temperature:	290°C
Initial temperature:	75°C
Initial time:	2 min.
Temperature program:	75-150°C at 15°C/min.
	150-270°C at 2.5°C/min.
Final time:	7 min.
Carrier gas velocity:	40 cm/sec at 200°C

NOTE: The GC conditions may be optimized for compound separation and sensitivity. Once optimized, the same GC conditions must be used for the analysis of all standards, blanks, LCS/LCSD aliquots, and samples.

2.0 OPERATING INFORMATION

2.1 Congener Solutions

Mixes of individual congeners that will allow separation of all 209 congeners on the DB-1 column have not been developed.

2.2 Elution Order Data

The congener mixes developed for the SPB-Octyl column were analyzed on the DB-1 column. Although some congeners in these mixes co-elute, the mixes allow determination of retention times for many congeners on the DB-1 column. These retention times (RT) are shown in Appendix A, Table A-1.

2.3 Window-Defining Congeners

The beginning and ending congeners at each level of chlorination (LOC) are the same as for the SPB-Octyl column.

2.4 Scan Descriptors

The 6-function scan descriptors are shown in Appendix A, Table A-2.

TABLE A-1. RETENTION TIME REFERENCES, QUANTITATION REFERENCES, AND RELATIVE RETENTION TIMES FOR CHLORINATED BIPHENYL CONGENERS ON A DB-1 COLUMN

Labeled or Native CB[1]	Analyte Name[2]	Retention Time and Quantitation References	Analyte Name	RT	RRT	RRT QC Limits[3]
$^{13}C_{12}$-2-MoCB[4]	PCB-1L	$^{13}C_{12}$-4-MoCB[4,5]	PCB-3L	09:17	0.8855	0.8776-0.8935
2-MoCB	PCB-1	$^{13}C_{12}$-2-MoCB[4]	PCB-1L	09:17	1.0000	0.9964-1.0072
3-MoCB	PCB-2	$^{13}C_{12}$-4-MoCB[4,5]	PCB-3L	10:22	0.9889	0.9809-0.9968
$^{13}C_{12}$-4-MoCB[4,5]	PCB-3L	$^{13}C_{12}$-2,2',5,5'-TeCB[7]	PCB-52L	10:29	0.5561	0.5473-0.5650
4-MoCB	PCB-3	$^{13}C_{12}$-4-MoCB[4,5]	PCB-3L	10:29	1.0000	0.9968-1.0064
$^{13}C_{12}$-2,2'-DiCB[4]	PCB-4L	$^{13}C_{12}$-4,4'-DiCB[4,5]	PCB-15L	11:08	0.7591	0.7477-0.7705
2,2'-DiCB	PCB-4	$^{13}C_{12}$-2,2'-DiCB[4]	PCB-4L	11:08	1.0000	0.9925-1.0075
2,6-DiCB	PCB-10	$^{13}C_{12}$-4,4'-DiCB[4,5]	PCB-15L	11:10	0.7614	0.7500-0.7727
2,5-DiCB	PCB-9	$^{13}C_{12}$-4,4'-DiCB[4,5]	PCB-15L	12:08	0.8273	0.8216-0.8330
2,4-DiCB	PCB-7	$^{13}C_{12}$-4,4'-DiCB[4,5]	PCB-15L	12:09	0.8284	0.8227-0.8341
2,3'-DiCB	PCB-6	$^{13}C_{12}$-4,4'-DiCB[4,5]	PCB-15L	12:31	0.8534	0.8477-0.8591
2,4'-DiCB[6]	PCB-8	$^{13}C_{12}$-4,4'-DiCB[4,5]	PCB-15L	12:43	0.8670	0.8614-0.8727
2,3-DiCB	PCB-5	$^{13}C_{12}$-4,4'-DiCB[4,5]	PCB-15L	12:46	0.8705	0.8648-0.8761
$^{13}C_{12}$-2,2',6-TrCB[4]	PCB-19L	$^{13}C_{12}$-2,4,4'-TrCB[5]	PCB-28L	13:31	0.7990	0.7892-0.8089
2,2',6-TrCB	PCB-19	$^{13}C_{12}$-2,2',6-TrCB[4]	PCB-19L	13:31	1.0000	0.9975-1.0049
3,5-DiCB	PCB-14	$^{13}C_{12}$-4,4'-DiCB[4,5]	PCB-15L	13:36	0.9273	0.9216-0.9330
2,4,6-TrCB	PCB-30	$^{13}C_{12}$-2,4,4'-TrCB[5]	PCB-28L	14:06	0.8335	0.8286-0.8384
3,3'-DiCB	PCB-11	$^{13}C_{12}$-4,4'-DiCB[4,5]	PCB-15L	14:11	0.9670	0.9614-0.9727
3,4'-DiCB	PCB-13	$^{13}C_{12}$-4,4'-DiCB[4,5]	PCB-15L	14:26	0.9841	0.9784-0.9898
3,4-DiCB	PCB-12	$^{13}C_{12}$-4,4'-DiCB[4,5]	PCB-15L	14:27	0.9852	0.9795-0.9909
2,2',5-TrCB[6]	PCB-18	$^{13}C_{12}$-2,4,4'-TrCB[5]	PCB-28L	14:36	0.8631	0.8581-0.8680
$^{13}C_{12}$-4,4'-DiCB[4,5]	PCB-15L	$^{13}C_{12}$-2,2',5,5'-TeCB[7]	PCB-52L	14:40	0.7781	0.7692-0.7869
4,4'-DiCB	PCB-15	$^{13}C_{12}$-4,4'-DiCB[4,5]	PCB-15L	14:40	1.0000	0.9977-1.0043
2,2',4-TrCB	PCB-17	$^{13}C_{12}$-2,4,4'-TrCB[5]	PCB-28L	14:43	0.8700	0.8650-0.8749
2,3',6-TrCB	PCB-27	$^{13}C_{12}$-2,4,4'-TrCB[5]	PCB-28L	15:06	0.8926	0.8877-0.8975
2,3,6-TrCB	PCB-24	$^{13}C_{12}$-2,4,4'-TrCB[5]	PCB-28L	15:06	0.8926	0.8877-0.8975
2,2',3-TrCB	PCB-16	$^{13}C_{12}$-2,4,4'-TrCB[5]	PCB-28L	15:26	0.9123	0.9074-0.9172
2,4',6-TrCB	PCB-32	$^{13}C_{12}$-2,4,4'-TrCB[5]	PCB-28L	15:29	0.9153	0.9103-0.9202
$^{13}C_{12}$-2,2',6,6'-TeCB[4]	PCB-54L	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	16:02	0.6139	0.6075-0.6203
2,2',6,6'-TeCB	PCB-54	$^{13}C_{12}$-2,2',6,6'-TeCB[4]	PCB-54L	16:02	1.0000	0.9979-1.0042
2',3,5-TrCB	PCB-34	$^{13}C_{12}$-2,4,4'-TrCB[5]	PCB-28L	16:03	0.9488	0.9438-0.9537
2,3,5-TrCB	PCB-23	$^{13}C_{12}$-2,4,4'-TrCB[5]	PCB-28L	16:07	0.9527	0.9478-0.9576
2,4,5-TrCB	PCB-29	$^{13}C_{12}$-2,4,4'-TrCB[5]	PCB-28L	16:18	0.9635	0.9586-0.9685

TABLE A-1. RETENTION TIME REFERENCES, QUANTITATION REFERENCES, AND RELATIVE RETENTION TIMES FOR CHLORINATED BIPHENYL CONGENERS ON A DB-1 COLUMN (CON'T)

Labeled or Native CB[1]	Analyte Name[2]	Retention Time and Quantitation References	Analyte Name	RT	RRT	RRT QC Limits[3]
2,3',5-TrCB	PCB-26	$^{13}C_{12}$-2,4,4'-TrCB[5]	PCB-28L	16:29	0.9744	0.9695-0.9793
2,3',4-TrCB	PCB-25	$^{13}C_{12}$-2,4,4'-TrCB[5]	PCB-28L	16:36	0.9813	0.9764-0.9862
2,4',5-TrCB	PCB-31	$^{13}C_{12}$-2,4,4'-TrCB[5]	PCB-28L	16:52	0.9970	0.9921-1.0020
$^{13}C_{12}$-2,4,4'-TrCB[5,8]	PCB-28L	$^{13}C_{12}$-2,2',5,5'-TeCB[7]	PCB-52L	16:55	0.8974	0.8930-0.9019
2,4,4'-TrCB[6]	PCB-28	$^{13}C_{12}$-2,4,4'-TrCB[5]	PCB-28L	16:55	1.0000	0.9980-1.0039
2,2',4,6-TeCB	PCB-50	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	16:55	0.6477	0.6414-0.6541
2,3,4-TrCB	PCB-21	$^{13}C_{12}$-2,4,4'-TrCB[5]	PCB-28L	17:21	1.0256	1.0207-1.0305
2,2',5,6'-TeCB	PCB-53	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	17:26	0.6675	0.6611-0.6739
2,3,3'-TrCB	PCB-20	$^{13}C_{12}$-2,4,4'-TrCB[5]	PCB-28L	17:22	1.0266	1.0217-1.0315
2',3,4-TrCB	PCB-33	$^{13}C_{12}$-2,4,4'-TrCB[5]	PCB-28L	17:24	1.0286	1.0236-1.0335
2,2',4,6'-TeCB	PCB-51	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	17:42	0.6777	0.6713-0.6841
2,3,4'-TrCB	PCB-22	$^{13}C_{12}$-2,4,4'-TrCB[5]	PCB-28L	17:43	1.0473	1.0424-1.0522
2,2',3,6-TeCB	PCB-45	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	18:00	0.6892	0.6828-0.6956
3,3',5-TrCB	PCB-36	$^{13}C_{12}$-2,4,4'-TrCB[5]	PCB-28L	18:16	1.0798	1.0749-1.0847
2,2',3,6'-TeCB	PCB-46	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	18:24	0.7045	0.6981-0.7109
3,4',5-TrCB	PCB-39	$^{13}C_{12}$-2,4,4'-TrCB[5]	PCB-28L	18:37	1.1005	1.0956-1.1054
$^{13}C_{12}$-2,2',5,5'-TeCB[7]	PCB-52L	$^{13}C_{12}$-2,2',5,5'-TeCB[7]	PCB-52L	18:51	1.0000	0.9956-1.0044
2,2',5,5'-TeCB[6]	PCB-52	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	18:51	0.7218	0.7154-0.7281
2,3',4,6-TeCB	PCB-69	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	18:52	0.7224	0.7160-0.7288
2,3',5',6-TeCB	PCB-73	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	18:57	0.7256	0.7192-0.7320
2,2',4,5'-TeCB	PCB-49	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	19:00	0.7275	0.7211-0.7339
2,2',3,5-TeCB	PCB-43	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	19:04	0.7301	0.7237-0.7364
3,4,5-TrCB	PCB-38	^{13}C12-2,4,4'-TrCB[5]	PCB-28L	19:12	1.1350	1.1300-1.1399
2,2',4,4'-TeCB	PCB-47	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	19:15	0.7371	0.7307-0.7435
2,4,4',6-TeCB	PCB-75	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	19:20	0.7403	0.7339-0.7466
2,2',4,5-TeCB	PCB-48	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	19:20	0.7403	0.7339-0.7466
2,3,5,6-TeCB	PCB-65	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	19:31	0.7473	0.7409-0.7537
2,3,4,6-TeCB	PCB-62	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	19:36	0.7505	0.7441-0.7569
3,3',4-TrCB	PCB-35	$^{13}C_{12}$-2,4,4'-TrCB[5]	PCB-28L	19:41	1.1635	1.1586-1.1685
$^{13}C_{12}$-2,2',4,6,6'-PeCB[4]	PCB-104L	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	19:45	0.7037	0.6977-0.7096
2,2',4,6,6'-PeCB	PCB-104	$^{13}C_{12}$-2,2',4,6,6'-PeCB[4]	PCB-104L	19:45	1.0000	0.9983-1.0034
2,2',3,5'-TeCB6	PCB-44	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	19:55	0.7626	0.7562-0.7690
$^{13}C_{12}$-3,4,4'-TrCB[4]	PCB-37L	$^{13}C_{12}$-2,4,4'-TrCB[5]	PCB-28L	20:03	1.1852	1.1803-1.1901
3,4,4'-TrCB	PCB-37	$^{13}C_{12}$-3,4,4'-TrCB[4]	PCB-37L	20:03	1.0000	0.9983-1.0033
2,3,3',6-TeCB	PCB-59	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	20:05	0.7690	0.7626-0.7754
2,2',3,4'-TeCB	PCB-42	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	20:07	0.7703	0.7639-0.7766

TABLE A-1. RETENTION TIME REFERENCES, QUANTITATION REFERENCES, AND RELATIVE RETENTION TIMES FOR CHLORINATED BIPHENYL CONGENERS ON A DB-1 COLUMN (CON'T)

Labeled or Native CB[1]	Analyte Name[2]	Retention Time and Quantitation References	Analyte Name	RT	RRT	RRT QC Limits[3]
2,3',5,5'-TeCB	PCB-72	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	20:36	0.7888	0.7824-0.7951
2,3',4',6-TeCB	PCB-71	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	20:36	0.7888	0.7824-0.7951
2,3,4',6-TeCB	PCB-64	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	20:37	0.7894	0.7830-0.7958
2,2',3,4-TeCB	PCB-41	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	20:39	0.7907	0.7843-0.7971
2,2',3,6,6'-PeCB	PCB-96	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	20:48	0.7411	0.7352-0.7470
2,3',4,5'-TeCB	PCB-68	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	20:52	0.7990	0.7926-0.8054
2,2',3,3'-TeCB	PCB-40	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	20:58	0.8028	0.7996-0.8060
2,3,3',5-TeCB	PCB-57	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	21:21	0.8175	0.8143-0.8207
2,2',4,5',6-PeCB	PCB-103	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	21:22	0.7613	0.7553-0.7672
2,3',4,5-TeCB	PCB-67	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	21:38	0.8283	0.8251-0.8315
2,2',4,4',6-PeCB	PCB-100	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	21:41	0.7726	0.7666-0.7785
2,3,3',5'-TeCB	PCB-58	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	21:43	0.8315	0.8283-0.8347
2,3,4',5-TeCB	PCB-63	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	21:51	0.8366	0.8334-0.8398
2,2',3,5,6'-PeCB	PCB-94	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	22:05	0.7868	0.7809-0.7928
2,4,4',5-TeCB	PCB-74	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	22:07	0.8468	0.8437-0.8500
2,3,4,5-TeCB	PCB-61	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	22:11	0.8494	0.8462-0.8526
2,3',4',5-TeCB	PCB-70	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	22:20	0.8551	0.8519-0.8583
2',3,4,5-TeCB	PCB-76	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	22:25	0.8583	0.8551-0.8615
2,2',3',4,6-PeCB	PCB-98	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	22:28	0.8005	0.7975-0.8034
2,3',4,4'-TeCB6	PCB-66	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	22:29	0.8609	0.8577-0.8641
2,2',4,5,6'-PeCB	PCB-102	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	22:32	0.8029	0.7999-0.8058
2,2',3,5',6-PeCB	PCB-95	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	22:34	0.8040	0.8011-0.8070
2,2',3,5,6-PeCB	PCB-93	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	22:36	0.8052	0.8023-0.8082
3,3',5,5'-TeCB	PCB-80	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	22:45	0.8711	0.8679-0.8743
2,2',3,4,6-PeCB	PCB-88	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	22:49	0.8129	0.8100-0.8159
2,2',3,4',6-PeCB	PCB-91	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	22:55	0.8165	0.8135-0.8195
2,3,3',4-TeCB	PCB-55	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	22:57	0.8787	0.8756-0.8819
2,3',4,5',6-PeCB	PCB-121	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	23:04	0.8219	0.8189-0.8248
2,3,3',4'-TeCB	PCB-56	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	23:24	0.8960	0.8928-0.8992
2,3,4,4'-TeCB	PCB-60	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	23:24	0.8960	0.8928-0.8992
$^{13}C_{12}$-2,2',4,4',6,6'-HxCB[4]	PCB-155L	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	23:43	0.7104	0.7054-0.7154
2,2',4,4',6,6'-HxCB	PCB-155	$^{13}C_{12}$-2,2',4,4',6,6'-HxCB[4]	PCB-155L	23:43	1.0000	0.9986-1.0028
2,2',3,3',6-PeCB	PCB-84	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	23:44	0.8456	0.8426-0.8486
2,2',3,5,5'-PeCB	PCB-92	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	23:50	0.8492	0.8462-0.8521
2,2',3,4,6'-PeCB	PCB-89	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	23:53	0.8510	0.8480-0.8539
2,2',3,4',5-PeCB	PCB-90	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	24:07	0.8593	0.8563-0.8622

TABLE A-1. RETENTION TIME REFERENCES, QUANTITATION REFERENCES, AND RELATIVE RETENTION TIMES FOR CHLORINATED BIPHENYL CONGENERS ON A DB-1 COLUMN (CON'T)

Labeled or Native CB[1]	Analyte Name[2]	Retention Time and Quantitation References	Analyte Name	RT	RRT	RRT QC Limits[3]
$^{13}C_{12}$-2,2',4,5,5'-PeCB[7]	PCB-101L	$^{13}C_{12}$-2,2',4,5,5'-PeCB[7]	PCB-101L	24:11	1.0000	0.9966-1.0034
2,2',4,5,5'-PeCB[6]	PCB-101	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	24:11	0.8616	0.8587-0.8646
2,3,3',5',6-PeCB	PCB-113	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	24:23	0.8688	0.8658-0.8717
3,3',4,5'-TeCB	PCB-79	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	24:27	0.9362	0.9330-0.9394
2,2',4,4',5-PeCB	PCB-99	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	24:28	0.8717	0.8688-0.8747
2,2',3,4',6,6'-HxCB	PCB-150	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	24:52	0.7449	0.7399-0.7499
2,3',4,4',6-PeCB	PCB-119	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	24:54	0.8872	0.8842-0.8901
2,3,3',5,6-PeCB	PCB-112	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	25:00	0.8907	0.8878-0.8937
2,3,3',4,5'-PeCB	PCB-108	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	25:09	0.8961	0.8931-0.8990
2,2',3,5,6,6'-HxCB	PCB-152	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	25:17	0.7574	0.7524-0.7624
2,2',3,3',5-PeCB	PCB-83	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	25:20	0.8919	0.8890-0.8949
2,2',3',4,5-PeCB	PCB-97	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	25:22	0.9038	0.9008-0.9068
2,2',3,4,5-PeCB	PCB-86	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	25:27	0.9068	0.9038-0.9097
$^{13}C_{12}$-3,4,4',5-TeCB[9]	PCB-81L	$^{13}C_{12}$-2,2',5,5'-TeCB[7]	PCB-52L	25:32	1.3546	1.3457-1.3634
3,4,4',5-TeCB[10]	PCB-81	$^{13}C_{12}$-3,4,4',5-TeCB[4,5,9]	PCB-77L	25:32	1.0000	0.9987-1.0026
2',3,4,5,6'-PeCB	PCB-125	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	25:36	0.9121	0.9091-0.9151
2,3,4',5,6-PeCB	PCB-117	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	25:37	0.9127	0.9097-0.9157
2,2',3,4,5'-PeCB	PCB-87	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	25:38	0.9133	0.9103-0.9163
3,3',4,5-TeCB	PCB-78	$^{13}C_{12}$-3,3',4,4'-TeCB[4,5,9]	PCB-77L	25:40	0.9598	0.9566-0.9630
2,2',3,4,6,6'-HxCB	PCB-145	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	25:42	0.7698	0.7649-0.7748
2,3,4,4',6-PeCB	PCB-115	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	25:44	0.9169	0.9139-0.9198
$^{13}C_{12}$-2,3,3',5,5'-PeCB[8]	PCB-111L	$^{13}C_{12}$-2,2',4,5,5'-PeCB[7]	PCB-101L	25:51	1.0689	1.0655-1.0724
2,3,3',5,5'-PeCB	PCB-111	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	25:51	0.9210	0.9181-0.9240
2,2',3,4,4'-PeCB	PCB-85	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	25:51	0.9210	0.9181-0.9240
2,3,4,5,6-PeCB	PCB-116	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	25:48	0.9192	0.9163-0.9222
$^{13}C_{12}$-3,3',4,4'-TeCB4,5,9	PCB-77L	$^{13}C_{12}$-2,2',5,5'-TeCB7	PCB-52L	26:07	1.3855	1.3767-1.3943
3,3',4,4'-TeCB6,10	PCB-77	$^{13}C_{12}$-3,3',4,4'-TeCB4,[5,9]	PCB-77L	26:07	1.0000	0.9987-1.0026
2,2',3,3',6,6'-HxCB	PCB-136	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	26:10	0.7793	0.7743-0.7843
2,3',4,5,5'-PeCB	PCB-120	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	26:12	0.9335	0.9305-0.9365
2,2',3,4',5,6'-HxCB	PCB-148	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	26:14	0.7858	0.7808-0.7908
2,3,3',4',6-PeCB	PCB-110	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	26:16	0.9359	0.9329-0.9388
2,2',4,4',5,6'-HxCB	PCB-154	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	26:44	0.8008	0.7983-0.8033

TABLE A-1. RETENTION TIME REFERENCES, QUANTITATION REFERENCES, AND RELATIVE RETENTION TIMES FOR CHLORINATED BIPHENYL CONGENERS ON A DB-1 COLUMN (CON'T)

Labeled or Native CB[1]	Analyte Name[2]	Retention Time and Quantitation References	Analyte Name	RT	RRT	RRT QC Limits[3]
2,2',3,3',4-PeCB	PCB-82	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	26:48	0.9549	0.9519-0.9578
2,2',3,5,5',6-HxCB	PCB-151	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	27:18	0.8178	0.8153-0.8203
2,2',3,3',5,6'-HxCB	PCB-135	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	27:31	0.8243	0.8218-0.8268
2',3,4,5,5'-PeCB	PCB-124	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	27:36	0.9834	0.9804-0.9863
2,2',3,4,5',6-HxCB	PCB-144	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	27:38	0.8278	0.8253-0.8303
2,3,3',4',5-PeCB	PCB-107	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	27:40	0.9857	0.9828-0.9887
2,2',3,4',5,6-HxCB	PCB-147	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	27:44	0.8308	0.8283-0.8333
2,3,3',4,6-PeCB	PCB-109	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	27:45	0.9887	0.9857-0.9917
2,2',3,4',5',6-HxCB	PCB-149	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	28:01	0.8392	0.8367-0.8417
2,2',3,3',5,6-HxCB	PCB-134	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	28:35	0.8562	0.8537-0.8587
2,2',3,4,5,6'-HxCB	PCB-143	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	28:34	0.8557	0.8532-0.8582
$^{13}C_{12}$-2',3,4,4',5-PeCB[9]	PCB-123L	$^{13}C_{12}$-2,2',4,5,5'-PeCB[7]	PCB-101L	27:53	1.1530	1.1496-1.1564
2',3,4,4',5-PeCB[10]	PCB-123	$^{13}C_{12}$-2',3,4,4',5-PeCB[9]	PCB-123L	27:53	1.0000	0.9988-1.0024
2,2',3,4,4',6-HxCB	PCB-139	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	28:01	0.8392	0.8367-0.8417
2,3,3',4,5-PeCB	PCB-106	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	28:04	1.0000	0.9970-1.0030
$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	$^{13}C_{12}$-2,2',4,5,5'-PeCB[7]	PCB-101L	28:04	1.1606	1.1571-1.1640
2,3',4,4',5-PeCB[6,10]	PCB-118	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	28:04	1.0000	0.9988-1.0024
2,2',3,4,4',6'-HxCB	PCB-140	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	28:12	0.8447	0.8422-0.8472
$^{13}C_{12}$-2,3,4,4',5-PeCB[9]	PCB-114L	$^{13}C_{12}$-2,2',4,5,5'-PeCB[7]	PCB-101L	28:38	1.1840	1.1806-1.1875
2,3,4,4',5-PeCB[10]	PCB-114	$^{13}C_{12}$-2,3,4,4',5-PeCB[9]	PCB-114L	28:38	1.0000	0.9988-1.0023
2',3,3',4,5-PeCB	PCB-122	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	28:48	1.0261	1.0232-1.0291
2,2',3,3',4,6-HxCB	PCB-131	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	28:52	0.8647	0.8622-0.8672
2,2',3,4,5,6-HxCB	PCB-142	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	28:59	0.8682	0.8657-0.8707
2,2',3,3',5,5'-HxCB	PCB-133	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	28:59	0.8682	0.8657-0.8707
2,2',3,3',4,6'-HxCB	PCB-132	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	29:32	0.8847	0.8822-0.8872

TABLE A-1. RETENTION TIME REFERENCES, QUANTITATION REFERENCES, AND RELATIVE RETENTION TIMES FOR CHLORINATED BIPHENYL CONGENERS ON A DB-1 COLUMN (CON'T)

Labeled or Native CB[1]	Analyte Name[2]	Retention Time and Quantitation References	Analyte Name	RT	RRT	RRT QC Limits[3]
2,3,3',5,5',6-HxCB	PCB-165	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	29:21	0.8792	0.8767-0.8817
$^{13}C_{12}$-2,2',3,4',5,6,6'-HpCB[4]	PCB-188L	$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB[4,5,9]	PCB-189L	29:22	0.9511	0.7327-0.7411
2,2',3,4',5,6,6'-HpCB	PCB-188	$^{13}C_{12}$-2,2',3,4',5,6,6'-HpCB[4]	PCB-188L	29:22	1.0000	0.9989-1.0023
2,2',3,4',5,5'-HxCB	PCB-146	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	29:24	0.8807	0.8782-0.8832
$^{13}C_{12}$-2,3,3',4,4'-PeCB[9]	PCB-105L	$^{13}C_{12}$-2,2',4,5,5'-PeCB[7]	PCB-101L	29:30	1.2198	1.2130-1.2267
2,3,3',4,4'-PeCB[6,10]	PCB-105	$^{13}C_{12}$-2,3,3',4,4'-PeCB[9]	PCB-105L	29:30	1.0000	0.9989-1.0023
2,3,3',4,5',6-HxCB	PCB-161	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	29:32	0.8847	0.8822-0.8872
2,2',4,4',5,5'-HxCB[6]	PCB-153	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	29:48	0.8927	0.8902-0.8952
2,2',3,4,4',6,6'-HpCB	PCB-184	$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB[4,5,9]	PCB-189L	29:49	0.7482	0.7440-0.7524
3,3',4,5,5'-PeCB	PCB-127	$^{13}C_{12}$-2,3',4,4',5-PeCB[5,9]	PCB-118L	29:57	1.0671	1.0641-1.0701
2,3',4,4',5',6-HxCB	PCB-168	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	29:59	0.8982	0.8957-0.9006
2,2',3,4,5,5'-HxCB	PCB-141	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	30:31	0.9141	0.9116-0.9166
2,2',3,3',5,6,6'-HpCB	PCB-179	$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB[4,5,9]	PCB-189L	30:33	0.7666	0.7624-0.7708
2,2',3,4,4',5-HxCB	PCB-137	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	30:51	0.9241	0.9216-0.9266
2,2',3,3',4,5'-HxCB	PCB-130	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	30:57	0.9271	0.9246-0.9296
2,2',3,3',4,6,6'-HpCB	PCB-176	$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB[4,5,9]	PCB-189L	31:01	0.7783	0.7742-0.7825
$^{13}C_{12}$-2,2',3,4,4',5'-HxCB[7]	PCB-138L	$^{13}C_{12}$-2,2',3,4,4',5'-HxCB[7]	PCB-138L	31:20	1.0000	0.9973-1.0027
2,2',3,4,4',5'-HxCB[6]	PCB-138	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	31:20	0.9386	0.9361-0.9411
2,3,3',4',5',6-HxCB	PCB-164	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	31:22	0.9396	0.9371-0.9421
2,3,3',4',5,6-HxCB	PCB-163	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	31:28	0.9426	0.9401-0.9451
2,3,3',4,5,6-HxCB	PCB-160	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	31:33	0.9451	0.9426-0.9476

TABLE A-1. RETENTION TIME REFERENCES, QUANTITATION REFERENCES, AND RELATIVE RETENTION TIMES FOR CHLORINATED BIPHENYL CONGENERS ON A DB-1 COLUMN (CON'T)

Labeled or Native CB[1]	Analyte Name[2]	Retention Time and Quantitation References	Analyte Name	RT	RRT	RRT QC Limits[3]
2,3,3',4,4',6-HxCB	PCB-158	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	31:35	0.9461	0.9436-0.9486
2,2',3,4,5,6,6'-HpCB	PCB-186	$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB[4,5,9]	PCB-189L	31:36	0.7930	0.7888-0.7972
2,2',3,3',4,5-HxCB	PCB-129	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	31:48	0.9526	0.9501-0.9551
$^{13}C_{12}$-3,3',4,4',5-PeCB[4,9]	PCB-126L	$^{13}C_{12}$-2,2',4,5,5'-PeCB[7]	PCB-101L	31:49	1.3156	1.3088-1.3225
3,3',4,4',5-PeCB[6,10]	PCB-126	$^{13}C_{12}$-3,3',4,4',5-PeCB[4,9]	PCB-126L	31:49	1.0000	0.9990-1.0021
2,3,4,4',5,6-HxCB	PCB-166	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	32:13	0.9651	0.9626-0.9675
$^{13}C_{12}$-2,2',3,3',5,5',6-HpCB[7,8]	PCB-178L	$^{13}C_{12}$-2,2',3,3',5,5',6-HpCB[7]	PCB-178L	32:14	1.0000	0.9974-1.0026
2,2',3,3',5,5',6-HpCB	PCB-178	$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB[4,5,9]	PCB-189L	32:14	0.8089	0.8068-0.8110
2,2',3,3',4,5',6-HpCB	PCB-175	$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB[4,5,9]	PCB-189L	32:33	0.8168	0.8147-0.8189
2,3,3',4,5,5'-HxCB	PCB-159	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	32:43	0.9800	0.9775-0.9825
2,2',3,4',5,5',6-HpCB[6]	PCB-187	$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB[4,5,9]	PCB-189L	32:46	0.8223	0.8202-0.8243
2,2',3,4,4',5,6'-HpCB	PCB-182	$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB[4,5,9]	PCB-189L	32:47	0.8227	0.8206-0.8248
2,2',3,3',4,4'-HxCB[6]	PCB-128	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	32:52	0.9845	0.9820-0.9870
2,3,3',4',5,5'-HxCB	PCB-162	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	33:00	0.9885	0.9860-0.9910
2,2',3,4,4',5',6-HpCB	PCB-183	$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB[4,5,9]	PCB-189L	33:06	0.8306	0.8285-0.8327
$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	$^{13}C_{12}$-2,2',3,4,4',5'-HxCB[7]	PCB-138L	33:23	1.0654	1.0628-1.0681
2,3',4,4',5,5'-HxCB[10]	PCB-167	$^{13}C_{12}$-2,3',4,4',5,5'-HxCB[5,9]	PCB-167L	33:23	1.0000	0.9990-1.0020
2,2',3,4,5,5',6-HpCB	PCB-185	$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB[4,5,9]	PCB-189L	33:43	0.8461	0.8440-0.8482
2,2',3,3',4,5,6'-HpCB	PCB-174	$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB[4,5,9]	PCB-189L	34:07	0.8561	0.8540-0.8582

TABLE A-1. RETENTION TIME REFERENCES, QUANTITATION REFERENCES, AND RELATIVE RETENTION TIMES FOR CHLORINATED BIPHENYL CONGENERS ON A DB-1 COLUMN (CON'T)

Labeled or Native CB[1]	Analyte Name[2]	Retention Time and Quantitation References	Analyte Name	RT	RRT	RRT QC Limits[3]
2,2',3,4,4',5,6-HpCB	PCB-181	$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB[4,5,9]	PCB-189L	34:11	0.8578	0.8557-0.8599
2,2',3,3',4',5,6-HpCB	PCB-177	$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB[4,5,9]	PCB-189L	34:22	0.8624	0.8603-0.8645
2,2',3,3',4,4',6-HpCB	PCB-171	$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB[4,5,9]	PCB-189L	34:40	0.8699	0.8678-0.8720
$^{13}C_{12}$-2,3,3',4,4',5-HxCB[9]	PCB-156L	$^{13}C_{12}$-2,2',3,4,4',5'-HxCB[7]	PCB-138L	34:40	1.1064	1.1037-1.1090
2,3,3',4,4',5-HxCB[10]	PCB-156	$^{13}C_{12}$-2,3,3',4,4',5-HxCB[9]	PCB-156L	34:40	1.0000	0.9990-1.0019
$^{13}C_{12}$-2,2',3,3',5,5',6,6'-OcCB[4]	PCB-202L	$^{13}C_{12}$-Cl8-PCB-1945	PCB-194L	34:56	0.8265	0.8245-0.8285
2,2',3,3',5,5',6,6'-OcCB	PCB-202	$^{13}C_{12}$-2,2',3,3',5,5',6,6'-OcCB[4]	PCB-202L	34:56	1.0000	0.9990-1.0019
$^{13}C_{12}$-2,3,3',4,4',5'-HxCB[9]	PCB-157L	$^{13}C_{12}$-2,2',3,4,4',5'-HxCB[7]	PCB-138L	34:57	1.1154	1.1128-1.1181
2,3,3',4,4',5'-HxCB[10]	PCB-157	$^{13}C_{12}$-2,3,3',4,4',5'-HxCB[9]	PCB-157L	34:57	1.0000	0.9990-1.0019
2,2',3,3',4,5,6-HpCB	PCB-173	$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB[4,5,9]	PCB-189L	35:04	0.8800	0.8779-0.8821
2,2',3,3',4,5',6,6'-OcCB	PCB-201	$^{13}C_{12}$-Cl8-PCB-1945	PCB-194L	35:25	0.8379	0.8360-0.8399
2,2',3,4,4',5,6,6'-OcCB	PCB-204	$^{13}C_{12}$-Cl8-PCB-194[5]	PCB-194L	35:36	0.8423	0.8403-0.8442
2,2',3,3',4,5,5'-HpCB	PCB-172	$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB[4,5,9]	PCB-189L	35:41	0.8954	0.8934-0.8975
2,3,3',4,5,5',6-HpCB	PCB-192	$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB[4,5,9]	PCB-189L	35:51	0.8996	0.8975-0.9017
2,2',3,3',4,4',6,6'-OcCB	PCB-197	$^{13}C_{12}$-Cl8-PCB-1945	PCB-194L	35:55	0.8498	0.8478-0.8517
2,2',3,4,4',5,5'-HpCB[6]	PCB-180	$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB[4,5,9]	PCB-189L	36:07	0.9063	0.9042-0.9084
2,3,3',4',5,5',6-HpCB	PCB-193	$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB[4,5,9]	PCB-189L	36:20	0.9118	0.9097-0.9138
2,3,3',4,4',5',6-HpCB	PCB-191	$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB[4,5,9]	PCB-189L	36:34	0.9176	0.9155-0.9197
2,2',3,3',4,5,6,6'-OcCB	PCB-200	$^{13}C_{12}$-Cl8-PCB-194[5]	PCB-194L	36:49	0.8711	0.8691-0.8730
$^{13}C_{12}$-3,3',4,4',5,5'-HxCB[4,9]	PCB-169L	$^{13}C_{12}$-2,2',3,4,4',5'-HxCB[7]	PCB-138L	37:19	1.1910	1.1883-1.1936
3,3',4,4',5,5'-HxCB[6,10]	PCB-169	$^{13}C_{12}$-3,3',4,4',5,5'-HxCB[4,9]	PCB-169L	37:19	1.0000	0.9991-1.0018

TABLE A-1. RETENTION TIME REFERENCES, QUANTITATION REFERENCES, AND RELATIVE RETENTION TIMES FOR CHLORINATED BIPHENYL CONGENERS ON A DB-1 COLUMN (CON'T)

Labeled or Native CB[1]	Analyte Name[2]	Retention Time and Quantitation References	Analyte Name	RT	RRT	RRT QC Limits[3]
2,2',3,3',4,4',5-HpCB[6]	PCB-170	$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB[4,5,9]	PCB-189L	37:44	0.9469	0.9448-0.9490
2,3,3',4,4',5,6-HpCB	PCB-190	$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB[4,5,9]	PCB-189L	37:56	0.9519	0.9498-0.9540
2,2',3,3',4,5,5',6-OcCB	PCB-198	$^{13}C_{12}$-Cl8-PCB-194[5]	PCB-194L	38:34	0.9125	0.9105-0.9144
2,2',3,3',4,5,5',6'-OcCB	PCB-199	$^{13}C_{12}$-Cl8-PCB-194[5]	PCB-194L	38:43	0.9160	0.9140-0.9180
2,2',3,3',4,4',5,6'-OcCB	PCB-196	$^{13}C_{12}$-Cl8-PCB-194[5]	PCB-194L	39:05	0.9247	0.9227-0.9267
2,2',3,4,4',5,5',6-OcCB	PCB-203	$^{13}C_{12}$-Cl8-PCB-194[5]	PCB-194L	39:05	0.9247	0.9227-0.9267
$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB4,5,9	PCB-189L	$^{13}C_{12}$-2,2',3,3',5,5',6-HpCB7	PCB-178L	39:51	1.2363	1.2311-1.2415
2,3,3',4,4',5,5'-HpCB10	PCB-189	$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB4,5,9	PCB-189L	39:51	1.0000	0.9992-1.0017
2,2',3,3',4,4',5,6-OcCB6	PCB-195	$^{13}C_{12}$-Cl8-PCB-1945	PCB-194L	40:45	0.9641	0.9621-0.9661
$^{13}C_{12}$-2,2',3,3',4,5,5',6,6'-NoCB4	PCB-208L	$^{13}C_{12}$-Cl9-PCB-2064,5	PCB-206L	41:03	0.9149	0.9131-0.9168
2,2',3,3',4,5,5',6,6'-NoCB	PCB-208	$^{13}C_{12}$-2,2',3,3',4,5,5',6,6'-NoCB4	PCB-208L	41:03	1.0000	0.9992-1.0016
2,2',3,3',4,4',5,6,6'-NoCB	PCB-207	$^{13}C_{12}$-Cl9-PCB-2064,5	PCB-206L	41:32	0.9257	0.9238-0.9276
$^{13}C_{12}$-2,2',3,3',4,4',5,5'-OcCB5	PCB-194L	$^{13}C_{12}$-2,2',3,3',5,5',6-HpCB[7]	PCB-178L	42:16	1.3113	1.3061-1.3164
2,2',3,3',4,4',5,5'-OcCB	PCB-194	$^{13}C1_2$-Cl8-PCB-1945	PCB-194L	42:16	1.0000	0.9992-1.0016
$^{13}C_{12}$-2,3,3',4,4',5,5',6-OcCB4	PCB-205L	$^{13}C_{12}$-Cl8-PCB-1945	PCB-194L	42:44	1.0110	1.0091-1.0130
2,3,3',4,4',5,5',6-OcCB	PCB-205	$^{13}C_{12}$-2,3,3',4,4',5,5',6-OcCB4	PCB-205L	42:44	1.0000	0.9992-1.0016
$^{13}C_{12}$-2,2',3,3',4,4',5,5',6-NoCB4,5	PCB-206L	$^{13}C_{12}$-2,2',3,3',5,5',6-HpCB7	PCB-178L	44:52	1.3919	1.3868-1.3971
2,2',3,3',4,4',5,5',6-NoCB6	PCB-206	^{13}C12-Cl9-PCB-2064,5	PCB-206L	44:52	1.0000	0.9993-1.0015
$^{13}C_{12}$-2,2',3,3',4,4',5,5',6,6'-DeCB4,5	PCB-209L	$^{13}C_{12}$-2,2',3,3',5,5',6-HpCB7	PCB-178L	46:55	1.4555	1.4504-1.4607
2,2',3,3',4,4',5,5',6,6'-DeCB6	PCB-209	$^{13}C_{12}$-Cl10-PCB-2094,5	PCB-209L	46:55	1.0000	0.9993-1.0014

[1]Abbreviations for chlorination levels:

MoCB =	monochlorobiphenyl	HxCB =	hexachlorobiphenyl
DiCB =	dichlorobiphenyl	HpCB =	heptachlorobiphenyl
TrCB =	trichlorobiphenyl	OcCB =	octachlorobiphenyl
TeCB =	tetrachlorobiphenyl	NoCB =	nonachlorobiphenyl
PeCB =	pentachlorobiphenyl	DeCB =	decachlorobiphenyl

[2]Suffix "L" indicates labeled compound.

[3]For native CBCs determined by isotope dilution quantitation, RRT QC limits were constructed using -2 to +4 seconds around the retention time for the labeled compound. For native CBCs determined by internal standard quantitation, RRT QC limits were constructed using a ±2 percent window around the retention time for retention times in the range of 0.8-1.2 and a ±4 percent window around the retention time for retention times <0.8 and >1.2. These windows may not be adequate for analyte identification (See the note in Exhibit D – CBC, Section 11.1.1.4).

[4]Labeled LOC window-defining congener.

[5]Labeled LOC quantitation congener.

[6]National Oceanic and Atmospheric Administration (NOAA) congener of interest.

[7]Instrument internal standard.

[8]Cleanup standard.

[9]Internal standard for World Health Organization (WHO) toxic congener.

[10]WHO Toxic Congener.

TABLE A-2. SCAN DESCRIPTORS, LEVELS OF CHLORINATION, m/z INFORMATION, AND SUBSTANCES

Function and Chlorine Level	m/z[1]	m/z Type	m/z Formula	Substance
Fn-1	188.0393	M	$^{12}C_{12}$ H_9 ^{35}Cl	Cl-1 PCB
Cl-1	190.0363	M+2	$^{12}C_{12}$ H_9 ^{37}Cl	Cl-1P CB
	200.0795	M	$^{13}C_{12}$ H_9 ^{35}Cl	$^{13}C_{12}$ Cl-1 PCB
	202.0766	M+2	$^{13}C_{12}$ H_9 ^{37}Cl	$^{13}C_{12}$ Cl-1 PCB
	218.9856	lock	C_4 F_9	PFK
Fn-2	222.0003	M	$^{12}C_{12}$ H_8 $^{35}Cl_2$	Cl-2 PCB
Cl-2,3	223.9974	M+2	$^{12}C_{12}$ H_8 ^{35}Cl ^{37} Cl	Cl-2 PCB
	225.9944	M+4	$^{12}C_{12}$ H_8 $^{37}Cl_2$	Cl-2 PCB
	234.0406	M	$^{13}C_{12}$ H_8 $^{35}Cl_2$	$^{13}C_{12}$ Cl-2 PCB
	236.0376	M+2	$^{13}C_{12}$ H_8 ^{35}Cl ^{37} Cl	$^{13}C_{12}$ Cl-2 PCB
	242.9856	lock	C_6 F_9	PFK
	255.9613	M	$^{12}C_{12}$ H_7 $^{35}Cl_3$	Cl-3 PCB
	257.9584	M+2	$^{12}C_{12}$ H_7 $^{35}Cl_2$ ^{37}Cl	Cl-3 PCB
Fn-3	255.9613	M	$^{12}C_{12}$ H_7 $^{35}Cl_3$	Cl-3 PCB
Cl-3,4,5	257.9584	M+2	$^{12}C_{12}$ H_7 $^{35}Cl_2$ ^{37}Cl	Cl-3 PCB
	259.9554	M+4	$^{12}C_{12}$ H_7 ^{35}Cl $^{37}Cl_2$	Cl-3 PCB
	268.0016	M	$^{13}C_{12}$ H_7 $^{35}Cl_3$	$^{13}C_{12}$ Cl-3 PCB
	269.9986	M+2	$^{13}C_{12}$ H_7 $^{35}Cl_2$ ^{37}Cl	$^{13}C_{12}$ Cl-3 PCB
	280.9825	lock	C_6 F_{11}	PFK
	289.9224	M	$^{12}C_{12}$ H_6 $^{35}Cl_4$	Cl-4 PCB
	291.9194	M+2	$^{12}C_{12}$ H_6 $^{35}Cl_3$ ^{37}Cl	Cl-4 PCB
	293.9165	M+4	$^{12}C_{12}$ H_6 $^{35}Cl_2$ $^{37}Cl_2$	Cl-4 PCB
	301.9626	M	$^{13}C_{12}$ H_6 $^{35}Cl_4$	$^{13}C_{12}$ Cl-4 PCB
	303.9597	M+2	$^{13}C_{12}$ H_6 $^{35}Cl_3$ ^{37}Cl	$^{13}C_{12}$ Cl-4 PCB
	323.8834	M	$^{12}C_{12}$ H_5 $^{35}Cl_5$	Cl-5 PCB
	325.8804	M+2	$^{12}C_{12}$ H_5 $^{35}Cl_4$ ^{37}Cl	Cl-5 PCB
	327.8775	M+4	$^{12}C_{12}$ H_5 $^{35}Cl_3$ $^{37}Cl_2$	Cl-5 PCB
	337.9207	M+2	$^{13}C_{12}$ H_5 $^{35}Cl_4$ ^{37}Cl	$^{13}C_{12}$ Cl-5 PCB
	339.9178	M+4	$^{13}C_{12}$ H_5 $^{35}Cl_3$ $^{37}Cl_2$	$^{13}C_{12}$ Cl-5 PCB
Fn-4	289.9224	M	$^{12}C_{12}$ H_6 $^{35}Cl_4$	Cl-4 PCB
Cl-4,5,6	291.9194	M+2	$^{12}C_{12}$ H_6 $^{35}Cl_3$ ^{37}Cl	Cl-4 PCB
	293.9165	M+4	$^{12}C_{12}$ H_6 $^{35}Cl_2$ $^{37}Cl_2$	Cl-4 PCB
	301.9626	M+2	$^{13}C_{12}$ H_6 $^{35}Cl_3$ ^{37}Cl	$^{13}C_{12}$ Cl-4 PCB
	303.9597	M+4	$^{13}C_{12}$ H_6 $^{35}C_{12}$ $^{37}C_{12}$	$^{13}C_{12}$ Cl-4 PCB
	323.8834	M	$^{12}C_{12}$ H_5 $^{35}Cl_5$	Cl-5 PCB
	325.8804	M+2	$^{12}C_{12}$ H_5 $^{35}Cl_4$ ^{37}Cl	Cl-5 PCB
	327.8775	M+4	$^{12}C_{12}$ H_5 $^{35}C_{l3}$ $^{37}C_{12}$	Cl-5 PCB
	330.9792	lock	C_7 F_{15}	PFK
	337.9207	M+2	$^{13}C_{12}$ H_5 $^{35}Cl_4$ ^{37}Cl	$^{13}C_{12}$ Cl-5 PCB
	339.9178	M+4	$^{13}C_{12}$ H_5 $^{35}Cl_3$ ^{37}Cl [2]	$^{13}C_{12}$ Cl-5 PCB
	359.8415	M+2	$^{13}C_{12}$ H_4 $^{35}C_{l5}$ ^{37}Cl	Cl-6 PCB
	361.8385	M+4	$^{13}C_{12}$ H_4 $^{35}C_{l4}$ $^{37}C_{l2}$	Cl-6 PCB
	363.8356	M+6	$^{13}C_{12}$ H_4 $^{35}C_{l3}$ $^{37}C_{l2}$	Cl-6 PCB
	371.8817	M+2	$^{13}C_{12}$ H_4 $^{35}C_{l5}$ ^{37}Cl	$^{13}C_{12}$ Cl-6 PCB
	373.8788	M+4	$^{13}C_{12}$ H_4 $^{35}C_{l4}$ $^{37}C_{l2}$	$^{13}C_{12}$ Cl-6 PCB

TABLE A-2. SCAN DESCRIPTORS, LEVELS OF CHLORINATION, m/z INFORMATION, AND SUBSTANCES (CON'T)

Function and Chlorine Level	m/z[1]	m/z Type	m/z Formula	Substance
Fn-5	323.8834	M	$^{12}C_{12}$ H_5 $^{35}Cl_5$	Cl-5 PCB
Cl-5,6,7,8	325.8804	M+2	$^{12}C_{12}$ H_5 $^{35}Cl_4$ ^{37}Cl	Cl-5 PCB
	327.8775	M+4	$^{12}C_{12}$ H_5 $^{35}Cl_3$ $^{37}Cl_2$	Cl-5 PCB
	337.9207	M+2	$^{13}C_{12}$ H_5 $^{35}Cl_4$ ^{37}Cl	$^{13}C_{12}$ Cl-5 PCB
	339.9178	M+4	$^{13}C_{12}$ H_5 $^{35}Cl_3$ $^{37}Cl_2$	$^{13}C_{12}$ Cl-5 PCB
	354.9792	lock	C_9 F_{13}	PFK
	359.8415	M+2	$^{12}C_{12}$ H_4 $^{35}Cl_5$ ^{37}Cl	Cl-6 PCB
	361.8385	M+4	$^{12}C_{12}$ H_4 $^{35}Cl_4$ $^{37}Cl_2$	Cl-6 PCB
	363.8356	M+6	$^{12}C_{12}$ H_4 $^{35}Cl_3$ $^{37}Cl_3$	Cl-6 PCB
	371.8817	M+2	$^{13}C_{12}$ H_4 $^{35}Cl_5$ ^{37}Cl	$^{13}C_{12}$ Cl-6 PCB
	373.8788	M+4	$^{13}C_{12}$ H_4 $^{35}Cl_4$ $^{37}Cl_2$	$^{13}C_{12}$ Cl-6 PCB
	393.8025	M+2	$^{12}C_{12}$ H_3 $^{35}Cl_6$ ^{37}Cl	Cl-7 PCB
	395.7995	M+4	$^{12}C_{12}$ H_3 $^{35}Cl_5$ $^{37}Cl_2$	Cl-7 PCB
	397.7966	M+6	$^{12}C_{12}$ H_3 $^{35}Cl_4$ $^{37}Cl_3$	Cl-7 PCB
	405.8428	M+2	$^{13}C_{12}$ H_3 $^{35}Cl_6$ ^{37}Cl	$^{13}C_{12}$ Cl-7 PCB
	407.8398	M+4	$^{13}C_{12}$ H_3 $^{35}Cl_5$ $^{37}Cl_2$	$^{13}C_{12}$ Cl-7 PCB
	427.7635	M+2	$^{12}C_{12}$ H_2 $^{35}Cl_7$ ^{37}Cl	Cl-8 PCB
	429.7606	M+4	$^{12}C_{12}$ H_2 $^{35}Cl_6$ $^{37}Cl_2$	Cl-8 PCB
	431.7576	M+6	$^{12}C_{12}$ H_2 $^{35}Cl_5$ $^{37}Cl_3$	Cl-8 PCB
	439.8038	M+2	$^{13}C_{12}$ H_2 $^{35}Cl_7$ ^{37}Cl	$^{13}C_{12}$ Cl-8 PCB
	441.8008	M+4	$^{13}C_{12}$ H_2 $^{35}Cl_6$ $^{37}Cl_2$	$^{13}C_{12}$ Cl-8 PCB
	454.9728	QC	C_{11} F_{17}	PFK
Fn-6	427.7635	M+2	$^{12}C_{12}$ H_2 $^{35}Cl_7$ ^{37}Cl	Cl-8 PCB
Cl-8,9,10	429.7606	M+4	$^{12}C_{12}$ H_2 $^{35}Cl_6$ $^{37}Cl_2$	Cl-8 PCB
	431.7576	M+6	$^{12}C_{12}$ H_2 $^{35}Cl_5$ $^{37}Cl_3$	Cl-8 PCB
	439.8038	M+2	$^{13}C_{12}$ H_2 $^{35}Cl_7$ ^{37}Cl	$^{13}C_{12}$ Cl-8 PCB
	441.8008	M+4	$^{13}C_{12}$ H_2 $^{35}Cl_6$ $^{37}Cl_2$	$^{13}C_{12}$ Cl-8 PCB
	442.9728	QC	C_{10} F_{13}	PFK
	454.9728	lock	C_{11} F_{13}	PFK
	461.7246	M+2	$^{12}C_{12}$ H_1 $^{35}Cl_8$ ^{37}Cl	Cl-9 PCB
	463.7216	M+4	$^{12}C_{12}$ H_1 $^{35}Cl_7$ $^{37}Cl_2$	Cl-9 PCB
	465.7187	M+6	$^{12}C_{12}$ H_1 $^{35}Cl_6$ $^{37}Cl_3$	Cl-9 PCB
	473.7648	M+2	$^{13}C_{12}$ H_1 $^{35}Cl_8$ ^{37}Cl	$^{13}C_{12}$ Cl-9 PCB
	475.7619	M+4	$^{13}C_{12}$ H_1 $^{35}Cl_7$ $^{37}Cl_2$	$^{13}C_{12}$ Cl-9 PCB
	495.6856	M+2	$^{13}C_{12}$ H_4 $^{35}Cl_9$ ^{37}Cl	Cl-10 PCB
	499.6797	M+4	$^{12}C_{12}$ $^{35}Cl_7$ $^{37}Cl_3$	Cl-10 PCB
	501.6767	M+6	$^{12}C_{12}$ $^{35}Cl_6$ $^{37}Cl_4$	Cl-10 PCB
	507.7258	M+2	$^{13}C_{12}$ H_4 $^{35}Cl_9$ ^{37}Cl	$^{13}C_{12}$ Cl-10 PCB

[1]Isotopic masses used for accurate mass calculation:

^{1}H 1.0078

^{12}C 12.0000

^{13}C 13.0034

^{35}Cl 34.9689

^{37}Cl 36.9659

^{19}F 18.9984

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