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QUALITY ASSURANCE PROJECT PLAN

CENTREDALE MANOR RESTORATION PROJECT SUPERFUND SITE BASELINE RISK ASSESSMENT, INITIAL PROJECT PLANNING, AND SUPPORT

Volume II of II (SOPs)

Tasks 19–22 QAPP

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ATTACHMENT G

Pertinent Fixed Laboratory SOPs

BATTELLE DUXBURY SOPs

STANDARD OPERATING PROCEDURE (SOP) FOR

PCB Sample Preparation and Analysis Using Modified Method 1668, Revision A

Originated by: _____ Date: _____

Approved by: _____ Date: _____
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I. Scope

This SOP describes routine procedures for the preparation and analysis of samples for PCBs using modified EPA Method 1668, Revision A and covers the following:

- Personnel qualifications
- Sample Preservation, Storage, and Holding times
- Apparatus and Materials
- Reagents and Standards
- Quality Control Procedures
- Sample Preparation
- Concentration Procedures
- Extract Cleanup
- HRGC/HRMS Calibration
- HRGC/HRMS Analysis
- Quantitative Determination

II. Purpose

This procedure is for the determination of up to 209 PCBs in water, soil, sediment, and tissue matrices using high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) following guidance of EPA Method 1668, Revision A.

III. References

A. EPA Method 1668, Revision A: Chlorinated Biphenyl Congeners in Water, Soil, Sediment, and Tissue by HRGC/HRMS, December 1999, EPA-821-R-00-002.

B. SOP No. ASAT.II-005, Polychlorinated Dibenzo-p-dioxin/Polychlorinated Dibenzofuran (PCDD/PCDF) Desiccating Agent and Adsorbent Preparation and Storage.

IV. Definitions

A. Definitions and acronyms (in alphabetical order).

ASE – Accelerated Solvent Extraction.

Calibration verification standard (VER)-The mid-point calibration standard (CS-3) that is used to verify calibration. See Table 5 (Appendix A).

CS-0.2, CS-1, CS-2, CS-3, CS-4, CS-5-See Calibration standards and Table 5 (Appendix A).

Estimated method detection limit (EMDL)-The lowest concentration at which a PCB can be detected with common laboratory interferences present. EMDLs are listed in Table 2 (Appendix A).

Estimated minimum level (EML)-The lowest concentration at which a PCB can be measured reliably with common laboratory interferences present. EMLs are listed in Table 2 (Appendix A).

GC-Gas chromatograph or gas chromatography

HRGC-High resolution GC

HRMS-High resolution MS

Internal standard quantitation-A means of determining the concentration of (1) a naturally occurring (native) compound by reference to a compound other than its labeled analog and (2) a labeled compound by reference to another labeled compound.

IPR-Initial precision and recovery; four aliquots of a reference matrix spiked with the analytes of interest and labeled compounds and analyzed to establish the ability of the laboratory to generate acceptable precision and recovery

Isotope dilution quantitation-A means of determining a naturally occurring (native) compound by reference to the same compound in which one or more atoms has been isotopically enriched. In this Method, all 12 carbon atoms in the biphenyl molecule are enriched with carbon- 13 to produce $^{13}C_{12}$-labeled analogs of the chlorinated biphenyls. The $^{13}C_{12}$-labeled CBs are spiked into each sample and allow identification and correction of the concentration of the native compounds in the analytical process.

KD-Kuderna-Danish concentrator; a device used to concentrate the analytes in a solvent

Labeled injection internal standard-All five, or any one of the five, $^{13}C_{12}$-labeled PCB congeners spiked into the concentrated extract prior to injection of an aliquot of the extract into the HRGC/HRMS. The five Labeled injection internal standards in this Method are PCBs with IUPAC numbers 9, 52, 101, 138, and 194. This standard performs the same task as the recovery standard in Method 1613 for dioxins.

Laboratory control sample (LCS)-See Ongoing precision and recovery standard (OPR)

LOC – Level of chlorination.

MDL-Method detection limit

Method blank-An aliquot of reference matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with samples. The Method blank is used to determine if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

Minimum level of quantitation (ML)-The level at which the entire analytical system must give a recognizable signal and acceptable calibration point for the analyte. It is equivalent to the concentration of the lowest calibration standard, assuming that all Method-specified sample weights, volumes, and cleanup procedures have been employed.

MS-Mass spectrometer or mass spectrometry

OPR-Ongoing precision and recovery standard (OPR); a method blank spiked with known quantities of analytes. The OPR is analyzed exactly like a sample. Its purpose is to assure that the results produced by the laboratory remain within the limits specified in this Method for precision and recovery.

Perfluorokerosene (PFK)-A mixture of compounds used to calibrate the exact m/z scale in the HRMS.

PCB-polychlorinated biphenyl

Relative standard deviation (RSD)-The standard deviation times 100 divided by the mean. Also termed "coefficient of variation."

RRF-Relative Response factor. See Section I.5

Signal-to-noise ratio (S/N)-The height of the signal as measured from the mean (average) of the noise to the peak maximum divided by the width of the noise.

SICP-Selected ion current profile; the line described by the signal at an exact m/z.

Unique GC resolution or uniquely resolved-Two adjacent chromatographic peaks in which the height of the valley is less than 40 percent of the height of the shorter peak.

VER-See Calibration verification.

V. Procedures

A. Personnel Qualifications.

Personnel assigned to laboratory activities meet the educational, work experience, and training requirements for their positions. Records on personnel qualifications and training are maintained in personnel files accessible for review during audit activities. Training is conducted in accordance with standard operating procedures and is available to all laboratory personnel. Employees must demonstrate proficiency at specific tasks and this capability is documented and kept in a central file.

B. Sample Preservation, Storage, and Holding Times

B.1 Aqueous samples

B.1.1 Amber glass jars in size appropriate for the sample (Environmental Sampling Supply (ESS) QC class, or equivalent) are used for aqueous samples.

B.1.2. 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 chlorine is known or suspected to be in the samples, the preservative may be added to sampling jars before shipping to the field.

B.1.3 Measure pH of samples upon receipt at the lab by taking a few drops of sample using muffled Pasteur pipettes and placing on pH paper (Hydrion Papers wide range, or equivalent). Adjust sample pH to 2-3 with sulfuric acid.

B.1.4 Store aqueous samples in the dark at 0-4°C.

B.2 Solid samples

B.2.1 Amber glass wide mouth jars (ESS QC class, or equivalent) in a size appropriate for the amount of sample are used for solid samples.

B.2.2 Store solid, semi-solid, oily, and mixed-phase samples in the dark at <-10°C.

B.3 Fish and other tissue samples

B.3.1 Amber glass wide mouth jars (ESS QC class, or equivalent) in a size appropriate for the amount of tissue should be used for homogenized samples. Whole fish can be wrapped in pre-cleaned aluminum foil (muffled at 450°C for 8 hours).

B.3.2 Tissue samples must be frozen upon receipt at the laboratory and maintained in the dark at <- 10°C.

B.4 Holding times

B.4.1 There are no demonstrated maximum holding times associated with the PCBs in aqueous, solid, semi-solid, tissues, or other sample matrices. If stored in the dark at 0-4 °C and preserved as given above, aqueous samples may be stored for up to one year. Similarly, if stored in the dark at <- 10°C, solid, semi-solid, multi-phase, and tissue samples may be stored for up to one year.

B.4.2 Store sample extracts in the dark at <- 10°C until analyzed. If stored in the dark at <- 10°C, sample extracts may be stored for up to one year.

C. Apparatus and Materials

C.1 Equipment for sample preparation

C.1.1 Laboratory fume hood of sufficient size to contain the sample preparation equipment listed below.

C.1.2 Tissue homogenizer-VirTis Model 45 Macro homogenizer (Ultra-Turrax T25 Janke and Kunkel TKA-Labortechnik, or equivalent) with stainless steel Macro-shaft and Turbo-shear blade.

C.1.3 Equipment for determining percent moisture

C.1.3.1 Oven capable of maintaining a temperature of 110 ± 5 °C.

C.1.3.2 Desiccator.

C.1.4 Balances

C.1.4.1 Analytical-capable of weighing 0.1 mg.

C.1.4.2 Top loading-capable of weighing 10 mg.

C.2 Extraction apparatus

C.2.1 Aqueous samples

C.2.1.1 Graduated cylinder, 1-L capacity
C.2.1.2 Liquid/liquid extraction- Separatory funnels, 2000mL with Teflon stopcocks for extraction of a 1-L sample.

C.2.2 Tissue, filters, and solid/sludge samples by Soxhlet
C.2.2.1 Soxhlet-49x180mm body ID (50-mm ID), 55/50 top joint, 24/40 bottom joint, 250-mL round-bottom (Kontes K585050-0023, or equivalent).
C.2.2.2 Thimble-43x123 to fit Soxhlet (Kones K586500-0025, or equivalent)
C.2.2.3 Heating mantle-Hemispherical, (Glass-col, 100A0402 or equivalent)
C.2.2.4 Variable transformer-Powerstat (or equivalent), 110-volt, 10-amp

C.2.3 Tissue and solid/sludge samples by Accelerated Solvent Extraction
C.2.3.1 Dionex ASE 200
C.2.3.2 Dionex ASE 200 33 mL Cell with endcaps
C.2.3.3 Dionex ASE 200 Large vial (60 mL)
C.2.3.4 Dionex ASE 200 filters, Grade D28, Size 1.983

C.3 Filtration apparatus

C.3.1 Pressure filtration apparatus (Cole Palmer, catalogue number 02933-00, or equivalent).

C.3.2 Glass-fiber filter paper-Whatman Order No 1825-142 (0.7 µm, 142 mm diameter). Muffled for a minimum of 4 hours at 450°C.

C.4 Cleanup apparatus

C.4.1 Pipets
C.4.1.1 Disposable, Pasteur, 150-mm long x 5-mm ID (Fisher Scientific 13678-6A, or equivalent)
C.4.1.2 Disposable, serological, 50-mL (8- to 10- mm ID)

C.4.2 Glass chromatographic columns
C.4.2.1 150-mm long x 8-mm ID, (Kontes K-420155, or equivalent) with coarse-glass frit or glass-wool plug and 250-mL reservoir.
C.4.2.2 200-mm long x 15-mm ID, with coarse-glass frit or glass-wool plug and 250-mL reservoir.
C.4.2.3 300-mm long x 22-mm ID, with coarse-glass frit, 300-mL reservoir, and glass or fluoropolymer stopcock.
C.4.2.4 25-mL disposable pipet with an ID of 14mm (Pyrex or Kimble, or equivalent).

C.4.3 Oven-For baking and storage of adsorbents, capable of maintaining a constant temperature (±5 °C) in the range of 105-250 °C.

C.4.4 Separatory funnels, 250-mL with Teflon stopcocks.

C.4.5 Automated gel permeation chromatograph (Analytical Biochemical Labs, Inc, Columbia, MO, Model GPC Autoprep 1002, or equivalent)

C.4.5.1 Column 600-700 mm long x 25 mm ID glass, packed with 70 g of 200-400 mesh SX-3 Bio-beads (available pre-packed from OI Analytical no. 624-116, or equivalent).

C.4.5.2 Syringe-10-mL, with Luer fitting

C.4.5.3 Syringe filter holder-stainless steel, and glass-fiber or fluoropolymer filters (Gelman 4310, or equivalent)

C.4.5.4 UV detectors-254-nm, preparative or semi-preparative flow cell (Isco, Inc., Type 6; Schmadzu, 5-mm path length; Beckman-Altex 152W, 8-µL micro-prep flow cell, 2-mm path; Pharmacia UV-1, 3-mm flow cell; LDC Milton-Roy UV-3, monitor #1203; or equivalent)

C.5 Concentration apparatus

C.5.1 Rotary evaporator-Buchi/Brinkman-American Scientific No. E5045-10 or equivalent, equipped with a variable temperature water bath.

C.5.1.1 Vacuum source for rotary evaporator equipped with shutoff valve at the evaporator and vacuum gauge

C.5.1.2 Round-bottom flask-250-mL and 500-mL or larger, with groundglass fitting compatible with the rotary evaporator.

C.5.2 Kuderna-Danish (KD) concentrator

C.5.2.1 Concentrator tube-25-mL, graduated (Kontes K-570050-1025, or equivalent) with calibration verified. Ground-glass stopper (size 19/22 joint) is used to prevent evaporation of extracts.

C.5.2.2 Evaporation flask-250-mL (Kontes K-570001-0500, or equivalent), attached to concentrator tube with springs (Kontes K-662750-0012 or equivalent).

C.5.2.3 Snyder column-Three-ball macro (Kontes K-503000-0232, or equivalent).

C.5.2.4 Boiling chips

C.5.2.4.1 Glass or silicon carbide-Approximately 10/40 mesh, extracted with methylene chloride and baked at 450 °C for one hour minimum.

C.5.2.4.2 Fluoropolymer (optionally-Extracted with methylene chloride).

C.5.2.5 Water bath-Heated, with concentric ring cover, capable of maintaining a temperature within ±2 °C, installed in a fume hood.

C.5.3 Nitrogen blowdown apparatus-Equipped with sand bath or holding ring for concentrations at room temperture controlled in the range of 30 - 60 °C (N-Evap, Organomation Associates, Inc., South Berlin, MA, or equivalent), installed in a fume hood.

C.5.4 Sample vials

C.5.4.1 Amber glass, 2- to 40-mL with fluoropolymer-lined screw-cap for sample archive and standards.

C.5.4.2 Blowdowns: Concentrator tubes (Kimble, 15 mL disposable, or equivalent).

C.5.4.3 GC vials: Glass, 0.3-mL insert, conical, with fluoropolymer-lined screw or crimp cap. (MicroLiter Analytical Supplies, or equivalent)

C.6 HRGC/HRMS Systems – VG Autospec or VG Autospec Ultima

C.6.1 GC column. SPB-octyl (30±5-m x 0.25-mm ID; 0.25 µm film) (Supelco 2-4218, or equivalent. Note: *The SPB-octyl column is subject to rapid degradation when exposed to oxygen. The analyst should exclude oxygen from the carrier gas, should eliminate air leaks, and should cool the injector, column, and transfer line before opening the column to the atmosphere.*

C.6.1.1 The retention time for PCB209 must be greater than 55 minutes.

C.6.1.2 The column must uniquely resolve congeners 34 from 23 and 187 from 182, and congeners 156 and 157 must coelute within 2 seconds at the peak maximum. Unique resolution means a valley height less than 40 percent of the shorter of the two peaks that result when the diluted combined 209 congener solution (Section D.6.3.2) is analyzed.

C.6.1.3 The column must be replaced when any of the criteria in Sections C.6.1.1 – C.6.1.2 are not met.

D. Reagents and Standards

D.1 pH adjustment and back-extraction

D.1.1 Potassium hydroxide-Dissolve 20 g reagent grade KOH in 100 mL reagent water.

D.1.2 Sulfuric acid-Reagent grade (specific gravity 1.84)

D.1.3 Hydrochloric acid-Reagent grade, 6N.

D.1.4 Sodium chloride-Reagent grade, prepare at 5% (w/v) solution in reagent water.

D.2 Solution drying and evaporation.

D.2.1 Solution drying-Sodium sulfate, reagent grade, granular, anhydrous (Baker 3375, or equivalent). See SOP ASAT.II-005-00, Section J for procedures on sodium sulfate preparation.

D.2.2 Tissue or Sediment drying-Varian Hydromatrix, see SOP ASAT.II-005-00, Section I for preparation.

D.2.3 Prepurified nitrogen.

D.3 Extraction

D.3.1 Solvents-Acetone, toluene, cyclohexane, hexane, methanol, methylene chloride, isooctane, decane and nonane; distilled in glass, pesticide quality, lot-certified to be free of interferences.

D.4 Adsorbents and reagents for sample cleanup

D.4.1 Silica gel

D.4.1.1 See SOP ASAT.II-005-00, Sections C and D for preparation and storage procedures.

D.4.1.2 Acid silica gel-see SOP ASAT.II-005-00, Section C for preparation and storage procedures.

D.4.1.3 Basic silica gel-see SOP ASAT.II-005-00, Section D for preparation and storage procedures.

D.4.2 Carbon

D.4.2.1 Carbopak C-(Supelco 1-0258, or equivalent) -see SOP ASAT.II-005-00, Section F for preparation and storage procedures.

D.4.2.2 Celite 545-(Supelco 2-0199, or equivalent) -see SOP ASAT.II-005-00, Section G for preparation and storage procedures.

D.4.2.3 Thoroughly mix 18.0 g Carbopak C and 18.0 g Celite 545 to produce a 50% w/w mixture. See SOP ASAT.II-005-00, Section H for preparation and storage procedures. Activate the mixture at 130°C for a mimimum of 6 hours. Store in a desiccator.

D.4.3 Alumina, F-20 (Sigma/Aldrich, CAS#B44-28-1, 80-200 mesh) is activated by heating in a shallow dish to 400°C for at least 4 hours. Allow the alumina to cool in an oven (do not keep in open lab atmosphere for extended periods). Store the alumina in a covered glass container (with an activation date label) at 105°C, and use within 1 week of activation. Deactivate the alumina prior to use with 2% water as follows. Prepare the 2% deactivated alumina in batches by adding 5 mL of Milli-Q water (accurately measure with a syringe or volumetric pipette) to 250 g of alumina in a 1,000 mL round bottom glass flask. Seal with a Teflon stopper, shake vigorously by hand for 1 minute to begin the process. Vent the flask, put the stopper back on, seal with Teflon tape, and allow to vigorously mix on a shaker table for 2 hr (± 15 minutes). Pack columns within 2 hr of completing the deactiviation and use the same day. Unused deactivated alumina should be discarded.

D.5 **Reference matrices.**

D.5.1 Reagent water-Distilled in glass HPLC grade water (JT Baker Ultra Resi-Analyzed, or equivalent).

D.5.2 Solids reference matrix-Playground sand or similar material. Prepared by extraction with methylene chloride and/or baking at 450 °C for a minimum of 4 hours.

D.5.3 Tissue reference matrix-Corn or other vegetable oil.

D.6 **Standard solutions**. When not being used, store unopened standard solutions in the dark at room temperature in screw-capped vials with fluoropolymer-lined caps and opened standard solutions in screw-capped vials with fluoropolymer-lined caps in a freezer at ≤ -20°C. Place a mark on the vial at the level of the solution so that solvent loss by evaporation can be detected. Replace the solution if solvent loss has occurred.

D.6.1 Native (unlabeled) stock solutions

D.6.1.1 Native Toxics/LOC stock solution- purchase Accu-Standard M1668A-C-NT-LOC-WD-GCPC, or equivalent

D.6.1.2 Native 209 PCB congener stock solutions-Solutions containing PCB congeners to calibrate the SPB-octyl column.

D.6.1.2.1 Native congener mix stock solutions for separation of individual congeners on the SPB-octyl column. Purchase Accu-Standard M- 1668A-1, M-1668A-2, M-1668A-3, M-1668-4, and M-1668-5, or equivalent. The 5 individual solutions, when analyzed individually, allow resolution of all 209 congeners on the SPB-octyl column, and are used for establishing retention time and other data for each congener.

D.6.1.2.2 Combined 209 congener stock solution- Combine equal volumes of the five Accustandard mixes (D.6.1.2.1) to form a stock solution containing all 209 PCBs. This solution will be at 1/5 the concentration of the 5 individual solutions

D.6.2 Labeled compound stock solutions

D.6.2.1 Labeled Toxics/LOC/window-defining stock solution- purchase Cambridge Isotope Laboratories (CIL) EC-4977, or equivalent.

D.6.2.2 Labeled cleanup standard stock solution- purchase CIL EC-4978, or equivalent.

D.6.2.3 Labeled injection internal standard stock solution- purchase CIL EC-4979, or equivalent.

D.6.3 Calibration standards.

D.6.3.1 Purchase CIL EC-4976, or equivalent, for the CS-0.2 to CS-5 set of calibration solutions. The CS-3 standard (CIL EC-4976-3, or equivalent) is used for calibration verification (VER).

D.6.3.2 Diluted combined 209 congener solution. This solution combines the 5 individual mixes with the labeled compounds to allow single-point calibration of the congeners not included in the multi-point calibration, and establishes an average response factor for the coeluting- isomeric congeners. Combine an aliquot of the combined 209 congener solution (D.6.1.2.2) with an aliquot of the Labeled Toxics/ LOC/ window-defining stock solution (D.6.2.1), the Labeled cleanup standard stock solution (D.6.2.2), and the Labeled injection internal standard stock solution (D.6.2.3) to produce concentrations of 100 ng/mL for the labeled compounds and 25, 50, and 75 ng/mL for the mono-tri, tetra- hepta, and octa-deca PCBs, respectively.

D.6.3.3 GPC calibration solution. Solution containing 2.5 mg/mL corn oil, 0.05 mg/mL bis(2-ethylhexyl) phthalate (BEHP), 0.01 mg/mL methoxychlor, 0.002 mg/mL perylene, and 0.008 mg/mL sulfur (Accustandard CLP027PAK, or equivalent).

D.6.4 Standard Spiking Solutions

D.6.4.1 Native Toxics/LOC standard spiking solution-Used for determining initial precision and recovery (IPR) and ongoing precision and recovery (OPR) and for matrix spike samples. Dilute the Native Toxics/LOC stock solution (Section D.6.1.1) with acetone to produce a concentration of the Toxics at 1 ng/mL, as shown in Table 3

(Appendix A). Prepare only the amount necessary for each sample batch.

D.6.4.2 Labeled Toxics/LOC/window-defining standard spiking solution-This solution is spiked into each field and QC sample to measure recovery. Dilute the Labeled Toxics/LOC/ window-defining stock solution (Section D.6.2.1) with acetone to produce a concentration of the labeled compounds at 2 ng/mL, as shown in Table 3 (Appendix A). Prepare only the amount necessary for each sample batch.

D.6.4.3 Labeled cleanup standard spiking solution-This solution is spiked into each extract prior to cleanup to measure the efficiency of the cleanup process. Dilute the Labeled cleanup standard stock solution (Section D.6.2.2) in acetone to produce a concentration of the cleanup standards at 2 ng/mL, as shown in Table 3 (Appendix A).

D.6.4.4 Labeled injection internal standard spiking solution-This solution is added to each concentrated extract during final concentration procedures. Dilute the Labeled injection internal standard stock solution (Section D.6.2.3) in nonane to produce a concentration of the injection internal standards at 200 ng/mL.

D.6.5 QC Check Sample-A QC Check Sample should be obtained from a source independent of the calibration standards. Ideally, this check sample would be a certified Standard Reference Material (SRM) containing the PCBs in known concentrations in a sample matrix similar to the matrix under test. The National Institute of Standards and Technology (NIST) in Gaithersburg, Maryland is a good source for PCB SRMs.

E. Quality Control Procedures

E.1 Results of initial precision and recovery studies (IPR) utilizing procedures described in this SOP are included in Appendix B.

E.2 Results of method detection limit studies (MDL) using procedures described in this SOP are included in Appendix C.

E.3 The laboratory demonstrates, on an ongoing basis, through calibration verification, the analysis of the ongoing precision and recovery sample (OPR), and blanks that the analytical system is in control. These procedures are given in Sections J.4 through J.6.

E.4 To assess Method performance on the sample matrix, the laboratory spikes all samples with the Labeled Toxics/LOC/window-defining standard spiking solution (D.6.4.2) and all sample extracts with the Labeled cleanup standard spiking solution (D.6.4.3). The target recovery for these compounds is discussed in J.7.

E.5 QC Check Sample-The QC Check Sample (Section D.6.5) is analyzed periodically to assure the accuracy of calibration standards and the overall reliability of the analytical

process. Results of each QC Check Sample should be placed in the PCB QC check sample binder at completion of each project.

F. Sample Preparation

F.1 PCB Contamination Reduction Procedures. The following steps should be taken for sample preparation activities to minimize PCB background levels.

- The night before use, muffle at 450°C all already cleaned glassware, sodium sulfate which has been rinsed with methylene chloride, glasswool which has been rinsed with methylene chloride, and any glass pipette which will be used. If not possible to muffle the night before use, at a minimum immediately prior to use rinse glassware with methylene chloride or the solvent that will be used with the glassware.
- All water used for method blank and OPR samples or to make up any of the reagents should be reagent grade water (D.5.1) shipped in a glass bottle.
- All gas cylinders should be fitted with clean charcoal filters.
- All solutions must be made in cleaned, muffled containers.
- All GC vials should be muffled the night prior to use.

F.2 Determination of Percent Suspended Solids.

Note: This aliquot is used for determining the solids content of the sample not for determination of PCBs.

F.2.1 Aqueous liquids and multi-phase samples consisting of mainly an aqueous phase. Desiccate and weigh a glass fiber filter (C.3.2) to three significant figures. Filter 10.0 ± 0.02 mL of well-mixed sample through the filter. Dry the filter a minimum of 12 hours at 110 ± 5°C and cool in a desiccator. Re-weigh the filter. Calculate percent solids as follows:

$$\%\ solids = \frac{\text{weight of sample aliquot after drying (g) - weight } \textit{of filter (g)}}{10\ g} \times 100$$

F.2.2 Non-aqueous liquids, solids, semi-solid samples, and multi-phase samples in which the main phase is not aqueous; but not tissues. Weigh 5 to 10 g of sample to three significant figures in a tared sample jar. Dry a minimum of 12 hours at 110 ± 5°C, and cool in a desiccator. Calculate percent solids as follows:

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

F.3 Preparation of Aqueous Samples Containing One Percent Suspended Solids or Less.

F.3.1 Mark the original level of the sample on the sample bottle for later determination of exact volume. Shake the sample jar and inspect for particulates. If no or very few visible particles are present, transfer the sample to a 2-L separatory funnel.

F.3.2 For each sample or sample batch (to a maximum of 20 field samples) to be extracted during the same 12-hour shift, place two 1.0-L aliquots of reagent water (D.5.1) in individual separatory funnels. One will serve as the method blank and one as the OPR.

F.3.3 Spike 1.0 mL of the Labeled Toxics/LOC/window-defining standard spiking solution (D.6.4.2) into the sample in each separatory funnel. Cap the bottle and mix the sample by careful shaking. Allow the sample to equilibrate for 1 to 2 hours, with occasional shaking.

F.3.4 Spike 1.0 mL of the Native Toxics/LOC standard spiking solution (D.6.4.1) into the OPR and any matrix spike samples (as indicated in project workplans). For method detection limit (MDL) samples spike 250 µL of the Native Toxics/LOC standard spiking solution.

F.3.5 Add 3 x 20 mL methylene chloride to each empty sample bottle. With each addition, seal the bottle and shake 60 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel corresponding to the sample. After the third methlyene chloride addition, extract the sample by shaking the funnel for 2 minutes with periodic venting. Allow the organic layer to separate from the aqueous 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 (such as stirring the emulsion with a muffled glass rod). Drain the methylene chloride extract through a methylene chloride-rinsed glass funnel approximately one-half full of granular anhydrous sodium sulfate (D.2.1) supported on muffled glass wool into a solvent-rinsed 500-mL round bottom. Extract the water sample two more times with 60-mL portions of methylene chloride. (Note: if > 1L samples are extracted, use of 80% methlyene chloride/20% acetone for the second and third extraction may aid recovery of analytes.) Drain each portion through the sodium sulfate into the same 500-mL round bottom. After the third extraction, rinse the sodium sulfate with at least 20 mL of methylene chloride, and drain this rinse through the sodium sulfate into the 500-mL round bottom. Repeat this rinse at least twice. Concentrate the extract using the heating mantel technique (G.2) to approximately 30 mL. Proceed to H.1 for extract cleanup.

F.3.6 Determine the exact sample volume by filling each sample jars to the mark with tap water and measuring the volume of water in each jar using a graduated cylinder. Record the volume in the lab record book.

F.4 Preparation of Aqueous Samples Containing Greater than One Percent Solids

F.4.1 Mark the original level of the sample on the sample bottle for later determination of exact volume. Shake the sample jar and inspect for particulates. If there are lots of visible particles (up to one percent solids as determined by F.2.1) proceed as follows.

F.4.2 Prepare Soxhlets for extraction of filters. Place a thimble filled with muffled glass wool in a clean extractor. Fill the 250-mL round bottom ¾ full with toluene or methylene chloride (depending on project requirements). Pre-extract the glassware, which is muffled at 450°C the night before use, by heating the flask until the solvent is boiling. When properly adjusted, 1 to 2 drops of solvent will fall per second from the condenser tip into the receiver. Extract the apparatus for a minimum of 3 hours. After pre-extraction, cool and disassemble the apparatus. Archive the pre-extraction solvent at room temperature. Add silica to the thimble prior to the addition of sample.

F.4.3 For each sample batch (up to a maximum of 20 field samples) to be extracted in the same 12-hour shift, place two 1.0-L aliquots of reagent water (D.5.1) in clean sample bottles. One will serve as the method blank and one will serve as the OPR.

F.4.4 Filter all samples including the QC samples according to Section F.7. Load the filter from F.7.3 into the thimble without disturbing the silica. The filter will be Soxhlet extracted and the filtrate will be liquid-liquid extracted.

F.4.5 Spike 500 µL of the Labeled Toxics/LOC/window-defining standard spiking solution (D.6.4.2) into the each sample filtrate in the separatory funnel. Spike 500 µL of the Labeled Toxics/LOC/window-defining standard spiking solution (D.6.4.2) into the each sample filter in the Soxhlet.

F.4.6 Spike 500 µL of Native Toxics/LOC standard spiking solution (D.6.4.1) into the OPR and any matrix spike sample filtrates (as indicated in project workplan) in the separatory funnel. Spike 500 µL of Native Toxics/LOC standard spiking solution (D.6.4.1) into the OPR and any matrix spike sample filters (as indicated in project workplan) in the Soxhlet. For MDL samples, spike 125 µL into each filtrate and 125 µL into each filter.

F.4.7 Soxhlet extract the filters as follows. Reassemble the pre-extracted Soxhlet apparatus, and add a fresh charge of solvent to the receiver and reflux flask. Apply power to the heating mantle to begin refluxing. Extract for a minimum of 16 hours. Concentrate the extracts to approximately 10 mL using the rotary evaporator (G.1) or heating mantle (G.2).

F.4.8 Liquid-liquid extract each filtrate as follows. Add 3 x 20-mL methylene chloride to each empty sample bottle. With each addition, seal the bottle and shake 60 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel corresponding to the sample. After the third methylene chloride addition, extract the sample by shaking the funnel for 2 minutes with periodic venting. Allow the organic layer to separate from the aqueous 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 (such as stirring the emulsion with a muffled glass rod). Drain the methylene chloride extract through a methylene chloride-rinsed glass funnel approximately one-half full of

granular anhydrous sodium sulfate (D.2.1) supported on muffled glass wool into a solvent-rinsed 500-mL round bottom. Extract the water sample two more times with 60-mL portions of methylene chloride. (Note: If > 1L samples are extracted, use of 80% methylene chloride/20% acetone for the second and third extractions may aid recovery of analytes.) Drain each portion through the sodium sulfate into the same 500-mL round bottom. After the third extraction, rinse the sodium sulfate with at least 20 mL of methylene chloride, and drain this rinse through the sodium sulfate into the 500-mL round bottom. Repeat this rinse at least twice. Concentrate the extract using the heating mantel technique (G.2) to approximately 30 mL.

F.4.9 Determine the exact sample volume by filling each sample jars to the mark with tap water and measuring the volume of water in each jar using a graduated cylinder. Record the volume in the lab record book.

F.4.10 Combine the filter Soxhlet extract with the filtrate liquid-liquid extract by using the following steps. Using disposable pipettes transfer the ~30 mL liquid-liquid extract to the Soxhlet round bottom, and rinse the liquid-liquid round bottom with 3 x 6-mL methylene chloride. Add each rinse of the liquid-liquid round bottom to the Soxhlet round bottom. Proceed to Section H.1 for extract cleanup.

F.5 Preparation of Soils, Sediments, and Solids

F.5.1 Soxhlet Extraction of soils, sediments and solids

F.5.1.1 Prepare Soxhlets for extraction of solid samples. Place a thimble filled with muffled glass wool in a clean extractor. Fill the 250-mL round bottom ¾ full with toluene or methylene chloride (depending on project requirements). Pre-extract the glassware, which is muffled at 450°C the night before use, by heating the flask until the solvent is boiling. When properly adjusted, 1 to 2 drops of solvent will fall per second from the condenser tip into the receiver. Extract the apparatus for a minimum of 3 hours. After pre-extraction, cool and disassemble the apparatus. Archive the pre-extraction solvent at room temperature. Add silica to the thimble prior to the addition of sample.

F.5.1.2 Decant excess water. If necessary to remove water, centrifuge or filter the sample through a glass-fiber filter (per Section F.7). The filtrate will not be extracted. Weigh a well-mixed aliquot (up to 10-g wet weight) of each sample into a clean beaker or glass jar. When mixing the solid samples prior to weighing out an aliquot, compare the particle size to that of the Hydromatrix drying agent (D.2.2). The sample particles should be no bigger than the Hydromatrix particles. If the sample particles are bigger than the Hydromatrix, but can be crushed with a spatula, weigh out the sample aliquot and crush to size while mixing and drying the sample with Hydromatrix (see F.5.1.4 below). If there are large particles that cannot be broken up in the manner discussed above, contact the project manager for further options.

F.5.1.3 For each sample or sample batch (to a maximum of 20 samples) to be extracted during the same 12 hour shift, weigh two 10-g aliquots of the appropriate reference matrix (Section D.5) into clean beakers or glass jars. One will serve as the method blank, the other as the OPR.

F.5.1.4 Add enough Hydromatrix to each sample to cover the sample and to give a 2 cm layer above the sample. Mix well. If the sample is still moist, add small amounts of Hydromatrix until the sample becomes a free flowing, powdery mixture. Load each sample into the thimble without disturbing the silica layer.

F.5.1.5 Spike 1.0 mL of the Labeled Toxics/LOC/window-defining standard spiking solution (D.6.4.2) into each field and QC sample while in the extraction apparatus.

F.5.1.6 Spike 1.0 mL of the Native Toxics/LOC standard spiking solution (D.6.4.1) into the OPR and any matrix spike samples (as indicated in project workplan) while in the extraction apparatus. For MDL samples use 250 µL.

F.5.1.7 Reassemble the pre-extracted Soxhlet apparatus, and add a fresh charge of solvent to the receiver and reflux flask. Apply power to the heating mantle to begin refluxing. Extract for a minimum of 16 hours. After extraction, concentrate the extracts to approximately 30 mL following procedures in G.2. Proceed to Section H.1 for extract cleanup.

F.5.2 Accelerated Solvent Extraction (ASE) for Soils, Sediments and Solids

F.5.2.1 Clean ASE cells by removing the cells from the ASE system (make sure the cells are cool to the touch). Unscrew top endcap and discard the contents appropriately, then remove bottom endcap and filter. Wash cell bodies in detergent water and endcaps in very dilute detergent water (washing endcaps with large amounts of detergent makes it difficult to rinse detergent out of frit). Rinse all parts with tap water, then MilliQ DI water, and finally methanol. Make sure all ink has been removed from cell bodies. Cover all parts with methanol and sonicate for 10 minutes. Pour off methanol and replace with methylene chloride, sonicate for 10 minutes. Allow all parts to air dry.

F.5.2.2 Assemble bottom endcap to cell body and insert filter (C.2.3.4). A muffled, 25-mL disposable pipette (top cut off and edges fire polished) may aid in keeping the filter level while pushing the filter into the cell.

F.5.2.3 Weigh out samples following section F.5.1.2

F.5.2.4 Make method blank and OPR samples following section F.5.1.3

F.5.2.5 Add approximately 5 g Hydromatrix (D.2.2) to each sample and mix well. (approximate 5 g by weighing 5 g Hydromatrix into a small beaker and

marking the volume with a permanent marker. Use this line to measure 5 g for all other samples).

F.5.2.6 Load approximately ½ of the sample-Hydromatrix mixture into cell using a solids funnel. Pack contents tightly using cell packing rod.

F.5.2.7 Dilute the Labeled toxics/LOC/window defining stock solution (D.6.2.1) to 20 ng/mL in nonane. Spike 100 µL of the 20 ng/mL solution into each field sample and QC sample while in ASE cell.

F.5.2.8 Dilute the Native Toxics/LOC stock solution (D.6.1.1) to 10 ng/mL in nonane. Spike 100 µL of the 10 ng/mL solution into OPR and matrix spike samples (as indicated in project workplans) while in ASE cell. For MDL samples use 25 µL of the 10 ng/mL solution.

F.5.2.9 Load remaining sample using a solids funnel. Pack contents tightly using cell packing rod. Fill any empty space at the top of cell with Hydromatrix. Assemble top endcap.

F.5.2.10 Load ASE cells and ASE large vials onto ASE, making sure the sample ID's on both cell and vial are correct. Use ASE manufacturer guidelines when loading and labeling both cells and vials. Check that the Dionex solvent controller has all solvent containers full and in the correct position. Empty and recap all waste rinse vials. Check that the nitrogen supply is adequate to complete all samples (1000 psi for 24 samples, 500 psi for 12 samples).

F.5.2.11 Before extracting samples load method "Rinse.met" on ASE system and press rinse two times. The rinse method will rinse the lines with acetone.

F.5.2.12 On the ASE system load method "Dioxinsed.met". Fill any size ASE cell full with muffled Accusand (Accusand specialty sand, industrial quality). Use this cell as a "dummy sample" and place in slot 1 on the ASE system. This sample is used to check for proper ASE function and will not be analyzed. Load the remaining samples in the slots following the slot containing the Accusand.

F.5.2.13 Check "Dioxinsed.met" for the following parameters:

Pressure at 2000 psi
Temperature at 150°C
Pre-heat time at 0
Heat time at 7 minutes
Static time at 5 minutes
Flush volume at 60%
Purge time at 60 seconds
Static cycles at 2
Toluene at 100%
5 mL rinse between each sample on schedule

F.5.2.14 Start the extraction. Check the "dummy sample" for proper operation before leaving system unattended.

F.5.2.15 After extraction, transfer each sample extract (excluding the dummy sample) into a 250-mL round bottom flask as follows. For each sample prepare a funnel ¾ full with sodium sulfate (D.2.1) supported by muffled glasswool and rinse the funnel with toluene. Pour each sample extract through a sodium sulfate-filled funnel into an individual 250-mL round bottom flask. Each ASE vial is then rinsed with 3 x 4-mL toluene with each rinse also being transferred to its respective 250-mL round bottom through the sodium sulfate. The sodium sulfate is then rinsed with at least 20 mL toluene to complete the transfer. Using rotary evaporator concentration (G.1), concentrate each sample until the round bottom just appears wet -do NOT let the round bottom go dry. Add 4 mL hexane to each sample, cap with glass stoppers and seal with teflon tape. Proceed to Section H.1 for extract cleanup.

F.6 Fish and Other Tissues

F.6.1 **Homogenization.** Samples are homogenized while still frozen, where practical. Each analysis requires 10 g of tissue (wet weight), therefore, the laboratory should homogenize at least 20 g of tissue if possible. When whole fish analysis is necessary, the entire fish is homogenized. Homogenize samples using a tissue homogenizer (C.1.2). To assure homogeneity, grind three times. Store homogenized tissue at <-10°C.

F.6.2 **Percent lipid determination**-If required by the project, lipid content can be determined. The following procedure utilizes the same solvent system (methylene chloride:hexane) that was used in EPA's National Dioxin Study so that lipid contents are consistent with that study. If lipid determination is required, extract a separate aliquot from that used to determine PCBs so as to avoid loss of PCBs in concentration to near dryness. Soxhlet extract as per Sections F.6.3.2, F.6.3.5, and F.6.3.8. Concentrate by KD (G.3) or rotovap (G.1) to approximately 1 mL. To complete removal of solvent use nitrogen blowdown until a constant residue weight is obtained. Re-dissolve the residue and transfer to another vial. Use several rinses to assure that all material is transferred. Allow receiver and boiling chips to dry. Weigh receiver and boiling chips. Calculate lipid content as follows:

*Percent Lipid = (weight of residue (g)/ weight of tissue (g))*100*

F.6.3 Preparation of fish and other tissue by soxhlet.

F.6.3.1 Prepare Soxhlets for extraction of tissue samples. Place a thimble filled with muffled glass wool in a clean extractor. Fill the 250-mL round bottom ¾ full with methylene chloride:hexane (1:1). Pre-extract the glassware, which is muffled at 450°C the night before use, by heating the flask until the solvent is boiling. When properly adjusted, 1 to 2 drops of solvent will fall

per second from the condenser tip into the receiver. Extract the apparatus for a minimum of 3 hours. After pre-extraction, cool and disassemble the apparatus. Archive the pre-extraction solvent at room temperature. Add silica to the thimble prior to the addition of sample.

F.6.3.2 Transfer approximately 10 g (wet weight) of homogenized tissue to a clean, sample jar or beaker.

F.6.3.3 Prepare a Method blank by adding approximately 1-2 g of the tissue reference matrix (Section D.5.3) to a clean sample jar.

F.6.3.4 Prepare an OPR sample by adding 1-2 g of the tissue reference matrix (Section D.5.3) to a separate clean sample jar.

F.6.3.5 Add enough Hydromatrix (D.2.2) to each sample to cover the sample and to give a 2 cm layer above the sample. Mix well. If the sample is still moist, add small amounts of Hydromatrix until the sample becomes a free flowing powdery mixture. Load each sample into the thimble without disturbing the silica layer.

F.6.3.6 Spike 1.0 mL of the Labeled Toxics/LOC/window-defining standard spiking solution (Section D.6.4.2) into each sample while in the extraction apparatus.

F.6.3.7 Spike 1.0 mL of the Native Toxics/LOC standard spiking solution (Section D.6.4.1) into the OPR and any matrix spike aliquots (as indicated in project workplan) while in extraction apparatus. Spike 250 µL for MDL samples.

F.6.3.8 Reassemble the pre-extracted Soxhlet apparatus and add a fresh charge of methylene chloride:hexane (1:1) to the receiver and reflux flask. Apply power to the heating mantle to begin refluxing. Extract for a minimum of 16 hours.

F.6.3.9 Concentrate extracts to approximately 20 mL using the heating mantel procedure in G.2 and proceed to Section H.1 for extract cleanup.

F.6.4 Preparation of fish and other tissue by ASE

F.6.4.1 Clean ASE cells by removing the cells from the ASE system (make sure the cells are cool to the touch). Unscrew top endcap and discard the contents appropriately, then remove bottom endcap and filter. Wash cell bodies in detergent water and endcaps in very dilute detergent water (washing endcaps with large amounts of detergent makes it difficult to rinse detergent out of frit). Rinse all parts with tap water, then MilliQ DI water, and finally methanol. Make sure all ink has been removed from cell bodies. Cover all parts with methanol and sonicate for 10 minutes. Pour off methanol and replace with methylene chloride, sonicate for 10 minutes. Allow all parts to air dry.

F.6.4.2 Assemble bottom endcap to cell body and insert filter (C.2.3.4). A muffled, 25-mL disposable pipette (top cut off and edges fire polished) may aid in keeping the filter level while pushing the filter into the cell.

F.6.4.3 Weigh out samples and prepare the method blank and OPR following sections F.6.3.2 through F.6.3.4.

F.6.4.4 Add approximately 5 g Hydromatrix (D.2.2) to each sample and mix well. (approximate 5 g by weighing 5 g Hydromatrix into a small beaker and marking the volume with a permanent marker. Use this line to measure 5 g for all other samples).

F.6.4.5 Load approximately ½ of the sample-Hydromatrix mixture into cell using a solids funnel. Pack contents tightly using cell packing rod.

F.6.4.6 Dilute the Labeled toxics/LOC/window defining stock solution (D.6.2.1) to 20 ng/mL in nonane. Spike 100 µL of the 20 ng/mL solution into to each field sample and QC sample while in ASE cell.

F.6.4.7 Dilute the Native Toxics/LOC stock solution (D.6.1.1) to 10 ng/mL in nonane. Spike 100 µL of the 10 ng/mL solution into OPR and matrix spike samples (as indicated in project workplans) while in ASE cell. For MDL samples use 25 µL of the 10 ng/mL solution.

F.6.4.8 Load remaining sample using a solids funnel. Pack contents tightly using cell packing rod. Fill any empty space at the top of cell with Hydromatrix. Assemble top endcap.

F.6.4.9 Load ASE cells and ASE large vials onto ASE, making sure the sample ID's on both cell and vial are correct. Use ASE manufacturer guidelines when loading and labeling both cells and vials. Check that the Dionex solvent controller has all solvent containers full and in the correct position. Empty and recap all waste rinse vials. Check that the nitrogen supply is adequate to complete all samples (1000 psi for 24 samples, 500 psi for 12).

F.6.4.10 Before extracting samples load method "Rinse.met" on ASE system and press rinse two times. The rinse method will rinse the lines with acetone.

F.6.4.11 On the ASE system load method "Dioxintissue.met". Fill any size ASE cell full with muffled Accusand (Accusand specialty sand, industrial quality). Use this cell as a "dummy sample" and place in slot 1 on the ASE system. This sample is used to check for proper ASE function and will not be analyzed. Load the remaining samples in the slots following the slot containing the Accusand.

F.6.4.12 Check "Dioxintissue.met" for the following parameters:
Pressure at 2000 psi

Temperature at 100°C
Pre-heat time at 0
Heat time at 5 minutes
Static time at 5 minutes
Flush volume at 60 %
Purge time at 60 seconds
Static cycles at 3
DCM at 50 %
Hexane at 50 %
5 mL rinse between each sample on schedule

F.6.4.13 Start the extraction. Check the "dummy sample" for proper operation before leaving system unattended.

F.6.4.14 After extraction, transfer each sample extract (excluding the dummy sample) into a 250-mL round bottom flask as follows. For each sample prepare a funnel ¾ full with sodium sulfate (D.2.1) supported by muffled glasswool and rinse the funnel with hexane. Pour each sample extract through a sodium sulfate-filled funnel into an individual 250-mL round bottom flask. Each ASE vial is then rinsed with 3 x 4-mL hexane with each rinse also being transferred to its respective 250-mL round bottom through the sodium sulfate. The sodium sulfate is then rinsed with at least 20 mL hexane to complete the transfer. Using heating mantel concentration (G.2), concentrate each sample to approximately 20 mL hexane-do NOT let the round bottom go dry. Add 4 mL hexane to each sample, cap with glass stoppers and seal with teflon tape. Proceed to Section H.1 for extract cleanup.

F.7 Filtration

F.7.1 Assemble the filtration unit (C.3.1) using glass fiber filter paper (C.3.2). Run a minimum of 1 L of reagent water (D.5.1) through the filter unit prior to the first sample to make sure the unit is leak free and operating properly. Use the manufacturer's suggested program to operate the filtration unit.

F.7.2 Filter the method blank first. Rinse the jar which contained the method blank with reagent water (D.5.1) and add this rinse to the filter unit.

F.7.3 Remove the filter from the filter unit after all liquid had passed through. Place the filter in a Soxhlet apparatus.

F.7.4 Transfer the filtrate to a 2-L separatory funnel if filtrate is to be extracted or to a jar if the filtrate is to be retained until samples have been analyzed.

F.7.5 Disassemble the filtration unit and rinse with reagent water (D.5.1) to clean. Reassemble the filter unit with a new filter before proceeding with filtering the next sample per Sections F.7.2 to F.7.5.

G. Concentration Procedures

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.*

G.1 **Rotary evaporation**-Concentrate the extracts in separate round-bottom flasks. Assemble the rotary evaporator according to manufacturer's instructions, and warm the water bath to 45 °C. On a daily basis, pre-clean the rotary evaporator by concentrating 100 mL of clean extraction solvent through the system. Archive both the concentrated solvent and the solvent in the catch flask for a contamination check if necessary. Between samples, three 2- to 3-mL aliquots of solvent should be rinsed down the feed tube into a waste beaker. Attach the round-bottom flask containing the sample extract to the rotary evaporator. Slowly apply vacuum to the system, and begin rotating the sample flask. Lower the flask into the water bath, and adjust the speed of rotation and the temperature as required to complete concentration in 15 to 20 minutes. At the proper rate of concentration, the flow of solvent into the receiving flask will be steady, but no bumping or visible boiling of the extract will occur.

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

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.

G.2 **Heating mantle** -Concentrate the extracts in separate round-bottom flasks. Add one or two clean boiling chips to the round-bottom flask, and attach a three-ball macro Snyder column. Pre-wet the column by adding approximately 1 mL of solvent through the top. Place the round-bottom flask in a heating mantle, and apply heat as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter, but the chambers will not flood. When the liquid has reached an apparent volume of approximately 20 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.

G.3 **Kuderna-Danish (KD)**-Concentrate the extracts in separate 250-mL KD flasks equipped with Snyder columns, then with 25-mL concentrator tubes. The KD technique is used for solvents such as methylene chloride and hexane. Toluene is concentrated using the KD technique to ~3-6 mL followed by nitrogen blowdown to 20 –50 µL. Add 1 to 2 clean boiling chips to the receiver. Attach a three-ball macro Snyder column. Prewet the column by adding approximately 1 mL of solvent through the top. Place the KD apparatus in a hot water bath so that the entire lower rounded surface of the flask is bathed with steam. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood. When the liquid has reached an apparent volume of 1 mL, remove the KD 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 solvent. Remove the three-ball Snyder column, add a fresh boiling chip, and attach a two ball micro Snyder column to the concentrator tube. Prewet the column by adding approximately 0.5 mL of solvent through the top. Place the

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

H. Extract Cleanup

H.1 Back-extraction with base and acid.

H.1.1 Transfer sample and QC extracts to individual 250-mL separatory funnels with the following numbers of hexane rinses depending on the type of flask being used: 3 x 4 mL for 250-mL round bottom or for KD flask, 3 x 10 mL for 500-mL round bottom. Spike 1.0 mL of the Labeled cleanup standard spiking solution (D.6.4.3) into the separatory funnels containing the sample and QC extracts .

H.1.2 Partition the extract against 50 mL of sulfuric acid solution (D.1.2). Shake for 2 minutes with periodic venting into a hood. Remove and discard the aqueous layer. Repeat the acid washing until no color is visible in the aqueous layer, to a maximum of four washings.

H.1.3 Partition the extract against 50 mL of sodium chloride solution (D.1.4) in the same way as with acid. Discard the aqueous layer.

H.1.4 Partition the extract against 50 mL of potassium hydroxide (D.1.1) in the same way as with acid. Repeat the base washing until no color is visible in the aqueous layer, to a maximum of four washings. Minimize contact time between the extract and the base to prevent degradation of the PCBs.

H.1.5 Repeat the partitioning against sodium chloride solution two times and discard the aqueous layer each time.

H.1.6 Pour each extract through a funnel containing 7 to 10 cm of hexane-rinsed granular anhydrous sodium sulfate (D.2.1). Rinse the separatory funnel with 3 x 10-mL hexane, and pour through the funnel containing sodium sulfate. Rinse sodium sulfate with 2 x 20-mL hexane. Collect each extract in a 250-mL or 500-mL round-bottom flask. Re-concentrate the sample and QC aliquots using KD (G.3) and proceed to Section H.2

H.2 Silica gel cleanup.

H.2.1 Acid/base silica columns are prepared by cutting the tops from 25-mL muffled pipettes, plugging with muffled glass wool, adding activated silica to the 22-mark, base silica to the 18-mark, silica to the 16-mark, acid silica to the 1-mark and approximately ½ inch of muffled sodium sulfate on top. See Section D.4.1 for silica gel preparation.

H.2.2 Pre-elute the column with 20 mL of hexane. Discard the eluate. Check the column for channeling. If channeling is present, discard the column and prepare another.

H.2.3 As the last of the hexane rinse approaches the top of the packing of the acid/base column the sample extracts are applied to the columns using muffled Pasteur pipettes with 3 x 4-mL hexane rinses of the KD flask or roundbottom.

H.2.4 The columns are eluted with 75 mL hexane and the hexane is collected in a round bottom flask.

H.2.5 The sample extracts are KD concentrated (G.3) to ~0.5 mL in a 90°C water bath and brought up in 1 mL hexane through the Snyder column.

H.2.6 If the acid silica remains discolored throughout the column a second acid/base silica column should be processed. If a second silica column is not required, proceed to Section H.3

H.3. **Alumina column cleanup – Optional**. Check project workplan or with project manager to determine if this cleanup step is required.

H.3.1 Sample extracts must be in methylene chloride when loading onto the alumina column, so solvent exchange may be needed if extracts are not already in methylene chloride.

H.3.2 Prepare columns as follows. Pack the tip of a 19/21-mm chromatography column with a small piece of glass wool. Add ~ 10 mL methylene chloride and tap glass wool with clean glass rod to remove bubbles. Drain to waste. Add ~25 mL methylene chloride to the column. Weigh 20g of 2% deactivated alumina (D.4.3) into a beaker. Add ~30 mL methylene chloride and swirl to remove bubbles. Slowly pour alumina slurry into the column while rinsing the beaker with methylene chloride. Place ~ 1g of sodium sulfate on top of the alumina. Tap the column to remove bubbles. Drain column to top of packing and discard solvent. Column is now ready for use. Columns must be used the day they are prepared.

H.3.3 Put a clean glass collection flask under the column. Load the 1 to 2 mL sample extract (must be in methylene chloride) onto the column. Slowly drain and stop at the top of the alumina packing. Rinse the sample vial with about 1 mL methylene chloride and load onto column. Slowly drain and stop at the top of the packing. Repeat for a total of three rinses of the extract container. Elute the column with 100mL methylene chloride.

H.4 **Gel Permeation Chromatography (GPC) – Optional**. Check project workplan or with project manager to determine if this cleanup step is required

H.4.1 Column calibration

H.4.1.1 Load 5 mL of the GPC calibration solution (D.4.3) into the sample loop. Inject the GPC calibration solution and record the signal from the detector. The elution pattern will be corn oil, BEHP, methoxychlor, perylene, and sulfur.

H.4.1.2 Set the "dump time" to allow >85% removal of BEHP and >85% collection of methoxychlor.

H.4.1.3 Set the "collect time" to the time of the sulfur peak maximum.

H.4.1.4 Verify calibration with the GPC calibration solution after every 20 extracts at a minimum. Calibration is verified if the recovery of the methoxychlor is greater than 85%. If calibration is not verified, the system should be recalibrated using the GPC calibration solution prior to processing any additional samples, and the impact of the unverified calibration on the previous sample batch must be discussed with the project manager so that a decision can be made as to the usability of the samples.

H.4.2 Extract cleanup. GPC requires that the column not be overloaded. The column (C.4.5.1) is designed to handle a maximum of 0.5 g of material in a 5-mL extract, and has been shown to handle 1.5 g of lipid from a tissue sample in a 5-mL extract. If the extract is known or expected to contain more than these amounts, 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.

H.4.2.1 Using KD concentration (G.3), solvent exchange the sample extract into methylene chloride. Bring to 2 mL in methylene chloride. Transfer the extract to a GPC vial with 3 x 1-mL methylene chloride rinses. Add 1 mL methylene chloride to the GPC vial to bring the total volume to 6 mL methylene chloride and mark the meniscus on the GPC vial.

H.4.2.2 Check the GPC system to insure that there is sufficient solvent in the reservoirs to accommodate processing all the samples in the batch.

H.4.2.3 Filter the extract or load through the filter holder (C.4.5.3) to remove particles. Load the first 6.0-mL sample extract onto the column.
Note: *5 mL will be injected to the GPC and 1 mL will be flushed to waste. This split must be taken into account in determining sample concentrations and should be documented in the project lab record book.*

H.4.2.4 Elute the extract according to the calibration data determined in H.4.1. Collect the eluate in a clean 400- to 500-mL beaker. Allow the system to rinse for additional 10 minutes before injecting the next sample.

H.4.2.5 Rinse the sample loading tube thoroughly with methylene chloride between extracts to prepare for the next sample.

H.4.2.6 If an extract is encountered that could overload the GPC column to the extent that carry-over could occur, a 6.0-mL methylene chloride blank should be run through the system to check for carry-over.

H.4.2.7 Concentrate the eluate into 1 mL hexane by KD (G.3) and proceed to H.5.

H.5 Carbon column

H.5.1 Carbon/Celite columns are prepared by cutting the top from a 25-mL muffled pipette, plugging one end with muffled glass wool, adding 3.6 g of carbon/Celite mixture (D.4.2) and plugging with muffled glass wool.

H.5.2 The columns are rinsed with 20 mL toluene, 20 mL methylene chloride, and 20 mL hexane. The rinses are added as each rinse approaches the top of the packed bed to avoid any mixing and dilution of solvents.

H.5.3 When the solvent is within 1 mm of the column packing, the sample extracts are applied using muffled Pasteur pipettes with 2 x 1-mL and 1 x 2-mL hexane rinses of the KD flask. The column is then eluted with 25 mL hexane and collected in a round bottom flask. The hexane rinse should contain the mono- and di-ortho PCBs. As the last of the hexane passes through the column, the column is rinsed with 15 mL methanol and collected in a 4-dram vial. The methanol rinse is not analyzed but will be archived until completion of successful analysis of samples. The column is then eluted with 15 mL toluene and collected in the same round bottom as the hexane eluant. The toluene rinse should contain PCBs 77, 126, and 169.

H.5.4 The combined hexane/toluene fractions from the carbon column are KD concentrated to ~ 4 mL in a 100°C water bath and brought up to 5 mL hexane through the Snyder column. Proceed to final blowdown (H.6).

H.6 Final Blowdown

H.6.1 Muffled concentrator tubes (C.5.4.2) are rinsed with methylene chloride by vortexing for ~30 seconds and discarding the rinse. After the concentrator tubes have dried, 20 µL of nonane is pipetted into the tubes. The tubes are lightly tapped to remove any air bubbles and the meniscus marked. Hexane (200 µL) is then pipetted into the tubes and the tubes are lightly tapped to remove any air bubbles. The meniscus of the hexane is then marked. Leaving the solvents in the concentrator tubes, the sample extracts are transferred from the KD flasks (from the carbon column cleanup procedure) into the concentrator tubes using muffled Pasteur pipettes with 3 x 1-mL hexane rinses of the KD flasks. The sample extracts are then blown down under nitrogen in a 45 °C sand bath to the hexane-marked meniscus. The KD flasks are then rinsed with 1 x 1-mL methylene chloride and the rinses transferred to the concentrator tubes. The extracts are again blown down under nitrogen to the hexane-marked meniscus. The KD flasks are then rinsed with 1 x 0.5-mL methylene chloride and the rinses transferred to the concentrator tubes. The extracts are again blown down to the hexane-marked meniscus. The sample extracts are then spiked with 10 µL Labeled injection internal standard spiking solution (D.6.4.4) and vortexed for approximately 10 seconds. The sample extracts are ready to be transferred to a GC vial per H.7. If the sample extracts cannot be transferred to GC vials immediately, store the extracts at < -10°C.

H.7 Transfer to GC Vial

H.7.1 If the sample extracts were stored, remove them from the freezer and allow to come to room temperature.

H.7.2 Put identifying labels on the pre-assembled GC vials with 300-µL inserts (C.5.4.3). There must be a gap in the label on the vial for liquid volume inspection. Using a micro syringe add 20 µL nonane to the 300-µL insert and mark the meniscus with a permanent marker.

H.7.3 Put squeeze bulbs on pipettes . Visually inspect concentrator tubes to insure that at least 200 µL volume is contained. If not, adjust to hexane-marked meniscus line with methylene chloride.

H.7.4 Vortex the concentrator tubes, making sure that the liquid rinses the side walls. A 200-µL volume will reach about halfway up the tube.

H.7.5 Transfer the contents of the concentrator tube to the correct GC vial insert. It is not possible with a disposable pipette to completely transfer all the material (about 10 µL will remain).

H.7.6 Cap the GC vial and place it in a plastic box meant for holding GC vials. Continue with the remaining samples. When all original samples have been transferred, start the nitrogen blow down procedure described in H.7.7 through H.7.9.

H.7.7 Push Teflon tubing onto the ¼" metal tube/valve assemblies, and install new glass pipettes on the N-Evap (C.5.3).

H.7.8 Check to see that the Nitrogen is turned on at the tank, and that the pressure is set to 40 psi on the second stage of the tank regulator, and ~ 5 psi on the regulator just before the N-Evap.

H.7.9 Uncap the GC vials and place them in the sample holder, with the label gap visible so that the solvent level may be inspected. Push the pipettes down until the tip just rests inside the vial opening. Check each blowdown valve to see that the flow is correct so that evaporation occurs, but the level of the liquid is not greatly deformed. Blow sample extracts down to the 20 µL nonane-marked meniscus. Never let the sample go to dryness. For some samples, a 50 µL final sample volume may be needed.

H.7.10 Vortex the GC vial and return it to the plastic box. Sample extracts should be stored in a freezer at <-10°C until HRGC/HRMS analysis.

I. Calibration

I.1 Recommended GC operating conditions:

Injector temperature:	140°C
Interface temperature:	300°C
Initial temperature:	70 °C
Initial time:	3 minutes
Temperature program:	70°C for 3 min, 70-120°C @ 10 °C/minute, 120-280°C @ 3°C/minute
Final time:	22 minutes

Note: *All portions of the column that connect the GC to the ion source should remain at or above the interface temperature specified above during analysis to preclude condensation of less volatile compounds.*

I.2 Retention time calibration for the CB congeners

I.2.1 At least once every 24 hours of analysis, inject the diluted combined 209 congener solution (D.6.3.2). Establish the beginning and ending retention times for the scan descriptors in Appendix D. Check the column performance criteria per Section C.6.1.1 to C.6.1.3. If acceptable, store the retention time (RT) and relative retention time (RRT) for each congener in the data system. Appendix E contains chromatograms showing expected retention order of all PCBs in the diluted combined 209 congener solution.

I.2.2 On an ongoing basis with every 12 hour CS3 (VER), monitor the retention times of analytes for any shifts which might indicate changes in GC resolution. Also monitor the CS3 (VER) solution for continued co-elution of PCBs 156 and 157.

I.3 Mass spectrometer (MS) resolution

I.3.1 Using PFK (or other reference substance) and a molecular leak, tune the instrument to meet the minimum required resolving power of 10,000 (% valley) at m/z 330.9792 or any other significant PFK fragment in the range of 300 to 350. For each descriptor (see Appendix D), monitor and record the resolution and exact m/z's of three to five reference peaks covering the mass range of the descriptor.

Obtain a selected ion current profile (SICP) at the two exact m/z's specified in Appendix D and at ≥ 10,000 resolving power at each LOC for the native congeners and congener groups and for the labeled congeners. Because of 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 ≥ 8,000 throughout the mass range and must be ≥ 10,000 in the center of the mass range for each function. The level of PFK metered into the HRMS during analyses should be adjusted so that the amplitude of m/z 330.9792 is approximately 1 volt.

I.3.2 If the HRMS has the capability to monitor resolution during the analysis, it is acceptable to terminate the analysis when the resolution falls below the minimum to save re-analysis time.

I.4 Ion abundance ratios, minimum levels, and signal-to-noise ratios.

I.4.1 Inject a 1- or 2-µL aliquot of the CS-1 calibration solution (Table 5 – see Appendix A) using the GC conditions in I.1. Measure the SICP areas for each congener or congener group, and compute the ion abundance ratios at the exact m/z's specified in Appendix D. Compare the computed ratio to the theoretical ratio given in Table 8 (see Appendix A). All compounds in the CS-1 standard must have ion abundance ratios within the QC limits in Table 8 of Method 1668A (see Appendix A); otherwise, the mass spectrometer must be adjusted and this test repeated until the m/z ratios fall within the limits specified. If the adjustment alters the resolution of the mass spectrometer, resolution must be verified (I.3.1) prior to repeat of the test.

I.4.2 The mass spectrometer will be operated in a mass-drift correction mode, using PFK to provide lock m/z's. The lock mass for each group of m/z's is shown in Appendix D. Each lock mass must be monitored and must not vary by more than ±20% throughout its respective retention time window. Variations of lock mass by more than 20% indicate the presence of co-eluting interferences that raise the source pressure and may significantly reduce the sensitivity of the mass spectrometer. A lock mass interference or suppression in a retention time region in which PCBs and labeled compounds do not elute may be ignored. If such a suppression interferes with quantitation of analytes, the project manager should be notified and a decision made as to usability of the data. Possible corrective actions include dilution and re-analysis of the extract or additional cleanup and, at a minimum, the effect of the suppression on the sample results will be discussed in the report.

I.4.3 The peaks representing the PCBs and labeled compounds in the CS-1 calibration standard must have signal-to-noise ratios (S/N) $\geq$ 10 with the exception of the high mass for PCB 15 which should have a S/N $\geq$ 2.5; otherwise, the mass spectrometer must be adjusted and this test repeated.

I.5 Initial Calibration

I.5.1 Generate a 5- or 6- point curve using the solutions listed in D.6.3.1, at a minimum of every 6 months. Use a volume identical to that chosen in Section I.4, and the conditions in Section I.1. Calculate response factors for each analyte in the calibration solutions according the procedures in I.5.1.1 below. Use the quantitation relationships listed in Table 2 (Appendix A). Compute the average (mean) RRF and the RSD of the 5 (or 6) RRF. The response factors from the multi-point curve will be used to calculate sample concentrations when only the Toxics/LOC PCBs are target analytes.

I.5.1.1 The response of each analyte relative to its labeled internal standard is determined by using the area responses of both the primary and secondary exact m/z's specified in Appendix C, for each calibration standard, as follows:

$$RRF = \frac{(A1 + A2)\ Cs}{(A1s + A2s)\ C}$$

Where:

A1 and A2 = The areas of the primary and secondary m/z's for the compound of interest.

A1s andA2s = The areas of the primary and secondary m/z's for the appropriate labeled standard.

Cs = Concentration of the appropriate labeled standard

C= Concentration of the compound of interest.

I.5.2 Linearity

I.5.2.1 If the RRF for any Native Toxics/LOC CB is ≤ 20% RSD, the average RRF may be used for that congener; otherwise, the complete calibration curve for that congener must be used over the calibration range.

I.5.2.2 If the RRF for any Labeled Toxic/LOC/window defining standard or labeled cleanup standard is ≤ 35% RSD, the average RF may be used for that congener; otherwise the complete curve for that congener must be used over the calibration range.

I.5.3 Single Point Calibration for all 209 PBCs.

I.5.3.1 Along with every multi-point initial calibration, the diluted, combined 209 congener solution (D.6.3.2) should be analyzed using the volume chosen in Section I.4 and the conditions in Section I.1. Calculate RRFs for all compounds in the solution using the equation in I.5.1.1 and the quantitation relationships in Table 2 (Appendix A). The RRFs from this single calibration will be used for all analytes (including the Toxic/LOC PCBs) in instances when more than just the Toxic/LOC PCBs are to be determined. Response factors generated in this single point calibration are acceptable if they are within ± 35% of response factors generated in the multi-point curve for non-coeluting congeners which are in both the multi-point and the single-point calibration solutions.

J. HRGC/HRMS Analysis

J.1 Establish the operating conditions given in Section I.1. Inject 1 or 2 µL (consistent with volume used in calibration) of the concentrated extract using on-column or splitless injection (consistent with technique used in calibration).

J.2 MS resolution-Static resolving power checks and documentation should be performed at the beginning and at the end of each shift per Sections I.3.1. If analyses are performed on successive shifts, only the beginning of shift static resolving power check is required. If the requirements in Section I.3.1 cannot be met, the problem must be corrected before analyses can proceed.

J.3 Check windows and column performance per Section I.2

J.4 Verify calibration by injecting the CS-3 (VER) standard (D.6.3.1) using the procedure in Section J.1. The ion abundance ratios for all PCBs should be within the limits in Table 8 (see Appendix A); otherwise, adjust the mass spectrometer until the ion abundance ratios fall within the limits specified when the verification test is repeated. If the adjustment alters the resolution of the mass spectrometer, verify resolution (I.3.1) prior to repeat of the verification test. The GC peak representing each native PCB and labeled compound in the VER standard must be present with a S/N of at least 10; otherwise, adjust the mass spectrometer and repeat the verification test. Compute the concentration of the Toxics/LOC PCBs (Section L.1). These concentrations are computed based on the calibration data in Section I. For each compound in the CS-3 (VER), compare the concentration with the calibration verification limit in Table 6 (see Appendix A). If all compounds meet the acceptance criteria, calibration has been verified and analysis of standards and sample extracts may proceed. If, however, any compound fails its respective limit, the measurement system is not performing properly. In this event, correct the problem and repeat the resolution (J.2) and verification (J.4) tests, or recalibrate (Section I.5). Alternatively, average daily response factors from the continuing calibrations bracketing sample analyses may be used to calculate analyte concentrations.

J.5 Ongoing Precision and Recovery (OPR). Once the calibration has been verified, analyze the ongoing precision and recovery (OPR) extract prior to analysis of samples from the same batch. Compute the percent recovery of the Toxics/LOC PCBs and each labeled compound using the equations in Section L.1. For the Toxics/LOC PCBs and labeled compounds, compare the recovery to the OPR limits given in Table 6 (Appendix A). If all compounds meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may proceed. If, however, any individual concentration falls outside of the range given, correct the problem causing the outlying data if possible. At a minimum, causes of any problems should be investigated and any results outside acceptable criteria should be discussed in the report. Impact on sample results should also be discussed in the report.

J.6 Blank. Analyze the Method blank extracted with each sample batch immediately following analysis of the OPR to demonstrate freedom from contamination and freedom from carryover from the OPR. If any analyte is found in the blank at greater than the minimum level (EML in Table 2 – see Appendix A) the project manager should be notified. Certain PCBs are occasionally detected above the EML and should be flagged as such and background contribution to sample results discussed in the report. Any gross exceedences or wide-spread contamination should be evaluated by the project manager and a decision as to usability of the sample set should be made on a case-by-case basis. Once the method blank has been successfully analyzed, begin analysis of the remaining samples. On a continuing basis, the method blank should be reanalyzed after each calibration verification.

J.7 Analyze the sample extracts.

J.7.1 Labeled Compound Recovery. Target recovery of each labeled compound is listed in Table 6 (Appendix A). If the recovery of any compound falls outside of these limits, the project manager should be notified and attempts to identify the reason for outlying recoveries should be made. Depending on the project requirements, client requirements and the severity of the outlying data, a project manager will decide how to treat the outlying data. Possible options include:

- evaluating the cleanup column archive rinses for lost labeled standards
- diluting samples and reanalyzing to minimize interferences
- documenting possible sources of outlying data in the report to the client and discussing the impact any exceedences have on reported results.

K. Qualitative determination

A PCB or labeled compound is identified in a standard, blank, or sample when all of the criteria in Sections K.1 through K.4 are met.

K.1 The signals for the two exact m/z's in Appendix D must be present and must maximize within the same two scans.

K.2 The signal-to-noise ratio (S/N) for the GC peak at each exact m/z must be greater than or equal to 2.5 for each PCB detected in a sample extract, and greater than or equal to 10 for all PCBs in the calibration and verification standards (CS-1 and higher standards with the exception noted in I.4.3).

K.3 Ion abundance ratios of the two exact m/z's specified in Appendix D should be within the limit in Table 8 (see Appendix A). At the analysts discretion, peaks which meet all other identification criteria except ion ratio may be calculated as an Estimated Maximum Possible Concentration by determining peak areas which would provide theoretically correct ion ratios and using these areas in the calculation of analyte concentrations per L.1.

K.4 The relative retention time of the peak for a PCB should be within the RRT established in the calibration unless the analyst can identify a justifiable reason for a shift in the retention times (e.g., chromatographic interference).

L Quantitative Determination

L.1 Compute the concentrations of native PCBs and labeled compounds within a sample using the RRFs from the calibration data (Section I.5), the quantitation relationships in Table 2 (Appendix A) and the following equation. Be sure to account for any project-specific splits of the sample extract or splits as a result of GPC cleanup as appropriate.

$$C\ (pg/g\ or\ pg/L) = \frac{(A1 + A2) * Cs}{(A1s + A2s) * RRF * V}$$

where:
V = sample weight or volume in g or L, and the other terms are as defined in Section I.5.1.

L.2 If the SICP area at either quantitation m/z for any congener exceeds the calibration range of the system, notify the project manager. Actions taken for levels exceeding the calibration range will be project specific and must be approved by the project manager. Such actions may include the following

- At a minimum, levels exceeding the calibration should be flagged and discussed in the report.
- The sample extract may be diluted by the factor necessary to bring the concentration within the calibration range. Adjust the concentration of the Labeled injection internal standard to 100 pg/μL in the extract, if necessary. Adjust the PCB congener concentrations, detection limits, and minimum levels to account for the dilution.
- If the PCBs cannot be measured reliably by dilution of the sample extract, a diluted aqueous sample or a smaller portion of a soil, tissue, or mixed-phase sample may be analyzed.

L.3 If requested, the total concentration of all congeners at a given level of chlorination (i.e., total TrCB, total PeCB, total HxCB) may be reported by summing the concentrations of all congeners identified at that LOC, including both the Toxics and other congeners.

VI. Revision History

This is the first version of this SOP.

Appendix A
Relevant Tables from Method 1668A

Table 2. Retention times (RT), RT references, relative retention times (RRTs), estimated method detection limits (EMDLs), and estimated minimum levels (EMLs) for the 209 CB congeners on SPB-Octyl.

Cl No.[1]	IUPAC No.[2,3]	RT Ref[4]	RTs[5]	RRT[6]	RRT limits[7]	Window (sec)[8]	Quantitation reference[9]	Detection limits and minimum levels - Matrix and concentration[10] Water (pg/L) EMDL	Water (pg/L) EML	Other (ng/kg) EMDL	Other (ng/kg) EML	Extract (pg/μL) EML
Compounds using 9L ($^{13}C_{12}$-2,5-DiCB) as Labeled injection internal standard												
CB congener												
Monochlorobiphenyls												
1	1	1L	13:44	1.0012	0.9951-1.0073	10	1L	82	200	8	20	10
1	2	3L	16:08	0.9878	0.9847-0.9908	6	1L/3L	4	10	0.4	1	0.5
1	3	3L	16:21	1.0010	0.9980-1.0041	6	3L	88	200	9	20	10
Dichlorobiphenyls												
2	4	4L	16:40	1.0010	0.9960-1.0060	10	4L	172	500	17	50	20
2	10	4L	16:53	1.0140	1.0110-1.0170	6	4L/15L	22	50	2	5	2
2	9	4L	18:55	1.1361	1.1331-1.1391	6	4L/15L	20	50	2	5	2
2	7	4L	19:07	1.1481	1.1451-1.1512	6	4L/15L	15	50	2	5	2
2	6	4L	19:26	1.1672	1.1642-1.1702	6	4L/15L	13	50	1	5	2
2	5	4L	19:48	1.1892	1.1862-1.1922	6	4L/15L	11	50	1	5	2
2	8	4L	19:56	1.1972	1.1942-1.2002	6	4L/15L	121	500	12	50	20
2	14	15L	21:42	0.9267	0.9246-0.9288	6	4L/15L	31	100	3	10	5
2	11	15L	22:42	0.9694	0.9673-0.9715	6	4L/15L	105	200	10	20	10
2	13	15L	23:03	0.9843	0.9822-0.9865	6	4L/15L	28	100	3	10	5
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	0.9972-1.0043	10	15L	183	500	18	50	20
Trichlorobiphenyls												
3	19	19L	20:19	1.0008	0.9967-1.0049	10	19L	42	100	4	10	5
3	30	19L	22:15	1.0961	1.0936-1.0985	6	19L/37L	175	500	17	50	20
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	86	200	9	20	10
3	27	19L	23:06	1.1379	1.1355-1.1404	6	19L/37L	59	200	6	20	10
3	24	19L	23:14	1.1445	1.1420-1.1470	6	19L/37L	53	200	5	20	10
3	16	19L	23:25	1.1535	1.1511-1.1560	6	19L/37L	35	100	4	10	5
3	32	19L	24:57	1.2291	1.2266-1.2315	6	19L/37L	84	200	8	20	10
3	34	19L	25:17	1.2455	1.2430-1.2479	6	19L/37L	74	200	7	20	10
3	23	19L	25:26	1.2529	1.2504-1.2553	6	19L/37L	50	200	5	20	10

Cl No.[1]	IUPAC No.[2,3]	RT Ref[4]	RTs[5]	RRT[6]	RRT limits[7]	Window (sec)[8]	Quantitation reference[9]	Detection limits and minimum levels - Matrix and concentration[10] Water (pg/L) EMDL	Water (pg/L) EML	Other (ng/kg) EMDL	Other (ng/kg) EML	Extract (pg/µL) EML
3	29	19L	25:47	1.2701	1.2660-1.2742	10	19L/37L					
3	26	19L	25:48	1.2709	1.2668-1.2750	10	19L/37L	83	200	8	20	10
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	55	200	5	20	10
3	31	37L	26:25	0.8476	0.8460-0.8492	6	19L/37L	152	500	15	50	20
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	192	500	19	50	20
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	51	200	5	20	10
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	90	200	9	20	10
3	36	37L	29:05	0.9332	0.9316-0.9348	6	19L/37L	79	200	8	20	10
3	39	37L	29:30	0.9465	0.9449-0.9481	6	19L/37L	85	200	9	20	10
3	38	37L	30:10	0.9679	0.9663-0.9695	6	19L/37L	83	200	8	20	10
3	35	37L	30:42	0.9850	0.9834-0.9866	6	19L/37L	77	200	8	20	10
3	37	37L	31:11	1.0005	0.9989-1.0021	6	37L	132	500	13	50	20
	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	20:118	1.0741	1.0608-1.0873	30	9L					
3	37L	52L	31:10	1.0803	1.0716-1.0890	30	52L					
Compounds using 52L ($^{13}C_{12}$-2,2',5,5'-TeCB) as Labeled injection internal standard												
	CB congener											
	Tetrachlorobiphenyls											
4	54	54L	23:51	1.0007	0.9972-1.0042	10	54L	118	500	12	50	20
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	58	200	6	20	10
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	51	200	5	20	10
4	45/51	54L	26:57	1.1308	1.1273-1.1343	10	54L/81L/77L					
4	46	54L	27:18	1.1455	1.1434-1.1476	6	54L/81L/77L	101	200	10	20	10
4	52	54L	28:45	1.2063	1.2042-1.2084	6	54L/81L/77L	191	500	19	50	20

Cl No.[1]	IUPAC No.[2,3]	RT Ref[4]	RTs[5]	RRT[6]	RRT limits[7]	Window (sec)[8]	Quantitation reference[9]	Detection limits and minimum levels - Matrix and concentration[10] Water (pg/L) EMDL	Water (pg/L) EML	Other (ng/kg) EMDL	Other (ng/kg) EML	Extract (pg/µL) EML
4	73	54L	28:52	1.2112	1.2091-1.2133	6	54L/81L/77L	160	500	16	50	20
4	43	54L	28:58	1.2154	1.2133-1.2175	6	54L/81L/77L	94	200	9	20	10
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	115	500	11	50	20
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	76	200	8	20	10
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	195	500	19	50	20
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	57	200	6	20	10
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	61	200	6	20	10
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	119	500	12	50	20
4	40	54L	30:01	1.2594	1.2559-1.2629	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	70	200	7	20	10
4	72	81L	31:59	0.8336	0.8323-0.8349	6	54L/81L/77L	158	500	16	50	20
4	68	81L	32:18	0.8419	0.8406-0.8432	6	54L/81L/77L	149	500	15	50	20
4	57	81L	32:46	0.8540	0.8527-0.8553	6	54L/81L/77L	125	500	12	50	20
4	58	81L	33:05	0.8623	0.8610-0.8636	6	54L/81L/77L	127	500	13	50	20
4	67	81L	33:13	0.8658	0.8645-0.8671	6	54L/81L/77L	147	500	15	50	20
4	63	81L	33:30	0.8732	0.8719-0.8745	6	54L/81L/77L	138	500	14	50	20
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	171	500	17	50	20
4	74	81L ~~54L~~ ①	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					
4	66	81L	34:15	0.8927	0.8914-0.8940	6	54L/81L/77L	162	500	16	50	20
4	55	81L	34:28	0.8983	0.8970-0.8997	6	54L/81L/77L	120	500	12	50	20
4	56	81L	35:03	0.9136	0.9123-0.9149	6	54L/81L/77L	98	200	10	20	10
4	60	81L	35:16	0.9192	0.9179-0.9205	6	54L/81L/77L	131	500	13	50	20
4	80	81L	35:32	0.9262	0.9248-0.9275	6	54L/81L/77L	175	500	18	50	20

① Set at 81L to be consistent with other co-eluting isomers and to achieve the RRT listed in the table. NBS 6/2/01

Cl No.[1]	IUPAC No.[2,3]	RT Ref[4]	RTs[5]	RRT[6]	RRT limits[7]	Window (sec)[8]	Quantitation reference[9]	Detection limits and minimum levels - Matrix and concentration[10] Water (pg/L) EMDL	Water (pg/L) EML	Other (ng/kg) EMDL	Other (ng/kg) EML	Extract (pg/µL) EML
4	79	81L	37:16	0.9713	0.9700-0.9726	6	54L/81L/77L	173	500	17	50	20
4	78	81L	37:52	0.9870	0.9857-0.9883	6	54L/81L/77L	171	500	17	50	20
4	81	81L	38:23	1.0004	0.9991-1.0017	6	81L	177	500	18	50	20
4	77	77L	39:02	1.0004	0.9991-1.0017	6	77L	169	500	17	50	20
	Labeled compounds											
4	54L	52L	23:50	0.8261	0.8203-0.8319	20	52L					
4	81L	52L	38:22	1.3299	1.3241-1.3356	20	52L					
4	77L	52L	39:01	1.3524	1.3466-1.3582	20	52L					
Compounds using 101L ($^{13}C_{12}$-2,2',4,5,5'-PeCB) as Labeled injection internal standard												
	CB congener											
	Pentachlorobiphenyls											
5	104	104L	29:46	1.0000	0.9972-1.0028	10	104L	228	500	23	50	20
5	96	104L	30:17	1.0174	1.0146-1.0202	10	104L/123L/114L/118L/105L/126L	210	500	21	50	20
5	103	104L	32:11	1.0812	1.0795-1.0829	6	104L/123L/114L/118L/105L/126L	225	500	23	50	20
5	94	104L	32:29	1.0913	1.0896-1.0929	6	104L/123L/114L/118L/105L/126L	121	500	12	50	20
5	95	104L	33:00	1.1086	1.1058-1.1114	10	104L/123L/114L/118L/105L/126L					
5	100	104L	33:06	1.1120	1.1092-1.1148	10	104L/123L/114L/118L/105L/126L					
5	93	104L	33:14	1.1165	1.1137-1.1193	10	104L/123L/114L/118L/105L/126L	221	500	22	50	20
5	102	104L	33:21	1.1204	1.1176-1.1232	10	104L/123L/114L/118L/105L/126L					
5	98	104L	33:26	1.1232	1.1204-1.1260	10	104L/123L/114L/118L/105L/126L					
5	95/100/93/102/98	104L	33:13	1.1159	1.1131-1.1187	15	104L/123L/114L/118L/105L/126L					
5	88	104L	33:48	1.1355	1.1321-1.1389	12	104L/123L/114L/118L/105L/126L					
5	91	104L	33:55	1.1394	1.1366-1.1422	10	104L/123L/114L/118L/105L/126L	118	500	12	50	20
5	88/91	104L	33:52	1.1377	1.1344-1.1411	12	104L/123L/114L/118L/105L/126L					
5	84	104L	34:14	1.1501	1.1484-1.1517	6	104L/123L/114L/118L/105L/126L	124	500	12	50	20
5	89	104L	34:44	1.1669	1.1652-1.1685	6	104L/123L/114L/118L/105L/126L	195	500	19	50	20
5	121	104L	34:57	1.1741	1.1725-1.1758	6	104L/123L/114L/118L/105L/126L	209	500	21	50	20
5	92	123L	35:26	0.8639	0.8627-0.8651	6	104L/123L/114L/118L/105L/126L	115	500	12	50	20
5	113 123L	~~104L~~ ①	36:01	0.8781	0.8761-0.8801	10	104L/123L/114L/118L/105L/126L					
5	90	~~104L~~	36:03	0.8789	0.8769-0.8809	10	104L/123L/114L/118L/105L/126L	241	1000	24	100	50
5	101	~~104L~~	36:04	0.8793	0.8773-0.8813	10	104L/123L/114L/118L/105L/126L					
5	113/90/101	~~104L~~	36:03	0.8789	0.8769-0.8809	10	104L/123L/114L/118L/105L/126L					
5	83	~~104L~~	36:39	0.8935	0.8911-0.8960	12	104L/123L/114L/118L/105L/126L					
5	99	~~104L~~	36:41	0.8944	0.8923-0.8964	10	104L/123L/114L/118L/105L/126L	217	500	22	50	20
5	83/99	~~104L~~	36:40	0.8939	0.8915-0.8964	12	104L/123L/114L/118L/105L/126L					

① Needs to be 123L to achieve the RRT listed in the table. MES 6/2/01

Cl No.[1]	IUPAC No.[2,3]	RT Ref[4]	RTs[5]	RRT[6]	RRT limits[7]	Window (sec)[8]	Quantitation reference[9]	Detection limits and minimum levels - Matrix and concentration[10] Water (pg/L) EMDL	Water (pg/L) EML	Other (ng/kg) EMDL	Other (ng/kg) EML	Extract (pg/µL) EML
5	112 123L	~~104L~~ (1)	36:51	0.8984	0.8972-0.8996	6	104L/123L/114L/118L/105L/126L	245	1000	25	100	50
5	119	104L	37:12	0.9069	0.9037-0.9102	16	104L/123L/114L/118L/105L/126L					
5	108 (4)	104L	37:12	0.9069	0.9037-0.9102	16	104L/123L/114L/118L/105L/126L					
5	86	104L	37:17	0.9090	0.9057-0.9122	16	104L/123L/114L/118L/105L/126L	149	500	15	50	20
5	97	104L	37:17	0.9090	0.9057-0.9122	16	104L/123L/114L/118L/105L/126L					
5	125	104L	37:21	0.9106	0.9074-0.9139	16	104L/123L/114L/118L/105L/126L					
5	(4) 87	104L	37:25	0.9122	0.9102-0.9143	10	104L/123L/114L/118L/105L/126L					
5	108/119/86/97/125/87	104L	37:19	0.9098	0.9065-0.9130	16	104L/123L/114L/118L/105L/126L					
5	117	104L	37:57	0.9252	0.9228-0.9277	12	104L/123L/114L/118L/105L/126L					
5	116	104L	38:02	0.9273	0.9248-0.9297	12	104L/123L/114L/118L/105L/126L	104	200	10	20	10
5	85	104L	38:05	0.9285	0.9265-0.9305	10	104L/123L/114L/118L/105L/126L					
5	117/116/85	104L	38:00	0.9265	0.9240-0.9289	12	104L/123L/114L/118L/105L/126L					
5	110	104L	38:16	0.9330	0.9309-0.9350	10	104L/123L/114L/118L/105L/126L					
5	115	104L	38:18	0.9338	0.9317-0.9358	10	104L/123L/114L/118L/105L/126L	243	1000	24	100	50
5	110/115	104L	38:17	0.9334	0.9313-0.9354	10	104L/123L/114L/118L/105L/126L					
5	82	104L	38:40	0.9427	0.9415-0.9439	6	104L/123L/114L/118L/105L/126L	133	500	13	50	20
5	111	104L	38:52	0.9476	0.9464-0.9488	6	104L/123L/114L/118L/105L/126L	243	1000	24	100	50
5	120	104L	39:21	0.9594	0.9581-0.9606	6	104L/123L/114L/118L/105L/126L	147	500	15	50	20
5	107 (2)	104L	40:39	0.9911	0.9890-0.9931	10	104L/123L/114L/118L/105L/126L					
5	124	104L	40:40	0.9915	0.9894-0.9935	10	104L/123L/114L/118L/105L/126L	200	1000	27	100	50
5	(2) 107/124	104L	40:39	0.9911	0.9890-0.9931	10	104L/123L/114L/118L/105L/126L					
5	109 (3)	~~104L~~	40:54	0.9972	0.9959-0.9984	6	104L/123L/114L/118L/105L/126L	103	200	10	20	10
5	123	123L	41:02	1.0004	0.9992-1.0016	6	123L	150	500	15	50	20
5	106	123L	41:10	1.0037	1.0024-1.0049	6	104L/123L/114L/118L/105L/126L	143	500	14	50	20
5	118	118L	41:22	1.0004	0.9992-1.0016	6	118L	193	500	19	50	20
5	122	118L	41:49	1.0113	1.0101-1.0125	6	104L/123L/114L/118L/105L/126L	117	500	12	50	20
5	114	114L	41:58	1.0004	0.9992-1.0016	6	114L	120	500	12	50	20
5	105	105L	42:43	0.9996	0.9984-1.0008	6	105L	109	200	11	20	10
5	127	105L	44:09	1.0332	1.0320-1.0343	6	104L/123L/114L/118L/105L/126L	278	1000	28	100	50
5	126	126L	45:58	1.0004	0.9993-1.0015	6	126L	136	500	14	50	20
	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					

(1) Needs to be 123L to achieve the RRT listed in the table. MBS 6/2/01

(2) 107 = Accustandard IUPAC ID. Compound = 2,3,3',4,5'-PeCB (CAS 70362-41-3) whose current IUPAC = 108.

(3) 109 = Accustandard ID. Compound = 2,3,3',4',5-PeCB (CAS 70424-68-9) whose current IUPAC = 107.

(4) 108 = Accustandard ID. Compound = 2,3,3',4,6-PeCB (CAS 74472-35-8) whose current IUPAC = 109.

Cl No.[1]	IUPAC No.[2,3]	RT Ref[4]	RTs[5]	RRT[6]	RRT limits[7]	Window (sec)[8]	Quantitation reference[9]	Detection limits and minimum levels - Matrix and concentration[10] Water (pg/L) EMDL	Water (pg/L) EML	Other (ng/kg) EMDL	Other (ng/kg) EML	Extract (pg/μL) EML
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 Labeled injection internal standard												
	CB congener											
	Hexachlorobiphenyls											
6	155	155L	35:44	1.0000	0.9977-1.0023	10	155L	339	1000	34	100	50
6	152	155L	36:07	1.0107	1.0093-1.0121	6	155L/156L/157L/167L	238	1000	24	100	50
6	150	155L	36:15	1.0145	1.0131-1.0159	6	155L/156L/157L/167L	328	1000	33	100	50
6	136	155L	36:44	1.0280	1.0266-1.0294	6	155L/156L/157L/167L	91	200	9	20	10
6	145	155L	37:00	1.0354	1.0340-1.0368	6	155L/156L/157L/167L	317	1000	32	100	50
6	148	155L	34:26	1.0756	1.0742-1.0770	6	155L/156L/157L/167L	324	1000	32	100	50
6	151	155L	39:10	1.0961	1.0938-1.0984	10	155L/156L/157L/167L	112	500	11	50	20
6	135	155L	39:17	1.0993	1.0970-1.1017	10	155L/156L/157L/167L					
6	154	155L	39:21	1.1012	1.0989-1.1035	10	155L/156L/157L/167L					
6	151/135/154	155L	39:15	1.0984	1.0961-1.1007	10	155L/156L/157L/167L					
6	144	155L	39:47	1.1133	1.1119-1.1147	6	155L/156L/157L/167L	167	500	17	50	20
6	147	155L	40:09	1.1236	1.1213-1.1259	10	155L/156L/157L/167L	179	500	18	50	20
6	149	155L	40:12	1.1250	1.1227-1.1273	10	155L/156L/157L/167L					
6	147/149	155L	40:10	1.1241	1.1217-1.1264	10	155L/156L/157L/167L					
6	134	155L	40:27	1.1320	1.1297-1.1343	10	155L/156L/157L/167L	134	500	13	50	20
6	143	155L	40:30	1.1334	1.1311-1.1357	10	155L/156L/157L/167L					
6	134/143	155L	40:29	1.1329	1.1306-1.1353	10	155L/156L/157L/167L					
6	139	155L	40:47	1.1413	1.1390-1.1437	10	155L/156L/157L/167L	196	500	20	50	20
6	140	155L	40:48	1.1418	1.1395-1.1441	10	155L/156L/157L/167L					
6	139/140	155L	40:47	1.1413	1.1390-1.1437	10	155L/156L/157L/167L					
6	131	155L	41:03	1.1488	1.1474-1.1502	6	155L/156L/157L/167L	121	500	12	50	20
6	142	155L	41:13	1.1535	1.1521-1.1549	6	155L/156L/157L/167L	311	1000	31	100	50
6	132	155L	41:36	1.1642	1.1618-1.1665	10	155L/156L/157L/167L	125	500	12	50	20
6	133	155L	41:57	1.1740	1.1726-1.1754	6	155L/156L/157L/167L	169	500	17	50	20
6	165	167L	42:23	0.8864	0.8853-0.8874	6	155L/156L/157L/167L	361	1000	36	100	50
6	146	167L	42:38	0.8916	0.8906-0.8926	6	155L/156L/157L/167L	182	500	18	50	20
6	161	167L	42:47	0.8947	0.8937-0.8958	6	155L/156L/157L/167L	352	1000	35	100	50
6	153	167L	43:17	0.9052	0.9035-0.9069	10	155L/156L/157L/167L	130	500	13	50	20
6	168	167L	43:21	0.9066	0.9048-0.9083	10	155L/156L/157L/167L					
6	153/168	167L	43:19	0.9059	0.9041-0.9076	10	155L/156L/157L/167L					
6	141	167L	43:34	0.9111	0.9101-0.9122	6	155L/156L/157L/167L	93	200	9	20	10
6	130	167L	44:01	0.9205	0.9195-0.9216	6	155L/156L/157L/167L	136	500	14	50	20

Cl No.[1]	IUPAC No.[2,3]	RT Ref[4]	RTs[5]	RRT[6]	RRT limits[7]	Window (sec)[8]	Quantitation reference[9]	Detection limits and minimum levels - Matrix and concentration[10] Water (pg/L) EMDL	Water (pg/L) EML	Other (ng/kg) EMDL	Other (ng/kg) EML	Extract (pg/µL) EML
6	137	167L	44:14	0.9251	0.9240-0.9261	6	155L/156L/157L/167L	300	1000	30	100	50
6	164	167L	44:22	0.9278	0.9268-0.9289	6	155L/156L/157L/167L	136	500	14	50	20
6	138	167L	44:42	0.9348	0.9324-0.9373	14	155L/156L/157L/167L					
6	163	167L	44:42	0.9348	0.9324-0.9373	14	155L/156L/157L/167L					
6	129	167L	44:47	0.9366	0.9341-0.9390	14	155L/156L/157L/167L	211	500	21	50	20
6	160	167L	44:53	0.9387	0.9369-0.9404	10	155L/156L/157L/167L					
6	138/163/129/160	167L	44:47	0.9366	0.9341-0.9390	14	155L/156L/157L/167L					
6	158	167L	45:05	0.9428	0.9418-0.9439	6	155L/156L/157L/167L	96	200	10	20	10
6	166	167L	45:59	0.9617	0.9599-0.9634	10	155L/156L/157L/167L					
6	128	167L	46:46	0.9651	0.9634-0.9669	10	155L/156L/157L/167L	124	500	12	50	20
6	128/166	167L	46:04	0.9634	0.9617-0.9651	10	155L/156L/157L/167L					
6	159	167L	46:59	0.9826	0.9815-0.9836	6	155L/156L/157L/167L	348	1000	35	100	50
6	162	167L	47:18	0.9892	0.9881-0.9902	6	155L/156L/157L/167L	355	1000	35	100	50
6	167	167L	47:49	1.0000	0.9990-1.0010	6	~~155L/156L/157L/167L~~ 167L only①	115	500	11	50	20
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	132	500	13	50	20
6	156/157	156L/157L	45:07	1.0000	0.9990-1.0010	6	156L/157L					
6	169	169L	52:31	0.9949	0.9940-0.9959	6	169L	161	500	16	50	20
	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	6	138L					
6	157L	138L	49:08	1.0996	1.0959-1.1033	20	138L					
6	156L/157L	138L	49:07	1.0992	1.0981-1.1003	6	138L					
6	169L	138L	52:30	1.1749	1.1738-1.1761	6	138L					
Compounds using 194L($^{13}C_{12}$-2,2',3,3',4,4',5,5'-OcCB) as Labeled injection internal standard												
	CB congener											
	Heptachlorobiphenyls											
7	188	188L	41:51	1.0000	0.9988-1.0012	6	188L	235	500	23	50	20
7	179	188L	42:19	1.0112	1.0100-1.0123	6	188L/189L	229	500	23	50	20
7	184	188L	42:45	1.0215	1.0203-1.0227	6	188L/189L	403	1000	40	100	50
7	176	188L	43:15	1.0335	1.0323-1.0346	6	188L/189L	385	1000	39	100	50
7	186	188L	43:45	1.0454	1.0442-1.0466	6	188L/189L	407	1000	41	100	50
7	178	188L	45:06	1.0777	1.0765-1.0789	6	188L/189L	221	500	22	50	20
7	175	188L	45:46	1.0936	1.0924-1.0948	6	188L/189L	383	1000	38	100	50
7	187	188L	46:02	1.1000	1.0988-1.1012	6	188L/189L	191	500	19	50	20

① Set to 167L only to be consistent with the way other native toxic/LOC PCBs are quantified. MES 6/2/01

Cl No.[1]	IUPAC No.[2,3]	RT Ref[4]	RTs[5]	RRT[6]	RRT limits[7]	Window (sec)[8]	Quantitation reference[9]	Detection limits and minimum levels - Matrix and concentration[10] Water (pg/L) EMDL	Water (pg/L) EML	Other (ng/kg) EMDL	Other (ng/kg) EML	Extract (pg/µL) EML
7	182	188L	46:14	1.1047	1.1035-1.1059	6	188L/189L	398	1000	40	100	50
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	401	1000	40	100	50
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	186	500	19	50	20
7	177	188L	47:30	1.1350	1.1338-1.1362	6	188L/189L	141	500	14	50	20
7	181	188L	47:52	1.1438	1.1426-1.1450	6	188L/189L	396	1000	40	100	50
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	374	1000	37	100	50
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	377	1000	38	100	50
7	192	189L	50:06	0.9093	0.9083-0.9102	6	188L/189L	420	1000	42	100	50
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	136	500	14	50	20
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	418	1000	42	100	50
7	170	189L	51:54	0.9419	0.9410-0.9428	6	188L/189L	162	500	16	50	20
7	190	189L	52:26	0.9516	0.9507-0.9525	6	188L/189L	234	500	23	50	20
7	189	189L	55:07	1.0003	0.9994-1.0012	6	189L	177	500	18	50	20
	Octachlorobiphenyls											
8	202	202L	47:32	1.0004	0.9986-1.0021	10	202L	442	1000	44	100	50
8	201	202L	48:31	1.0210	1.0193-1.0228	10	202L/205L	440	1000	44	100	50
8	204	202L	49:11	1.0351	1.0340-1.0361	6	202L/205L	447	1000	45	100	50
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	245	1000	25	100	50
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	203	500	20	50	25
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	429	1000	43	100	50
8	203	205L	53:26	0.9245	0.9236-0.9253	6	202L/205L	444	1000	44	100	50
8	195	205L	54:55	0.9501	0.9493-0.9510	6	202L/205L	427	1000	43	100	50
8	194	205L	57:19	0.9916	0.9908-0.9925	6	202L/205L	170	500	17	50	20
8	205	205L	57:49	1.0003	0.9994-1.0012	6	205L	449	1000	45	100	50
	Nonachlorobiphenyls											
9	208	208L	54:33	1.0003	0.9994-1.0012	6	208L	455	1000	46	100	50

Cl No.[1]	IUPAC No.[2,3]	RT Ref[4]	RTs[5]	RRT[6]	RRT limits[7]	Window (sec)[8]	Quantitation reference[9]	Detection limits and minimum levels - Matrix and concentration[10] Water (pg/L) EMDL	Water (pg/L) EML	Other (ng/kg) EMDL	Other (ng/kg) EML	Extract (pg/µL) EML
9	207	208L	55:32	1.0183	1.0174-1.0193	6	208L/206L	453	1000	45	100	50
9	206	206L	59:37	1.0003	0.9994-1.0011	6	206L	451	1000	45	100	50
	Decachlorobiphenyl											
10	209	209L	61:15	1.0003	0.9995-1.0011	6	209L	153	500	15	50	20
	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					
Labeled clean-up 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					
Labeled injection internal standards												
2	9L	138L	18:54	0.4648	0.4596-0.4699	25	~~178L~~ (A)					
4	52L	138L	28:51	0.7094	0.7043-0.7145	25	~~178L~~					
5	101L	138L	36:03	0.8865	0.8814-0.8916	25	~~178L~~					
6	138L	138L	44:41	1.0988	1.0783-1.1193	100	~~178L~~					
8	194L	138L	57:18	1.4090	1.4039-1.4141	25	~~178L~~					

1. Number of chlorines on congener.
2. Suffix "L" indicates labeled compound.
3. Multiple congeners in a box indicates a group of congeners that co-elute 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. Retention time reference that is used to locate target congener.
5. Retention time of target congener.
6. RRT between the RT for the congener and RT for the reference.
7. Nominal limits based on an ± 0.5% of the RRT, adjusted for the nearest eluted isomer.
8. RT window width for congener or group of two or more congeners.

(A) Labeled injection internal standards added at the end of cleanup are not quantified against 178L cleanup standard due to potential losses of a cleanup standard during the cleanup procedures. MEB 6/2/01.

9. Labeled congeners that form the quantitation reference. Areas from the exact m/z's of the congeners listed in the quantitation reference are summed, and divided by the number of congeners in the quantitation reference. For example, for congener 10, the areas at the exact m/z's for 4L and 15L are summed and the sum is divided by 2 (because there are 2 congeners in the quantitation reference).
10. EMDLs and EMLs with common laboratory interferences present. Without interferences, EMDLs and EMLs will be, respectively, 5 and 10 pg/L for aqueous samples, 0.5 and 1.0 ng/kg for soil, tissue, and mixed-phase samples, and EMLs for extracts will be 0.5 pg/uL.

Table 3. Concentrations of native and labeled chlorinated biphenyls in stock solutions, spiking solutions, and final extracts

	Solution concentrations		
CB congener	**Stock (µg/mL)**	**Spiking (ng/mL)**	**Extract (ng/mL)**
Native Toxics/LOC[1]			
1	20	1.0	50
3	20	1.0	50
4	20	1.0	50
15	20	1.0	50
19	20	1.0	50
37	20	1.0	50
54	20	1.0	50
77	20	1.0	50
81	20	1.0	50
104	20	1.0	50
105	20	1.0	50
114	20	1.0	50
118	20	1.0	50
123	20	1.0	50
126	20	1.0	50
155	20	1.0	50
156	20	1.0	50
157	20	1.0	50
167	20	1.0	50
169	20	1.0	50
188	20	1.0	50
189	20	1.0	50
202	20	1.0	50
205	20	1.0	50
206	20	1.0	50
208	20	1.0	50
209	20	1.0	50
Native congener mix stock solutions[2]			
MoCB thru TrCB	2.5		
TeCB thru HpCB	5.0		
OcCB thru DeCB	7.5		
Labeled Toxics/LOC/window-defining[3]			
1L	1.0	2.0	100
3L	1.0	2.0	100
4L	1.0	2.0	100

	Solution concentrations		
CB congener	**Stock (µg/mL)**	**Spiking (ng/mL)**	**Extract (ng/mL)**
15L	1.0	2.0	100
19L	1.0	2.0	100
37L	1.0	2.0	100
54L	1.0	2.0	100
77L	1.0	2.0	100
81L	1.0	2.0	100
104L	1.0	2.0	100
105L	1.0	2.0	100
114L	1.0	2.0	100
118L	1.0	2.0	100
123L	1.0	2.0	100
126L	1.0	2.0	100
155L	1.0	2.0	100
156L	1.0	2.0	100
157L	1.0	2.0	100
167L	1.0	2.0	100
169L	1.0	2.0	100
188L	1.0	2.0	100
189L	1.0	2.0	100
202L	1.0	2.0	100
205L	1.0	2.0	100
206L	1.0	2.0	100
208L	1.0	2.0	100
209L	1.0	2.0	100
Labeled clean-up[4]			
28L	1.0	2.0	100
111L	1.0	2.0	100
178L	1.0	2.0	100
Labeled injection internal[5]			
9L	5.0	1000	100
52L	5.0	1000	100
101L	5.0	1000	100
138L	5.0	1000	100
194L	5.0	1000	100

Diluted combined 209 congener[6]		
	Solution concentration (~~µg/mL~~) ng/ml (A)	
Standard	Native	~~Labeled~~
Native congeners		
MoCB thru TrCB	50	
TeCB thru HpCB	100	
OcCB thru DeCB	150	
Labeled Toxics/LOC/window-defining		100
Labeled Cleanup		100
Labeled Injection internal		100

1. Stock solution: Section 7.8.1; Spiking solution: Section 7.11
2. Section 7.8.1.2
3. Stock solution: Section 7.9.1; Spiking solution: Section 7.12
4. Stock solution: Section 7.9.2; Spiking solution: Section 7.13
5. Stock solution: Section 7.9.3; Spiking solution: Section 7.14
6. Section 7.10.2.2.2

(A) incorrect units are listed in 1668A. WES 6/2/01

Table 5. Concentration of CB congeners in calibration and calibration verification standards

		Solution concentration (ng/mL)					
CB congener	**IUPAC[1]**	**CS-0.2 (Hi sens)[2]**	**CS-1**	**CS-2**	**CS-3 (VER)**	**CS-4**	**CS-5**
Native Toxics/LOC							
2-MoCB	1	0.2	1.0	5.0	50	400	2000
4-MoCB	3	0.2	1.0	5.0	50	400	2000
2,2'-DiCB	4	0.2	1.0	5.0	50	400	2000
4,4'-DiCB	15	0.2	1.0	5.0	50	400	2000
2,2',6'-TrCB	19	0.2	1.0	5.0	50	400	2000
3,4,4'-TrCB	37	0.2	1.0	5.0	50	400	2000
2,2',6,6'-TeCB	54	0.2	1.0	5.0	50	400	2000
3,3',4,4'-TeCB	77	0.2	1.0	5.0	50	400	2000
3,4,4',5-TeCB	81	0.2	1.0	5.0	50	400	2000
2,2',4,6,6'-PeCB	104	0.2	1.0	5.0	50	400	2000
2,3,3',4,4'-PeCB	105	0.2	1.0	5.0	50	400	2000
2,3,4,4',5-PeCB	114	0.2	1.0	5.0	50	400	2000
2,3',4,4',5-PeCB	118	0.2	1.0	5.0	50	400	2000
2',3,4,4',5-PeCB	123	0.2	1.0	5.0	50	400	2000
3,3',4,4',5-PeCB	126	0.2	1.0	5.0	50	400	2000
2,2',4,4',6,6'-HxCB	155	0.2	1.0	5.0	50	400	2000
2,3,3',4,4',5-HxCB	156	0.2	1.0	5.0	50	400	2000
2,3,3',4,4',5'-HxCB	157	0.2	1.0	5.0	50	400	2000
2,3',4,4',5,5'-HxCB	167	0.2	1.0	5.0	50	400	2000
3,3',4,4',5,5'-HxCB	169	0.2	1.0	5.0	50	400	2000
2,2',3,4',5,6,6'-HpCB	188	0.2	1.0	5.0	50	400	2000
2,3,3',4,4',5,5'-HpCB	189	0.2	1.0	5.0	50	400	2000
2,2',3,3',5,5',6,6'-OcCB	202	0.2	1.0	5.0	50	400	2000
2,3,3',4,4',5,5',6-OcCB	205	0.2	1.0	5.0	50	400	2000
2,2',3,3',4,4',5,5',6-NoCB	206	0.2	1.0	5.0	50	400	2000
2,2',3,3',4',5,5',6,6'-NoCB	208	0.2	1.0	5.0	50	400	2000
DeCB	209	0.2	1.0	5.0	50	400	2000
Labeled Toxics/LOC/window-defining							
$^{13}C_{12}$-2-MoCB	1L	100	100	100	100	100	100
$^{13}C_{12}$-4-MoCB	3L	100	100	100	100	100	100
$^{13}C_{12}$-2,2'-DiCB	4L	100	100	100	100	100	100
$^{13}C_{12}$-4,4'-DiCB	15L	100	100	100	100	100	100
$^{13}C_{12}$-2,2',6'-TrCB	19L	100	100	100	100	100	100
$^{13}C_{12}$-3,4,4'-TrCB	37L	100	100	100	100	100	100
$^{13}C_{12}$-2,2',6,6'-TeCB	54L	100	100	100	100	100	100
$^{13}C_{12}$-3,3',4,4'-TeCB	77L	100	100	100	100	100	100
$^{13}C_{12}$-3,4,4',5-TeCB	81L	100	100	100	100	100	100
$^{13}C_{12}$-2,2',4,6,6'-PeCB	104L	100	100	100	100	100	100
$^{13}C_{12}$-2,3,3',4,4'-PeCB	105L	100	100	100	100	100	100
$^{13}C_{12}$-2,3,4,4',5-PeCB	114L	100	100	100	100	100	100
$^{13}C_{12}$-2,3',4,4',5-PeCB	118L	100	100	100	100	100	100
$^{13}C_{12}$-2',3,4,4',5-PeCB	123L	100	100	100	100	100	100
$^{13}C_{12}$-3,3',4,4',5-PeCB	126L	100	100	100	100	100	100

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		Solution concentration (ng/mL)					
CB congener	**IUPAC[1]**	**CS-0.2 (Hi sens)[2]**	**CS-1**	**CS-2**	**CS-3 (VER)**	**CS-4**	**CS-5**
$^{13}C_{12}$-2,2',4,4',6,6'-HxCB	155L	100	100	100	100	100	100
$^{13}C_{12}$-2,3,3',4,4',5-HxCB	156L	100	100	100	100	100	100
$^{13}C_{12}$-2,3,3',4,4',5'-HxCB	157L	100	100	100	100	100	100
$^{13}C_{12}$-2,3',4,4',5,5'-HxCB	167L	100	100	100	100	100	100
$^{13}C_{12}$-3,3',4,4',5,5'-HxCB	169L	100	100	100	100	100	100
$^{13}C_{12}$-2,2',3,4',5,6,6'-HpCB	188L	100	100	100	100	100	100
$^{13}C_{12}$-2,3,3',4,4',5,5'-HpCB	189L	100	100	100	100	100	100
$^{13}C_{12}$-2,2',3,3',5,5',6,6'-OcCB	202L	100	100	100	100	100	100
$^{13}C_{12}$-2,3,3',4,4',5,5',6-OcCB	205L	100	100	100	100	100	100
$^{13}C_{12}$-2,2',3,3',4,4',5,5',6-NoCB	206L	100	100	100	100	100	100
$^{13}C_{12}$-2,2',3,3',4',5,5',6,6'-NoCB	208L	100	100	100	100	100	100
$^{13}C_{12}$-DeCB	209L	100	100	100	100	100	100
Labeled clean-up							
$^{13}C_{12}$-2,4,4'-TrCB	28L	100	100	100	100	100	100
$^{13}C_{12}$-2,3,3',5,5'-PeCB	111L	100	100	100	100	100	100
$^{13}C_{12}$-2,2',3,3',5,5',6-HpCB	178L	100	100	100	100	100	100
Labeled injection internal							
$^{13}C_{12}$-2,5-DiCB	9L	100	100	100	100	100	100
$^{13}C_{12}$-2,2',5,5'-TeCB	52L	100	100	100	100	100	100
$^{13}C_{12}$-2,2',4',5,5'-PeCB	101L	100	100	100	100	100	100
$^{13}C_{12}$-2,2',3',4,4',5'-HxCB	138L	100	100	100	100	100	100
$^{13}C_{12}$-2,2',3,3',4,4',5,5'-OcCB	194L	100	100	100	100	100	100

1. Suffix "L" indicates labeled compound
2. Additional concentration used for calibration of high sensitivity HRGC/HRMS systems

Table 6. QC acceptance crtiteria for chlorinated biphenyls in VER, IPR, OPR, and samples[1]

Congener	IUPAC number[2]	Test conc (ng/mL)[3]	VER[4] (ng/ml) (%) 35-65	IPR 30-70% RSD (%)	IPR X (%)	OPR 25-75 ng/ml (%)	Labeled compound recovery in samples (%)
2-MoCB	1	50	70-130	40	60-140	50-150	
4-MoCB	3	50	70-130	40	60-140	50-150	
2,2'-DiCB	4	50	70-130	40	60-140	50-150	
4,4'-DiCB	15	50	70-130	40	60-140	50-150	
2,2'6-TrCB	19	50	70-130	40	60-140	50-150	
3,4,4'-TrCB	37	50	70-130	40	60-140	50-150	
2,2'6,6'TeCB	54	50	70-130	40	60-140	50-150	
3,3',4,4'-TeCB	77	50	70-130	40	60-140	50-150	
3,4,4',5-TeCB	81	50	70-130	40	60-140	50-150	
2,2',4,6,6'-PeCB	104	50	70-130	40	60-140	50-150	
2,3,3',4,4'-PeCB	105	50	70-130	40	60-140	50-150	
2,3,4,4',5-PeCB	114	50	70-130	40	60-140	50-150	
2,3',4,4',5-PeCB	118	50	70-130	40	60-140	50-150	
2',3,4,4',5-PeCB	123	50	70-130	40	60-140	50-150	
3,3',4,4',5-PeCB	126	50	70-130	40	60-140	50-150	
2,2',4,4',6,6'-HxCB	155	50	70-130	40	60-140	50-150	
2,3,3',4,4',5-HxCB[5]	156	50	70-130	40	60-140	50-150	
2,3,3',4,4',5'-HxCB[5]	157	50	70-130	40	60-140	50-150	
2,3',4,4',5,5'-HxCB	167	50	70-130	40	60-140	50-150	
3,3',4,4',5,5'-HxCB	169	50	70-130	40	60-140	50-150	
2,2',3,4',5,6,6'-HpCB	188	50	70-130	40	60-140	50-150	
2,3,3',4,4',5,5'-HpCB	189	50	70-130	40	60-140	50-150	
2,2',3,3',5,5',6,6'-OcCB	202	50	70-130	40	60-140	50-150	
2,3,3',4,4',5,5',6-OcCB	205	50	70-130	40	60-140	50-150	
2,2',3,3',4,4',5,5',6-NoCB	206	50	70-130	40	60-140	50-150	
2,2',3,3,'4,5,5',6,6'-NoCB	208	50	70-130	40	60-140	50-150	
DeCB	209	50	70-130	40	60-140	50-150	
$^{13}C_{12}$-2-MoCB	1L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-4-MoCB	3L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,2'-DiCB	4L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-4,4'-DiCB	15L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,2',6-TrCB	19L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-3,4,4'-TrCB	37L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,2',6,6'-TeCB	54L	100	50-150	50	35-135	30-140	25-150
13C12-3,3',4,4'-TCB	77L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-3,4,4',5-TeCB	81L	100	50-150	50	35-135	30-140	25-150

Congener	IUPAC number[2]	Test conc (ng/mL)[3]	VER[4] (%)	IPR RSD (%)	IPR X (%)	OPR (%)	Labeled compound recovery in samples (%)
$^{13}C_{12}$-2,2',4,6,6'-PeCB	104L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,3,3',4,4'-PeCB	105L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,3,4,4',5-PeCB	114L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,3',4,4',5-PeCB	118L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2',3,4,4',5-PeCB	123L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-3,3',4,4',5-PeCB	126L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,2',4,4',6,6'-HxCB	155L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,3,3',4,4',5 -HxCB[5]	156L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,3,3',4,4',5'-HxCB[5]	157L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,3',4,4',5,5'-HxCB	167L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-3,3',4,4',5,5'-HxCB	169L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,2',3,4',5,6,6'-HpCB	188L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB	189L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,2',3,3',5,5',6,6'-OcCB	202L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,3,3',4,4',5,5',6-OcCB	205L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,2',3,3',4,4',5,5',6-NoCB	206L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,2',3,3',4,5,5',6,6'-NoCB	208L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,2',3,3',4,4',5,5',6,6'-DeCB	209L	100	50-150	50	35-135	30-140	25-150
Cleanup standard							
$^{13}C_{12}$-2,4,4'-TrCB	28L	100	60-130	45	45-120	40-125	30-135
$^{13}C_{12}$-2,3,3',5,5'-PeCB	111L	100	60-130	45	45-120	40-125	30-135
$^{13}C_{12}$-2,2',3,3',5,5',6-HpCB	178L	100	60-130	45	45-120	40-125	30-135

1. QC acceptance criteria for IPR, OPR, and samples based on a 20 μL extract final volume
2. Suffix "L" indicates labeled compound.
3. See Table 5.
4. Section 15.3.
5. PCBs 156 and 157 are tested as the sum of two concentrations

Table 8. Theoretical ion abundance ratios and QC limits

Chlorine atoms	m/z's forming ratio	Theoretical ratio	Lower QC limit	Upper QC limit
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+2)/(m+4)	0.69	0.59	0.79

Appendix B
IPR Results

LAB	BATC
SDG	48616-25
EXTRACT_METH	SEPF

PCB H2O IPR Data Table - IPR Recovery Data

SAMP_ID		NA	NA	NA	NA			
LAB_SAMP_ID		48616-25-02	48616-25-03	48616-25-04	48616-25-05			
FRACTION		TOTAL	TOTAL	TOTAL	TOTAL			
REEXTRACT								
EXTRACT_DATE		26-Oct-00	26-Oct-00	26-Oct-00	26-Oct-00			
ANALYSIS_DATE		22-Nov-00	23-Nov-00	23-Nov-00	23-Nov-00			
INSTR_RUN_ID		4828012B_3	4828012B_4	4828012B_5	4828012B_6			
DILUTION		1.000	1.000	1.000	1.000			
SAMP_WGT_VOL		1.00	1.00	1.00	1.00			
SAMP_WGT_VOL_UNIT		L	L	L	L			
QC_CODE	Spike Conc	IPR	IPR	IPR	IPR	Mean		
REP	NG/ML	1	1	1	1	% recovery	std. Dev.	%
PARAM_NAME		% recovery	% recovery	% recovery	% recovery	X	s	RSD
1	50	68	59	59	58	61	4.7	8
3	50	70	66	61	57	63	5.6	9
4	50	40	80	80	76	69	19.4	28
19	50	103	108	116	112	110	5.6	5
15	50	90	83	92	79	86	6.0	7
54	50	118	120	130	127	124	5.8	5
104	50	101	120	117	135	118	14.0	12
37	50	98	84	100	80	90	9.4	10
155	50	111	122	111	129	118	8.7	7
81	50	83	73	76	64	74	8.1	11
77	50	85	73	78	65	75	8.5	11
123	50	101	96	101	94	98	3.7	4
118	50	137	122	165	113	134	22.7	17
188	50	115	124	124	129	123	6.0	5
114	50	95	90	92	88	91	2.9	3
105	50	106	100	114	97	105	7.5	7
126	50	89	87	86	85	87	2.0	2
202	50	94	98	97	98	97	1.9	2
167	50	94	90	92	85	90	3.9	4
156	50	96	93	96	88	93	3.9	4
157	50	96	93	96	88	93	3.9	4
169	50	95	91	91	87	91	3.1	3
208	50	88	94	92	86	90	3.4	4
189	50	95	88	92	86	90	3.9	4
205	50	98	98	97	93	96	2.5	3
206	50	98	99	102	95	98	2.7	3
209	50	94	95	94	88	93	3.3	4
1 L	100	43	52	49	56	50	5.6	11
3L	100	41	53	40	48	46	6.1	13
4 L	100	61	79	77	83	75	9.7	13
19 L	100	52	62	55	60	57	4.6	8
15L	100	64	85	80	73	76	9.2	12
54 L	100	55	76	63	73	66	9.6	14
104 L	100	30	22	32	20	26	5.7	22
37L	100	57	103	93	82	84	19.9	24
155 L	100	57	63	85	72	69	12.0	17
81L	100	68	98	91	72	82	14.8	18
77L	100	60	101	90	60	78	20.8	27
123L	100	58	82	86	81	77	12.7	16
118L	100	57	81	85	80	76	12.8	17
188 L	100	52	55	62	59	57	4.5	8
114L	100	59	85	86	84	78	13.1	17
105L	100	60	85	89	83	79	13.2	17
126L	100	54	90	77	51	68	18.4	27
202 L	100	59	66	68	73	67	5.6	8
167L	100	66	98	101	108	93	18.5	20
156L	100	65	96	99	105	91	17.8	20
157L	100	65	96	99	105	91	17.8	20
169L	100	58	86	78	57	70	14.8	21
208L	100	59	77	80	86	75	11.6	15
189L	100	61	79	84	86	78	11.5	15
205 L	100	62	87	90	95	84	14.6	17
206L	100	58	80	81	83	76	11.6	15
209L	100	38	52	53	59	50	8.6	17
28L	100	85	111	110	107	103	12.2	12
111L	100	72	93	93	87	86	9.7	11
178L	100	58	65	62	55	60	4.4	7

Appendix C
MDL Results

LAB BATC
SDG 48616-25
EXTRACT_METH SEPF

PCB H2O MDL Data Table - MDL NG/ML and PG/SAMPLE Data

	SAMP_ID		NA	NA	NA	NA	NA	NA	NA				
	LAB_SAMP_ID		48616-25-06	48616-25-07	48616-25-08	48616-25-09	48616-25-10	48616-25-11	48616-25-12				
	FRACTION		TOTAL	TOTAL	TOTAL	TOTAL	TOTAL	TOTAL	TOTAL				
	INSTR_RUN_ID		4828013B_4	4828013B_5	4828013B_6	4828013B_7	4828014_2	4828014_3	4828014A_2				
	SAMP_WGT_VOL		1.00	1.00	1.00	1.00	1.00	1.00	1.00				
	SAMP_WGT_VOL_UNIT		L	L	L	L	L	L	L				
	QC_CODE	Spike Level	MDL	MDL	MDL	MDL	MDL	MDL	MDL				
	REP	NG/ML	1	1	1	1	1	1	1	Mean	std. Dev	MDL	MDL
PARAM_CODE	PARAM_NAME		RESULT (NG/ML)	RESULT (NG/ML)	RESULT (NG/ML)	RESULT (NG/ML)	RESULT (NG/ML)	RESULT (NG/ML)	RESULT (NG/ML)	(NG/ML)	s	(NG/ML)	(PG/SAMPLE)
2051-62-9	3	5.00	3.46	2.90	2.50	2.23	4.24	3.34	6.41	3.58	1.4	4.4	89
13029-08-8	4	5.00	6.39	5.19	5.44	3.46	6.37	7.86	7.03	5.98	1.4	4.5	90
38444-73-4	19	5.00	6.25	6.31	6.61	5.87	6.79	7.19	5.22	6.32	0.6	2.0	40
2050-68-2	15	5.00	7.83	7.80	7.64	3.44	6.31	7.18	7.38	6.80	1.6	4.9	99
56558-16-8	104	5.00	6.62	6.79	6.68	6.07	6.92	6.97	8.99	7.01	0.9	2.9	58
38444-90-5	37	5.00	6.45	6.40	6.61	3.86	5.83	5.84	6.28	5.90	0.9	3.0	59
70362-50-4	81	5.00	3.98	3.94	3.76	3.54	3.62	4.00	3.79	3.80	0.2	0.6	11
32598-13-3	77	5.00	4.18	3.89	3.66	3.72	3.82	3.92	3.77	3.85	0.2	0.5	11
65510-44-3	123	5.00	4.98	4.95	5.22	4.01	4.84	4.79	4.71	4.79	0.4	1.2	24
31508-00-6	118	5.00	18.92	18.12	22.60	4.53 *	17.09	17.22	17.05	18.50	2.1	7.2	144
74472-37-0	114	5.00	4.11	4.03	4.24	3.76	4.01	3.97	3.94	4.01	0.1	0.5	9
32598-14-4	105	5.00	8.52	8.40	9.13	3.91	8.11	7.79	8.11	7.71	1.7	5.4	109
57465-28-8	126	5.00	3.58	3.74	3.71	3.87	3.65	3.75	4.07	3.77	0.2	0.5	10
52663-72-6	167	5.00	4.18	4.26	4.35	3.87	4.25	4.41	4.67	4.28	0.2	0.8	15
38380-08-4	156	5.00	4.50	4.35	4.51	4.02	4.39	4.44	4.96	4.45	0.3	0.9	17
69782-90-7	157	5.00	4.50	4.35	4.51	4.02	4.39	4.44	4.96	4.45	0.3	0.9	17
32774-16-6	169	5.00	3.67	3.92	3.65	3.73	3.74	3.85	4.19	3.82	0.2	0.6	12
52663-77-1	208	5.00	4.07	4.07	4.17	4.20	4.99	4.77	4.26	4.36	0.4	1.2	23
39635-31-9	189	5.00	3.74	3.74	3.52	3.94	4.17	4.02	4.46	3.94	0.3	1.0	20
74472-53-0	205	5.00	4.18	4.10	4.03	4.27	4.31	4.30	4.29	4.21	0.1	0.3	7
40186-72-9	206	5.00	4.52	4.46	4.58	4.44	5.33	5.28	4.90	4.79	0.4	1.2	24
2051-24-3	209	5.00	4.14	4.19	4.12	4.51	4.27	4.22	5.01	4.35	0.3	1.0	20

* The PCB118 result was a procedurally and technically identified and explained outlier. It can clearly be rationalized and justified to be dropped from the analysis.
3.365 is used, rather than 3.143, to multiply the standard deviation by for 6 replicates (n-1 = 5)

Appendix D
HRMS Descriptors

Experiment: M1668B (8 Functions)

Operator	: User
Date	: 30-JAN-2001 23:37:31
Instrument	: AutoSpec

Function 1

Type	: SIR Voltage
Calibration file used	: 4828039_1
High mass	: 236.0
Low mass	: 188.0
Resolution	: 10000
Ionisation mode	: EI+
Accelerating Voltage	: 8000.0V
Magnet 1 control	: Current
Start Time	: 17:00
End Time	: 23:00
Fast lock	: On
Number of channels	: 10
Cycle time (ms)	: 1000

windows changed 01-30-01 JM

Channel	Mass	Ch Time (ms)	I/ch Time (ms)
1	188.0393	80	20
2	190.0363	80	20
3	200.0795	80	20
4	202.0766	80	20
5 (Lock)	218.9856	80	20
Primary Span Lock (Peaks) 2.00			
Secondary Span Lock (Peaks) 2.00			
Lock Level (mV) 0			
Step Lock (Peaks) 0.020			
6	218.9856	80	20
7	222.0003	80	20
8	223.9974	80	20
9	234.0406	80	20
10	236.0376	80	20

Septum flow	: Off
Repeats	: 1

Experiment: M1668B (8 Functions)

Operator	: User
Date	: 30-JAN-2001 23:37:31
Instrument	: AutoSpec

Function 2

Type	: SIR Voltage
Calibration file used	: 4828039_2
High mass	: 304.0
Low mass	: 222.0
Resolution	: 10000
Ionisation mode	: EI+
Accelerating Voltage	: 8000.0V
Magnet 1 control	: Current
Start Time	: 23:00
End Time	: 29:50
Fast lock	: On
Number of channels	: 14
Cycle time (ms)	: 1000

Channel	Mass	Ch Time (ms)	I/ch Time (ms)
1	222.0003	50	20
2	223.9974	50	20
3	234.0406	50	20
4	236.0376	50	20
5	242.9856	50	30
6	255.9613	50	20
7	257.9584	50	20
8	268.0016	50	20
9	269.9986	50	20
10 (Lock)	280.9824	50	30
Primary Span Lock (Peaks) 2.00			
Secondary Span Lock (Peaks) 2.00			
Lock Level (mV) 0			
Step Lock (Peaks) 0.020			
11	289.9224	50	20
12	291.9194	50	20
13	301.9626	50	20
14	303.9597	50	20

Septum flow	: Off
Repeats	: 1

Experiment: M1668B (8 Functions)

Operator	: User
Date	: 30-JAN-2001 23:37:31
Instrument	: AutoSpec

Function 3

Type	: SIR Voltage
Calibration file used	: 4828039_3
High mass	: 339.9
Low mass	: 256.0
Resolution	: 10000
Ionisation mode	: EI+
Accelerating Voltage	: 8000.0V
Magnet 1 control	: Current
Start Time	: 29:50
End Time	: 36:20
Fast lock	: On
Number of channels	: 14
Cycle time (ms)	: 1000

Channel	Mass	Ch Time (ms)	I/ch Time (ms)
1	255.9613	50	20
2	257.9584	50	20
3	268.0016	50	20
4	269.9986	50	20
5	280.9824	50	30
6	289.9224	50	20
7	291.9194	50	20
8	301.9626	50	20
9	303.9597	50	20
10 (Lock)	318.9792	50	30
Primary Span Lock (Peaks) 2.00			
Secondary Span Lock (Peaks) 2.00			
Lock Level (mV) 0			
Step Lock (Peaks) 0.020			
11	325.8804	50	20
12	327.8775	50	20
13	337.9207	50	20
14	339.9178	50	20

Septum flow	: Off
Repeats	: 1

Experiment: M1668B (8 Functions)

Operator	: User
Date	: 30-JAN-2001 23:37:31
Instrument	: AutoSpec

Function 4

Type	: SIR Voltage
Calibration file used	: 4828039_4
High mass	: 373.9
Low mass	: 289.9
Resolution	: 10000
Ionisation mode	: EI+
Accelerating Voltage	: 8000.0V
Magnet 1 control	: Current
Start Time	: 36:20
End Time	: 43:20
Fast lock	: On
Number of channels	: 14
Cycle time (ms)	: 1000

Channel	Mass	Ch Time (ms)	I/ch Time (ms)
1	289.9224	50	20
2	291.9194	50	20
3	301.9626	50	20
4	303.9597	50	20
5	325.8804	50	20
6	327.8775	50	20
7	330.9792	50	30
8	337.9207	50	20
9	339.9178	50	20
10 (Lock)	354.9792	50	30
Primary Span Lock (Peaks) 2.00			
Secondary Span Lock (Peaks) 2.00			
Lock Level (mV) 0			
Step Lock (Peaks) 0.020			
11	359.8415	50	20
12	361.8385	50	20
13	371.8817	50	20
14	373.8788	50	20

Septum flow	: Off
Repeats	: 1

Experiment: M1668B (8 Functions)

Operator	: User
Date	: 30-JAN-2001 23:37:31
Instrument	: AutoSpec

Function 5

Type	: SIR Voltage
Calibration file used	: 4828039_5
High mass	: 407.8
Low mass	: 325.9
Resolution	: 10000
Ionisation mode	: EI+
Accelerating Voltage	: 8000.0V
Magnet 1 control	: Current
Start Time	: 43:20
End Time	: 50:45
Fast lock	: On
Number of channels	: 14
Cycle time (ms)	: 1000

Channel	Mass	Ch Time (ms)	I/ch Time (ms)
1	325.8804	50	20
2	327.8775	50	20
3	337.9207	50	20
4	339.9178	50	20
5	354.9792	50	30
6	359.8415	50	20
7	361.8385	50	20
8	371.8817	50	20
9	373.8788	50	20
10 (Lock)	380.9760	50	30
Primary Span Lock (Peaks) 2.00			
Secondary Span Lock (Peaks) 2.00			
Lock Level (mV) 0			
Step Lock (Peaks) 0.020			
11	393.8025	50	20
12	395.7995	50	20
13	405.8428	50	20
14	407.8398	50	20

Septum flow	: Off
Repeats	: 1

Experiment: M1668B (8 Functions)

Operator	: User
Date	: 30-JAN-2001 23:37:31
Instrument	: AutoSpec

Function 6

Type	: SIR Voltage
Calibration file used	: 4828039_6
High mass	: 441.8
Low mass	: 359.8
Resolution	: 10000
Ionisation mode	: EI+
Accelerating Voltage	: 8000.0V
Magnet 1 control	: Current
Start Time	: 50:45
End Time	: 54:55
Fast lock	: On
Number of channels	: 14
Cycle time (ms)	: 1000

Channel	Mass	Ch Time (ms)	I/ch Time (ms)
1	359.8415	50	20
2	361.8385	50	20
3	371.8817	50	20
4	373.8788	50	20
5	380.9760	50	30
6	393.8025	50	20
7	395.7995	50	20
8	405.8428	50	20
9	407.8398	50	20
10	427.7635	50	20
11	429.7606	50	20
12 (Lock)	430.9728	50	30
Primary Span Lock (Peaks) 2.00			
Secondary Span Lock (Peaks) 2.00			
Lock Level (mV) 0			
Step Lock (Peaks) 0.020			
13	439.8038	50	20
14	441.8008	50	20

Septum flow	: Off
Repeats	: 1

Experiment: M1668B (8 Functions)

Operator	: User
Date	: 30-JAN-2001 23:37:31
Instrument	: AutoSpec

Function 7

Type	: SIR Voltage
Calibration file used	: 4828039_7
High mass	: 475.8
Low mass	: 393.8
Resolution	: 10000
Ionisation mode	: EI+
Accelerating Voltage	: 8000.0V
Magnet 1 control	: Current
Start Time	: 54:55
End Time	: 60:20
Fast lock	: On
Number of channels	: 14
Cycle time (ms)	: 1000

Channel	Mass	Ch Time (ms)	I/ch Time (ms)
1	393.8025	50	20
2	395.7995	50	20
3	405.8428	50	20
4	407.8398	50	20
5	416.9760	50	30
6	427.7635	50	20
7	429.7606	50	20
8	439.8038	50	20
9	441.8008	50	20
10 (Lock)	442.9728	50	30
Primary Span Lock (Peaks) 2.00			
Secondary Span Lock (Peaks) 2.00			
Lock Level (mV) 0			
Step Lock (Peaks) 0.020			
11	461.7246	50	20
12	463.7216	50	20
13	473.7648	50	20
14	475.7619	50	20

Septum flow	: Off
Repeats	: 1

Experiment: M1668B (8 Functions)

Operator	: User
Date	: 30-JAN-2001 23:37:31
Instrument	: AutoSpec

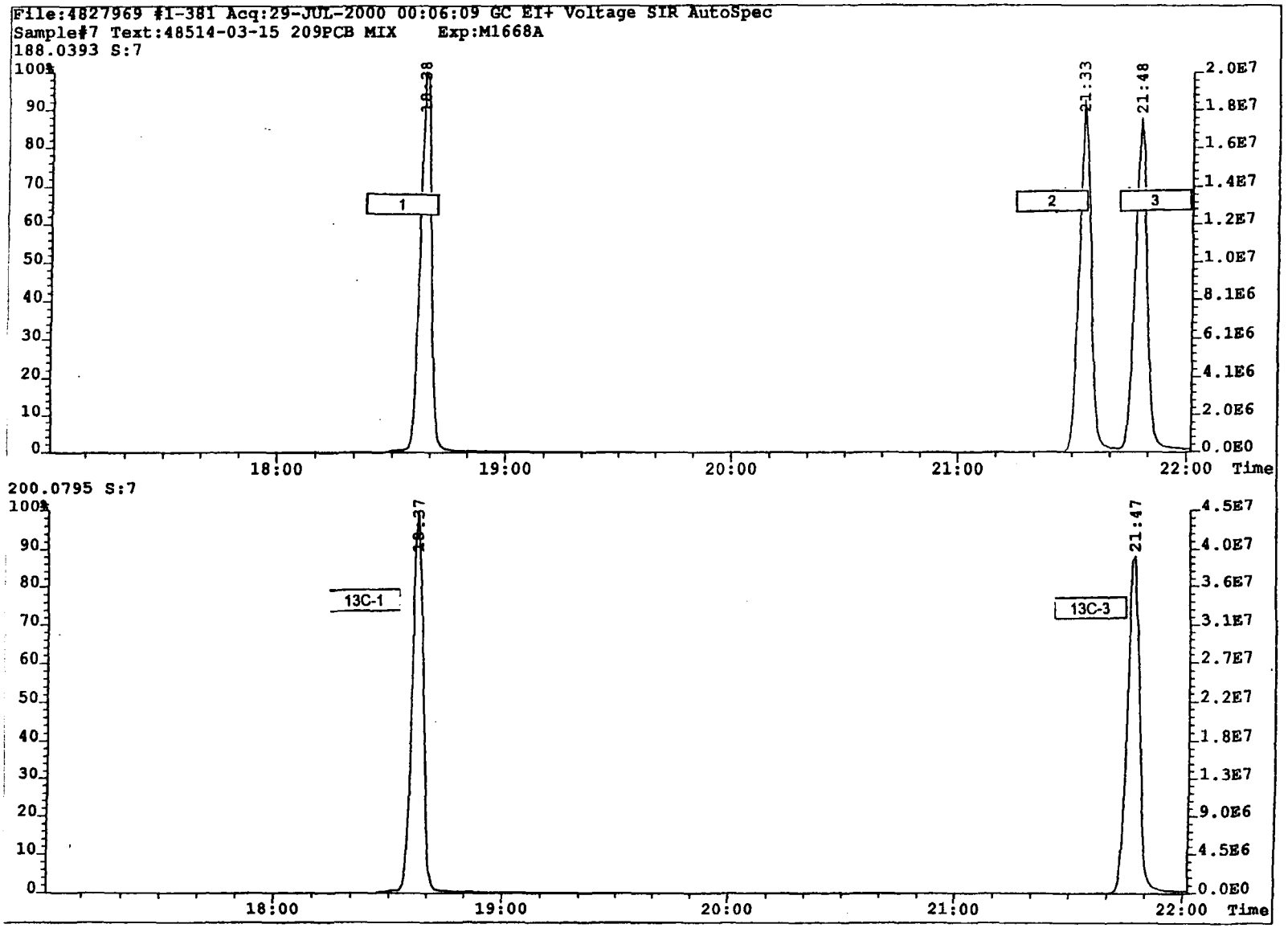
Function 8

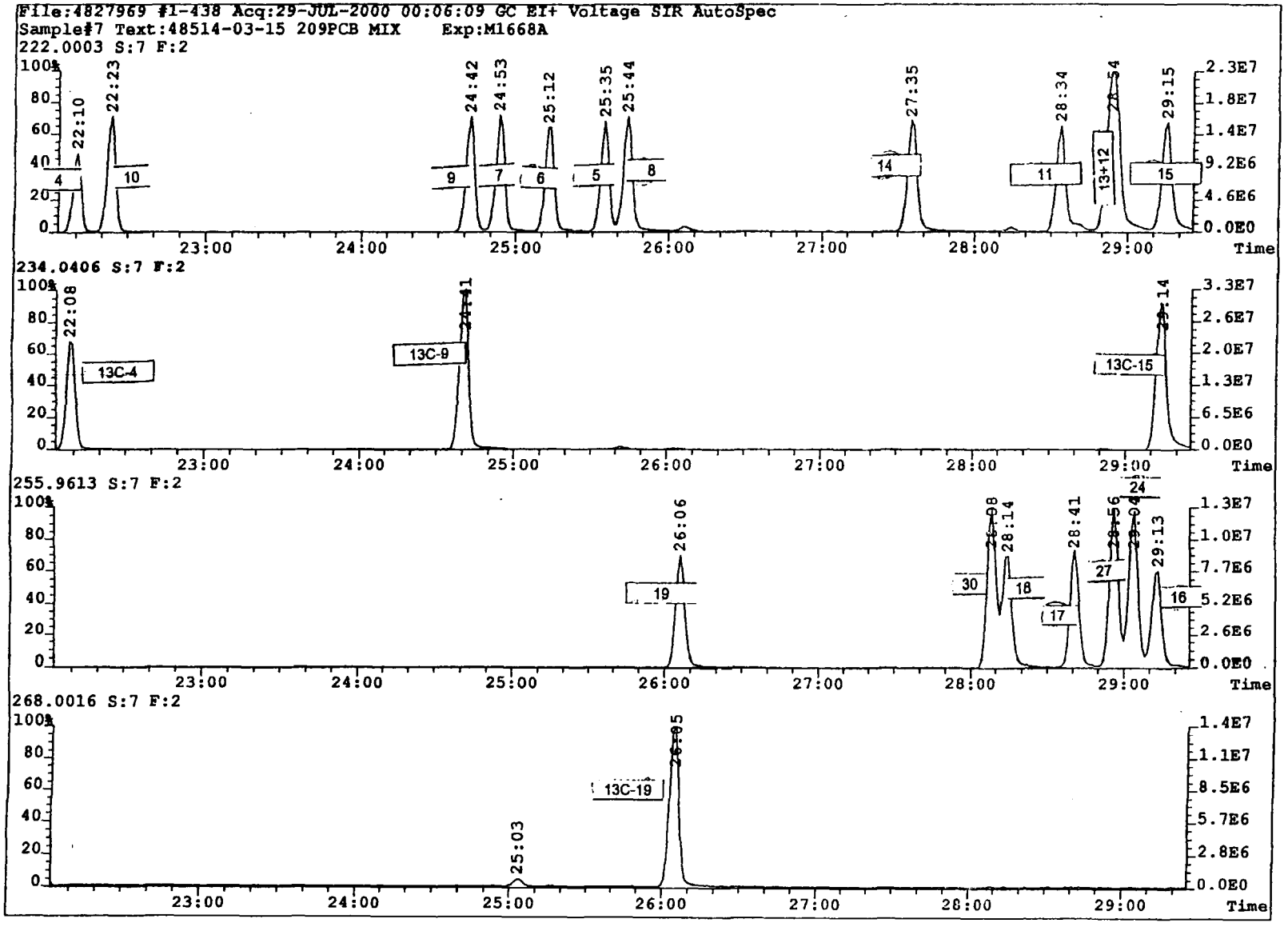
Type	: SIR Voltage
Calibration file used	: 4828039_8
High mass	: 511.7
Low mass	: 495.7
Resolution	: 10000
Ionisation mode	: EI+
Accelerating Voltage	: 8000.0V
Magnet 1 control	: Current
Start Time	: 60:20
End Time	: 63:00
Fast lock	: On
Number of channels	: 8
Cycle time (ms)	: 1000

Channel	Mass	Ch Time (ms)	I/ch Time (ms)
1	495.6856	100	30
2	497.6826	100	20
3	499.6797	100	20
4 (Lock)	504.9696	100	40
Primary Span Lock (Peaks) 2.00			
Secondary Span Lock (Peaks) 2.00			
Lock Level (mV) 0			
Step Lock (Peaks) 0.020			
5	504.9696	100	30
6	507.7258	100	20
7	509.7229	100	20
8	511.7199	100	20

Septum flow	: Off
Repeats	: 1

Appendix E
Chromatograms Showing Elution of all 209 PCBs


File:4827969 #1-381 Acq:29-JUL-2000 00:06:09 GC EI+ Voltage SIR AutoSpec
Sample#7 Text:48514-03-15 209PCB MIX Exp:M1668A
188.0393 S:7
1
2
3
21:33
21:48
200.0795 S:7
13C-1
13C-3
21:47
Time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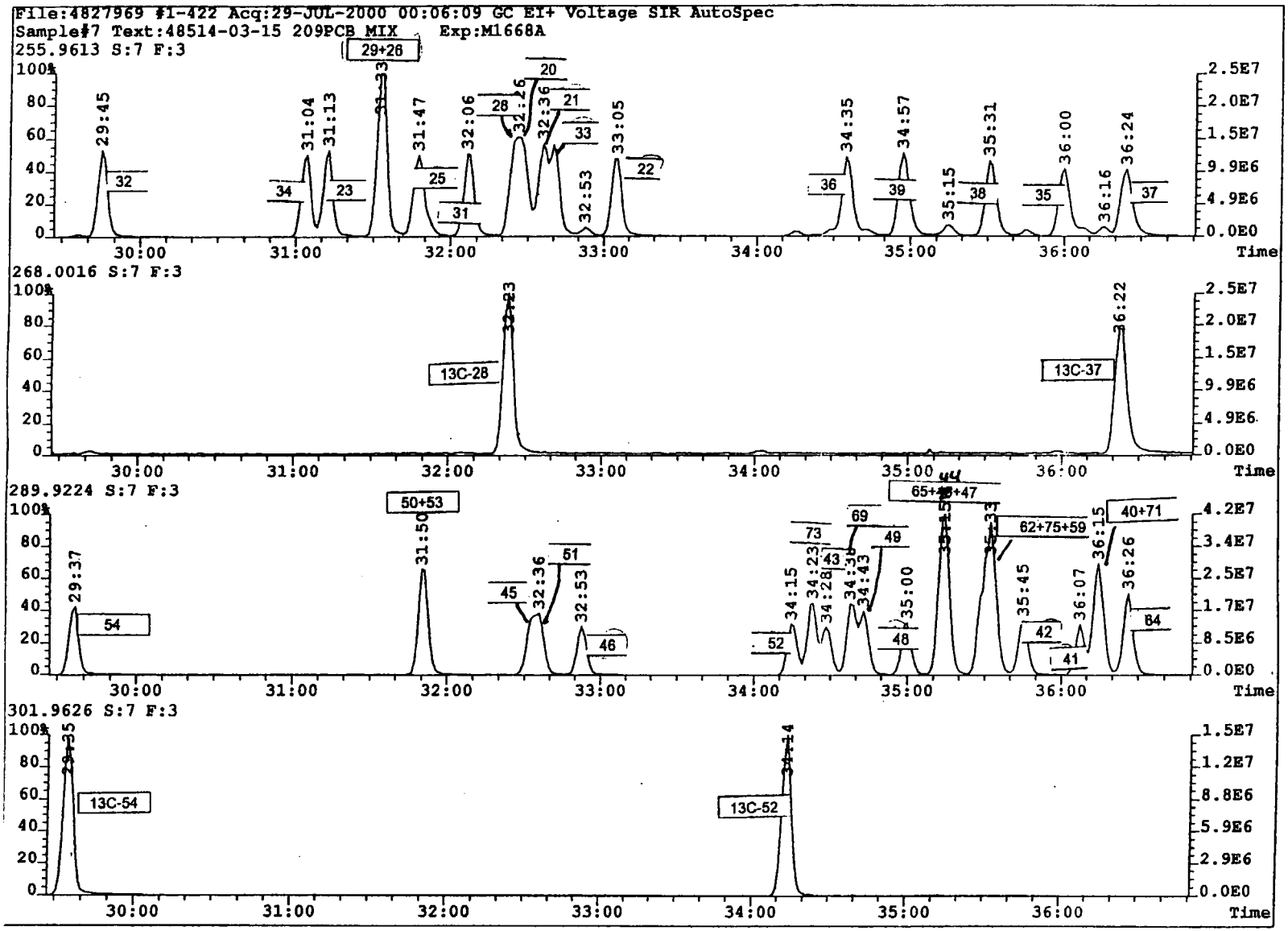
Sample#7 Text:48514-03-15 209PCB MIX Exp:M1668A
222.0003 S:7 F:2
234.0406 S:7 F:2
13C-4
13C-9
13C-15
255.9613 S:7 F:2
268.0016 S:7 F:2
13C-19
Time



Manual Number:

Battelle SOP Number: ASAT.II-009-00 (DRAFT)

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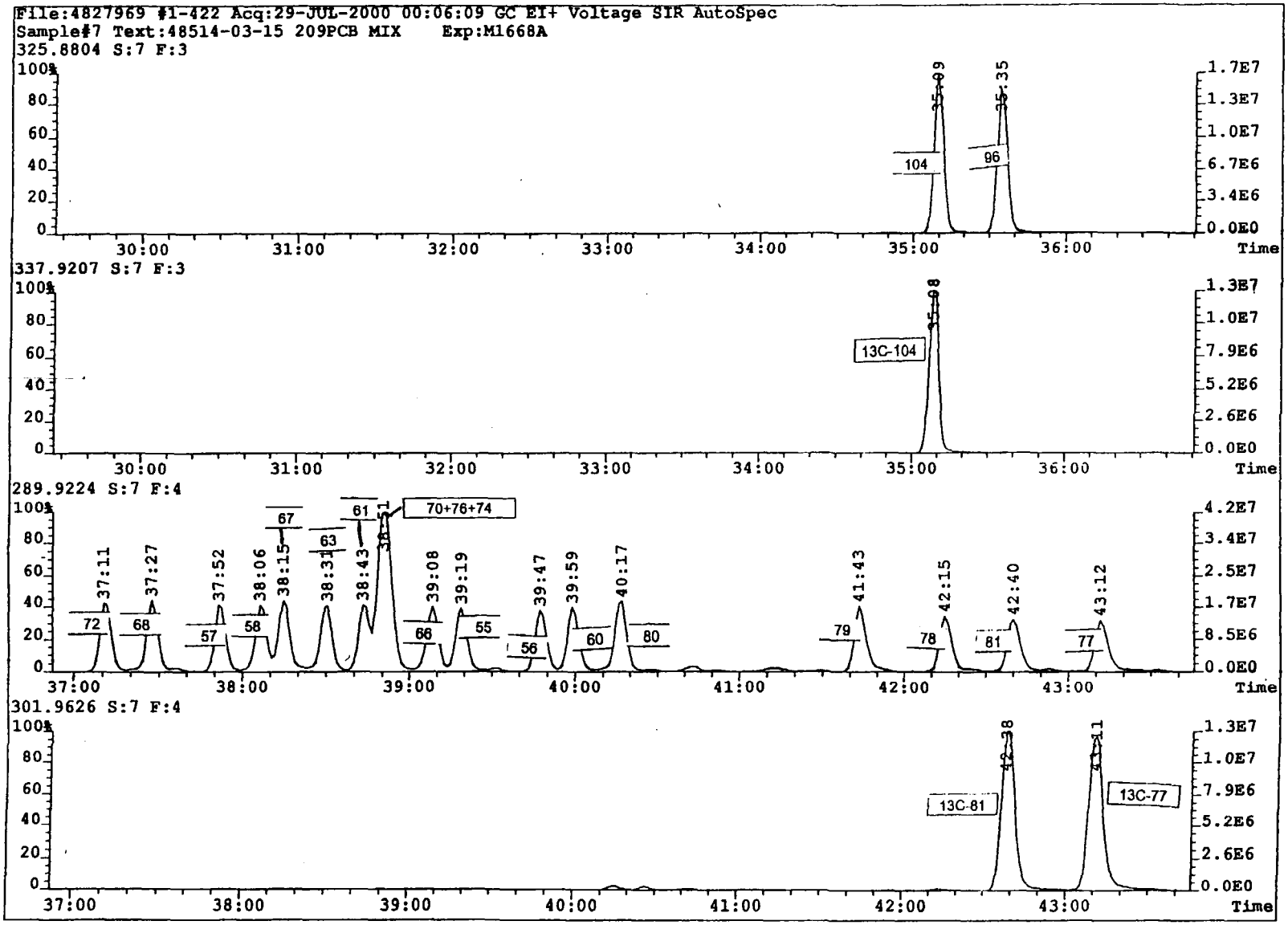
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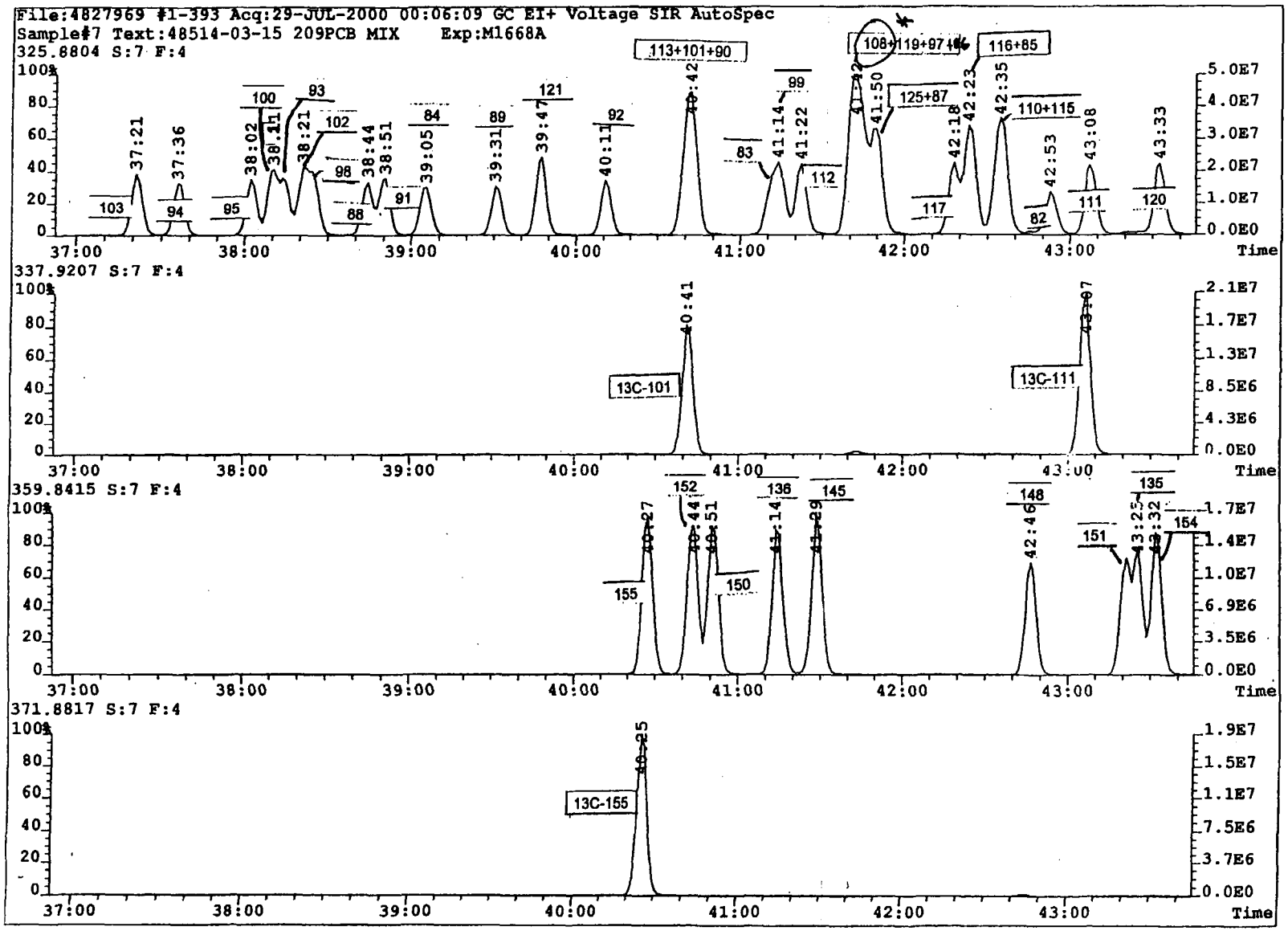
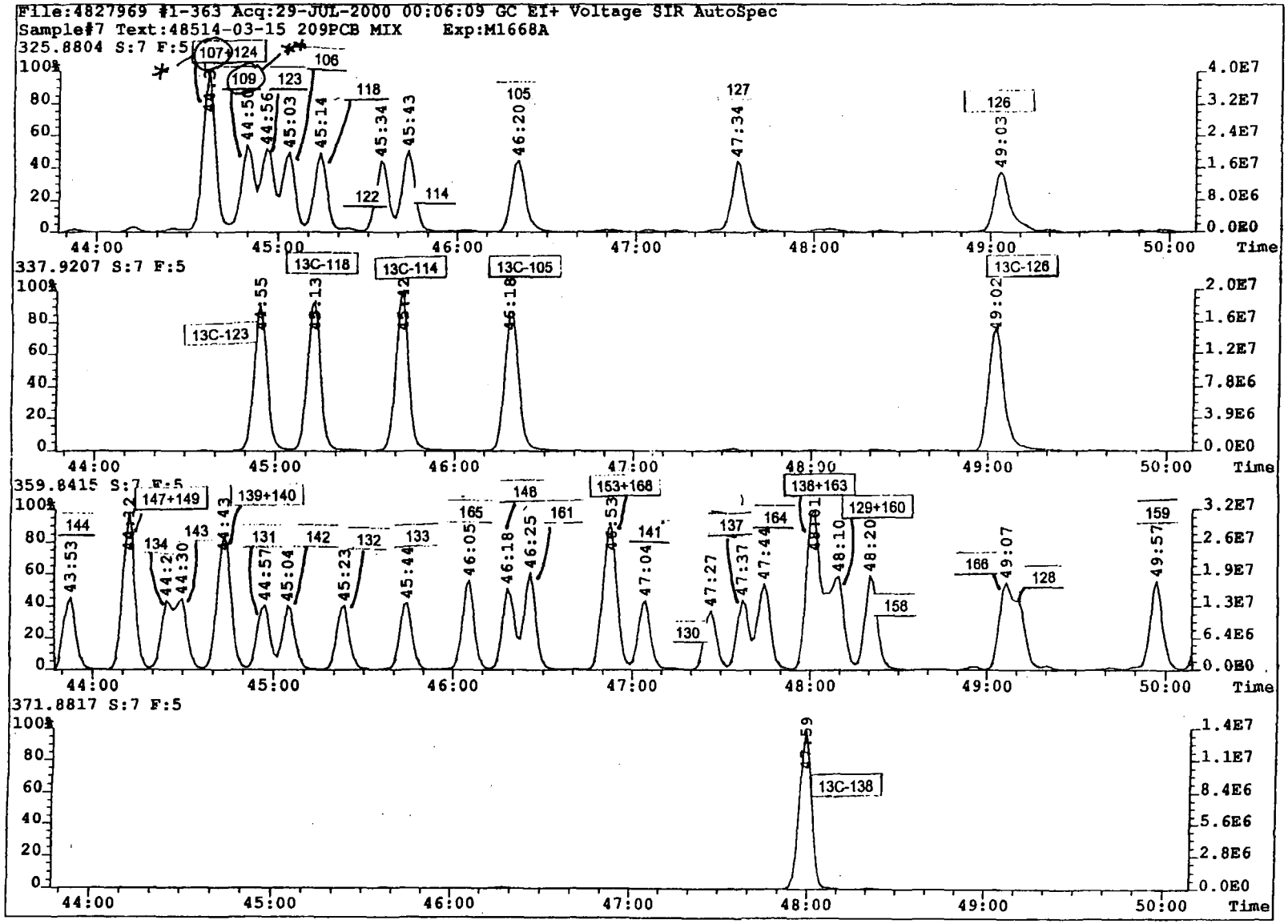
Sample#7 Text:48514-03-15 209PCB MIX Exp:M1668A
325.8804 S:7 F:3
104
96
337.9207 S:7 F:3
13C-104
289.9224 S:7 F:4
70+76+74
72
68
57
58
67
63
61
66
55
56
60
80
79
78
81
77
37:11
37:27
37:52
38:06
38:15
38:31
38:43
39:08
39:19
39:47
39:59
40:17
41:43
42:15
42:40
43:12
301.9626 S:7 F:4
13C-81
13C-77
Time



* 108 = Accustandard ID.

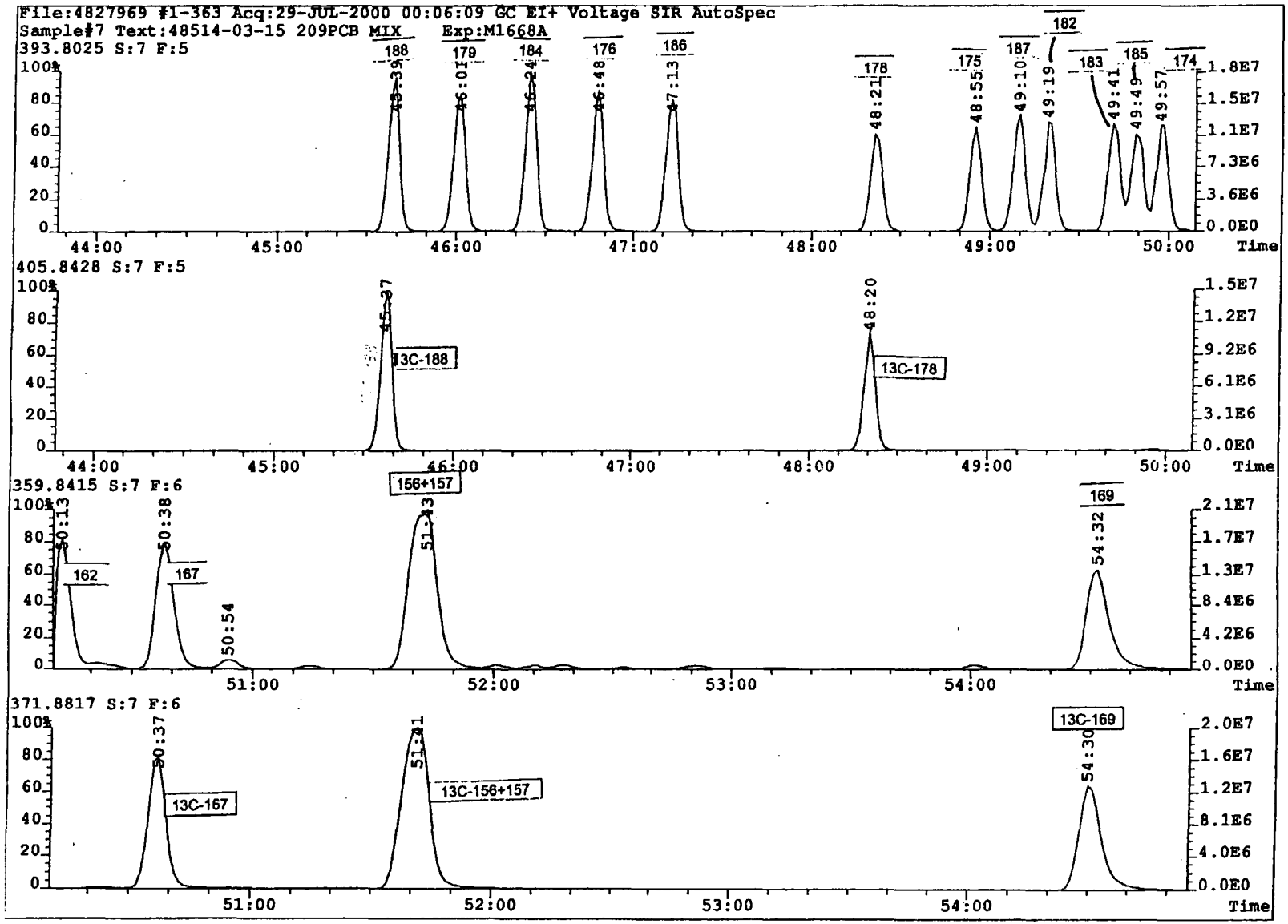
2,3,3',4,6 - PeCB = currently IUPAC = 109

WBS 6/2/01



* = 107 = Accustandard ID. 2,3,3',4,5'-PeCB is currently IUPAC 108

** = 109 = Accustandard ID. 2,3,3',4',5-PeCB is currently IUPAC 107.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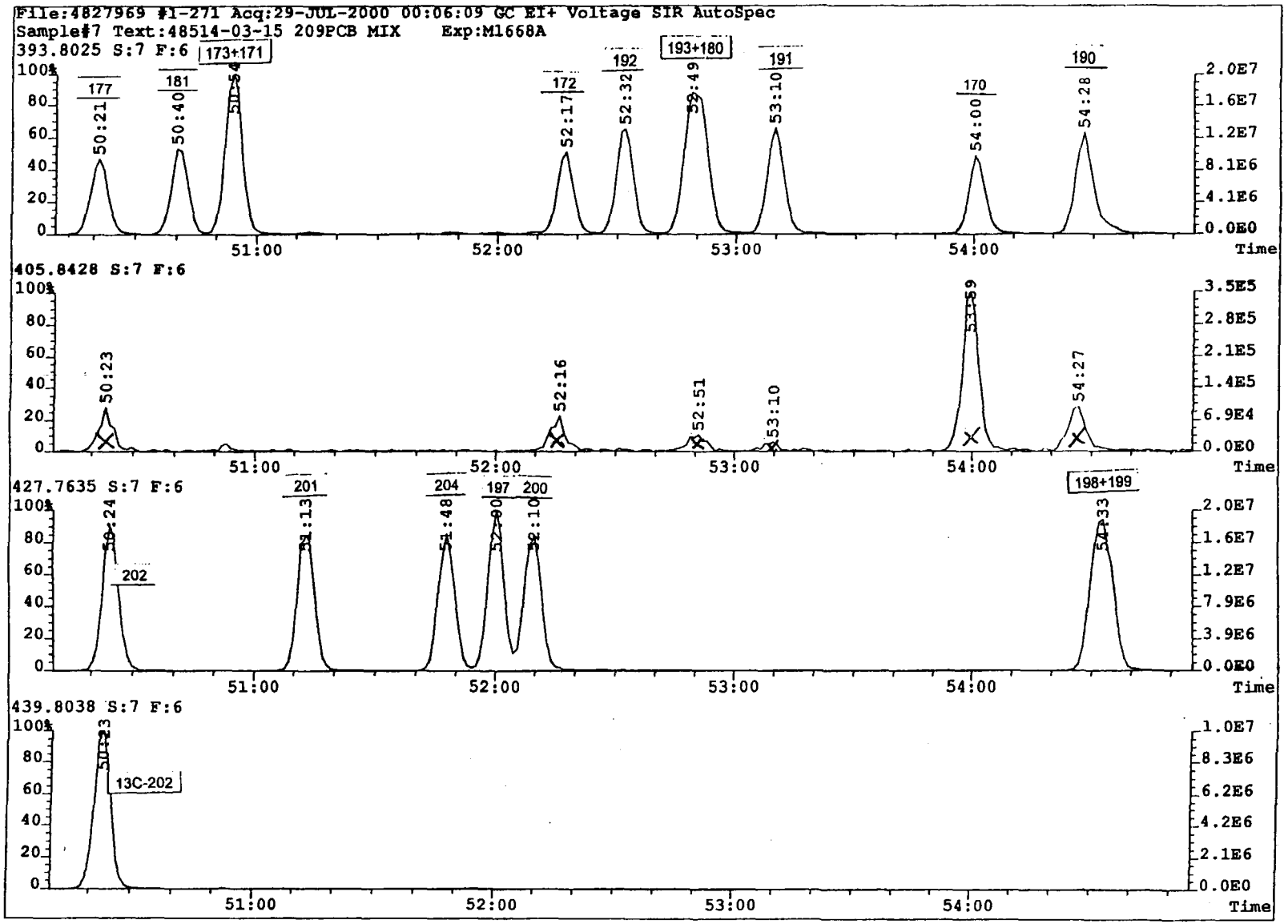
Sample#7 Text:48514-03-15 209PCB MIX Exp:M1668A
393.8025 S:7 F:5
188
179
184
176
186
178
175
187
182
183
185
174
405.8428 S:7 F:5
13C-188
13C-178
359.8415 S:7 F:6
162
167
156+157
169
371.8817 S:7 F:6
13C-167
13C-156+157
13C-169
Time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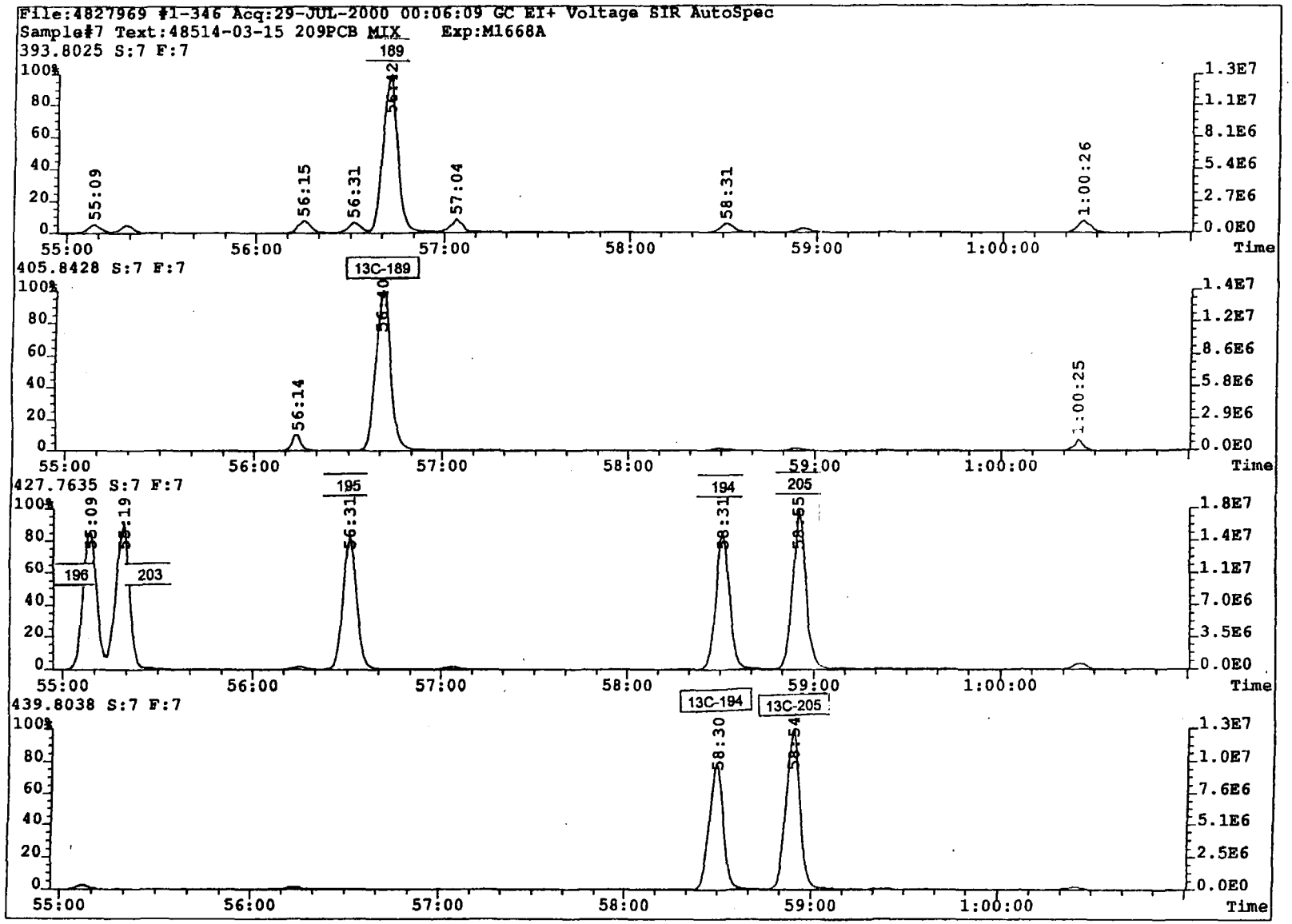
Sample#7 Text:48514-03-15 209PCB MIX Exp:M1668A
393.8025 S:7 F:6
177
181
173+171
172
192
193+180
191
170
190
50:21
50:40
52:17
52:32
53:10
54:00
54:28
405.8428 S:7 F:6
50:23
52:16
52:51
53:10
54:27
427.7635 S:7 F:6
202
201
204
197
200
198+199
439.8038 S:7 F:6
13C-202
Time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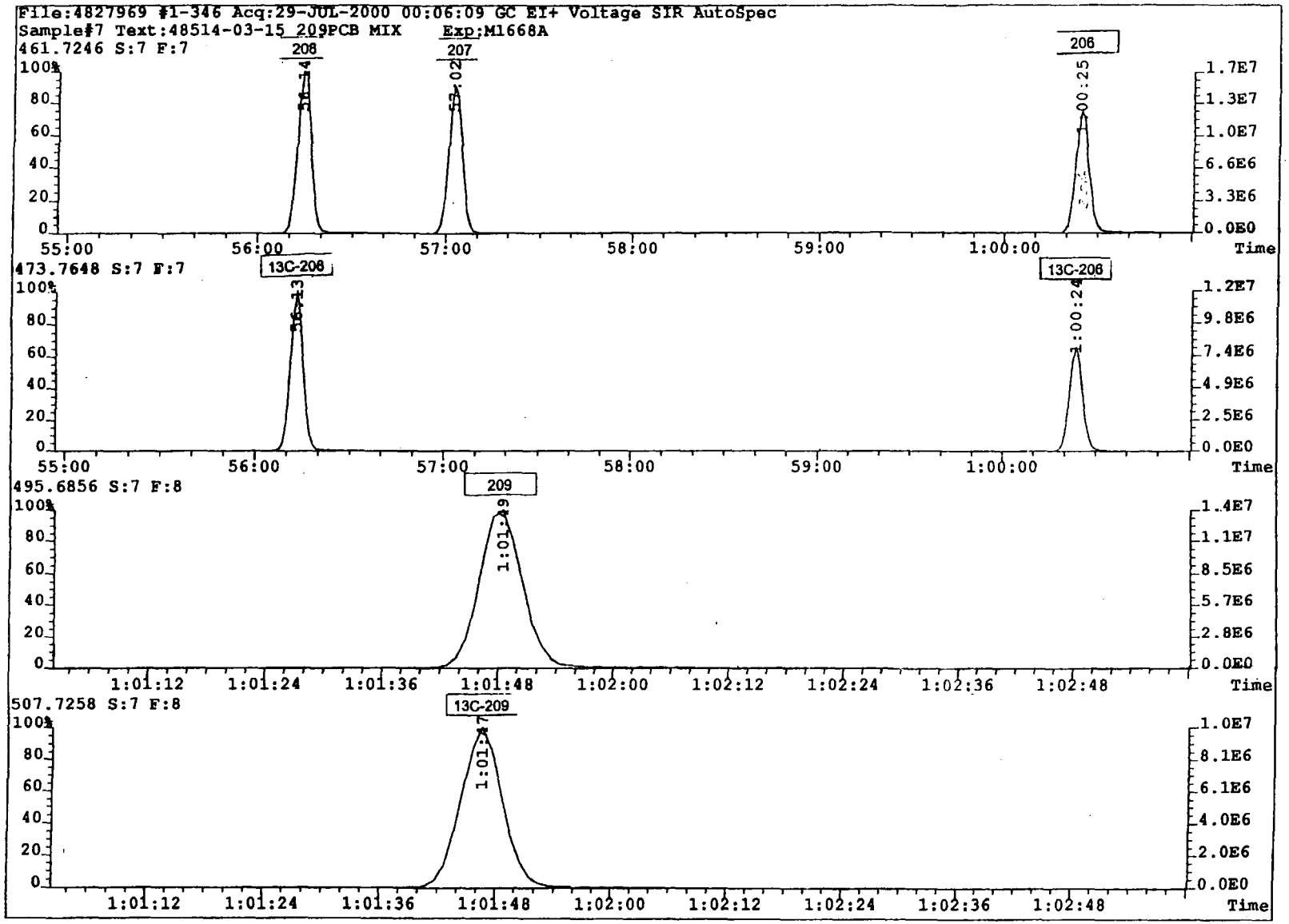
Sample#7 Text:48514-03-15 209PCB MIX Exp:M1668A
393.8025 S:7 F:7
189
55:09
56:15
56:31
57:04
58:31
1:00:26
405.8428 S:7 F:7
13C-189
56:14
1:00:25
427.7635 S:7 F:7
196
203
195
194
205
439.8038 S:7 F:7
13C-194
13C-205
58:30
Time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Sample#7 Text:48514-03-15 209PCB MIX Exp:M1668A
461.7246 S:7 F:7
208
207
206
473.7648 S:7 F:7
13C-208
13C-206
495.6856 S:7 F:8
209
507.7258 S:7 F:8
13C-209
Time

Battelle Ocean Sciences
Standard Operating Procedures

for

USE OF THE CAHN MODEL 25 AND CAHN MODEL 28 ELECTROBALANCES

Summary of changes in this version: Section 3.0 has been replaced by new text that describes the two current uses of Cahn balances: weighing procedures for neat materials and the gravimetric determination of oil weights.

1.0 APPLICATION

The CAHN 25 and CAHN 28 Electrobalances are very sensitive closed loop electromechanical weight and force measurement instruments that can be used to measure weight changes as small as 100 nanograms (0.0000001g), with a minimum amount of leveling or other special preparations. Operators should note that drafts, direct sunlight and excessive static may adversely affect the sensitivity of the balance. The balances should be used on a solid surface or weighing table, away from drafts and other disturbances.

2.0 CALIBRATION

The CAHN model 25 and CAHN model 28 electrobalances should be calibrated prior to use.

2.1 CAHN 25

1. Turn on the instrument. Allow the balance to warm up for a minimum of 30 minutes. The instrument should normally be left on continuously, however, a 30 minute warm-up is sufficient. The instrument must be recalibrated every time the power is lost, or if the instrument has been shut off.

2. Choose either the small or large weighing pans (the balance must be recalibrated if pans are changed during the weighing procedure). Clean weighing pans with CH_2Cl_2, allow to dry and replace on stirrups. Always use forceps when handling weighing pans, weights, and pans. The stirrups should be lifted from below the yoke for removal.

3. Deactivate the tare mechanism by pressing the tare button twice in rapid succession (the red indicator light above the tare button should go off).

4. Set the instrument to the 200 mg range. Adjust the digital readout to zero using the coarse and fine zero knobs.

5. Reactivate the tare mechanism by pressing the tare button once. The red tare indicator light should go on, wait until the white tare button illuminator light goes off. Now the balance is ready to calibrate. (If the balance does not zero on the first tare, the tare button may be depressed again until the digital readout is zero).

6. Place the 200 mg calibration weight (NBS Class "S") on the left pan (A) and close the weighing chamber door. Adjust the digital readout to 199.99 mg using the calibrate knob. Open the chamber door and carefully remove the calibration weight with forceps. Calibration weights are stored in the NBS supplied container. This helps to keep the weights free from dust.

7. Each time the balance is calibrated, the operator should record the calibration weight (e.g. 199.99 mg), date, and initials on the CAHN Electrobalance Calibration Form (Attachment 1). These records are kept with the balance in a "Balance Operation and Maintenance" book, located with the instrument.

8. Select the desired range and push tare to zero the balance. You are now ready to weigh your sample.

   Note: Procedure to switch ranges: Remove any sample from the pan. Deactivate the tare (two quick pushes of the tare button) and change to the desired range. Push the tare button once. You are now ready to weigh in the new range without recalibration.

9. If you plan to use the CAHN 25 in the "non-tare" mode, by using the zero adjustments or counterbalancing please familiarize yourself with the procedures in the CAHN 25 manual first (located with the balance).

2.2 CAHN 28

1. Turn on the instrument. Allow the balance to warm up for a minimum of 30 min. The instrument should normally be left on continuously; however, a 30-min warm-up is sufficient. The instrument must be recalibrated every time the power is lost, or if the instrument has been shut off.

2. Choose either the small or large weighing pans (the balance must be recalibrated if pans are changed during weighing procedures). Clean the selected weighing pans with methylene chloride, allow to dry, and place on stirrups. Always use forceps when handling weighing pans, weights, and pans. The stirrups should be lifted from below the yoke for removal.

2.2.1 Calibration Of The A Loop

To calibrate the balance on the A loop, it must be in the correct range. The correct range on the A loop is in the 200 mg to 1.0 μg range.

1. Place a weighing pan on the A loop.

2. Place a similar weighing pan on the tare loop.

3. Press the range button until the balance is in the 200 mg or 250 mg range.

4. Zero the balance by pressing the TARE button.

5. Place the 200 mg calibration weight on the A loop pan.

6. Once the digital display has stabilized, push the CAL button once; 200.000 will appear on the meter. Record the calibration weight, date, and initials on the CAHN Electrobalance Calibration Form (Attachment 1).

2.2.2 Calibration Of The B Loop

The B loop is not routinely used at Battelle but does allow for material(s) in a higher weight range to be weighed (1.5 - 3.5 g). The same calibration procedure used for the A loop (Section 2.2.1) should be used when calibrating the B loop. Use a 1000 mg NBS class "S" weight for calibration of the B loop (refer to the balance manual Section 3.3.2, page 7).

3.0 OPERATION

The CAHN model 25 and CAHN model 28 are normally used only in the preparation of analytical standards (from solid neat material) and the gravimetric determination of oil weights.

3.1 PREPARATION OF ANALYTICAL STANDARDS

Clean the weighing pans as described in Section 2.1 (step 2). Push tare button once. Using a micro spatula, weigh a portion of neat material directly onto the left weighing pan (A). Close the weighing chamber door. Allow the balance to equilibrate and read the weight from the digital display. Record the weight on the Solution Preparation Form. Carefully remove the weighing pan and gently rinse the neat material into a volumetric flask, using the appropriate solvent. Use of a small funnel for this transfer step is recommended to minimize material loss during transfer to the volumetric flask.

If the maximum weight limit in a particular weight range is exceeded, the digital display will blink repeatedly. Remove and clean the weighing pan. Change to a higher weight range and weigh a new portion of neat material.

3.2 GRAVIMETRIC DETERMINATION OF OIL WEIGHT

Clean the weighing pans as described in Section 2.1 (step 2). Push tare button once. Remove the weighing pan and place on hotplate. Draw an aliquot (usually 25, 50, or 100 uL) from the sample (total sample volume previously determined by volumetric or syringe measurement) and gently displace it onto the weighing pan. Just before the liquid has completely evaporated, remove the pan and replace it on the stirrups. Close the chamber door and allow the digital readout to equilibrate. Record the weight, remove and clean the weighing pan, tare the balance, and continue.

4.0 MAINTENANCE

The overall calibration and maintenance of the CAHN 25 and CAHN 28 balances is the responsibility of the Organics Sample Preparation Group Supervisor (supervisor) or designee. Daily cleaning and calibration is the responsibility of the operator. All routine and non-routine maintenance should be documented on the CAHN Electrobalance Maintenance Form (Attachment 2), located in the maintenance section of the CAHN Balance Logbook. Routine maintenance generally only includes cleaning the balance weighing chamber. When cleaning the weighing chamber, the operator should remove the weighing pans and stirrups. Using a Kimwipe or a balance cleaning brush, gently remove any dust or spilled sample from the weighing chamber. Replace the stirrups and weighing pans, close the chamber door and calibrate the balance (Section 2). If non-routine maintenance is required then consult the balance manual (CAHN 25: Section 4 Maintenance and Service, CAHN 28: Section 5 Maintenance and Troubleshooting) for assistance. The manual is located in the Organics Laboratory. If inhouse maintenance is not effective then the balance should be professionally serviced.

5.0 TRAINING

The trainee will read this SOP in its entirety. An experienced operator will then demonstrate calibration, operation and routine maintenance procedures to the trainee. Following this demonstration, the experienced operator and trainee will each weigh a series of five NBS Class "S" weights, usually 5, 10, 20, 30, and 50 mg. If the results of the trainee are within $\pm 2\%$ of the readings obtained by the certified instructor, then a Certificate of Training will be issue to the trainee. Percent difference is calculated as:

$$[(W_a - W_b)/W_a] * 100$$

where:

W_a = Weight determined by certified operator
W_b = Weight determined by trainee

The certificate is provided to the Quality Assurance Unit.

6.0 SAFETY

The CAHN 25 and CAHN 28 are very sensitive electrobalances and should be handled carefully if moved. Exercise caution when plugging in the instrument and make sure it is unplugged before removing the cover.

7.0 ATTACHMENTS

Attachment 1. Example of CAHN Electrobalance Calibration Form.

Attachment 2. Example of CAHN Electrobalance Maintenance Form.

Attachment 3. Example of Battelle Ocean Sciences Certificate of Training Form.

APPROVALS

Author		4/8/96
Laboratory Manager		4/8/96
QA Coordinator		4-9-96
Environmental Chemistry Section Manager	Name	April 9, 1996 Date

ATTACHMENT 1

CAHN ELECTROBALANCE CALIBRATION FORM

NBS Class "S" Weight Used	Weight after Calibration	Date and Initials

CAHN ELECTROBALANCE MAINTENANCE FORM

Description	Date and Initials

ATTACHMENT 3

Battelle Ocean Sciences
CERTIFICATE OF TRAINING

SOP No._____

SOP Title_____

Trainee_____

Instructor_____

Date SOP Read_____

Date Training Completed_____

The above mentioned trainee has satisfactorily completed the training requirements associated with this SOP. Supporting document (if applicable) is attached.

Results

NBS Class "S" Weight	Instructor's Results	Trainee's Results	Percent Difference

Comments

Approved by_____ **Date**_____

ORIGINAL

Battelle Duxbury Operations
Standard Operating Procedures

for

OPERATION AND MAINTENANCE OF HEWLETT-PACKARD 5970B, 5972A and 5973A GAS CHROMATOGRAPH/MASS SELECTIVE DETECTOR (GC/MS) USING HEWLETT-PACKARD SOFTWARE

Summary of changes in this version: Corrective action documentation for failed sample runs has been addressed. Verification of operation after maintenance has been addressed.

1.0 APPLICATION

This SOP details the daily operation and routine maintenance of the Hewlett-Packard 5970B and 5972A Gas Chromatograph/Mass Selective Detector (GC/MS) which is used for the identification and quantification of volatile and semi-volatile analytes in sample extracts. Additional references to the procedures presented in this SOP can be found in the Hewlett-Packard manuals located in the GC/MS facility.

2.0 MASS SPECTROMETER CALIBRATION

The mass spectrometer must be tuned prior to starting a new sequence to ensure that the electronic mass to charge (m/z) values are precise to within +/- 0.5 atomic mass units (AMU) and that the relative abundances of ions across the mass range 50 - 650 AMU meet accepted criteria. The calibration solution used to tune the mass spectrometer may be perfluorotributylamine (PFTBA) and/or decafluorotriphenylphosphine (DFTPP); both of which are commercially available and accepted as calibration solutions. PFTBA tuning can be performed automatically through autotuning or manually with operator control of tune parameter settings. The instrument is PFTBA tuned prior to DFTPP tuning. All MSDs at Battelle are tuned using PFTBA unless DFTPP is specified in the project work plan.

2.1 PFTBA TUNING

Hewlett-Packard's EnviroQuant software can automatically tune the instrument using PFTBA to achieve certain predefined performance criteria. Use of the autotune program minimizes operator-to-operator variation in tuning procedures and can maximize instrument sensitivity. The autotune program sets target values for six PFTBA ions(m/z 51, 69, 131, 219, 414, 502, and 614) and adjusts the various tuning parameters to achieve the relative values listed in Table 1. These target values are the levels that the masses would have if the instrument were tuned so that a spectrum of DFTPP conformed to EPA criteria.

2.1.1 The AUTOTUNE program is always used as the default tuning method after source cleanings.

2.1.2 AUTOTUNE programs should be run with the "standard values/settings" as a starting point.

2.1.3 The AUTOTUNE program adjusts the voltages of the repeller, ion focus lens, entrance lens, electron multiplier, and x-ray lens. It also adjusts the AMU gain and offset and the mass gain and offset.

TABLE 1. Hewlett-Packard Autotune Settings

m/z	Ion Abundance[a]
51	1 - 6 %
69	100%
131	30 - 50%
219	30 - 60%
414	1 - 2 %
502	1 - 6%
614	detectable

[a]Relative to base peak m/z 69

2.1.4 The relative ion abundances of the **three** masses m/z 69, 219, and 502 from the tune report should be compared to the target abundances listed in Table 1. If the abundances are not within range, tuning should be restarted using standard autotune. If the abundances are still not within range, the instrument can be tuned manually, as described in Section 2.3, below, to achieve the desired ion abundances. In all cases, a hard-copy of the final tune report will be placed in the GC/MS Tune Logbook, as specified in SOP No. 6-011.

2.2 MANUAL TUNING

In all cases, manual tuning is the tune method of choice (except after source cleanings where standard autotuning is used). Manual tuning can be used when PFTBA and/or DFTPP tuning criteria can not be achieved by the procedures in Sections 2.1 and 2.2, above. Manual tuning may be performed as a more time efficient method of generating a tune report. Each of the various instrumental parameters (e.g. repeller, entrance lens) may be varied within the manual tuning procedures. Manual tune parameters are always saved as a file called ATUNE.U on the EnviroQuant system. For more information regarding tuning procedures refer to the section on tuning in the Hewlett-Packard manuals.

2.3 DFTPP TUNING

Certain Projects require tuning of the GC/MS with DFTPP. This tuning procedure is more rigorous than PFTBA tuning, and strict specifications for DFTPP ions and ion abundances (Table 2) must be met prior to the start of a sequence and analysis of samples.

2.3.1 Perform a PFTBA tune as specified in Section 2.1 or 2.2 above.

2.3.2 Set up an acquisition as specified in Section 3.0, below. Inject 2 µL DFTPP (25 µg/mL). Where the acquisition is completed, run EnviroQuant program under Tuner/Evaluate DFTPP. This program will extract the spectra of DFTPP and automatically compare it to the values listed in Table 2.

2.3.3 If all key ions fall within the ranges given in Table 2, the instrument meets DFTPP tuning criteria, and sample analysis and quantitation may proceed. A copy of DFTPP Evaluation Report is kept in the GC/MS Tune Logbook as specified in SOP No. 6-011.

2.3.4 If DFTPP criteria are not met for all key ions, remedial action must be taken. Requantify the DFTPP peak as described in Sections 2.3.2 and 2.3.3, above, taking particular care in the placement of the cursor on the apex of the peak. If key ion abundances are still not met, additional remedial action, adjustment, and re-calibration with PFTBA and DFTPP is necessary before re-analysis.

TABLE 2. DFTPP Tuning Criteria

m/z	Ion Abundance
51	30 - 60%
68	< 2% of m/z 69
69	< 100%
70	< 2% of m/z 69
127	40 - 60%
197	< 1%
198	100%
199	5 - 9%
275	10 - 30%
365	>1%
441	Present < m/z 443
442	> 40%
443	17 - 23% of m/z

[a] Relative to base peak m/z

3.0 OPERATION

3.1 DATA ACQUISITION

Data acquisition software is controlled by the programs METHOD and SEQUENCE. Each different type of analysis performed on the GC/MS requires a specific set of acquisition parameters, including heated zones, and GC temperature, pressure, and autosampler programs. The acquisition parameters are stored in

a unique acquisition parameter file or method file. A hard copy of the acquisition parameter (method) file can be printed on request. The software program under the SEQUENCE menubar allows for creation of the sequence file. The sequence file is a list containing the names of samples to be analyzed, along with their assigned data file names, project numbers, vial positions, and acquisition methods. A hard copy of the sequence list is generated and kept in the GC/MS Logbook as specified in SOP No. 6-011.

If a sample needs to be rerun, (i.e., bad injection, failed calibration, power outage, etc.), this will be documented on the sequence. The analyst's initials and the date should accompany the corrective action taken and the reason a corrective action is needed.

3.2 METHOD - MASS SPECTROMETER CONDITIONS

The GC/MS can acquire data in linear scanning (full-scan) mode where all masses in a given range are scanned equally or in Selected Ion Monitoring (SIM) mode where selected ions are scanned while all other masses are ignored. SIM analysis is used for the detection of trace level analytes in environmental samples. SIM provides greater sensitivity and selectivity than full scan analysis. Full scan analysis is most often used when samples concentrations of target analytes are expected to be high. Full scan analysis is also used to detect, confirm (through library search), and quantify unknown analytes in environmental samples. The mode of acquisition is project-specific and is detailed in the project work plan.

3.2.1 The electron multiplier voltage should be set approximately 100 volts above the value calculated by the TUNE program. A scan-start time/solvent delay will be chosen which allows time for the solvent peak to elute before the first scan occurs. A minimum of three (3) minutes is typically required for a 30 or 60-m GC column. The mass range scanned is 50 - 450 m/z units for full scan analysis, unless stated otherwise in project protocols.

3.2.2 Certain analyses require the increased sensitivity of selected ion monitoring mode (SIM). Operating in SIM, the mass spectrometer monitors a specific m/z value for a predetermined length of time (dwell time), and then switches to another mass, rather than monitoring all the m/z values in a certain mass range.

3.3 GAS CHROMATOGRAPH AND ELECTRONIC PRESSURE CONTROL (EPC) CONDITIONS

The following GC conditions will be used unless stated otherwise in project protocols:

Injection port temp:	300°C
Detector temp:	280°C
Initial oven temp:	40°C
Initial hold time:	1 minute
Ramp rate:	6°C/minute
Final oven temp:	290°C
Final hold time:	20 - 30 minutes or until benzo(g,h,i)perylene elutes
GC Column:	DB5, 60 m, 0.25 id, 0.25 μm film (or equivalent – i.e. 30 m)

Injection port liner:	silanized straight liner (4 mm id) with glass wool
Glass wool position:	~ 1.8 cm from end of liner
Column position:	~ 0.8 cm from end of column nut
Septa:	Merlin microseal (preferable) or 9 mm septa
EPC Initial Pressure:	30 psi (for 60-m column, 15 psi for 30-m column)
EPC Initial hold time:	1.0 minutes
EPC Level 1 rate:	99 psi/min
EPC Final pressure:	psi equivalent to 1 mL/min

3.4 QUANTITATION - DATA PROCESSING

The extent to which acquired data is processed is project-specific, and depends on contract deliverables, analysis type, and acquisition type.

3.4.1 The identification and quantification of analytes in a sample is controlled by the quantitation database file located in the Data Analysis section of the EnviroQuant software. The database file is a three tiered (page) file containing information such as:

- compound names
- retention time windows
- quantifiation ions (primary and secondary)
- compound amounts
- internal standard designations
- surrogate standard designations
- calculated average response factors (RF)
- expected retention times
- integration parameters
- relative ion ratios
- compound responses
- internal standard amounts
- surrogate standard amounts

3.4.2 The retention time window range for all parent compounds in the database file should be ± 0.20 minutes. The retention time window for the alkyl homologues will be set at varying ranges depending on the alkyl homologue pattern. The slope sensitivity in the integration parameter file (rteint.p) is typically set at -0.020.

3.4.3 The first analyte listed in a database file is always an internal standard. Internal standards are designated as compound type "I". All analytes listed after the internal standard will be quantified versus that internal standard. In the case of multiple internal standards, once another analyte is designated as an internal standard, it will be used to quantify all analytes that follow it in the list.

3.4.4 The correct amount of internal standard spiked into the sample is input onto page one of the quantitation database file for the respective internal standard.

3.4.5 If the recovery of the surrogate internal standards are to be calculated, the spike amounts are input onto page two of the respective surrogate compound in the quantitation database file. Designate the surrogate compound as a type "S" on page two of the quantitation database file for the respective surrogate.

3.4.6 The pull down programs *Quant*, and *Calculate and Generate a Report*, allow for sample quantification. The following steps can be performed to quantify a sample or standard:

- Load the sample data file and proper quantitation method into memory by using the *File* pull down menus.
- Confirm that the correct amount of internal standard(s) is entered into the quantitation database file by using the pull down menu *Edit Compounds* located under the *InitCal* menubar.
- Check to see that the proper units are entered into the quantitation database. All data are quantified in total nanogram (ng) unless stated otherwise in project protocols. The units may be changed globally using the pull down menus *InitCal*, *Global Update*, and the option *"Other"*. Type in the runstring -- amount_units$="ng" -- to change the units of all target analytes to ng.
- Save the method by using the *File* pull down menus.
- Quantify the sample by using the *Quant* and *Calculate and Generate a Report* pull down menus. If the file has never been quantified the options *Generate Report* and *QEdit Quant Result* will be grayed out. Select the *summary report* option and select *printer* as the destination. This print out contains the all the analytes (targets + internal standards and surrogates) found in the quantitation database, their respective retention times, area abundances, quantitation ions, and nanogram amounts detected.
- The file naming convention for the quantified file will not change (see SOP 6-011). The only difference will be that there is a quantitation report associated with the file number.

3.4.7 Samples with the same amount of internal and surrogate standards spiked into them can be quantified in a batch mode. The following procedures are followed to quantify a group of similar samples:

- Load one of the sample data files and the proper quantitation method into memory by using the *File* pull down menus.
- Confirm that the correct amount of internal standard(s) is entered into the quantitation database file by using the pull down menu *Edit Compounds* located under the *InitCal* menubar.
- Check to see that the proper units are entered into the quantitation database. All data are quantified in total nanogram (ng) unless stated otherwise in project protocols. Sample sizes, dilutions, and any additional correction factors are applied in spreadsheet. The units may be changed globally using the pull down menus *InitCal* and *Global Update* and selecting *"Other"*. Type in the runstring -- amount_units$="ng" to change the units of all target analytes to ng.
- Save the method by using the *File* pull down menus.
- Using the *Tools* pull down menu, select *DOLIST* followed by *Summary Quant w/Calc* and *OK*. A window appears and allows for files to be selected for quantification. Select the files to be quantified and click *process*.
- The batch of samples will automatically be quantified and spooled to the printer for reporting.

3.4.8 All quantified samples are inspected for proper integration and verification of computer selection by entering the *QEdit Quant Result* program under the *Quant* menu bar. This program allows for reintegration of peaks if necessary and straight baseline integration of the alkyl homologue groups. Analytes that do not meet GC/MS peak criteria (SOP 5-157) may be deleted in this program. QDel's, as they are referred to, are recorded on the hard copy summary report generated above and placed in the Unused Data section of the project data package.

3.4.9 When all the summary reports are reviewed, final reports are generated. At the command line cursor type the runstring -- DOLIST$_{sp}$`QT$_{sp}$0,1,"S"'. Select from the window the files that require final report generation and click *process*. Hard copies of the final reports will be generated to the printer. The reports are spot checked for errors and dated and initialed by the analyst.

3.4.10 The EnviroQuant software allows for the creation of Excel databases to streamline final data table preparation. First a quantified data file is loaded and then a *detail.xls* file is created using the *Quant*, and *Generate Report* pull down menus. Select the *detail, text only* option along with selecting *File*. Type *detail.xls* in the box provided. The database template can then be created from the *Cust Rpt* and *Create/Modify Template/Database* menus. A list of files can be loaded into the database in a batch mode using the *Jobs* and *Queue List of Files* menus. Consult the facility manager for assistance in these procedures.

3.5 DATA STORAGE

Data is stored by the computer on a hard disc with a minimum capacity of 500 megabytes. When the hard disk becomes full, system operation will stop and no files can be acquired. Hard disk space may be made by transferring files from one network disk to another network disc (see SOP 6-011). Data, sequence, method, tune and database files are archived routinely on 60m DDS-1 data cartridges according to procedures outlined in SOP 6-011. Files that have been archived will be purged from the disk drive in order to created more space.

3.6 GC/MS CALIBRATION PROCEDURES

Certain calibration procedures are common to all types of analyses. These procedures include peak identification in standard solutions, generation of a calibration curve, and linearity checks of the initial and continuing calibrations.

3.6.1 Each of the standard solutions that are part of the calibration must be quantified as specified in Section 3.4, above.

3.6.2 Once all of the standards have been quantified correctly, the calibration curve can be generated. The following steps are used to update an existing a calibration curve (consult the facility manager and/or the Hewlett-Packard Software Manuals for assistance in creating a calibration file from scratch):

- Load the proper method and the low standard data file into memory using the *File* pull down menu.
- Input the low standard quantitation results into the quantitation database by using the *InitCal*, followed by *Update Levels* menu bars.

- When the window emerges, select *Recalibrate*, followed by choosing the appropriate standard level from the pull down menubar.
- In the same window select *Replace* for Responses and Retention Times.
- Finally, press *Do Update*.
- Follow the above procedures for each of the remaining calibration levels.
- When all the levels of the curve are loaded into the quantation database save the method using the *File* menubar.
- Generate the response factors to the screen, and then to the printer by using the *InitCal*, *Response Factors to Excel*, and *print* options. Save the method again. This report contains the calculated average response factors that will be used for future standard and sample quantification. The calculated relative standard deviation (RSD) for each of the target analytes in the standard must meet the criteria specified in SOP 5-157 prior to sample quantification.
- If alkyl homologues are to be quantified versus the response factor of the parent compound then the runstring -- $MACRO_{sp}$`BAT_RF,G' -- must be entered at the blinking cursor at the command line.
- Save the method again to ensure the response factors are retained in the quantitation database file. The quantitation database may now be used to quantify samples as specified in Section 3.4 above.

3.6.3 Continuing calibration standards are quantified, reviewed in *Quant*, *QEdit Quant Result* and checked for linearity relative to the initial calibration using the *ConCal*, and *Evaluate Data file as Continuing Cal to Printer* pull down menus. This report lists the percent difference between the average response factor generated in the initial calibration and the response factor determined from the continuing calibration, for each analyte. Results from the continuing calibration should be compared to the calibration criteria specified in SOP 5-157.

4.0 MAINTENANCE

Maintenance will be performed by the facility manager or other trained operators. All routine and non-routine maintenance will be recorded in the GC/MS Maintenance Log as specified in SOP 6-011.

4.1 PUMP MAINTENANCE

The rough pumps, turbomolecular pump (5970B MSD), and diffusion pumps (5972A MSD) should be serviced every six months. The pump maintenance is performed by HP service engineers approximately every six months under the preventative maintenance (PM) service contract. Pump fluid levels may be checked by GC/MS personnel periodically to ensure system performance.

4.2 FORELINE TRAPS (5972A MSD) AND HELIUM GAS TRAPS

The adsorbent pellets (molecular sieve) contained in the foreline traps should be replaced every six to twelve months or as needed. The foreline traps may be inspected during source cleaning events when the mass spectrometer is vented. Gas traps should be inspected routinely for adsorbent discoloration (e.g. black to yellow). The traps should be replaced when this color change is evident.

4.3 GC MAINTENANCE

Several of the GC maintenance procedures require exposing the column, injection port and mass spectrometer to air. To minimize damage of the mass spectrometer and column; the oven, injection port and transfer line should be cooled to room temperature whenever routine maintenance requires opening the system to air. Never leave the heated zones at their setpoints without carrier gas flowing through the column.

4.3.1 The injection port septum is typically a Merlin microseal. The frequency at which the seal is replaced is generally dependent on the number of injections. Replace the Merlin when the EPC no longer maintains pressure. Cool the oven and injection port to ambient temperature. Turn off the EPC. Unscrew the Merlin and remove the microseal with forceps. Using forceps gently insert a new microseal into the top of the port and place the thin Teflon washer into the Merlin nut. Set the EPC pressure to 15 psi. Hand tighten the nut to a position (~ 3 mm) just beyond the point where there is a noticeable pressure change (e.g. <0.2 psi to 15 psi). Tightening the nut too much will cause the seal to leak and the EPC to shut down.

4.3.2 Injection port liners should be changed routinely (every 1 - 3 batches). Cool the injection port. Remove the injection port liner-retaining nut. Remove the injection port liner and silicone o-ring with forceps. Unscrew the column nut from the bottom of the injection port. Unscrew the large bottom injection port nut inside the oven. Remove any debris from the injection port barrel and retaining nut by cleaning them with a cotton swab dipped in solvent. Clean the washer, gold seal, and nut with solvent before reinstalling. Reinstall the bottom injection port nut. Hand tightening the nut and further tighten 1/4 turn with the appropriate wrench. Using forceps, carefully install a clean, silanized liner with a new silicone o-ring into the injection port. Replace the ferrule if it has become disfigured or splintered. Next, clip the column (~ 2 - 8cm) as needed and readjust the column position prior to inserting the column nut into the injection port. Insert the column nut into the bottom injection port and hand tighten followed by tightening 1/4 turn with the appropriate wrench. Reinstall the retaining nut on the top of the port. Hand tighten the nut followed by tightening 1/4 turn with the HP wrench. Do not over tighten this nut as it will cause the top of the glass liner to chip. Wait 5 - 10 minutes before bringing the oven and injection port back up to operating temperature.

4.3.3 Whenever peak shape, resolution or sensitivity warrants, the precolumn (if installed) should be replaced. If this fails to correct the chromatographic problems, 30 - 50 cm of the analytical column should be removed. If column performance is not improved, the analytical column should be replaced. Replacing the column requires the instrument to be vented to the atmosphere, and involves instrument down time. Complete directions for column replacement are found in the Hewlett-Packard manuals.

4.4 MASS SPECTROMETER MAINTENANCE

4.4.1 Approximately every four months, or as needed, inspect and refill the calibration sample vial with PFTBA. in accordance with the instructions in the Hewlett-Packard manuals.

4.4.2 Approximately every 6 months or when needed, vacuum the dust from the grills of the fans on the back of the mass spectrometer.

4.4.3 The ion source should to be cleaned when usage-dependent surface deposits degrade the ion source function below user-determined limits. This will usually be indicated by an increase in the ion focus voltage and a decrease in the repeller voltage necessary to obtain proper abundances of masses m/z 219 and 502 during PFTBA tuning. Detailed, step by step instructions on ion source cleaning are found in the Hewlett-Packard hardware manuals.

4.5 VERIFICATION OF PERFORMANCE

After instrument maintenance has been performed, the analyst must verify that the instrument is back in working order and that any problems requiring maintenance have been corrected. This verification is accomplished by verifying instrument calibration. Either a calibration check standard or a new initial calibration curve that meets the acceptance criteria of this SOP will verify instrument operation.

5.0 TRAINING

1. The trainee must read, understand, and be able to perform all the functions listed in this SOP.

2. An experienced operator must provide a complete demonstration of all aspects of calibration, tuning, acquisition and data processing to the trainee.

3. An experienced operator must supervise and assist the trainee during training and test-sample analysis for each type of analysis to be conducted.

4. A trainee must demonstrate competence by performing independent PFTBA tuning. Furthermore, a previously analyzed sample must be rerun and reprocessed by the trainee. Results should be within +/- 15% of previous values to be acceptable.

5. Upon successful completion of the test sample, the new operator is qualified to operate the GC/MS for that type of sample. A Certificate of Training (Attachment 1) will be issued to the new operator and provided to the QA Office.

6.0 SAFETY

1. Certain areas of the GC and MSD are heated zones. Use caution around these areas and always drop the set temperatures to room temperature before performing hardware maintenance.

2. Proper care should be exercised for the use and disposal of syringes.

3. High voltages exist in several well-marked areas of the GC/MS and data systems and operators should avoid contact with these areas unless the instrument is turned off and power disconnected.

4. Potential health hazards may be associated with analytical standards, solvents, and samples to be analyzed (e.g. PCBs, pesticides, and other carcinogenic compounds). Protective clothing such as safety glasses, lab coats, and gloves may be required. Care should be taken when performing source cleanings as the source parts contain concentrated sample and standard residues. Proper disposal should be followed for used pump oils and used foreline trap pellets. Refer to the project protocol and Material Safety Data Sheets for specific safety guidelines.

5. Proper protocol should be used when handling gas cylinders, high-pressure lines, and gas filters.

7.0 ATTACHMENTS

Attachment 1. Battelle Duxbury Operations Certificate of Training

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APPROVALS

Author:		8·6·99
Laboratory Manager:		8·6·99
Quality Systems Manager:		8-10-99
Environmental Chemistry Section Manager:	AD Uhl for S S Durell	8-9-99
	Name	**Date**

ATTACHMENT 1

Battelle Duxbury Operations

CERTIFICATE OF TRAINING

for

SOP No. _____

TITLE: _____

_____

_____

TRAINEE: _____

INSTRUCTOR: _____

DATE OF COMPLETION OF PROFICIENCY TEST: _____

_____

TEST RESULTS

APPROVED BY: _____ **DATE:** _____

Battelle Duxbury Operations
Standard Operating Procedures

for

OPERATION AND MAINTENANCE OF GAS CHROMATOGRAPHS

Summary of Changes in this version: References to the HP LAS Data system have been replaced with the newer Xchrom data system. Internal Standard types have been modified to reflect new analysis methods. Calibration mid check re-occurrence has been changed. References to analysis detectors that are no longer in use have been deleted. This SOP now references the newer 6890 model GC. References to magnetic tape storage have been changed to reflect the data archiving SOP. Corrective Action documentation for failed sample runs has been addressed.

1.0 OBJECTIVE

This Standard Operating Procedure (SOP) covers the daily operation and routine maintenance of the Chemistry Department's non-GC/MS gas chromatographs (GCs). The Department maintains several gas chromatographs, comprised of Hewlett-Packard Model 5890A and 6890 gas chromatographs. These GCs are used for the analysis of a variety of organic compounds including petroleum hydrocarbons, PCBs, pesticides, sterols, and organotins.

This SOP is intended to be used as a guideline for operation of these instruments. For additional information, refer to the specific Instrument Manuals. Additional project-specific information relevant to the operation and maintenance of the GCs may be specified in Quality Assurance Project Plans (QAPPs).

2.0 CALIBRATION

Before the sample is injected into the GC, the detector must be calibrated to determine the response for the analytes of interest. Demonstration of detector response linearity is required before sample analysis can begin. Calibration checks must be analyzed minimally every 10 samples, or 12 hours of run time, whichever is more frequent.

All analytical chromatographic data are quantified by either the internal standard or external standard method. The calibration and quantification method will be by the method of internal standards unless otherwise specified in the QAPP.

The internal standard method of analysis is based on measuring the response of analyte(s) relative to a quantification internal standard (QIS). The QIS(s) is typically a recovery internal standard(s) (RIS) added to the sample after sample processing in the laboratory, but may, if specified in the QAPP, be a surrogate internal standard (SIS) added prior to sample processing is completed and before instrumental analysis. The RIS is used to determine the recovery of the SIS(s), and the thus the efficiency of the sample processing.

In practice, an average response factor, or linear regression calibration equation, is determined using the response of analyte(s) of interest relative to the QIS in a series of calibration standard solutions. Actual samples are fortified with the QIS prior to instrumental analysis. The analysis of the sample is accomplished by measuring the response of the analyte relative to the QIS, and calculating the analyte concentration using the average response factor, or linear regression calibration equation, obtained from the calibration process. The internal standard calibration procedure is discussed in more detail in Section 2.1.

The external standard method of analysis is based on measuring the response of an analyte in a sample, and comparing it to the response of the same compound in the analysis of standard(s) of known concentration(s). In practice, an average response factor, or linear regression calibration equation, is determined for analyte(s) of interest in a series of calibration standard solutions. A sample is prepared to a known volume and analyzed. The concentration of the analyte(s) in the sample is determined by applying the average response factor, or linear regression calibration equation, obtained from the calibration process, to the response of the analyte(s) in the sample analyses. Accurate determination and recording of sample preinjection volumes (PIV) are necessary when using this method. The external standard calibration procedure is discussed in more detail in Section 2.2.

Instrument calibration is the responsibility of the analyst. Calibration records are maintained in project-specific data packages. In the following sections common calibration procedures are described. However, project-specific procedures may be selected and will then be specified in the QAPP.

2.1 INTERNAL STANDARD CALIBRATION

2.1.1 Initial Calibration

Most GC analyses are quantified by the method of internal standards. To use this approach select one or more internal standards that are similar in analytical behavior to the compounds of interest. Further, the measurement of the internal standard should not be affected by method or matrix interferences. Because of these limitations no one internal standard is applicable to all samples. The following listing includes examples of internals standard(s) for the corresponding analyses. However, project-specific internal standards may be selected and will be specified in the QAPP.

Analysis Type	Internal Standard
Petroleum hydrocarbons	*o*-terphenyl, androstane,
PCB's, pesticides	Cl_3(39) 3,4',5 Trichlorobiphenyl
	Cl_3(34) 2',3,5 Trichlorobiphenyl
	Cl_5(103) 2,2',4,5',6 Pentachlorobiphenyl
	Cl_5(112) 2,3,3',5,6 Pentachlorobiphenyl
	Cl_6(166) 2,3,4,4',5,6 Hexachlorobiphenyl
Coprostanol	androstane, androstanol
Organotins	dipropyltin, tripropyltin, dipentyltin, tripentyltin

Calibration standards are prepared at a minimum of one concentration level for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard a known constant amount of the appropriate internal standard(s) is added and the resulting standard diluted

to volume with an appropriate solvent. If only one calibration standard is prepared, it must be at a concentration corresponding to the expected concentration found in real samples.

Typically a five-point calibration is performed, but more or fewer calibration levels may be specified in the QAPP. These standards include the analytes of interest, as well as the appropriate internal standards if an internal standard calibration is to be performed. The concentration of the LOW standard should be approximately 2 to 5 times the detection limit of the instrument. The MID-range calibration standard should be near the expected concentration of the samples. The HIGH concentration standard should be approximately 5 to 10 times the concentration of the LOW standard, or high enough to span the expected concentration range. A wider calibration range may be appropriate for certain applications, and will then be specified in the QAPP. Commonly, calibration standards have the following concentrations (for individual PCB congeners and single peak chlorinated pesticides):

LOW	~ 0.008 ng/μL
MID LOW	~ 0.012 ng/μL
MID	~ 0.048 ng/μL
MID HIGH	~ 0.096 ng/μL
HIGH	~ 0.120 ng/μL

The concentrations of the SIS and RIS should be approximately at the same as the MID-level target analytes, the same in all calibration solutions, and approximately the same concentration as expected in the samples. A multilevel calibration that meets acceptance criteria must be analyzed before sample analysis begins.

Using injections of 1 to 5 μL (1 to 2 μL for most applications), analyze each calibration standard using the same instrumental analysis conditions as will be used for samples. The calibration is established and verified by one of two methods: linear regression or response factor. Instrument calibration is generally accomplished using the linear regression algorithm of the **Xchrom data system**; calibration verification may be performed using the correlation coefficient of the Xchrom data system linear regression calculation or by using response factors (RFs). The following are the applicable equations for the two different methods.

Linear Regression Method

- $Y = mX + b$

  which is equivalent to: $A_A/A_{IS} = [[m \times (C_A/C_{IS})] + b]$

- $C_A = [(A_A/A_{IS}) \times b] \times (C_{IS}/m)$

Response Factor Method

- $RF = (A_A \times C_{IS})/(A_{IS} \times C_A)$

- $C_A = (A_A \times C_{IS})/(A_{IS} \times RF_{AVG})$

where,

C_A = analyte amount
A_A = analyte peak area (or height)
A_{IS} = area (or height) of the QIS
C_{IS} = amount of QIS added to the sample
b = y-intercept of linear regression equation
m = slope of linear regression equation
RF = response factor of the analyte
RF_{AVG} = average response factor of the analyte from the initial multilevel calibration

If a multi-level calibration is performed, the linearity of the calibration for each target analyte is checked. If the response for a given analyte is acceptably linear over the calibrated range [acceptable correlation coefficient or percent relative standard deviation (%RSD) in RFs], the calibration is considered valid and can be used for sample quantification. Calibration acceptance criteria are discussed in Section 2.5.

The **Xchrom** data system automatically computes and reports the correlation coefficients for the linear regression equations as part of the calibration. However, if the RF calibration check method is used, the variation in the RF for each compound is determined as %RSD using the following equation:

$$\%RSD = \frac{SD_{RF}}{AVG\ RF} \times 100\%$$

%RSD = percent relative standard deviation
SD_{RF} = standard deviation in average RF for the calibration standards
AVG RF = average RF for the calibration standards

2.1.2 Continuing Calibration

The continued validity of the initial calibration must be verified by the analysis of calibration check standard(s). Typically, a MID-level calibration standard is analyzed for every 10 samples analyses. The initial calibration is considered valid if the RF of the calibration check standard (response factor method) is acceptably similar to the average RF determined for the initial calibration. The ongoing acceptance of the calibration can also be verified by quantifying the calibration check standard as a sample and calculating the difference between the measured amount and the known (true) standard amount. Calibration check standard acceptance criteria are discussed in Section 2.5.

In either case, the comparability of the calibration check standard for each analyte versus the initial calibration is computed, and expressed as percent difference (%DIFF), using the following equation:

$$\%DIFF = \frac{(RF_C \times RF_I)}{RF_I} \times 100\%$$

%DIFF = percent difference
RF_I = average RF for the initial calibration standards or true value
RF_C = RF for the calibration check standard or measured value

If a single-point calibration is used, no initial calibration criteria apply but the calibration is continuously verified by determining the %DIFF in the RF or measured value for each analyte in the calibration check

analyses versus the RF or true value from the initial calibration. The RF calculated for a single-point analysis is equivalent to the slope determined in a single-point calibration with the Xchrom datasystem. As with multilevel calibrations, sample analyses should be bracketed by calibrations that are acceptably similar.

2.2 EXTERNAL STANDARD CALIBRATION

Each calibration standard is analyzed using the same instrumental analysis conditions as used for samples and the calibration is established and verified by one of two methods (linear regression or response factor). Instrument calibration is generally accomplished using the linear regression algorithm of the Xchrom data system; calibration verification may be performed using the correlation coefficient of the Xchrom data system linear regression calculation or by using calibration factors (CFs). The following are the applicable equations for the two methods.

Linear Regression Method

- $Y = mX + b$

  which is equivalent to: $A_A = [(m \times C_A) + b]$

- $C_A = (A_A \times b)/(m)$

Response/Calibration Factor Method

- $CF = (A_A)/(C_A)$
- $C_A = (A_A)/(CF_{AVG})$

If a multi-level calibration is performed, the linearity of the calibration for each target analyte is checked as indicated in Section 2.1. The continued validity of the initial calibration must be verified by the analysis of calibration check standard(s), as described in Section 3.1. Calibration acceptance criteria are discussed in Section 2.5.

2.3 CALIBRATION/QUANTIFICATION OF MULTICOMPONENT COMPOUNDS

Depending upon the QAPP instructions, either a multi- or single-level calibration is analyzed when samples are quantified for multi-component mixtures such as Aroclors, technical chlordane, or toxaphene. The instrument calibration and analysis procedures for multi-level multicomponent standards are discussed in SOP 5-128.

2.4 PREPARATION OF CALIBRATION STANDARDS

The preparation of calibration standards may be performed by properly trained Battelle personnel, in accordance with SOP No. 5-027.

2.5 CALIBRATION ACCEPTANCE CRITERIA

Unless otherwise documented in the QAPP or analysis-specific SOPs, the following acceptance criteria apply.

Initial calibration - Linear Regression Method: correlation coefficient of >0.99 for a 3-point calibration, and >0.995 for a 5-point calibration. Response/Calibration Factor Method: %RSD of the average analyte RF or CF should be ≤25%.

If these criteria are not met corrective action is taken, a new calibration run, and the samples re-analyzed. Corrective action includes verifying that the proper analytical conditions are used, that appropriate standards are used, and routine maintenance. However, corrective action and re-analysis will be at the discretion of the Project Manager. A decision to continue analysis without corrective action must be documented, justified, and approved by the Project Manager, or his/her designee.

Calibration check - Response/Calibration Factor Method: %DIFF between the RF or CF from the calibration check and the average analyte RF or CF of the initial calibration must be ≤25%.

If these criteria are not met, the check standard can be re-analyzed. If the check standard fails to meet the criteria a second time, corrective action will be taken, and the check standard and associated samples re-analyzed. A new initial calibration may need to be run, and affected samples re-analyzed. Corrective action includes verifying that the proper analytical conditions are used, that appropriate standards are used, and routine maintenance. However, corrective action and re-analysis may be at the discretion of the Project Manager. A decision to continue analysis without corrective action must be documented, justified, and approved by the Project Manager, or his/her designee.

3.0 OPERATION

The individual should refer to the H-P 5890/6890 instrument manuals for instruction in the keyboard operation of the appropriate chromatographs and ancillary equipment (including integrators and autosamplers). These manuals have separate sections describing the operation and maintenance of the GCs, including each GC detector, and this source of information should be familiar to the analyst and referred to when needed. It is beyond the scope of this SOP to list step-by-step instructions for the programming, activation, operation, and all possible maintenance of these instruments. Each manual is kept in the instrument room near the appropriate chromatograph.

3.1 EQUIPMENT/SUPPLIES

3.1.1 Instrumentation

The GCs currently used in the GC laboratory are 5890 & 6890 GCs. A separate Instrument Log Book is maintained for each GC. Instrumentation consists of:

> H-P 5890 & 6890 Gas Chromatographs, equipped with combinations of split/splitless and on-column capillary injectors, FIDs, ECDs, flame photometric detector (FPD), and 7673A/6890 series autosamplers. Many of the instruments have dual injection capabilities,

where both injection ports are used simultaneously. The front injection port is connected to the front detector and the back injection port to the back detector.

Xchrom Data System, Digital & Analog data acquisition servers, Dell Power Edge 2100/200 network server, Xchrom chromatography software, disk drives, and printers.

3.1.2 Analytical Columns

A variety of capillary and packed columns may be used in the GC analyses. 30 m X 0.25 or 0.32 mm i.d. (inner diameter) fused silica capillary columns will be used for most applications. DB-1, DB-5, or DB-1701 (J&W Scientific), or equivalent, column stationary phase will generally be used. Other columns (e.g., 0.53 mm i.d. capillary or packed columns) may also be used as specified in QAPPs. Unless otherwise specified in the QAPP or analysis-specific SOPs the following columns will be installed for analysis

FID	30 m DB-5 0.32 mm ID, 0.25 μm film
FPD	30 m DB-5 0.25 mm ID
ECD	Primary column: 60 m DB-5 0.25 mm ID, 0.25 μm film Confirmation column: 60 m DB1701 0.25 mm ID, 0.25 μm film

3.1.3 Compressed Gases

FID, FPD:	Helium, ultra high purity (UHP) grade, or equivalent; Hydrogen, UHP grade, or equivalent; Air, zero grade, or equivalent.
ECD:	Hydrogen or Helium, UHP grade, or equivalent; Argon/Methane (95%/5%), UHP grade, or equivalent.

3.2 STANDARD CONDITIONS

Standard conditions for each type of analysis are specified in the analytical SOPs or in the QAPP. Analysis conditions are recorded on each analysis acquisition schedule and filed in the Instrument Log Book with each analysis sequence, as described in SOP 6-025 and as discussed in Section 3.8.

3.2.1 Injection Mode

All capillary gas chromatographic analyses will be performed in the splitless mode unless otherwise specified in the project protocols.

3.2.2 Flowrates

Optimum GC flowrates will vary with instrument, detector, and the analysis to be performed. Please refer to the applicable instrument manuals and/or analytical SOPs for more information on flowrate and gas pressure settings.

3.2.3 Temperature Programs

Gas chromatograph injector and detector temperature, and oven temperature programs are project/analysis specific. Refer to the appropriate analysis/quantification SOP for specific GC analytical conditions. These conditions may be modified in the QAPP for specific applications.

3.3 INSTRUMENT SETUP AND STARTUP

- Check tank and line pressures. Change compressed gas tanks if any are below 300 psi.
- Make sure all applicable GC equipment are on (including detector flames for FID and FPD) and communicating properly.
- Set GC chromatographic conditions according to applicable analytical SOP or project work plan.
- Place calibration standards and samples in autosampler tray in the order they are to be analyzed.
- Set up the sample data acquisition schedule on the data system for the samples to be analyzed.
- Start the chromatographic analysis by starting the gas chromatograph, autosampler, and data acquisition through the particular controller (e.g., autosampler, integrator, or instrument control) for the GC to be used.

3.4 DATA ACQUISITION

The chromatographic data generated during standard and sample analysis is automatically stored on the Xchrom data system network server hard disk for postrun analysis. The chromatographic data acquired with the Xchrom Data System is automatically dated and time stamped. Hard copies of chromatograms as well as peak area and/or height, and quantification reports may be obtained from the Data System.

Additionally, the GC signal may be plotted in real-time by an integrator connected to the GC or by viewing the acquisition status through the data system software. This will allow for rapid monitoring of the GC analysis, but in most cases will not be used for quantification (unless otherwise specified in QAPP).

The creation of the acquisition schedule is discussed in more detail in SOP 6-025. Information that must be entered include the following:

- Xchrom data system Sequence ID. The Instrument Log Book will be checked to determine which was the last analysis ID used, so that the next ID can be assigned to the first analysis in the sequence.
- Xchrom data system Method ID.
- Laboratory sample ID and project number.

A hard-copy of the data acquisition schedule is printed out, signed by the analyst, and placed in the Instrument Log Book, in accordance with the GC documentation SOP (6-025).

3.5 INSTRUMENTAL ANALYSIS

- Calibrate the GC system prior to analysis (Section 2).
- Immediately after the calibration, begin analyzing samples using the same GC conditions. Ideally, the same sample volume should be introduced into the GC as was used in the analysis of standards. A calibration check standard should be analyzed every 10 samples, as discussed in Section 2, or as specified in the QAPP.
- If a sample needs to be rerun, (i.e., bad injection, failed calibration, power outage, etc.), this will be documented on the sequence. The analyst's initials and the date should accompany the corrective action taken and the reason a corrective action is needed.

3.6 DATA REDUCTION - ANALYTE IDENTIFICATION AND QUANTIFICATION

The chromatographic data is stored on the Xchrom Data System for postrun data reduction. Analyte identification and quantification procedures are described in the applicable analytical SOPs or project plan. The Xchrom-specific procedures for data reduction are discussed in the Xchrom data system manuals and SOP 3-132.

3.7 DATA STORAGE

The chromatographic data generated during standard and sample analysis is initially stored on the Data System hard disk for postrun analysis. The reduced data is later transferred to compact disc for archival by the Data System Manager. For more detailed archival instructions refer to SOP 6-036.

3.8 DOCUMENTATION

Maintaining proper project and instrument specific documentation is critical for GC analysis. The documentation and record keeping procedures associated with GC analysis are described in a separate SOP (6-025), but are summarized below. This section is not intended to replace the documentation and record keeping SOP, and it is essential that the GC analysts are familiar with and adhere to these requirements.

Instrument-Specific Documentation
An Instrument Log Book is maintained for each gas chromatograph. The information in this book will include acquisition schedules of all analyses performed on the instrument, and maintenance logs for the instrument. The instrument-specific documentation is discussed in more detail in the GC documentation SOP (6-025), and the analyst will be familiar with and adhere to these documentation requirements.

The GC conditions are documented on the acquisition schedule. Flow rates are not verified daily, but are recorded as the rate most recently set, preceded by the "~" symbol. The maintenance logs will include

information such as routine maintenance, column changes, service, and major hardware modification/changes.

4.0 MAINTENANCE

All maintenance activities, service, and GC instrument hardware changes/modifications are documented in instrument-specific Log Books located in the GC laboratory. These documentation and record keeping procedures are described in SOP 6-025. All analysts will be trained to performed routine maintenance, and each analyst will perform the maintenance needed to conduct the analysis he/she has been assigned.

Instrument maintenance and troubleshooting is discussed in the instrument manuals under the applicable sections. The manuals also described how to disassemble the various GC components and how to perform maintenance and servicing. Maintenance will be performed to correct a performance problem, but will also be performed routinely to prevent problems. The analysts will be familiar with the routine maintenance procedures described in the following sections.

4.1 SERVICING INJECTION PORT

The injection port of actively used GCs will be serviced routinely, as described below.

- Replace septum weekly after approximately 50 injections, unless a Merlin Microseal is used, or as needed. (The Merlin Microseal can be replaced about every 20,000 injections.)

  Remove top nut on injection port. Septum will either be stuck to this nut or left in injection port. Remove with forceps. Remove any pieces of septa from inside the nut. Install new septum. Replace nut. Tighten to "finger" tight plus ¼ turn.

- Replace injection port liner weekly, after approximately 50 samples, or as needed.

  Loosen the main injection port nut on the top of the injection port until it is free. Place to one side by lifting on attached gas lines. Remove liner with forceps. Remove any small pieces of septa from around liner. Position new o-ring on new liner approximately 1.5 cm from top of clean, silanized liner (o-ring will position itself when injector is reassembled). Insert a small amount of deactivated/silanized glass wool ¼ to ½ the way up from the bottom of the liner, if appropriate for the type of analysis to be performed. Install new liner and o-ring. Reposition nut, screw down until "finger tight" plus ¼ turn.

- Clean rest of injection port monthly, after approximately 100 samples, or as needed.

  With injection liner removed, remove any foreign material from injection port from top to bottom. To clean the lower parts of the injection port the analytical column must be removed from inside the oven, after which the injector nut can be removed. Upon re-installation replace the washer and seal.

.2 COLUMN MAINTENANCE

As a column begins to degrade (i.e., poor peak shape, loss of resolution, or loss of sensitivity) clipping may be needed to remove the compromised front end of the column.

Cool the over to <50°C, then turn off the oven fan. Remove the column and column nut from the injection port from inside the GC oven. To clip a column, place the column (which is already through the column nut and graphite ferrule) on a flat surface and score the column with a diamond tip scribe or similar device (remove ~6 inches of column). Then snap the column to ensure a clean break. Never cut through the column, it will crush it and not leave a perpendicular cut. Inspect the column tip to determine if any burrs or crushing occurred during the cut. If the cut is good, install the column appropriately; if it is not good then repeat the cutting procedure. Check the integrity of the column connections using a leak detector.

4.3 PRECOLUMN INSTALLATION/REPLACEMENT

Capillary analytical columns may be fitted with a 5 m precolumn. The precolumn is a length of deactivated, uncoated fused silica column that is connected to the analytical column by a butt connector. When degradation in chromatography is observed, the precolumn is replaced without any loss in the length or performance of the analytical column.

Cool the oven to <50°C and turn off the oven fan. Remove the column and column nut from the injection port from inside the GC oven. Install a 5 meter length of precolumn. Inspect both ends of the precolumn and the front end of the analytical column to ensure that the ends are trimmed cleanly. Connect one end of the precolumn to the analytical column using a butt connector or instrument specific connector and the other end of the precolumn to the injection port, following the column installation instructions in the appropriate instrument manual. Check the integrity of the column connections prior to use.

4.4 GAS CYLINDER REPLACEMENT

Replace cylinder when tank pressure drops below 300 psi.

4.5 OTHER MAINTENANCE

Other maintenance that will be performed routinely, and/or as needed, include the following:

- Autosampler Rinse Vials - make sure the solvent rinse vials are full prior to starting a new analysis sequence. Replace the solvent weekly and rinse the vials at the same time. These activities do not need to be recorded in the maintenance records.

- Autosampler Syringe - replace and/or align as needed when it does not operate properly (e.g., plunger sticks or aspirates/dispenses air, needle is no longer straight or properly aligned). Consult autosampler manual for more information.

- Ferrule Replacement - several ferrules (mostly graphite ferrules) are installed in the injector, detector, and column interfaces from inside the GC oven. These ferrules ensure a good seal,

- Ferrule Replacement - several ferrules (mostly graphite ferrules) are installed in the injector, detector, and column interfaces from inside the GC oven. These ferrules ensure a good seal, and with time they will need to be replaced. Check for gas leaks on a regular basis, and inspect the ferrules whenever the fitting is disconnected. Replace as needed.

- Gas Drying/Purification Traps - Instrument performance and gas quality will dictate the types of gas purifiers to be used, and when they are to be replaced. The date of installation of the gas purification trap will be recorded on the trap at the time of installation. The following gas purification traps may be appropriate:

  OMI-2 (Supelco), or equivalent - indicating purifier used to remove oxygen, water, and trace contaminants from helium, hydrogen, nitrogen, and argon/methane. This trap is *not* to be used for the air supply. The trap is ideally placed in the gas line prior to the gas distribution manifold. Replace as needed (when indicator turns brown) but at a minimum once a year.

  Hydrocarbon trap - used for air supply. The trap is ideally placed in the gas line prior to the gas distribution manifold. Replace as needed, but at a minimum once a year.

  Moisture trap - used for air supply. The trap is placed in the gas line prior to or after the gas distribution manifold. Replace as needed, but at a minimum once a year.

- Column Bakeout/Cleaning - with time, high molecular weight compounds will deposit onto the column and may create analysis problems. Many of these compounds may be removed by raising the temperature of the oven 10 to 50°C above the highest temperature of the sample analysis temperature used, and allowing the GC to remain at this temperature for 1 h or more. The temperature of the detector should also be raised, ideally to the same or a slightly higher temperature than the oven temperature, to prevent contaminant deposition in the detector. Before raising the temperature consult the manuals to determine column and detector maximum temperatures.

- Column Changing - columns are changed when a different type of column is needed for the analysis to be performed, and when the current column no longer performs adequately. Instructions on column installation, including proper positioning, can be found in the instrument manuals.

- Detector Bakeout and Cleaning - with time compounds will deposit in the detector and may create analysis problems. Some of these compounds may be removed by raising the temperature of the detector to 10 to 50°C above the standard operating temperature, and allowing the GC to remain there for 1 h or more. Significantly longer bakeout periods may sometimes be needed. Before raising the temperature consult the manual to determine detector maximum temperature.

  Should detector "bakeout" not adequately clean the detector it may need to be disassembled and subjected to more rigorous cleaning. Consult the manual for detector cleaning.

For all other maintenance, and for more detail on the above mentioned maintenance, refer to the appropriate instrument manual.

4.6 SERVICE

Battelle has service contracts on all gas chromatographs. If there are operational problems that cannot be resolved by routine maintenance or by consulting the instrument manuals, the analyst will inform the GC Group Leader about the problem. They will jointly review the problem, consider corrective actions, and decide if it is appropriate to call Hewlett-Packard for service. Each instrument problem will be dealt with on a case by case basis, including when to place a service call. All service calls are documented in the instrument-specific Log Books.

4.7 VERIFICATION OF PERFORMANCE

After instrument maintenance has been performed the analyst must verify that the instrument is back in working order and that any problems requiring maintenance have been corrected. This verification is accomplished by verifying instrument calibration. Either a calibration check standard or a new initial calibration curve that meets the acceptance criteria of this SOP will verify instrument operation.

5.0 TRAINING

An analyst performing the procedures described in this document must initially work under the supervision of qualified, trained staff. The analyst must have read and been given proper training and thorough understanding of this SOP, the GC documentation SOP (6-025), and instrument instruction manuals. Appropriate training consists of direct tutorial by the GC Group Leader, or other qualified individual(s) designated by the GC Group Leader. This training will include a demonstration of proficiency in the areas indicated on the Certificate of Training (Attachment 1).

Individuals may work independently once they have satisfactorily completed supervised training. Once training has been completed a Certificate of Training will be completed and provided to the Quality Assurance Unit.

6.0 SAFETY

- Heated zones of the GC are hot and to be avoided.
- Compressed gas cylinders must be secured at all times.
- Specific safety concerns of any particular analysis procedures will be listed in the project work plan or procedure/analysis SOP.

7.0 ATTACHMENTS

Attachment 1. Example of Certificate of Training

APPROVALS

Author:		8.6.99
Laboratory Manager:		8.6.99
Quality Systems Manager:		8-10-99
Environmental Chemistry Section Manager:	for G S Purell	8-9-99
	Name	**Date**

ATTACHMENT 1

Battelle Duxbury Operations

CERTIFICATE OF TRAINING

FOR

SOP NO. **3-116-04**

TITLE: **OPERATION AND MAINTENANCE OF GAS CHROMATOGRAPHS**

TRAINEE: _____

Task	**Independent Operation Demonstrated**	
Read SOP 3-116	_____	_____
Read SOP 6-025	_____	_____
High Pressure Gas System	_____	_____
Gas Chromatograph Programming, Initialization, and Routine Operation	_____	_____
Routine Maintenance	_____	_____
Data Acquisition	_____	_____
Data Electronic Transfer and Calibration Check	_____	_____
Data Analysis and Quantification	_____	_____
Data Package Compilation	_____	_____
	Date	**Observer/Training Supervisor**

APPROVED: _____ _____

Name **Date**

Battelle Duxbury Operations
Standard Operating Procedures

for

ANALYTICAL INSTRUMENT DATA ACQUISITION USING THE LABSYSTEMS X-CHROM DATA SYSTEM

Summary of changes in this version: References to the Metals mercury detectors have been deleted. The SOP has been changed to reflect the new data archiving procedures. Analysis channels have been modified to reflect a new dual channel GC/ECD and also the deletion of the Metals analysis channel which is now used for temporarily restoring archived data for viewing.

1.0 APPLICATION

This Standard Operating Procedure (SOP) describes the use of the Labsystems X-Chrom data system with gas chromatography (GC), and liquid chromatography (LC). The data system has the capability to acquire, store, integrate and report the results of analyses. This SOP describes how to set up a sequence file, acquire data, perform post-run analysis, generate chromatographic reports, and archive data.

2.0 CALIBRATION

The data system requires no calibration after installation.

3.0 OPERATION

Proper operation of the Data System is performed in four steps: (1) Creation of a method file, which provides the Data System with information about run time, acquisition rates, and guides the Data System through peak identification and integration, (2) generation of an analysis file, which identifies the samples to be run, (3) data acquiring, and (4) data reduction and report generation.

Because use of the X-Chrom system is integral to instrument operation, SOPs 3-116 (GC), 3-115 (HPLC), and 6-025 (GC documentation procedures) should be referenced.

3.1 SYSTEM LOG-ON

Access to the Data System is accomplished by clicking the X-Chrom prompt on the PC window menu. The X-Chrom data system is linked to the Battelle Duxbury network and is access-specific to a Battelle employee with the appropriate login name and password. If entered properly, system access is granted.

3.2 CREATING A METHOD FILE

A method file is a set of instructions that provides the Data System with the information necessary to perform analytical instrument data acquisition, data calibration, and sample analysis. If the specific instrument is capable of instrument control, the method file also allows the analyst to control the instrument from the PC. Instrument identification, run time and data sampling rates are specified. In addition, the method file is used after data from an instrumental analysis has been acquired for calibration and quantification of the samples. The information in the method file instructs the Data System in peak identification and integration techniques. Many options are available to the user, most of which are beyond the scope of this document. Advanced analysis techniques are described in the *Labsystems X-Chrom Users Manuals* which are kept with the Data System at all times. Refer to these manuals for instruction on advance analysis and quantification techniques (e.g. PCB aroclor analysis). (Please note that italics are used to identify specific terms defined and used in the Labsystems X-Chrom software.)

To prepare a method, from the main window move the mouse arrow over the *File* header and hit enter. The highlight should be brought to the *New...* header and clicked. The *File Type* should be chosen as *Method* with the appropriate channel and project (projects are defined as the type of analysis being performed) listed (see Section 3.5). Then click the *OK* button. The six or eight buttons at the top of the Method window define the method. Six buttons are used if there is no instrument control, and eight buttons are used if instrument control is available.

The *Acquisition* button (pictured with a needle) is the first button. This allows the user to program the minimum peak width, the acquisition rate, the acquisition time, post injection delay, time between runs, and voltage offset along with a dual channel option.

The second button is the *Peak Detection* button (pictured by an integral sign). This buttons allows for various peak detection run parameters to be assigned.

The third button is the *System Suitability Parameter* button (pictured by an integrated peak). This section can be used to define the parameters set forth by various Method protocols.

The fourth button, *Calibration Information* (pictured by a graph) allows the user to define the calibration type, the curve type, the parameter (height or area) used for the calibration, the analytes of interest, retention times, etc.

The next two buttons, *Reporting* (pictured by a printer) and *Processing Schemes* (pictured by cogwheels) determine the report format, report device, and number of copies. Also included are the choices of post-injection and post-processing schemes.

Buttons seven and eight, *Instrument Control* (pictured by a GC) and *Autosampler Control* (pictured by an autosampler) are only present for methods which have instrument control associated with the channel. These sections prompt the user to set the parameters used in the sample run.

The method is saved, once completed, by pressing the *SAVE* key (pictured as a diskette). The window may be closed or iconized upon completion of the method. The user can then return to the main menu.

A sample of the method printout is provided as Attachment 1.

3.3 GENERATING AN ANALYSIS FILE

An analysis file is a listing of samples to be run on the GC instrument and also provides the Data System with the information necessary to perform data calibration and sample analysis (e.g. internal standard amounts and calibration standards). An analysis file is prepared from the main window by moving the mouse arrow over the *File* header and clicking. The highlight should be brought to the *New...* header and clicked. The *File Type* should be chosen as *Analysis* with the appropriate channel and project chosen. Then the OK button should be clicked. The analysis file has three sections that need to be addressed. *Analysis Information* is used to define the Project title under Analysis Name, the analyst under Analyst Name, and the Project and Batch number under Comments. Other information may also be put in these boxes. The middle section of the analysis file, *Sample Information,* need not be filled in as the next section of this file restates this section.

The last section, *Sample Sequence*, is the listing of the samples to be run. This section needs to include the Sample Name, Sample ID, the Bottle #, the Sample Type, the Method, and the STD (amount of the quantitation internal standard). There are other columns that may be included by the analyst as he/she sees necessary.

A sample of the analysis printout is provided as Attachment 2.

3.4 ACQUIRING DATA

Data from a series of instrumental analyses is collected by instructing the Data System to acquire a fixed number of runs as pre-determined in the sequence file, activating the electronic link between the data system and the instrument, and initiating the run.

Once the method and sequence are prepared the user need only to *Start Run* from the main window to initiate the instrument and start the auto sampler. The injection triggers the system to begin acquiring the signal. For instruments without an autosampler pressing the start button on the remote starter box activates the Data System. Data are acquired in the analysis file.

3.5 FILE NAMING

Each instrument connected to the X-Chrom system is assigned a unique alpha-numeric labeling system for all Method and Sequence Files in the following format:

XYNNnn

where:

X = File type code - An alphabetic character which designates the file type. In method files X will be "M", and in sequence files X will be "S".

Y = An alphabetic character in the range A-Z, which will initially be A and increment every 100 method and sequence file names.

NN = The channel number where channels 0 through 6 will have a leading zero.

nn = A number that will begin with 00 and increment to 99 with each method or sequence file name. A new method or analysis file name must be assigned to each new method or analysis file.

For example, the first 100 method files and sequence files used for instrument #1, channel 1 and instrument #5, channel 12 will be labeled as follows:

Instrument	Channel	Method File	Sequence File
#1	1	MA0100-0199	SA0100-0199
#5	12	MA1200-1299	SA1200-1299

The result files are associated with the analysis file by bottle # and Injection #. Thus the result file name for the nineteenth sample (bottle) of sequence SA0123 will SA0123,19,1. This result would read the first injection of bottle nineteen in sequence SA0123. This file name prints on only the first page of the result report. Care should be taken not to mix pages while quantifying samples. Battelle Duxbury Operations Laboratory instrument designations are defined in Table 1.

When determining total hydrocarbon concentrations a copy of each sequence and the raw data file must first be made.

Copies of methods and analysis files can be designated with the use of suffixes (i.e. mc0110a). This is done in circumstances where multiple methods are employed in an analysis file, retention shifts, or other similar situations.

3.6 DATA REDUCTION

Sample data reduction, where peaks are identified and integrated, can be carried out in two places: from the Analysis file, which was previously created, or from the *View Chromatogram* from the main window of X-Chrom. It is suggested that the View Chromatogram method be employed when analyzing samples, so the chromatography can be examined. The *Integral* button at the top of the View Chromatogram window will apply the method integration parameters to the sample. The *ID* button will assign peak Ids and retention times (depending on the options chosen from the Format pull down menu) according to the method. If the method was not previously calibrated the user must wait to quantify the samples with a calibrated method.
Table 1. Instrument Designations

Instrument	Detector	Channel, project	H-P Instrument ID
#1 HP6890	A	CH1, pesticides	Chanl_1
#1 HP6890	B	CH2, pesticides	Chanl_2
#2 HP5890	A	CH3, pesticides	Chanl_3
#2 HP5890	B	CH4, pesticides	Chanl_4
#3 HP5890	A	CH5, pesticides	Chanl_5
#3 HP5890	B	CH6, pesticides	Chanl_6
#4 HP5890	A	CH7, hydrocarbons	Chanl_7
#4 HP5890	B	CH8, hydrocarbons	Chanl_8
#5 HP5890	A	CH12, hydrocarbons	Chanl_12
#5 HP5890	B	CH12, organotins	Chanl_12
#6 HP6890	A	CH13, hydrocarbons	Chanl_13
#6 HP6890	B	CH14, hydrocarbons	Chanl_14
#7 HP5890	A	CH15, pesticides	Chanl_15
#7 HP5890	B	CH16, pesticides	Chanl_16
Archive restoration	NA	CH11,	Chanl_11
HP1050 HPLC	A	CH9, hplc	Chanl_9
HP1050 HPLC	B	CH10, hplc	Chanl_10

Any printer on the Battelle network may be used to print the X-Chrom files. Peak integration methods are described in analysis-specific SOPs.

3.7 CHROMATOGRAPHIC PLOTS

Full or partial scale plots for any chromatogram may be generated after acquisition is complete. Plots can be generated individually by the user by using the *Print* button in View Chromatogram or as an option on the *Report* button on the X-Chrom main window.

3.8 REPORTING

Data acquired with the data system is reported on a project-specific basis. At a minimum, data reported for a set of analyses should include:

- a copy of the method file (Attachment 1) which includes peak identification, calibration information, and correlation coefficients,
- the sequence file or analysis printout (Attachment 2) which should be printed when the analysis is completed to show the standard amounts for the analysis, and
- the result file (Quant) reports (Attachment 3) which should have the analyte name, the area/height, and the calculated amount, a chromatographic plot (only if required by the project work plan or if needed for data an lysis) and the method ID used to quantify the sample.

3.9 ARCHIVING DATA

The System Manager or Assistant System Manager will do all archiving and retrieval of electronic data. Once the Quality Assurance Unit has approved the data, the Project Task Manager should deliver a list of sequence, and method files to the System Manager for archival. Archiving of data will be performed according to SOP 6-036. Backup of the data on the data system occurs monthly. The data archives will be documented in a logbook maintained by the System Manager.

4.0 MAINTENANCE

Service call procedures and maintenance records of all aspects of non-routine maintenance are kept in the Labsystems X-Chrom Data System logbook, which is kept by the System Manager. Routine maintenance for each instrument is documented as described in SOP 6-025. No routine maintenance other than clean out and backup is required for this system. The System Manager is responsible for all operation and scheduling of non-routine maintenance of the main system. In the event of a malfunction, the System Manager will contact Labsystems and arrange for prompt service. All maintenance is documented in the X-Chrom Server and Chemistry Data Archival Log Book.

5.0 TRAINING

A training certificate indicating proficiency in use of the Data System will be granted when a user is able to log onto the system, create acquisition and analysis files, acquire data, analyze data, and produce chromatographic reports without the assistance of a trained user. The GC/HPLC Laboratory Group Manager, or designee will issue a Certificate of Training (Attachment 4), to the new operator. The original training certificate is provided to the Quality Assurance Unit.

6.0 SAFETY

The Data System operates on 110 V line voltage. In order to avoid malfunction or possible injury, no liquids, which might accidentally spill onto the terminal, should be brought near the consoles.

7.0 ATTACHMENTS

Attachment 1.	Example of Method Printout
Attachment 2.	Example of Analysis Printout
Attachment 3.	Example of Quant Printout
Attachment 4.	Example of Certificate of Training

APPROVALS

Author	for GWD	7·26·99
Laboratory Manager		7·26·99
QA Coordinator		7-27-99
Environmental Chemistry Section Manager	Name	7/28/99 Date

Attachment 1. Example of Method Printout

Printed at 12:16pm on 21 July 1999 Page 1
Project: organotins
Instrument: chanl_12
Method: mb1292

Method Report

Acquisition Parameters

Acq. Rate	7.500000
Peak Width	2.00
Acq. Time	960.00
Post Inj. Delay	0.00
Post Acq. Delay	0.00
Voltage Offset	30.000000
Offset Baseline	Yes
Acq Type	0

Peak Detection

Start RT	Start Thres	End Thresh	Suppress	Reject Are
0.00	0.2000	0.1000	On	0.00
8.00	0.9538	0.4769	Off	0.00
14.00	0.6510	0.3255	On	0.00

System Suitability

Method Type	US
Use Default Parameters	true
Column Length	1.00
Void Volume Mode	Rt. time
Void Volume Time	0.00
Width Upper Limit	0.70
Width Lower Limit	0.50
Moments Upper Limit	0.10
Tailing Factor Height	0.05
Asymmetry Ratio Height	0.10
Asymmetry Upper Limit	0.25
Asymmetry Lower Limit	0.05
Column Efficiency Coefficient	16.00
Resolution Ratio Coefficient	2.00
Fit Lower Limit	0.90
Width Type	Base

Attachment 1. Example of Method Printout (Continued)

Printed at 12:16pm on 21 July 1999 Page 2
Project: organotins
Instrument: chanl_12
Method: mb1292

Calibration Information

Cal Type	Internal standard
Cal Base	Height
Uncal Type	Named peak
Const Rsp Area	
Named Pk	
Old Named Pk	
Amts Calc	Absolute, User modified
User Eq Area	
User Eq Height	
Curve Type	Linear
Force Thro Origin	Yes
X Trans	None (use raw data)
Y Trans	None (use raw data)
Weighting	No weighting (all weights = 1)
Mod Date	
1st Rsp Height	
Extrap High type	Linear extrapolation
Extrap Low type	Linear extrapolation
Extrap High PC	9.999000E+003
Extrap Low PC	9.999000E+003
RV Curve Type	Linear
RV Force Thro Zero	false

Components

Peak Name	RT	(-)	(+)	Internal Std	Int Std	Peak ID
TPT	9.60	9.10	10.10	No	DPT	First
TTBT	9.88	9.68	9.96	No	DPT	Largest
TBT	10.98	10.78	11.06	No	DPT	Largest
DPT	11.18	11.11	11.26	Yes		Largest
DBT	12.13	11.93	12.33	No	DPT	Largest
TPET	12.71	12.51	12.79	No	DPT	Largest
MBT	13.46	13.23	13.54	No	DPT	Largest

Peak Name	Group Name
TPT	
TTBT	
TBT	
DPT	
DBT	
TPET	

Attachment 1. Example of Method Printout (Continued)

Printed at 12:16pm on 21 July 1999 Page 3
Project: organotins
Instrument: chanl_12
Analysis: sb1292

Peak Name Group Name
MBT

Group Identification

>>> error <<<
Could not print table: Group Identification
for data id: organotins,chanl_12.mb1292,0,0,1;
because: Table is empty

Calibration Standards

Peak Name	L1	L2	L3	L4
TPT	1.5654	1.5654	1.5654	1.5654
TTBT	0.0549	0.2196	0.5490	2.1960
TBT	0.0510	0.2041	0.5103	2.0412
DPT	1.0316	1.0316	1.0316	1.0316
[illegible]	0.0512	0.2048	0.5121	2.0484
[illegible]ET	1.1998	1.1998	1.1998	1.1998
MBT	0.0515	0.2060	0.5149	2.0596

Calibration Values

Sample No.	Peak Name	Std. Amoun	Std. Area	Standard I
2	TPT	1.565	143.053	L1
2	TTBT	0.055	7.022	L1
2	TBT	0.051	4.742	L1
2	DPT	1.032	183.951	L1
2	DBT	0.051	5.678	L1
2	TPET	1.200	79.949	L1
2	MBT	0.052	7.406	L1
3	TPT	1.565	148.542	L2
3	TTBT	0.220	20.993	L2
3	TBT	0.204	17.970	L2
3	DPT	1.032	187.099	L2
3	DBT	0.205	20.044	L2
3	TPET	1.200	79.863	L2
3	MBT	0.206	27.894	L2
4	TPT	1.565	153.108	L3
4	TTBT	0.549	53.224	L3
4	TBT	0.510	53.346	L3
4	DPT	1.032	214.681	L3
4	DBT	0.512	65.347	L3

Attachment 1. Example of Method Printout (Continued)

Printed at 12:16pm on 21 July 1999 Page 4
Project: organotins
Instrument: chanl_12
Method: mb1292

Sample No.	Peak Name	Std. Amoun	Std. Area	Standard I
4	TPET	1.200	96.049	L3
4	MBT	0.515	88.213	L3
5	TPT	1.565	150.940	L4
5	TTBT	2.196	224.108	L4
5	TBT	2.041	238.555	L4
5	DPT	1.032	217.778	L4
5	DBT	2.048	278.848	L4
5	TPET	1.200	94.142	L4
5	MBT	2.060	397.810	L4

Sample No.	Injection Data Id.
2	organotins,chanl_12.sb1292,2,1,
2	organotins,chanl_12.sb1292,2,1,
2	organotins,chanl_12.sb1292,2,1,
2	organotins,chanl_12.sb1292,2,1,
2	organotins,chanl_12.sb1292,2,1,
2	organotins,chanl_12.sb1292,2,1,
2	organotins,chanl_12.sb1292,2,1,
3	organotins,chanl_12.sb1292,3,1,
3	organotins,chanl_12.sb1292,3,1,
3	organotins,chanl_12.sb1292,3,1,
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4	organotins,chanl_12.sb1292,4,1,
4	organotins,chanl_12 sb1292,4,1,
4	organotins,chanl_12.sb1292,4,1,
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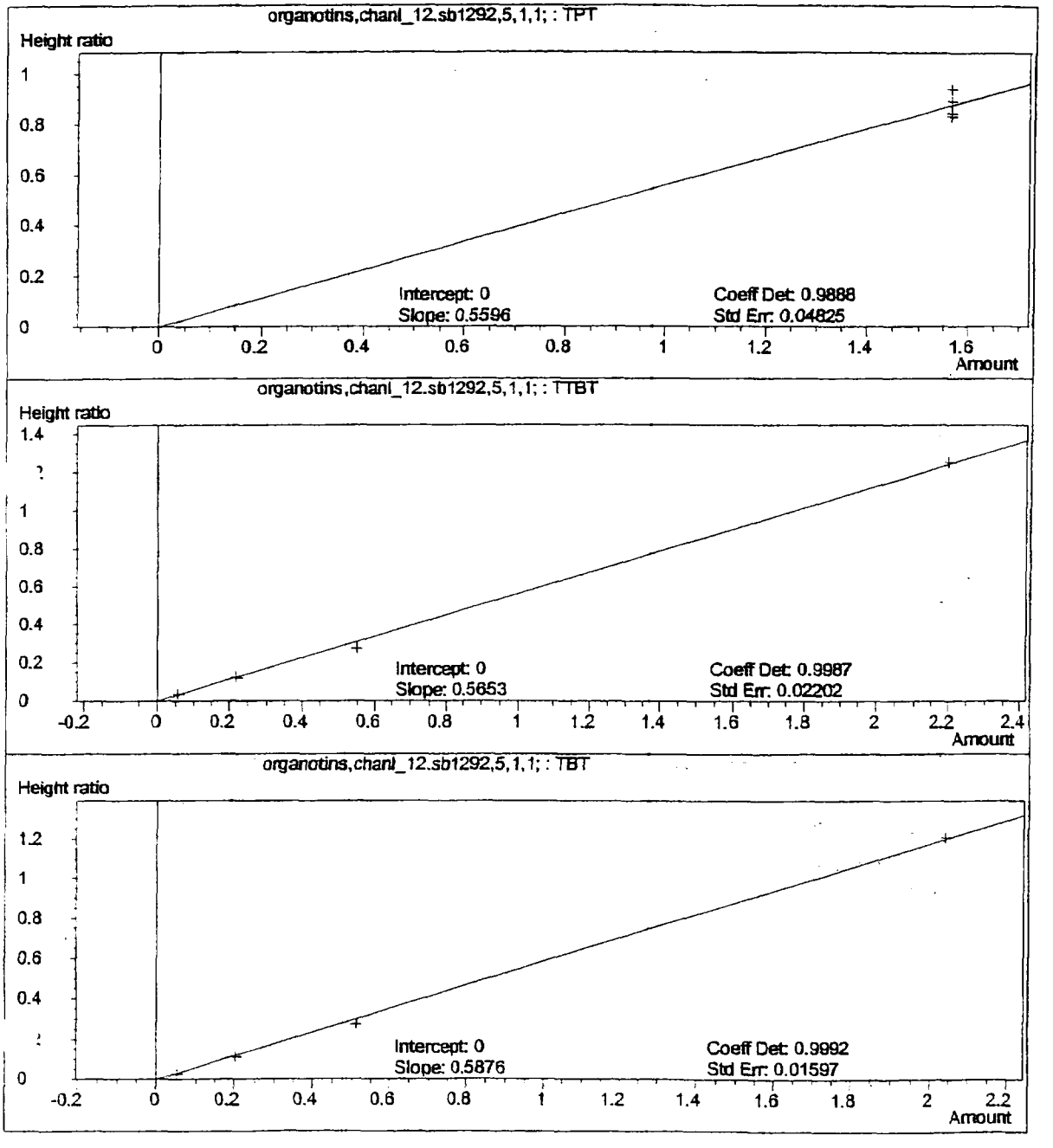
5	organotins,chanl_12.sb1292,5,1,
5	organotins,chanl_12.sb1292,5,1,
5	organotins,chanl_12.sb1292,5,1,

Reporting

Column Id.	Report Column?	Position	Width	Decimal Places
PK_NO	false	0	10	2

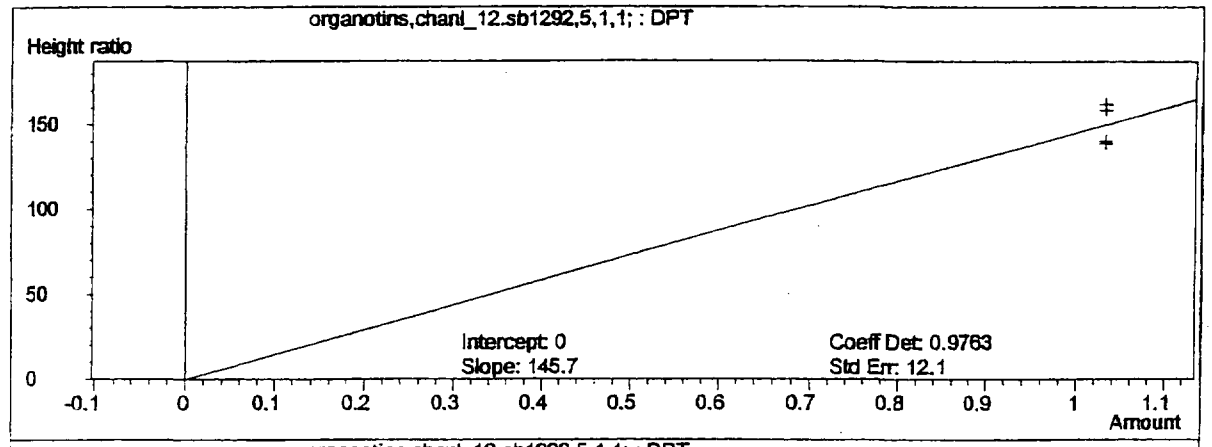
Attachment 1. Example of Method Printout (Continued)

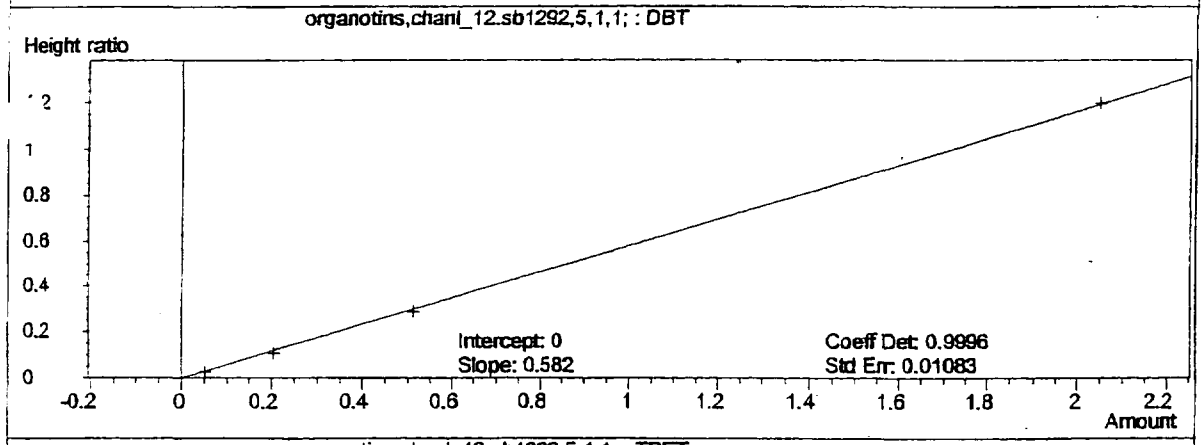
```
Printed at 12:18pm on 21 July 1999                                  Page   1
Project:    organotins
Instrument: chanl_12
Analysis:   sb1292
Sample:     EY32
Injection:  1
```

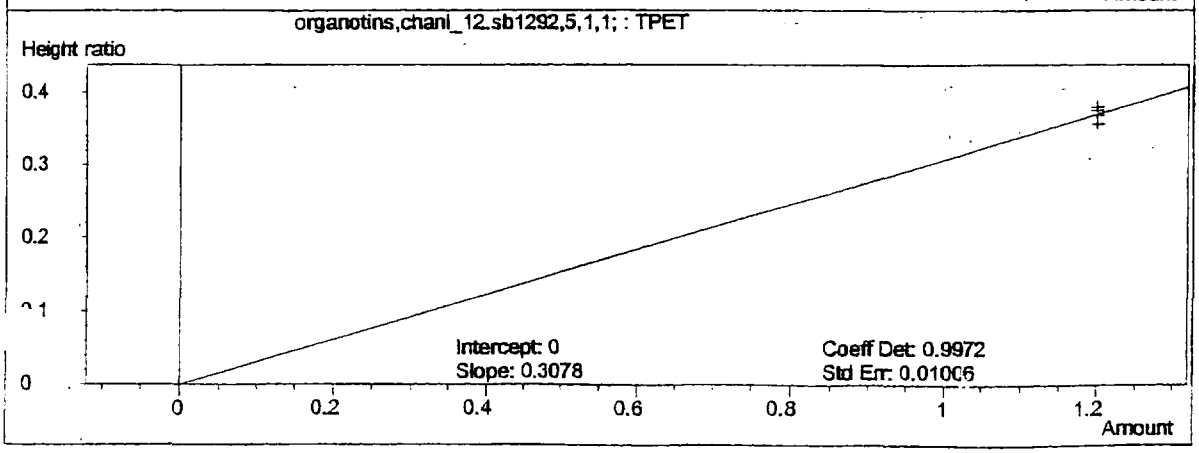


Attachment 1. Example of Method Printout (Continued)

```
Printed at 12:18pm on 21 July 1999                                  Page   2
Project:    organotins
Instrument: chanl_12
Analysis:   sb1292
Sample:     EY32
Injection:  1
```

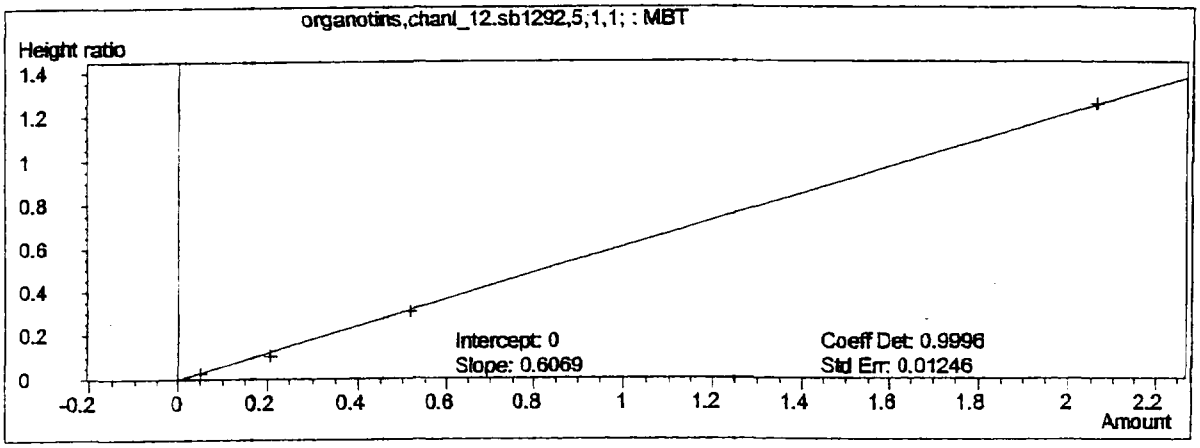






Attachment 1. Example of Method Printout (Continued)

Printed at 12:18pm on 21 July 1999 Page 3
Project: organotins
Instrument: chanl_12
Analysis: sb1292
Sample: EY32
Injection: 1



Calibration Values

Sample No.	Peak Name	Std. Amoun	Std. Area	Standard I
2	TPT	1.565	143.053	L1
2	TTBT	0.055	7.022	L1
2	TBT	0.051	4.742	L1
2	DPT	1.032	183.951	L1
2	DBT	0.051	5.678	L1
2	TPET	1.200	79.949	L1
2	MBT	0.052	7.406	L1
3	TPT	1.565	148.542	L2
3	TTBT	0.220	20.993	L2
3	TBT	0.204	17.970	L2
3	DPT	1.032	187.099	L2
3	DBT	0.205	20.044	L2
3	TPET	1.200	79.863	L2
3	MBT	0.206	27.894	L2
4	TPT	1.565	153.108	L3
4	TTBT	0.549	53.224	L3
4	TBT	0.510	53.346	L3
4	DPT	1.032	214.681	L3
4	DBT	0.512	65.347	L3
4	TPET	1.200	96.049	L3
4	MBT	0.515	88.213	L3
5	TPT	1.565	150.940	L4
5	TTBT	2.196	224.108	L4
5	TBT	2.041	238.555	L4
5	DPT	1.032	217.778	L4
5	DBT	2.048	278.848	L4

Attachment 2. Example of Analysis Printout

Printed at 10:12am on 22 July 1999 Page 1
Project: organotins
Instrument: chanl_12
Analysis: sb1292

Analysis Report

Analysis Header

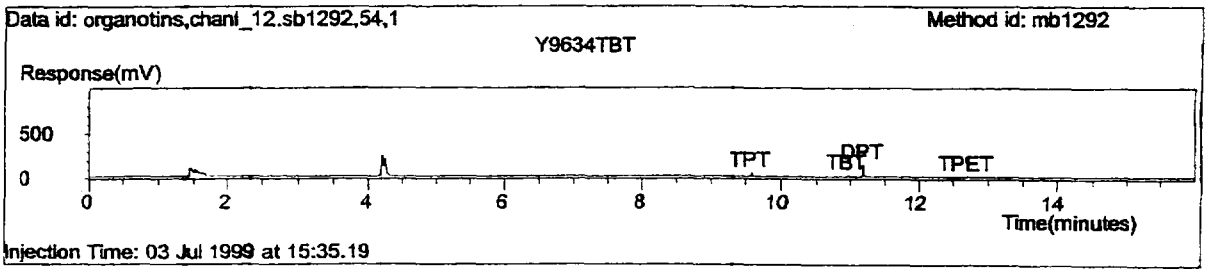
Analyst Name Jonathan R Thorn
Analysis Name Striplin
Comment G003791-0001 Tissue batches 99-357 and 358

Sample Sequence

#	Sample Id.	Method	STD	Sample Type	DPT
1	Hexane	mb1292		Sample	1.0000
2	EY29	mb1292	L1	Standard	1.0316
3	EY30	mb1292	L2	Standard	1.0316
4	EY31	mb1292	L3	Standard	1.0316
5	EY32	mb1292	L4	Standard	1.0316
6	WV32PB	mb1292		Sample	515.8000
7	WV33BS	mb1292		Sample	515.8000
8	WV34MS	mb1292		Sample	515.8000
9	WV35MSD	mb1292		Sample	515.8000
10	Y9330TBT	mb1292		Sample	515.8000
11	Y9338TBT	mb1292		Sample	515.8000
12	Y9346TBT	mb1292		Sample	515.8000
13	Y9367TBT	mb1292		Sample	515.8000
14	Hexane	mb1292		Sample	1.0000
15	EY31	mb1292		Sample	1.0316
16	Y9377TBT	mb1292		Sample	515.8000
17	Y9386TBT	mb1292		Sample	515.8000
18	Y9389TBT	mb1292		Sample	515.8000
19	Y9363TBT	mb1292		Sample	515.8000
20	Y9357TBT	mb1292		Sample	515.8000
21	Y9635TBT	mb1292		Sample	515.8000
22	Y9684TBT	mb1292		Sample	515.8000
23	Y9686TBT	mb1292		Sample	515.8000
24	Hexane	mb1292		Sample	1.0000
25	EY31	mb1292		Sample	1.0316
26	Y9674TBT	mb1292		Sample	515.8000
27	Y9667TBT	mb1292		Sample	515.8000
28	WV36PB	mb1292		Sample	515.8000
29	WV37BS	mb1292		Sample	515.8000
30	WV38MS	mb1292		Sample	515.8000
31	WV39MSD	mb1292		Sample	515.8000

Attachment 3. Example of Quant Report Printout

Printed at 04:48pm on 20 July 1999 Page 1
Project: organotins
Instrument: chanl_12
Analysis: sb1292
Sample: Y9634TBT
Injection: 1



Peak Information

Peak Name	RT	Raw Height	Amount
TPT	9.60	42.507	295.644
TBT	10.98	15.542	102.959
DPT	11.19	132.515	515.800
TPET	12.72	6.405	80.996

Attachment 4

Battelle Duxbury Operations
CERTIFICATE OF TRAINING

SOP No. 3-132-04

Title Analytical Instrument Data Acquisition Using the Labsystems X-CHROM Data System

Trainee _____

Instructor _____

Date SOP Read _____

Date Training Completed _____

The above mentioned trainee has satisfactorily completed the training requirements associated with this SOP. Supporting document (if applicable) is attached.

Comments

Approved by_____ **Date**_____

Battelle Duxbury Operations
Standard Operating Procedure

for

USE OF ELECTRONIC BALANCES

Summary of changes in this version: The use of a computer for direct-capture of data and electronic entry into spreadsheets is added. The acceptance criteria for calibration checks is modified. The identification and labeling of each NIST-traceable weight set is described. Note that this SOP still requires that balances be calibrated using two weights that bracket the expected weight of the samples.

1.0 APPLICATION

Two types of electronic balances are covered by this SOP: top loading and those with a pan in an enclosed chamber. These balances are capable of weighing within a wide range of accuracy (0.01mg to 3000 g), and the selection of the balance to be used for any task should be determined by the precision required. Balances which have a pan in an enclosed chamber, such as the Mettler AC100 and Mettler AT201, are capable of weighing within four or five decimal places (0.0001 to 0.00001 g) in ranges up to 205 g. The top-loading balances, including various models of Ohaus, Mettler, and Sartorius balances, are capable of weighing to one, two, or three decimal places (0.1, 0.01 or 0.001 g) to a maximum weight of 3000 g.

2.0 CALIBRATION

Balances are calibrated annually by a professional balance service using NIST-traceable calibration weights.

Balance calibration must be checked prior to each day's use. Calibration must also be checked if the balance is moved from one location to another. Balances are customarily left plugged in and "on" or in "standby" mode. If the balance has been unplugged or turned "off" then a warm-up period of 30-90 minutes, depending on the balance, is required prior to calibration.

Balance calibration is checked by qualified operators using NIST Class 1 and 2 weights. The weights selected as calibration checks should bracket the anticipated weight of the sample. The results of the calibration checks are recorded on the Balance Calibration form (Attachment 1) located in the Balance Logbook specific to the balance being used. Based on the guidance of ASTM E617-91 most balances at Battelle are used as class 2 or 3 balances. As such, the accuracy of each balance must be verified at the level of accuracy for which measurements will be made. (In other words, to the last readable decimal place).

Example 1:

> The Mettler AC100 is a 5-place balance that reads to 0.00000 g. If weights will be measured at the 5th decimal place then the acceptance criteria for the calibration check is ± 0.00001 g.

Example 2:

The Ohaus PE3600 reads 0.000 g. or 00.00 g. depending on the selected sensitivity setting. The calibration checks for these two settings must be ± 0.001 g or 0.01 g, respectively.

Minor calibration adjustments are possible on some models (see Attachment 2). If the required accuracy level cannot be achieved even after adjustments, contact the Laboratory Manager who will arrange for professional servicing of the balance.

3.0 OPERATION

3.1 Routine Operation

The balance must be located on a stable surface. Most balances have a level indicator; in these cases, adjustments to level the balance are made using adjustable "feet." The balance should also be kept away from drafts, vibrations and direct sunlight.

Zero the balance by pressing "Tare" or "Zero." Place the material/object to be weighed on the pan of the balance. Read the weight and record, if necessary. With many of the electronic balances, it is possible to connect with a computer interface so that weights may be recorded directly into spreadsheets.

To weigh only the contents of a vessel, place the vessel on the balance pan and press "Tare" or "Zero." Add the material to be weighed to the vessel, and record the weight.

Operating options vary from balance to balance, but may include: automatic door operation, vibration compensation, weight units, and computer interface. The manufacturer's instructions, located in the balance logbook for each balance, define the options relative to each instrument. Attachment 2 provides a summary of operations.

3.2 Electronic Data Entry

Balance weights may be sent directly to a spreadsheet for some balances. The program is accessed on Windows-based computers by selecting sequentially:

Start → Programs → WINWEDGE → WINWEDGE → File →Open → Sample Weights or Lipid Weights

then, Activate → Normal Mode → Open spreadsheet for the analysis batch

Once the spreadsheet is open, place the sample on the balance and wait until the balance measurement has stabilized. Place the computer cursor in the correct spreadsheet cell (e.g., Tare Weight) and hit the **ENTER PRINT** bar on the balance. Verify that the weight is entered into the correct cell.

4.0 MAINTENANCE

The balance must be cleaned after each use using a soft brush, a moist, soft cloth, or lint-free tissue. If necessary, remove pan and wash with soapy water. Thoroughly dry the pan before replacing. Other surfaces of the balance should be cleaned if needed as well as the bench area around the balance. Do not use solvents or abrasives on the balance surfaces.

The Lab Manager or designee is responsible for balance maintenance. Each balance is cleaned, inspected, and certified annually by a professional balance service (Bay State Scale and Systems, Inc., Cambridge, MA, (800) 696-8232 or equivalent). A record of this maintenance and calibration is kept in the logbook for each balance. If any other maintenance, such as calibration adjustment (see Attachment 2) is performed, it must be documented on the Balance Maintenance form (Attachment 3). A brief description of the maintenance procedure, date, and initials of the operator should be documented on the form.

Battelle owns several sets of NIST-traceable Class 1 and 2 weights. Each set of weights is uniquely identified with a unique weight set ID (Table 1). One set of weights will be calibrated annually by a professional balance service (Denver Instrument Co., Arvada, CO, (303) 431-7255, or equivalent) and used to verify that all weights in the other sets fall within the acceptable range for their class. A record of this calibration is kept in the Chemistry Section files (Attachment 4). A sticker indicating the calibration date is placed on each set of weights.

Table 1. Weight IDs for the Calibration Weights Sets Used in the Analytical Laboratory

Weight ID	Full Name (manufacturer)[1]	Storage Location
CS#1	Christian Becker set 1	Organic Prep Lab # 1
CS#2	Christian Becker	Organic Prep Lab # 3
P#1	Permas	Oil/weathering
A#1	Ainsworth	Lower Level Chem North

5.0 TRAINING

Trainees must read this SOP. A certified operator will then demonstrate the use of each type of balance: top-loading and enclosed pan. The instructor and trainee will then each weigh a series of five NIST Class 1 or 2 weights on each type of balance and record the results on the Certificate of Training (Attachment 5). If the results of the trainee are within 2% of those of the instructor, a training certificate will be issued. Original copies of each individual's training certificate will be kept in the Quality Assurance Unit training files.

6.0 SAFETY

The use of the balances presents no specific safety hazards. However, care must be exercised when handling hazardous or potentially hazardous materials or samples.

7.0 ATTACHMENTS

Attachment 1. Example of Balance Calibration Form

Attachment 2. Calibration Adjustments for Specific Instruments

Attachment 3. Example of Balance Maintenance Form

Attachment 4. Example for NIST-traceable weight calibration verification form

Attachment 5. Example of Battelle Duxbury Operations Certificate of Training

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APPROVALS

Author	Richard L Motta	11/12/98
Laboratory Manager		11·12·98
QA Coordinator	Rosana Burke	11-12-98
Environmental Chemistry Section Manager	Name	11/19/98 Date

ATTACHMENT 1
Balance Calibration Form

Balance Brand/Model: _____ **Serial Number:** _____

NIST Class 1 or 2 Weight (g) 1	Tared Display (g)	Measured Weight (g)	Recalibration Required (Y/N)2	Results of Recalibration	Date and Initials
Ex: 1 g	0.00000	1.00001	N	NA	

Comments:

_____ _____

1 NIST calibration weight ID _____ assigned to this balance. If another weight set is used this must be documented.

2 Recalibration is required if the measured weight is not ±0.nnn1 g where nnn is equal to the decimal display of the balance.

ATTACHMENT 2

Calibration Adjustments for Specific Instruments

- **Ohaus GT4000, GT4000D:** If after placing a calibrated weight on the pan, the difference between the calibrated weight and the reading is >5%, proceed as follows:

  1. Turn the balance ON and allow it to warm up with no weight on the pan for at least 30 minutes.

  2. Press and hold the ON/rezero switch. Release the switch as soon as CAL is displayed. The display will show "C O", indicating that no weight should be on the platform.

  3. Momentarily push the ON/rezero switch.

  4. The balance will then display a number which corresponds to the value of the calibration weight which should be placed in the center of the pan.

     NOTE: If no calibration weight is available, press the OFF switch to save the existing calibration.

  5. Place the appropriate calibration weight on the center of the pan and momentarily press the ON/rezero switch.

  6. Wait until the balance displays the indicated calibration weight value. This indicates that the balance is now calibrated.

- **Mettler AC100:** Must be calibrated if the balance is moved to a new location. To calibrate, a set of NIST-traceable weights totaling 70 g (50 g + 20 g) is necessary.

1. Checking the Calibration: The balance must be connected to a power supply for at least 30 minutes and must be leveled.
- Press control bar (19-Figure 1): 0.0000 g
  (If not exactly zero, press bar again)
- Place test weights (total of 70 g) on pan. Read display.

If the Balance indicates 70.0000 g, the calibration is okay; if not, the balance must be recalibrated. Set the weight display using the calibration screw (1-Figure 2) as described in Step 2. After every correction, recheck display. See Step 3.

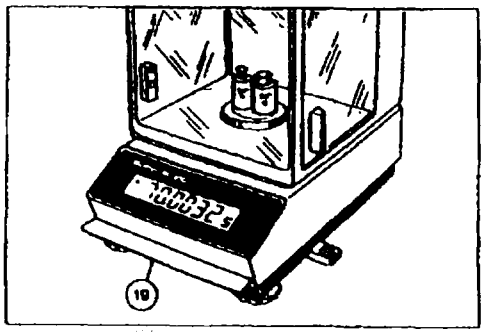


Figure 1

Attachment 2 (continued)

2. Resetting the weight display:
   - Lift off the weights
   - Insert screwdriver into the opening (1-Figure 2) and turn the calibration screw located there: Clockwise if the displayed weight is more than 70.0000 g; Counterclockwise if the displayed weight is less than 70.0000 g.
   One turn of the screw changes the weight display by about 0.006 g.

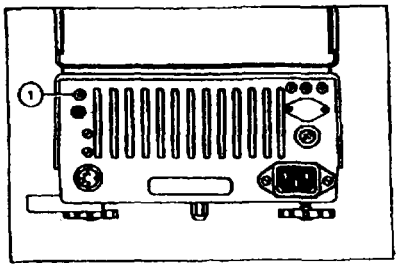


Figure 2

3. Checking the new setting:
   After every correction made using the calibration screw, recheck the result:
   - With test weights removed, press control bar (19-Figure 1): 0.0000 g.
   - Place test weights on pan; read result.
   If the balance now indicates 70.0000 g, it is ready to use-- if not, it must be corrected further by using the calibration screw and checking again until the display is correct.

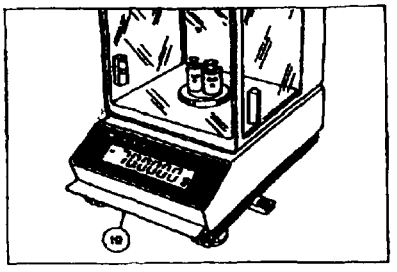


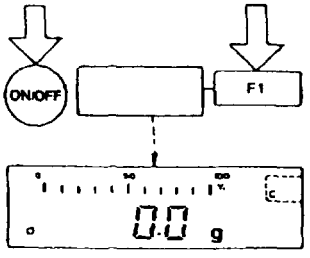
Figure 1

- **Sartorius PT600:** Requires an accurate (certified) calibration weight of 500 g.

1. Remove the protective cap located on the front right of the balance and slide the access switch in the direction of the arrow.

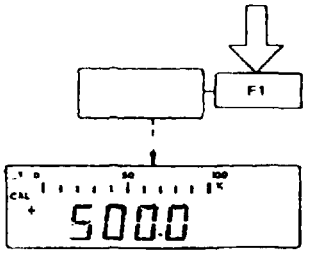
2. With the balance turned off, hold down the F 1 key and briefly press the ON/OFF key.

   Upon completion of the automatic self-test, release the F 1 key as soon as "C" is displayed.



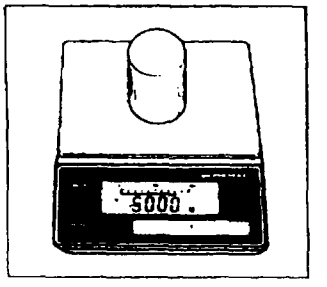
3. Unload the balance and press the tare control to zero the display.

4. When the display shows a zero readout, press the F 1 key. "CAL" and the calibration weight readout will now be displayed.



5. Place the calibration weight in the center of the weighing pan. Now the weight unit is displayed which indicates the end of the calibration procedure. Relock the calibration access function by

Attachment 2 (continued)



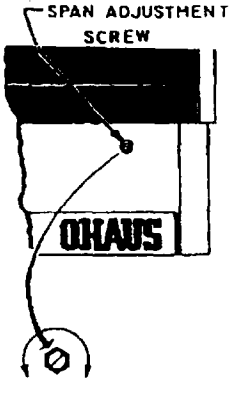
sliding the access switch back to its original setting, and replace the protective cap. After calibration, use the ON/OFF key to turn the balance off then on again so that you will have direct access again to the dedicated application programs preselected by the menu code.

- **Ohaus GT480:** If after placing a calibrated weight on the pan, the difference between the calibrated weight and the reading is >5%, proceed as follows:

  1. Remove all weight from the platform.
  2. Initialize the calibration menu by pressing and holding **ON/TARE** until **CAL** is displayed, then release immediately. **C 0 g** will then be displayed; indicating that no weight should be on the platform.
  3. Momentarily press **ON/TARE.** The display will blank, then show - **C** - momentarily before showing C followed by the value of the calibration weight to be place on the platform.
  4. Place the required weight on the center of the platform.
  5. Momentarily press the ON/TARE and DO NOT remove the calibration weight until the balance displays a weight reading.
  6. The balance should now be calibrated.

- **Ohaus 3000D (Brainweigh):** Calibration must be checked/adjusted if the balance is moved from one location to another.

  1. Turn the balance **ON** and allow a minimum of twenty minutes for it to thoroughly "warm up".
  2. Zero the balance by pressing the TARE BAR.
  3. Place a known (certified) mass of at least half the capacity of the balance in the center of the platform.
  4. Should the display not agree with the known mass, the **SPAN** must be adjusted. The SPAN **ADJUSTMENT** screw is located above the **TARE BAR** on the right side (when viewed from the front). Using the screwdriver supplied with the balance, slowly rotate the screw until the displayed weight is equal to that of the known mass.



Attachment 2 (continued)

5. Remove the known mass and depress the TARE BAR. Replace the known mass on the platform. If the displayed mass is not equal to that of the known mass, repeat step 4.

- **Mettler PE3600:** Must be calibrated if the balance is moved to a new location.

  1. The balance must be connected to the power source for 30 minutes before calibrating it.
  2. Press the control bar until zero appears in the display.
  3. Place the test weight on the pan and read the display after the ring symbol (stability detector) has been blanked out. If the balance displays the weight of the test weight accurately, the calibration is okay. If not, the calibration must be corrected.
  4. Remove the test weights from the pan. Press the control bar until the display indicates "-----", then release the control bar.
  5. "- C -"appears in the display.
  6. Place test weight (1000 g for the P3600) on the pan. The balance automatically performs its own calibration. After calibration is completed, the display indicates "1000.0 g".

- **Sartorius AC210S:** Must be calibrated if the balance is moved to a new location.

  1. The balance must be connected to a power source for 30 minutes prior to use.
  2. Pressing "T" tares (zeros) the balance.
  3. The weight can be sent to an electronic spreadsheet by pressing ⊙ once the sample weight has stabilized.

- **Ohaus Explorer EOD120:** Must be calibrated if the balance is moved to a new location.

  1. The balance must be connected to a power source for 30 minutes prior to use.
  2. Pressing "O/T" tares (zeros) the balance.
  3. The weight can be sent to an electronic spreadsheet by pressing "Enter" once the sample weight has stabilized.

ATTACHMENT 3
Balance Maintenance Form

Balance Brand/Model:_____ **Serial Number:**_____

Description	Date	Name

ATTACHMENT 4

Verification of NIST-traceable weight ranges

Standard Weights (Nominal weight and acceptable range)	MEASURED WEIGHTS[1]		Acceptable? (Yes/No)
	Standard Weights ID _____	Working Weights ID _____	
CLASS 1			
5 kg ± 12.0 mg			
3 kg ± 7.5 mg			
2 kg ± 5.0 mg			
1 kg ± 2.5 mg			
500 g ± 1.20 mg			
300 g ± 0.75 mg			
200 g ± 0.50 mg			
100 g ± 0.25 mg			
50 g ± 0.120 mg			
30 g ± 0.074 mg			
20 g ± 0.074 mg			
CLASS 2			
10 g ± 0.074 mg			
5 g ± 0.054 mg			
3 g ± 0.054 mg			
2 g ± 0.054 mg			
1 g ± 0.054 mg			
500 mg ± 0.025 mg			
300 mg ± 0.025 mg			
200 mg ± 0.025 mg			
100 mg ± 0.025 mg			
50 mg ± 0.014 mg			
30 mg ± 0.014 mg			
20 mg ± 0.014 mg			
10 mg ± 0.014 mg			
5 mg ± 0.014 mg			
3 mg ± 0.014 mg			
2 mg ± 0.014 mg			
1 mg ± 0.014 mg			

[1] Balance ID used to check weights: _____. Please note that not all weights listed may be applicable.

ATTACHMENT 5

Battelle Duxbury Operations
CERTIFICATE OF TRAINING

SOP No. _____

SOP Title _____

Trainee _____

Instructor _____

Date SOP Read _____

Date Training Completed _____

The above mentioned trainee has satisfactorily completed the training requirements associated with this SOP. Supporting document (if applicable) is attached.

Results: Enclosed Pan Balance

NIST Class 1 or 2 Weight	Trainee Results	Instructor Results	Difference

Results: Top-Loading Balance

NIST Class 1 or 2 Weight	Trainee Results	Instructor Results	Difference

Approved by_____ **Date**_____

Battelle Duxbury Operations
Standard Operating Procedures

for

QUALITY ASSURANCE FACILITIES INSPECTIONS

Summary of changes in this version: . The reporting procedures (Section 5.0) are identical to those described in SOP 4-015. Specific references to the section number are deleted.

1.0 OBJECTIVE

Facilities inspections are conducted by the Quality Assurance Unit (QAU) to assure management of proper use and maintenance of equipment and facilities as defined by Battelle policies, standard operating procedures (SOP), and government regulations. These facilities may include analytical, database, benthic, histology, and field operations. This SOP describes the policies, procedures, responsibilities, and records associated with the conduct and reporting of facilities inspections.

2.0 PREPARATION

It is the responsibility of the inspector to prepare for each inspection. This preparation should include a thoroughly review of the requirements of this SOP and those of the specific SOPs that are used in the area to be inspected. Compliance with site policies (Section 3.2) should always be assessed.

3.0 PROCEDURES

Facilities inspections are conducted by QAU personnel. During the inspection, selected work areas within the facility are inspected for compliance with the standards discussed below. Areas are chosen by QAU staff based on several criteria, including whether work is being performed in the area and the date of the last inspection of that area. Specific areas may be targeted if a problem is suspected but in general each active facility should be inspected annually.

A facilities audit may include a review of an entire operation or it may focus on one aspect of the facility. In the latter case one or more specific standard operating procedures are used to direct the inspection.

3.1 GENERAL INSPECTION GUIDELINES

Each facility inspection is an evidence-gathering exercise. The job of the inspector is to gather objective evidence that confirms that (1) the facility is in compliance with laboratory policies and procedures, or (2) that it is not. Objective evidence takes the form of exhibits (documentation), direct observation of practice, and interviews with staff. It is the responsibility of inspectors to be objective, to confirm their observations, and to review evidence with the staff to clear up false impressions.

3.2 GENERAL POLICIES

All facilities must comply with the following general site policies.

- Adequate space should be provided for work and storage areas. Laboratory areas should be free from clutter.
- Facilities should be designed and used so that there is a degree of separation that will prevent any function or activity from having an adverse effect on any other function or activity.
- Appropriate personal protective equipment should be worn by personnel.
- Equipment used or involved in data generation activities should be inspected, cleaned, maintained, and calibrated as defined in SOPs or equipment logs. Written records should be kept for all inspection, maintenance, and calibration operations.
- Current, approved SOPs relative to the work being performed should be immediately available to personnel in each work area.
- Personnel should be appropriately qualified or adequately trained to perform their tasks. Written records of qualifications and training should be forwarded to the QAU training files by the appropriate managers.
- Disposal of contaminated or hazardous waste materials should be in accordance with SOPs.
- Raw data entries should be recorded directly, promptly, and legibly in ink. Each entry should be dated on the day of entry and signed or initialed by the person entering the data. Changes to entries should be made so as not to obscure the original entry, should indicate the reason for the change, and should be initialed and dated by the person making the change.

3.3 LABORATORY INSPECTION ELEMENTS

A full laboratory inspection should assess the following elements, in addition to the general requirements described in Section 3.2.

3.3.1 Test Substances, Solutions, Solvents, and Reagents

In analytical areas, any test substances, solutions, and reagents used in a facility are reviewed for conformance to the standards listed below.

- The receipt, handling, preparation, storage, and distribution of test substances, solutions, and reagents should be in accordance with project protocols or QA Project Plans (QAPPs) SOPs, the manufacturer's instructions, and Material Safety Data Sheet (MSDS) information.
- Test substances, reagents, and solutions should be labeled according to SOPs 5-217 and 5-027.

- Deteriorated or outdated materials should not be used and should be discarded on a routine basis in accordance with SOPs 6-010 and 5-114.

- Storage of test substances, reagents, and solutions should be adequate to maintain their purity, strength, and composition.

- The purity of solvents and reagents should be verified according to SOP 5-015.

3.3.2 Sample and Data Control

Sample naming, receipt, storage, and custody records should conform to the requirements of SOPs 6-007 and
6-010 and the following standards (when samples are collected or shipped by other organizations these records may not be available):

- Written records of the history of each sample should exist, including collection, preservation, transfer, storage, processing, analysis, and disposal.

- Written records of sample receipt should be maintained and should include the date and time of receipt, the condition of the samples upon receipt, and the identity of the persons releasing and receiving the samples.

- Each sample should be uniquely identified and should be traceable from collection through reporting.

- Samples should be held according to storage conditions defined in SOPs 6-010, the QAPP, or government regulations. Samples should be discarded according to SOPs 6-010 and 5-114.

3.3.3 Instruments and Equipment

The laboratory contains a wide array of instrument and equipment. Inspection of analytical instruments should be assessed in SOP-specific inspections (Section 3.6). The operation and maintenance of small equipment can be reviewed as part of a routine facilities inspection. This equipment includes, but is not limited to, balances (3-160 and 3-004), refrigerators and freezers (SOP 3-095), thermometers (SOPs 3-018 and 3-151), the deionized water system (SOP 3-084), HPLC (SOP 5-191), and non-data generating equipment (SOP 3-133). The following criteria are reviewed for these types of equipment.

- Standard operating procedures must be prepared and available for all instruments or equipment that generates data.

- Instrument and equipment logs should document calibration, use, and maintenance.

- All staff using the equipment should have documented training in the appropriate SOPs.

3.3.4 Control Charts

A review of control charts (SOP 7-028) should be conducted to ensure that they are current, that documentation follows the SOP requirements, that the appropriate quality control samples are being plotted for each analytical batch, and that corrective action is taken and documented when necessary.

3.3.5 Labware Cleaning

The inspector can verify compliance with the labware cleaning SOP (5-216) through observations and discussion with the glassware washer. The inspector should determine that the staff member understands the washing, rinsing, and heat treatments for the various types of labware used in the facility.

3.4 FIELD OPERATIONS INSPECTIONS

Inspections of field operations *in the field* are conducted on a project-specific basis according to SOP 4-027 Section 2.3. An inspector's presence must not jeopardize the field mission or create over-crowding or safety risks. Like laboratory inspections, field inspections focus on general policies (Section 3.2) and SOP requirements.

Shore-based field operations inspections typically review equipment calibration, maintenance and operations inspections. These are based on specific SOPs and are discussed in Section 3.6.

3.5 DATA MANAGEMENT INSPECTIONS

Inspections of data management activities should include a review of the following procedures.

- Receipt records of electronic or hardcopy data submitted for data management should include the date of receipt, login ID, and the submitter.
- Written procedures for data loading, format requirements, and storage should be available.
- Changes to data should be recorded such that the documentation is linked to the project data.

3.6 SOP-SPECIFIC INSPECTIONS

Facilities inspections in the analytical laboratory are often conducted as a series of SOP compliance inspections. For SOP-specific inspections the inspector should verify that

- the procedures described in the SOP are followed exactly as written
- all documentation described in the SOP is maintained as specified
- any quality control requirements specified by the SOP are implemented
- all staff using the SOP have documented training

4.0 SCHEDULE

At least one facilities inspection is conducted quarterly, or as necessary to review facilities operations. The appropriate Resource or Laboratory Manager is notified prior to the inspection to ensure that personnel will be available to answer questions during the inspection and to avoid scheduling conflicts. Unannounced inspections are conducted only if a problem is suspected. Facilities inspections will also be conducted at the request of management (Resource Managers or Laboratory Director).

5.0 REPORTING

Once the inspection is complete, the auditor prepares an inspection report that summarizes the results of the inspection. Any deficiencies are described and corrective actions are recommended. General and project-specific issues are identified as such and discussed separately in the inspection report.

The reporting procedures for project inspections are identical to those described in SOP 4-015. Outstanding issues are resolved through discussions with Lab, Project, or the Resource Manager, as necessary. All original audit reports are maintained in the QAU files.

6.0 TRAINING

An individual who is being trained to perform facilities inspections must first read this SOP. The trainee must then accompany an experienced inspector (instructor) on facilities inspections until it has been established to the instructor's satisfaction that the trainee is able to perform an inspection without supervision. A certificate of training (Attachment 1) is issued upon completion of training and is maintained in the QAU training files.

ATTACHMENTS

1. Example of Certificate of Training

APPROVALS

Author		12-10-99
Quality Systems Manager		12-10-99
Vice President, Battelle Duxbury Operations	Name	12-10-99 Date

Attachment 1

Battelle Duxbury Operations
Certificate of Training

SOP No. 4-009

SOP Title Quality Assurance Facilities Inspections

SOP read and understood _____ _____
Name Date

Description of Training	Date	Instructor

Battelle Duxbury Operations
Standard Operating Procedures

for

QUALITY ASSURANCE AUDITS OF REPORTED DATA

Summary of changes in this version: Define how the year 2000 will be used in assignment audit numbers. Clarify that the results of data audits are characterized as "acceptable," "acceptable once corrections are made," or "rejected" (Section 4). Clarify that data packages are not acceptable until every error is corrected and all documentation is complete.

1.0 INTRODUCTION

Audits of reported data (e.g., tables, figures, spreadsheets, databases) are conducted by the Quality Assurance Unit (QAU) to ensure that the data are accurate, traceable, defensible, complete and generated according to the project plan. This policy applies only to data generated for projects involving environmental measurements (i.e., involving the determination of chemical, physical, or biological factors related to the environment); financial and administrative data are excluded.

2.0 PROCEDURES

2.1 DATA SUBMISSION AND RECEIPT

All data to be audited must be validated (SOP 6-027) before being submitted to the QAU. The data validation process performed by technical personnel may include, but is not limited to, recalculation of reported data, verification of transcriptions, double keypunching, identification and removal of outliers or spurious data points, assessment of quality control (QC) results, performance of electronic QC checks, assessment of compliance with the project work plan, and evaluation of scientific validity.

Once validation is complete, the data are submitted for audit. Any original data and supporting documentation necessary to reconstruct the task or project should be provided. It is the responsibility of the person preparing the spreadsheets, report tables, or database output to ensure that the data are clearly labeled and that supporting data are complete and organized so that the auditor will be able to trace data without assistance. Documentation in the analytical data packages should include explanations of correction factors and all work plan or SOP deviations.

All formulas used in data calculations must be documented in one of the following formats

- full mathematical description in the work plan
- full mathematical description in an SOP and referenced by SOP number in the work plan
- full mathematical description in the data package or referenced by SOP number

Upon receipt in the QAU, the data package is logged in by project and data type and assigned a unique ID number in the format XX-yyyy

where: XX is the year (e.g., 00 for year 2000).
yyyy is a sequential four digit number starting with 0001

An example data tracking form is provided in Attachment 1.

3.0 DATA AUDIT PROCEDURES

Two types of data audits are performed at Battelle. The traditional statistical random audit (Section 3.1) is selected for data sets where the errors are likely to be randomly distributed throughout the data set (key-punched, transcribed, or hand-calculated data). Targeted data audits (Section 3.2) are conducted where the errors are more likely logical or global (unusual sample processing, computer generated or loaded data, data generated in spreadsheets). The project work plan should define the type of audit most appropriate for the data anticipated. If this specification is not made in the work plan then the audit strategy will be determined when the data are submitted to the QA unit for verification. Generally, targeted audits are performed on analytical data and report tables generated at Battelle while statistical audits are performed on subcontractor data, transcribed data, and electronically captured data. Both types of audits involve verifying selected reported values using the original (raw) data, previously audited data, and supporting documentation.

3.1 STATISTICAL RANDOM AUDIT

This procedure, which is based upon the Modified British Standard 6001 described by Hoover and Baldwin (1984)[1], is designed to ensure a 95% chance of detecting whether 1% or more reported values disagree with the original data.

Upon receipt of the data, the auditor photocopies the data to be audited and counts the total number of data points in the data set. A data set may be defined as all values reported for a project or task, or any subset of those values, at the discretion of the auditor. A description of data included in each data set and the number of data points per set is included in the audit report.

Once the data points have been counted and numbered, the auditor determines the number of points to be checked based on the Modified British Standard 6001 (Attachment 2). For example, if the data set contains a total of 303 data points, 50 of those points will be audited. The specific points to be audited are randomly selected using a DOS or Windows 95 random number generator, a calculator, or a random numbers table. These randomly selected points are highlighted on the photocopied data set and attached to it.

Alternative randomizing methods are employed when large data sets of electronic data are transferred from one format to another (e.g., from ASCII files to a client database such as ORACLE). In these cases, the data may be randomized electronically by the analyst prior to submittal. Frequently, these data are randomized by row rather than by individual data point. When an alternative randomizing procedure is appropriate then the randomizing method will be determined by the Project Manager and QA personnel and described in the audit report.

---

[1] Hoover, B.K. and J.K. Baldwin. 1984. *Meeting the Quality Assurance Challenges of the 1980s: Team Auditing by Toxicologists and QA Professionals.* J. Am. Coll. of Tox. 3:129-139.

3.2 TARGETED AUDIT

This procedure involves the review of specific data based on a knowledge of analytical data manipulation procedures. First, the auditor photocopies the data tables and determines if any data should be verified 100% and where data errors might logically occur (e.g., a sample that was split or re-fractionated after the initial preparation was complete). Minimum verification requirements are

- 100% verification of hand-calculated data, dilution factors, fractions, and all table header information.
- For organic chemistry analyses: at least one target analyte concentration, its relative qualifier, and one surrogate recovery for each sample, being sure to alternate between applicable recovery internal standards, and one complete calculation for each type of QC sample. (For the unusual cases where the target analyte list is less than 10 compounds per sample, either one target compound **or** one surrogate recovery is checked.
- For dissolved inorganic nutrients analyses: one value for each analytical sequence and one complete calculation for each type of QC sample.
- For database audits: verification of loading completeness, accuracy, and logical range checks for each parameter, and (if available) validation of QC checks performed by the analyst.

3.3 AUDITING THE DATA

The data audit is accomplished by checking each selected data point or sample to ensure that it accurately reflects the original data and is traceable throughout its history. For reported values that involve manual, spreadsheet, or instrument calculations where either transcription errors or incorrect manual entries can occur, the auditor repeats the full calculation. Values that result from manipulations involving complex software programs or from high-rate electronic data acquisition systems (e.g., statistical data or BOSS data) are checked vs. results files but are not reconstructed. In these cases, technical validation by someone familiar with the processing procedures is crucial; the type of QA audit performed will be determined jointly by the Project Manager and QA personnel.

The auditor also reviews the Quality Assurance Project Plan (work plan) to ensure that the processing, analytical, and reporting requirements have been met.

3.4 DATA REJECTION

When a data set is unacceptable, the data are returned to the Laboratory or Project Manager who is expected to oversee a review of the entire data set and the necessary corrections. The corrected data are returned to the QAU and the entire data set is re-audited.

3.4.1 Statistical Audits

Errors in reported data are defined as any values that cannot be derived from the original data, including, for example, miscalculations and transcription errors. For random audits (Section 3.1), if the auditor finds the number of errors in the highlighted data points to be greater than the acceptance criteria (as

defined in Attachment 2), the data are judged to be unacceptable ("rejected") and the audit terminated. Errors in data points other than those highlighted are noted by the auditor but are not used in determining the number of errors detected.

3.4.2 Targeted Audits

There are no statistical rejection criteria for target audits, however, a data package is rejected if

- the reported values cannot be traced back to the original data
- if more than 10% of the data are incorrect
- if two or more major error types are noted (e.g., more than one species or compound is missing from the report tables, all reported data are affected by a formula error).

3.5 SCHEDULING

Data audits may be performed at any stage of a project at the discretion of the Project Manager. Project Managers may deliver

- Spreadsheets (e.g., Excel) of all data values. These may be the final deliverable or an intermediate listing in advance of final tables.

- Final tables or appendices that will be inserted directly into report text

- The complete final report including all tables, figures, and text.

Ideally, the audit should be performed after final tables and figures have been prepared but before the accompanying text (if any) has been finalized, so that the text draws from verified data. If intermediate tables are provided for audit then the final product should be submitted to the QAU for verification against the previously audited data.

The Task Leader or Laboratory Manager is responsible for notifying the QAU of data delivery dates and for apprising the QAU of any major changes in the schedule. Data are usually audited on a first-come, first-serve basis; however, project deadlines and staff availability are also considered when scheduling audits. Any conflicts are resolved through discussions with the Resource Managers or designees.

4.0 REPORTING AND DOCUMENTATION

4.1 REPORTING

4.1.1 Audit Reports and Audit Files

Upon completion of the audit,

1. The copies of the data tables are marked to indicate the results of the audit as "acceptable," "acceptable once corrections are made," or "rejected." The tables are initialed and dated by the auditor. Any original tables that are "acceptable" are stamped (on the back) to indicate that they have been reviewed and accepted by the QAU.

2. The auditor prepares an audit report for every data audit. The audit report defines the data as acceptable, acceptable once corrections are made, or rejected. The audit report template is illustrated in Attachments 3a and 3b.

- If the data package is considered acceptable once corrections are made then the audit report specifically describes any errors that were found

- If the data were rejected, the audit report will not describe the specific errors identified; it will only describe the type and location of errors (e.g., weight data transcription, percent dominance calculation).

- All audit reports will also identify any documentation issues that must be addressed to ensure the traceability and defensibility of the data.

3. The report and the signature page of the data package are signed and dated by the auditor.

4. Audit reports are returned to the person responsible for generating the data (typically the analyst), who must address each error or documentation issue and document on the audit report the corrective action taken.

5. The audit report with completed responses is then signed and dated by the Project and Resource Managers and returned to the QAU within five working days.

6. Upon receipt of the audit report, the QA auditor verifies any corrections that were necessary and reviews the audit responses to ensure they are complete and appropriate. Final, corrected report tables are stamped on the back to indicate that they are accepted as final by QA.

7. When all audit findings are addressed the auditor signs the QAU Approval line on the data package title page.

8. Unresolved issues are addressed through discussions with the Project or Resource Manager, as necessary. In the Environmental Chemistry Section, the Laboratory Manager is designated to oversee responses to audit reports for the Resource Manager.

9. No audit file is considered "closed" until every error and audit issue has been addressed.

4.1.2 Reports to Management

The audit report constitutes the report to management for each project. Overall quality issues are discussed at quarterly laboratory and field reviews conducted by Battelle's Vice President. In addition, quality issues will be reported directly to the Resource Manager or Vice President whenever project data integrity is threatened.

4.2 DOCUMENTATION

The audited tables and supporting documentation are filed by client, project task, and audit number in the QA Office. The original audit reports are filed by audit number in the QAU audit files. The audit reports are maintained for 10 years unless otherwise specified in a contract.

The Data Package Tracking Log (Attachment 1) provides a record of all data packages received for audit and the audit number. Tracking includes the dates that data are received, relinquished for correction, returned for verification, and accepted (all audit issues addressed). In addition, an Excel spreadsheet of all data packages received for audit is on the Quality System Manager's hard drive (Active\QA\Status Reports). This file is used to track due dates and prompt follow-up. The Project Manager or designee will be contacted if the audit response is not received by the due date. Repeated failure to respond to audit reports is considered a performance issue, prompting the initiation of a formal Request for Corrective Action (SOP 4-035).

A project-specific Audit Log (Attachment 4) is maintained to provide a list of all audits conducted for a project.

5.0 PERSONNEL RESPONSIBILITIES

Quality assurance audits are performed by the QAU staff. Technical personnel may perform specific audit procedures (e.g., selecting and highlighting random numbers, checking calculations) under the direction of the QAU staff. QA or technical personnel who have been involved in the generation of a specific data set may not perform audits on that data set.

The QA Auditor is responsible for

- conducting a careful, thorough, unbiased audit according to this SOP
- following through on questions to ensure that evidence is identified for each audit comment
- preparing a concise, clearly written audit report that identifies each issue
- completing all QA office documentation described in this SOP

The Project Manager is responsible for

- defining in the project work plan the QAU audit activities, or the alternative validation activities, which will be performed for each project.
- performing a technical review of the data, and verifying that the data meet the requirements of the project.
- reviewing the data package for completeness and acceptable Quality Control results prior to the QA audit.
- reviewing the data package for technical reasonableness prior to submission for audit and for ensuring that documentation, including deviations, is complete.
- ensuring that all issues raised in the audit report are addressed and returning the audit response and corrected data to the QAU within the time specified in the audit report.
- reviewing audit responses and signing the completed audit report.

The Analyst/Task Leader is responsible for

- responding to the audit, addressing issues (after consultation with Project Manager, if necessary)
- ensuring that the data package is complete, validated, paginated,
- ensuring that all SOP or work plan deviations have been documented and approved by the Project Manager

The Laboratory Manager, as the Resource Manager in the Environmental Chemistry Section, is responsible for

- Follow-through on overdue audit reports
- noting general laboratory issues that should be addressed for the facility

The Resource Manager is responsible for reviewing and approving the audit response.

6.0 TRAINING

Personnel who are being trained to perform auditing tasks must first read this SOP. They may then perform specific tasks under the supervision of QAU personnel. Auditing tasks performed by the trainee will be reviewed and co-signed by QAU staff until it has been established that the trainee is able to perform these tasks without supervision. A certificate of training (Attachment 5) will be issued upon completion of training. The original certificate will be maintained in the QAU training files.

ATTACHMENTS

1. Example of Data Package Tracking Form
2. Modified British Standard 6001
3a. Example of Audit Report Cover Sheet
3b. Example of Data Audit Report Form
4. Example of Quality Assurance Audit Log Form
5. Example of Certificate of Training

APPROVALS

Author	Donna Buhl	11-29-99
Quality Systems Manager	Donna Buhl	11-29-99
Vice President Battelle Duxbury Operations	Q Graffeo Name	11-29-99 Date

Attachment 1
Example of Data Package Tracking Form

Initial Audit						Audit Response		
Description	Date in/ Initials	Date out/ Initials	Response Due	Dist'd to	Comments	Date in/ Initials	Date out/ Initials	Comments

Attachment 2

Modified British Standard 6001

Data Points in Data Set	Sample Size to be Inspected	Accepted Number of Errors	Errors for Rejection of Data Set
2-50	100% Inspection	-	-
51-90	12	0	1
91-150	20	0	1
151-280	32	1	2
281-500	50	1	2
501-1200	80	2	3
1201-3200	125	3	4
3201-10,000	200	5	6
10,001-35,000	315	7	8

Attachment 3a
Audit Report Cover Sheet

BATTELLE DUXBURY OPERATIONS
QUALITY ASSURANCE COVER SHEET

PROJECT TITLE:

PROJECT NUMBER:

CLIENT:

PROJECT MANAGER:

STUDY INITIATION DATE:

TYPE OF AUDIT:

PERSONS RESPONSIBLE FOR PHASE BEING AUDITED:

DATE OF AUDIT:

AUDITOR:

NUMBER OF PAGES ATTACHED:

DATE RESPONSE DUE:

DATE RESPONSE RECEIVED:

SUMMARY:

Signature of Auditor: _____

Date: _____

Attachment 3b
Example of Data Audit Report Form

BATTELLE DUXBURY OPERATIONS
AUDIT REPORT

Project Number/Title

Definition of Data Set

Audit Date

Number of Data Points in Data Set: **Number Checked**

Comments	Corrective Action/Date/Initials

Auditor _____ **Date** _____

Laboratory Manager _____ **Date** _____

Project Manager _____ **Date** _____

Attachment 4
Example of Quality Assurance Audit Log Form

BATTELLE DUXBURY OPERATIONS
QUALITY ASSURANCE AUDIT\INSPECTION LOG

Client_____ Project Number_____ Project Manager_____

Project Name_____ Start Date_____ Page ___ of ___

Audit Description	Auditor	Date(s)					Comments
		Audit	Audit Report	Response Expected	Response Received	Report To Mgmnt	

Battelle Duxbury Operations
Standard Operating Procedures

for

NON-CONFORMANCE AND CORRECTIVE ACTION

Summary of Changes in this version: The SOP scope is clarified. A discussion of root cause analysis is added. Responsibilities and authorities are defined. Documentation procedures are updated. The table of non-conformance examples is updated.

1.0 OBJECTIVE

One goal of Battelle's Quality Assurance Program is continuous improvement. The purpose of the corrective action program is to identify practices or activities that are adverse to the generation of quality products and to implement process improvements in response to these issues. This standard operating procedure (SOP) describes general corrective action procedures for all technical sections at Battelle - Field, Laboratory, Information Management, and Ecology. The requirements for corrective action are specific for some activities which are not addressed in this SOP:

- Control charts see SOP 7-028
- Quality control sample non-conformance see SOP 7-029
- SOP deviations see SOP 6-001
- Quality Assurance Project Plan deviations see SOP 6-021

2.0 DEFINITIONS

Observations are defined as incidences that require action or correction but that are not considered ongoing, operational problems.

Findings adverse to quality (findings) are defined as (1) repeated incidences of a deviation, (2) repeated errors due to a flaw in the data generation or validation process, or (3) inspection issues that require a change in laboratory procedures.

Corrective Action is defined as the series of activities undertaken to assess the root cause of a finding and the remedy implemented to minimize re-occurrence. Types of investigation activities, and suggested immediate and long-term corrective action are provided in Attachment 1.

A re-occurring *observation* is considered a *finding*. Normally, if the same error or incorrect procedure is noted during inspections or audits then the issue is considered a *finding* and a Request for Corrective Action (RCA - Attachment 2) is initiated by the QAU. The RCA will cite specific examples of the issue to facilitate investigation and correction.

> Example: If an error is noted in the calculation of a dilution factor, this is an *observation* that requires correction. If dilution factors errors found repeatedly, this is a *finding* that requires *corrective action.* In this example corrective action would include additional training for laboratory staff who must determine dilution factors.

3.0 PROCEDURES

3.1 INITIATION OF REQUESTS FOR CORRECTIVE ACTION

Non-conformance issues are documented by the QAU in audit or inspection findings to Laboratory, Project, and/or Resource Management. In addition, major issues should be discussed in person with management. However, where a pattern of practices or issues adverse to quality continues then a formal Request for Corrective Action (RCA) is initiated.

RCAs are initiated by the Quality Assurance Unit in response to:

- internal or external inspections performed by the QA unit, a client, or a government agency
- the audit of data (note that random errors or unusual occurrences are not considered findings)
- reviews of quality control data by either the QA unit or laboratory personnel
- awareness of an on-going issue

RCAs should be initiated by the Laboratory, Project, or Resource Manages as the result of

- inspections or reviews of laboratory activities
- project reviews by project management
- discussions with laboratory staff
- quality control failures

The person issuing the RCA must provide a complete description of the finding that requires corrective action on the RCA. Each RCA must specify the person responsible for responding and the time period considered adequate to investigate the issue and initiate corrective action. Copies of all RCAs should be forwarded to the QAU where a sequential number is assigned.

3.2 IDENTIFYING THE ROOT CAUSE

The principle of root cause analysis is that every failure has a preventable root cause. Instances of non-conformance may be systematic and indicative of a fundamental problem. The person responsible for addressing the RCA should conduct an informal root cause analysis to determine the reasons that the non-conformance occurred. A root cause analysis consists of isolating the cause and effect relationships for the non-conformance by:

- investigating the specific occurrence
- interviewing staff to determine if the issue is widespread
- defining who, what, when, and where
- repeatedly asking the question "why?"

3.3 RESPONSE TO REQUESTS FOR CORRECTIVE ACTION

Once the root cause of non-conformance is identified then the person responsible for investigating the finding and initiating corrective action must complete all three "response" sections of the RCA. Using the example cited in Section 2:

- The investigation activity performed to determine the cause of the dilution factor errors could be:

recalculated several dilution factors, reviewed spreadsheet templates for possible error.

- The immediate corrective action could be: *discussed dilution factor calculations with analysts, updated spreadsheet formula.*

- The long-term activity implemented to avoid re-occurrence could be: *initiated verification procedure that requires analysts to recalculate one dilution factor per spreadsheet and peer review of all complex dilution factors.*

The RCA is signed by the person responsible for implementing the corrective action and his/her Resource Manager, and returned to the QAU.

If the corrective action involves several staff members (e.g., analysts) or if policy or procedures are changed as a results of corrective action, then these changes must be documented in a memo to the appropriate staff. A copy of the memo is attached to the completed RCA.

3.4 RESPONSIBILITIES AND AUTHORITY FOR CORRECTION OF NON-CONFORMANCE

Management at all levels is responsible for reviewing activities and for identifying areas of repeated non-conformance that require formal RCAs. The Laboratory Manager is responsible for assessing laboratory quality control performance for repeated failures that warrant an RCA. Project Managers are responsible for identifying and investigating repeated failures during the conduct of a study. Resource Managers are ultimately responsible for addressing areas of non-conformance within their sections. They are responsible for

- assigning root cause analysis and RCA responses
- reviewing the results of investigations and the documentation on the RCA
- initiating any section-wide procedural changes required to address the root cause (e.g., including updating SOPs, conducting additional training, reassigning staff, or implementing new policies or procedures)

The Quality Systems Manager is responsible for reviewing audit and inspection reports to identify repeated performance issues and patterns of non-conformance.

The Laboratory, Project, and Resource Managers, and the Quality Systems Manager, are vested with the authority to **stop work** where repeated non-conformance will impact Battelle's ability to meet the Quality Policy defined in the Quality Management Plan.

3.5 DOCUMENTATION

All instances of non-conformance must be documented along with the corrective action implemented and the results of the corrective action. Note that the corrective action is not considered final until the issue is corrected and the affected area has returned to acceptable performance. The respective Laboratory, Resource, and/or Project Management must review this documentation. A copy should be maintained in the project or instrument files, as appropriate.

All formal Request for Corrective Action forms are filed sequentially (i.e., chronologically) in the Corrective Action Log maintained by the QAU. All RCAs that relate to specific projects must be copied to the project manager and included in the project files. Within one week of the receipt of the completed RCA the implementation must be verified by the QAU through a follow-up inspection and the

RCA the implementation must be verified by the QAU through a follow-up inspection and the verification documented on the RCA.

4.0 TRAINING

No specific training is required for this SOP. Project, Laboratory, and Section Managers should understand the principles related to corrective action and continuous improvement. The dated signature on the training form (Attachment 3) documents that this SOP has been read and understood.

ATTACHMENTS

1. Examples of Corrective Action
2. Request for Corrective Action form
3. Training Documentation

APPROVALS

Author		7-31-99
Quality Systems Manager		7-31-99
Vice President, Battelle Duxbury Operations	Name	7-31-99 Date

Attachment 1
Examples of Corrective Action

A. INVESTIGATION

A.1 Quality Control Excursions

1. Review calculations and transcriptions
2. Review original sample preparation and spiking records
3. Verify that instrument is within the calibration criteria
4. Identify any unusual issues related to sample matrix

A.2 Deviations (from SOPs , QAPjPs, etc)

1. Determine whether the deviation is isolated or re-occurring
2. Review the governing document to determine whether a revision should be prepared.
3. Discuss the deviation with lab personnel to determine if additional training is required

B. SHORT-TERM CORRECTIVE ACTION

B.1 Quality Control Excursions

1. Review errors with appropriate personnel; correct calculation or transcription errors
2. Review prep errors/procedures with laboratory personnel; identify practices that could contribute to poor QC results.
3. Perform instrument maintenance, recalibrate, and reanalyze samples
4. Determine with project manager whether reprocessing is required; document QC excursions

B.2 Deviations

1. Document the deviation.
2. Review project requirements and determine the appropriate corrective action (with the client, if appropriate).

C. LONG-TERM CORRECTIVE ACTION

C.1 Quality Control Excursions

1. Conduct additional training
2. Modify and document updated validation procedures
3. Increase frequency of instrument calibration checks
4. Modify clean-up procedures

C.2 Deviations

1. Conduct additional training
2. Update governing document

Attachment 2
Example of Request for Corrective Action Form

Date: Date Response Due: **To:** **From (Signature and Date):**
Description of finding which requires corrective action:
Describe investigation activities performed and the results:
Describe the immediate corrective action to be implemented:
Describe long-term corrective action to be implemented to minimize the re-occurrence of this issue:
Signature and Date: **Approval Signature and Date:** **(Operations Manager)** **Verified by Quality Assurance Office (Signature and Date):**

Attachment 3
Record of Training

for

NON-CONFORMANCE AND CORRECTIVE ACTION

The above mentioned SOP is relevant to your work. Your signature below attests to the fact that you have read this SOP and understand the requirements associated with this procedure.

Name (Printed)	Signature	Date

Battelle Duxbury Operations
Standard Operating Procedures

for

IDENTIFICATION AND QUANTIFICATION OF POLYCHLORINATED BIPHENYLS (BY CONGENER AND AROCLOR) AND CHLORINATED PESTICIDES BY GAS CHROMATOGRAPHY/ELECTRON CAPTURE DETECTION

Summary of changes in this version: : Iso-octane removed as a solvent; minor clarifications, and the justification of manual integrations. The analyte list has been updated.

1.0 OBJECTIVE

This Standard Operating Procedure (SOP) describes the identification and quantification of polychlorinated biphenyls (PCB) and chlorinated pesticides by capillary gas chromatography with ^{63}Ni electron capture detection (GC/ECD).

This SOP provides typical gas chromatography (GC) conditions for the detection of trace levels of PCBs and pesticides, methods for identifying the analytes, and procedures for analyte quantification. Table 1 lists the most frequently analyzed compounds and formulations, however, the list may be amended to fit specific projects.

2.0 PREPARATION

The GC/ECD must be operated and maintained as described in SOP No. 3-116. All documentation must comply with the procedures described in SOP No. 6-025.

2.1 GC PREPARATION

The GC is typically fitted with a 60-m x 0.25-mm internal diameter fused silica capillary column with a 0.25- m film thickness stationary phase. DB-5 (5% phenyl-, 95% methyl-polysiloxane) and DB-1701 (14% cyanopropylphenyl-, 86% methyl-polysiloxane), both from J&W Scientific, Inc., are examples of suitable column stationary phases. Suggested GC conditions are as follows (other columns and/or conditions may be specified in project plans):

Injection port temperature	280 °C
Detector temperature	300 °C
Initial temperature	60 °C
Initial hold	1 min

Table 1. Example PCB and Chlorinated Pesticide Analyte List

Suggested Internal Standards	Chlorinated Pesticides	Polychlorinated Biphenyls		
C12(14)	1 3 Dichlorobenzene	PCB1	PCB63	PCB176
C13(34)	1 4 Dichlorobenzene	PCB3	PCB74	PCB130
C15(103)	1 2 Dichlorobenzene	PCB4/10	PCB70/76	PCB138/160/163
C15(112)	Hexachloroethane	PCB7/9	PCB66	PCB158
C13(39)	1 2 4 Trichlorobenzene	PCB6	PCGB95	PCB129/126
C16(166)	Hexachlorobutadiene	PCB8/5	PCB91	PCB178
	1 2 4 5 Tetrachlorobenzene	PCB19	PCB56/60	PCB175
	Hexachlorocyclopentadiene	PCB12/13	PCB92	PCB187/182
	a BHC	PCB18	PCB84	PCB183
	Hexachlorobenzene	PCB17/15	PCB89	PCB128
	b BHC	PCB24/27	PCB101/90	PCB167
	g BHC	PCB16/32	PCB99	PCB185
	d BHC	PCB29	PCB119	PCB174
	Heptachlor	PCB26	PCB83	PCB177
	a Chlordene	PCB25	PCB97	PCB171/202
Aroclors	Aldrin	PCB31	PCB87/115/81	PCB156
	4 4 Dichlorobenzophenone	PCB28	PCB85	PCB173
Aroclor 1016	Dursban	PCB21	PCB136	PCB201/157
Aroclor 1221	Dacthal	PCB33/20	PCB110/77	PCB172
Aroclor 1232	Heptachlor epoxide	PCB53	PCB82	PCB197
Aroclor 1242	Oxychlordane	PCB51	PCB151	PCB180
Aroclor 1248	g Chlordane	PCB22	PCB135/144	PCB193
Aroclor 1254	4 4 DDMU	PCB45	PCB124	PCB191
Aroclor 1260	2 4 DDE	PCB46	PCB107/147	PCB200
Aroclor 1262	Endosulfan I	PCB52	PCB149/123	PCB169
Aroclor 1266	cis Chlordane	PCB43	PCB118	PCB170/190
	trans Nonachlor	PCB49	PCB134	PCB198
	Dieldrin	PCB47/75	PCB114	PCB199
	4 4 DDE	PCB48	PCB131	PCB203/196
	2 4 DDD	PCB44	PCB146	PCB189
	Endrin	PCB59	PCB153	PCB195/208
	Oxadiazinon	PCB42/37	PCB132	PCB207
	Endosulfan II	PCB41/64/71	PCB105	PCB194
	cis Nonachlor	PCB40	PCB141/179	PCB205
	4 4 DDD	PCB100	PCB137	PCB206
	2 4 DDD			PCB209
	Endrin aldehyde			
	Ethion			
	Endosulfan sulfate			
	4 4 DDT			
	Endrin ketone			
	Methoxychlor			
	Mirex			

Ramp 1 rate	10 °C /min to 140°C
Ramp 2 rate	0.9 °C /min to 220°C
Ramp 3 rate	5 °C /min to 290°C
Final hold	10 min
Carrier gas flow	1.5-2.5 mL/min (30-40 cm/sec)
Purge vent	1.5-2.5 mL/min
Split vent	40-80 mL/min
Purge on after 0.50 min	
EPC Program:	
Initial Pressure	30 psi
Initial Time	0.4 min
Rate 1	99 psi/min
Final Pressure	16.8 psi
Final Time	1 min

2.2 SAMPLE SPECIFICATIONS

Unless otherwise specified, for single column work a volume of 1 or 2 µL of standard and sample aliquots are injected into the GC. For simultaneous dual column work, where the injected standard and sample is split between two columns, a volume of 1- 3 µL is typically injected. Samples and standards should be prepared in hexane unless otherwise specified. Methylene chloride should *never* be introduced into the GC/ECD system because it will corrupt the detector.

2.3 ANALYTE IDENTIFICATION

Prior to sample analysis, the elution order of the analytes of interest must be determined. This is performed by analyzing the analytes individually, or in combination with analytes having known/pre-determined retention times. The elution order of the analytes listed in Table 1 have been verified for several columns using the GC conditions listed in Section 2.1 and can be reviewed in the Chemistry Department files.

3.0 PROCEDURES

3.1 CALIBRATION PROCEDURES

Before the sample is injected into the GC, the detector must be calibrated to determine the response for the analytes of interest. Demonstration of detector response, using a quadratic equation, is required before sample analysis. Calibration checks must be analyzed minimally every 10 samples during sample analysis. All analytical chromatographic data are quantified by either the internal standard or external standard method. The calibration and quantification method will be by the method of internal standards unless otherwise specified in the project plan.
The internal standard method of analysis is based on measuring the response of analyte(s) relative to a quantification internal standard (QIS). The QIS(s) is typically a recovery internal standard(s) (RIS) added after sample processing is completed and before instrumental analysis, but may, if specified in the project protocol, be a surrogate internal standard (SIS) added to the sample prior to sample processing in the laboratory. The RIS is used to determine the recovery of the SIS(s), and thus the efficiency of the sample processing.

The quadratic calibration equation is determined using the response of analyte(s) of interest relative to the QIS in a series of calibration standard solutions. Actual samples are fortified with the QIS after extraction but prior to analysis. The GC/ECD analysis of the sample is accomplished by measuring the response of the analyte relative to the QIS, and calculating the analyte concentration using the average response factor, or quadratic calibration equation, obtained from the calibration process. The internal standard calibration procedure is discussed in more detail in Section 3.2.

The external standard method of analysis is based on measuring the response of an analyte in a sample, and comparing it to the response of the same compound in the analysis of standard(s) of known concentration(s). In practice, an average response factor, or linear regression calibration equation, is determined for analyte(s) of interest in a series of calibration standard solutions. A sample is prepared to a known volume and analyzed. The concentration of the analyte(s) in the sample is determined by applying the average response factor, or linear regression calibration equation, obtained from the calibration process, to the response of the analyte(s) in the sample analyses. Accurate determination and recording of sample preinjection volumes (PIV) are necessary when using this method. The external standard calibration procedure is discussed in more detail in Section 3.3.

Instrument calibration is the responsibility of the analyst. Calibration records are maintained in project-specific data packages. In the following sections common calibration procedures are described. However, project-specific procedures may be selected and will then be specified in the project plan.

Initial Calibration

The initial calibration consists of the analysis of a series of calibration solutions, each at different concentrations, which approximately span the expected concentration range of the samples. Typically a five-point calibration is performed, but more or fewer calibration levels may be specified in the project plan. These standards include the analytes of interest, as well as the appropriate internal standards if an internal standard calibration is to be performed. The concentration of the LOW standard should be approximately 2 to 5 times the detection limit of the instrument. The MID-range calibration standard should be near the expected concentration of the samples. The HIGH concentration standard should be approximately 5 to 10 times the concentration of the LOW standard, or high enough to span the expected concentration range. A wider calibration range may be appropriate for certain applications, and will then be specified in the project plan. Commonly, the five-point calibration has the following concentrations (for individual PCB congeners and single peak chlorinated pesticides):

LOW	0.005 ng/µL
LOW MID	0.020 ng/µL
MID	0.050 ng/µL
HIGH MID	0.090 ng/µL
HIGH	0.120 ng/µL

The concentrations of the SIS and RIS should be approximately at the same as the MID-level target analytes, the same in all calibration solutions, and approximately the same concentration as expected in the samples. A multilevel (e.g., minimum three-point) calibration should be analyzed at the beginning of each set of continuous sample analyses.

Continuing Calibration

A calibration check, typically the MID calibration solution (if a typical five-point calibration is performed), is analyzed, at a minimum, every 24 hours (typically every 10 to 12 samples) while samples are being analyzed. All samples must be bracketed by two calibration checks that meet the calibration criteria defined in Section 3.4 or other project-specific calibration criteria.

3.2 INTERNAL STANDARD CALIBRATION

To use this approach one or more internal standards that are similar in analytical behavior to the compounds of interest are selected. The measurement of the internal standard should not be affected by the analytical method or matrix interferences. Because of these limitations no one internal standard is applicable to all samples, and internal standard selection, along with other analysis-specific information, should be specified in the project plan if non-standard compounds are to be used. Internal standards that may be appropriate include $Cl_3(39)$, $Cl_3(34)$, $Cl_5(103)$, $Cl_5(112)$, and $Cl_6(166)$. For most PCB and chlorinated pesticide analyses $Cl_3(34)$, $Cl_5(103)$ and/or $Cl_5(112)$ are added as the SISs and $Cl_3(39)$ and/or $Cl_6(166)$ are added as the RIS immediately prior to instrumental analysis (and used as the QISs).

Initial Calibration

Each calibration standard is analyzed using the same instrumental analysis conditions as used for samples and the calibration is established and verified by one of two methods (quadratic equation or response factor). Instrument calibration is generally accomplished using the quadratic equation of the Labsystems X-Chrom data system; calibration acceptance (Section 3.4) is determined using the correlation coefficient of the Labsystems X-Chrom quadratic calculation or by using response factors (RFs). The quadratic equation is a laborious method to verify, and has been verified. The details are not presented in this SOP. The following are the response factor equations.

Response Factor Method - Internal Standard Calibration

- $RF = (A_A * C_{IS})/(A_{IS} * C_A)$

Response Factor Method - Internal Standard Quantiification

- $C_A = (A_A * C_{IS})/(A_{IS} * RF_{AVG})$

where,

C_A	=	analyte amount/concentration.
A_A	=	analyte peak area (or height).
A_{IS}	=	area (or height) of the QIS.
C_{IS}	=	amount of QIS added to the sample.
b	=	y-intercept of linear regression equation.
m	=	slope of linear regression equation.
RF	=	response factor of the analyte.
RF_{AVG}	=	average response factor of the analyte from the initial multilevel calibration.

If a multi-level calibration is performed, the linearity of the calibration for each target analyte is checked. If the response for a given analyte is acceptably linear over the calibrated range [acceptable correlation coefficient or percent relative standard deviation (%RSD) in RFs], the calibration is considered valid and can be used for sample quantification. Calibration acceptance criteria are discussed in Section 3.4.

The Labsystems X-Chrom data system automatically computes and reports the correlation coefficients for the quadratic equations as part of the calibration. However, if the RF calibration check method is used, the variation in the RF over the calibration range for each compound is determined as %RSD of the average RF using the following equation:

$$\%RSD = \frac{SD_{RF}}{AVG\ RF} * 100\%$$

%RSD = percent relative standard deviation
SD_{RF} = standard deviation in average RF for the calibration standards
AVG RF = average RF for the calibration standards

Continuing Calibration
The continued validity of the initial calibration must be verified by the analysis of calibration check standard(s), as discussed in Section 3.1. The initial calibration is considered valid if the (1) "true" amount versus the "found" amount is acceptable, (2) the RF of the calibration check (response factor method) is acceptably similar to the average RF determined for the initial calibration, or (3) the new correlation coefficient (X-Chrom quadratic method) is acceptable. Calibration check acceptance criteria are discussed in Section 3.4. Unless the QAPP defines otherwise option (1) is used.

If the RF calibration check or the true concentration versus found methods are used, the comparability of the check standard RF or concentration for each analyte versus the RF or true concentration from the initial calibration is computed, and expressed as percent difference (%DIFF), using the following equation:

$$\%DIFF = \frac{(RF_C * RF_I)}{RF_I} * 100\%$$

%DIFF = percent difference
RF_I = average RF for the initial calibration standards
RF_C = RF for the calibration check standard

If a single-point calibration is used, no initial calibration check is performed but the calibration is continuously verified by determining the %DIFF in the RF (or calibration "slope") for each analyte in the calibration check analyses versus the RF from the initial calibration. The RF calculated for a single-point analysis is equivalent to the slope determined in a single-point calibration with the X-Chrom datasystem. As with multilevel calibrations, sample analyses should be bracketed by calibrations or checks that are acceptably similar.

3.3 EXTERNAL STANDARD CALIBRATION

Each calibration standard is analyzed using the same instrumental analysis conditions as used for samples and the calibration is established and verified by one of two methods (linear regression or response factor). Instrument calibration is generally accomplished using the linear regression algorithm of the Labsystems X-Chrom data system; calibration verification may be performed using the correlation coefficient of the X-Chrom linear regression calculation or by using calibration factors (CFs). The following are the applicable equations for the two methods.

Linear Regression Method - External Standard Quantification

- $Y = mX + b$

  which is equivalent to: $A_A = [(m * C_A) + b]$

- $C_A = (A_A * b)/(m)$

Response/Calibration Factor Method - External Standard Quantification

- $CF = (A_A)/(C_A)$
- $C_A = (A_A)/(CF_{AVG})$

If a multi-level calibration is performed, the linearity of the calibration for each target analyte is checked as indicated in Section 3.2. The continued validity of the initial calibration must be verified by the analysis of calibration check standard(s), as indicated in Section 3.1, and the continuing validity of the calibration is checked as specified in Section 3.2. Calibration acceptance criteria are discussed in Section 3.4.

3.4 CALIBRATION ACCEPTANCE CRITERIA

The following calibration acceptance criteria are suitable for most PCB and chlorinated pesticide work, and apply unless otherwise specified in the project plan.

Initial calibration - Linear Regression Method: correlation coefficient of >0.99 for a 3-point calibration, and >0.995 for a 5-point calibration. Response/Calibration Factor Method: %RSD of the average analyte RF or CF should be <25%.

If these criteria are not met corrective action is taken, a new calibration run, and the samples re-analyzed. Corrective action includes verifying that the proper analytical conditions are used, that appropriate standards are used, and routine maintenance. However, corrective action and re-analysis will be at the discretion of the Project Manager. A decision to continue analysis without corrective action must be documented, justified, and approved by the Project Manager, or his/her designee.

Calibration check - Quadratic Equation Method: correlation coefficient of >0.99 for a 3-point calibration, and >0.995 for a 5-point calibration when the check standard replaces the previously analyzed standard of the same concentration in the multilevel calibration or %DIFF between the "found" analyte concentration from the calibration check and the "true" analyte concentration of the initial calibration must be ≤25%. Response/Calibration Factor Method: %DIFF between the RF or CF from the calibration check and the average analyte RF or CF of the initial calibration must be ≤25%.

If these criteria are not met, the check standard can be re-analyzed. If the check standard fails to meet the criteria a second time, corrective action will be taken, and the check standard and associated samples re-analyzed. A new initial calibration may need to be run, and affected samples re-analyzed. Corrective action includes verifying that the proper analytical conditions are used, that appropriate standards are used, and routine maintenance. However, corrective action and re-analysis may be at the discretion of the Project Manager. A decision to continue analysis without corrective action must be documented, justified, and approved by the Project Manager, or his/her designee.

3.5 CALIBRATION/QUANTIFICATION OF MULTICOMPONENT COMPOUNDS

Depending upon individual project plans, either a multi- or single-level calibration (Sections 3.1 through 3.4) is analyzed when samples are quantified for multi-component mixtures such as Aroclors, technical chlordane, or toxaphene. If multi-level multicomponent standards are used for calibration, the detector

linearity should be verified similarly to single-component analyses, using either the X-Chrom datasystem's (multicomponent) analysis method or the response factor method. If one-point multicomponent standards are used for calibration, the detector linearity should be verified by analyzing a multilevel set of standards of representative single component PCB and/or pesticide analytes for initial and continuing calibration checks, just like for the single-component analyses described in Sections 3.1 through 3.4, unless otherwise documented in the project plan. Single-point calibration solutions are analyzed minimally at the beginning of each set of sample, unless otherwise specified in the project plan.

As with single component analyses, the calibration and quantification of multicomponent mixtures can be performed using the Labsystems X-Chrom system's linear regression method, and an X-Chrom program/procedure written specifically for Aroclors and other multicomponent analytes, or by the response factor method. Either method provides good data if applied correctly, and either method may be used.

When using the X-Chrom systems' multicomponent analysis linear regression method, a representative set of 5 to 10 peaks are selected in the multicomponent compound, and the total compound concentration in the standard(s) is divided by the number of peaks selected for the calibration, and entered for each peak in the method. Because of the equal weight given to each individual peak/component it is important to use only peaks/components that are (1) of significant relative size, (2) behave similarly to most other representative peaks/components in environmental samples, and (3) can be expected to be found in most environmental samples that contain the multicomponent compound. For instance, $Cl_4(44)$ is a fairly large peak in an Aroclor 1254 standard, but is frequently "lost" in environmental samples, or disproportionately small, and should therefore not be used when the X-Chrom systems quantification method is used. $Cl_4(44)$ can, however, be used in the response factor calibration and quantification method without significantly affecting the accuracy of the data because the peak of this congener is smaller than for most of the other PCB congeners that would be used, and it is weighed proportionately to the relative peak size in the Aroclor standard by this calibration/quantification method.

When using the response factor method the only difference between the calculation of the RF and CF for multicomponent mixtures and single peak analytes is the term A_A. When using the response factor method, as when using the X-Chrom method for multi-peak analysis, several (5 to 10, or more) pre-determined representative peaks are chosen which represents the multicomponent analyte. These peaks are typically either among the largest peaks in the Aroclor or congeners/peaks unique to that Aroclor. The areas of these peaks are added together to form the term A_A in the RF and CF formula. These same representative peaks will be identified in the samples (attempts should be made to identify and use as many of these pre-designated peaks as possible in each sample) for quantitation. The C_A term is to be the concentration of the multicomponent standard itself.

3.6 SAMPLE ANALYSIS

Samples are analyzed under the same analytical conditions as the calibration standards. Samples analyses must be bracketed by acceptable calibrations. Acceptance criteria for accepting peaks as analytes of interest are explained in Sections 3.6.1 through 3.6.3.

3.6.1 Retention Time Windows and Pattern Recognition

Retention time (RT) windows for each analyte should be determined with each set of samples. By definition (EPA Method 8000), the RT window is equal to plus or minus three times the standard deviation of an analyte's RT determined from the triplicate analysis of a calibration standard in a 72 hour

period. Rather than statistically determining the RT windows with each set of samples, they may be estimated as 0.1 min. for most PCB congener and chlorinated pesticide analysis. Retention times are verified, and, if appropriate, updated in the method by careful review of the calibration standards in each set of analyses. The experience of the analyst should weigh heavily in peak/analyte identification and chromatogram interpretation of actual sample, as matrix components can affect retention times on a sample-by-sample basis. For multicomponent mixtures and single peak analytes which may be related (i.e., individual PCB congeners, DDT family analytes), pattern recognition should weigh heavily in the interpretation of the chromatogram and analyte identification.

3.6.2 Peak Shape and Integration

Each peak should be individually examined for proper gaussian peak shape. However, because of the frequent complexity of chromatograms of environmental samples containing PCB and chlorinated pesticides, ideal peak shape and resolution is not always obtained. Peak identification and integration is at the discretion of the experienced analysts, with close communication and consultation with the Project Manager and/or GC Group Manager, as appropriate.

3.6.3 Peak Size

Peaks with a signal-to-noise ratio of five or less (as determine visually by the analysts during the chromatogram review process) should be regarded as not detected unless otherwise noted in project-specific protocols.

3.6.4 Manual Integration

Manual integration is an important component of sample analysis at Battelle to ensure that the most accurate data are generated. Given the complex nature of environmental samples. Battelle considers it critical that a trained analyst reviews each individual analyte in each sample using a chromatography data system, and manually alters and optimizes the integration as needed. Each analyst is trained in the proper integration of peaks.

The data system baseline integration can be used to quantify samples. If, during the review of the chromatogram, the analyst notices improper integration by the data system, he/she will manually reintegrate the peak using proper integration techniques.

Proper integration techniques will account for near co-eluted peaks, negative peaks, and other peak shape or baseline anomalies which often occur in environmental samples.

3.7 QUANTITATION

Instrument calibration verification may be accomplished by either the Labsystems X-Chrom data system's quadratic equation method or by the response factor method (Sections 3.2 and 3.3). However, analyte *quantification* is generally performed using the quadratic equation algorithm of the data system, but may be done using the RF method, if specified in the project plan. Unless otherwise noted, the internal standard method is used to calibrate and quantify PCBs and chlorinated pesticides in environmental samples. The internal standard methods for calculating analyte concentrations (C_A) by both the X-Chr m system's quadratic equation method and by the response factor method are described in Section 3.2. The quantitation calculations used for external standard calibration and quantification are

described in Section 3.3. Specific considerations for multicomponent analyses were presented in Section 3.5.

The equations presented in Sections 3.2 and 3.3 yield total amount (e.g., ng or µg, depending on the unit used for the internal standard) for the method of internal standard, and total amount or final extract concentration for the method of external standard (depending on the method used to enter amount/concentration of the calibration standards). To calculate actual original sample concentrations, sample volumes/weights, sample splits, and/or final pre-injection volumes need be considered, as appropriate. Non-standard concentration calculations (e.g., when a series of sample splits need be applied) should be thoroughly documented in the project plan or project files, and approved by the Project Manager.

3.8 CONFIRMATORY ANALYSIS

Some projects may require a GC/ECD confirmation of the identified analytes, which involves the analysis of those samples using a GC column with different analyte retention characteristics than the column used for primary analysis, in order to verify the initial identification of the analytes in the samples. The confirmatory analysis can be qualitative or quantitative. The DB-5 and DB-1701 columns (see Section 2.1) are a suitable column pair for primary and confirmatory analysis. Other columns (e.g., DB-17 and DB-608) may be used, as specified in the project plan.

When the confirmation is qualitative, a single calibration standard (typically a MID-level) is analyzed prior to the samples, and again minimally every 24 hours (typically every 10-12 samples) during sample analysis. These calibration analyses are used to determine retention times, and retention time windows, of the target analytes. Target analytes are then identified strictly based on retention time matching, and the data with identified analytes from the confirmatory analysis are compared to the data with identified *and* quantified analytes from the primary analysis to yield qualitatively confirmed data. The same thorough review of sample chromatograms is performed for the confirmatory analyses as for the primary analyses.

If the confirmation step is required to be quantitative in nature, all calibration and analysis procedures that apply to the primary analysis apply to the confirmatory analysis, and the analyses are treated as two separate quantitative analyses. Target analyte data from the primary and confirmatory analyses are compared. The confirmation procedure involves comparing the identifications and concentrations of the target analytes that were quantified in both sets of analyses for the same sample, and applying pre-established confirmation criteria. Analyte concentrations with a %RPD of $\leq$30% can be considered confirmed, unless other confirmation criteria are specified in the project plan. The %RPD is calculated as follows.

$$\%RPD = \frac{[C_P * C_C]}{C_A} * 100\%$$

%RPD = relative percent difference
C_P = concentration determined in the primary analysis
C_C = concentration determined in the confirmatory analysis
C_A = average of the primary and confirmatory analyses concentrations

4.0 CALCULATIONS

Following is a list of the formulae described in this SOP.

Description of Calculation	SOP Section Number
Response factor - internal standard calibration	3.2
Response factor - internal standard quantification	3.2
Relative standard deviation (%RSD)	3.2
Percent difference (%DIFF)	3.2
Linear Regression - external standard quantification	3.3
Response/Calibration factor - external standard quantification	3.3
Relative percent difference (%RPD)	3.8

5.0 QUALITY CONTROL

The GC Facility is operated and maintained in accordance with SOP No. 3-116. All operations are documented according to SOP No. 6-025. Hard copies of all calibrations and sample data are kept in three-ring binders and maintained with individual project files. Analysis of quality control samples, including procedural blanks, blank spikes, matrix spikes, control materials, and standard reference materials (SRM) are specified in the project plans. Acceptance criteria for calibrations stated in this SOP apply unless otherwise noted in the project plan.

In order to monitor the condition of the GC system, a solution containing endrin and/or 4,4'-DDT should be analyzed and the percent degradation determined. This check standard should be analyzed with each set of samples if the analysis is for pesticides that are susceptible to degradation. The sum of the areas of the degradation products (endrin aldehyde and endrin ketone for endrin; 4,4'-DDD and 4,4'-DDE for 4,4'-DDT) should be less than 20% of the total area of the degradation products and parent compound (i.e., area of endrin aldehyde+endrin ketone should be less than 20% of the sum of areas of endrin+endrin aldehyde+endrin ketone). If the area is greater than 20%, remedial action should be taken, such as clipping the front end of the column and/or replacing the injection port liner. However, as with calibration criteria exceedances, corrective action and re-analysis may be at the discretion of the Project Manager. Degradation check exceedances should, at a minimum, be brought to the attention of the Project Manager who may request corrective action and re-analysis, or may incorporate the degradation check data into the data package for data analysis/interpretation, as appropriate. The original chromatogram and the calculated % degradation products is maintained with the project data and a copy kept in the Instrument Control Log stored in the GC Instrument Lab.

6.0 TRAINING

The trainee must read and fully understand this SOP and have documented training for SOP Nos. 3-116 and 6-025. Individuals may work independently once they have analyzed and quantified a multilevel calibration, and a sample of known concentration (e.g., reference material or matrix spike). The samples are reviewed by an experienced, trained, analyst who determines if the analysis and quantitation were performed properly. When training is completed the trainee is issued a Certificate of Training (Attachment 1). This certificate is sent to the Quality Assurance Unit.

7.0 SAFETY

Protective clothing and eyeglasses should be worn when appropriate.

Proper care must be exercised when using syringes.

Certain areas of the GC/ECD system are heated and bodily contact with these areas should be avoided.

Care should be taken when using solvents in and around the GC system.

The ^{63}Ni sealed source contains radioactive material and should not be tampered with.

8.0 ATTACHMENTS

Attachment 1. Example of Battelle Duxbury Operations Certificate of Training.

APPROVALS

Author:	[signature]	8·6·99
Laboratory Manager:	[signature]	8·6·99
Quality Systems Manager:	[signature]	8-10-99
Environmental Chemistry Section Manager:	[signature] for GS Durell Name	8-9-99 Date

ATTACHMENT 1

Battelle Duxbury Operations
CERTIFICATE OF TRAINING

SOP No._____

SOP Title_____

Trainee_____

Instructor_____

Date SOP Read_____

Date Training Completed_____

The above mentioned trainee has satisfactorily completed the training requirements associated with this SOP. Supporting document (if applicable) is attached.

Comments

Approved by_____ **Date**_____

BATTELLE DUXBURY OPERATIONS
STANDARD OPERATING PROCEDURES

FOR

IDENTIFICATION AND QUANTIFICATION OF POLYNUCLEAR AROMATIC HYDROCARBONS BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY

Summary of changes in this version: Justification for manual integration has been added.

1.0 OBJECTIVE

This Standard Operating Procedure describes the identification and quantification of polynuclear aromatic hydrocarbons (PAH) by gas chromatography/mass spectrometry (GC/MS). Please note this method is a modification of EPA methods 8270C and 625 and therefore certain criteria (i.e. initial calibrations and daily verifications) are different than those referenced in EPA methods. Refer to SOPs 5-281 and 5-282 respectively for GC/MS analyses requiring Standard Methods.

1.1 SUMMARY OF METHOD

Polynuclear aromatic hydrocarbons are separated via high resolution capillary gas chromatography, and identified and quantified using electron impact mass spectrometry. A data system interfaced to the GC/MS is used to control acquisition and to store, retrieve, and manipulate mass spectral data. This method provides procedures for the identification and measurement of the selected PAH listed in Table 1 in full-scan mode and selected ion monitoring mode (SIM). Table 1 may be amended to meet project-specific protocols.

2.0 PREPARATION

The GC/MS must be operated and maintained as described in SOP No. 3-092. All documentation in the GC/MS Facility must comply with the procedures described in SOP No. 6-011.

2.1 GAS CHROMATOGRAPH/MASS SPECTROMETER

Prior to the analysis of analytical standards and/or samples the mass spectrometer must be tuned according to the procedures described in SOP No. 3-092. Perfluorotributlyamine (PFTBA) should be used to tune the mass spectrometer, unless otherwise specified in the project protocol.

2.1.1 The gas chromatograph is fitted with a 60-m x 0.25-mm DB-5MS (J & W Scientific, Inc.) or equivalent (0.25-µm film thickness) fused silica capillary column. The GC oven temperature program will be as follows, unless otherwise specified in the project-specific protocol:

Injection Port: 300 °C
Transfer Liner: 280 °C
Initial Temp: 40 °C

Initial Hold:	1 minute
Ramp Rate:	6 °C/min
Final Temp:	290 °C
Final Hold:	20 minutes or until benzo(g,h,i)perylene elutes

2.1.2 All gas chromatographs are fitted with Electronic Pressure Control (EPC). The EPC ramping program will be as follows, unless otherwise specified in the project-specific protocol:

Initial Pressure: 30 psi	Initial Time: 1 min
Level 1 Rate: 99 psi/min	Final Pressure: psi equivalent to flow 1 mL/min
Vacuum Compensation: On	

2.2 PREPARATION FOR FULL-SCAN ANALYSIS

A 1-μL injection volume will be analyzed, unless otherwise specified in the project protocol. The mass spectrometer should be operated in electron impact mode at a manifold pressure of less than 6 x 10^{-5} torr. The mass range scanned will be 50 to 450 atomic mass units (AMU) unless specifically stated otherwise in the project protocols. The quantification and confirmation ions used in the analysis of selected PAH are listed in Table 1.

2.3 PREPARATION FOR SELECTED ION MONITORING (SIM) ANALYSIS

The oven temperatures and electronic pressure programs outlined in sections 2.1.1 and 2.1.2 are used for SIM analysis unless stated otherwise in project protocols. A 2-μL injection volume will be analyzed, unless otherwise specified in the project protocol. The mass spectrometer should be operated in electron impact mode at a manifold pressure of less than 6 x 10^{-5} torr. The electron multiplier voltage should be set a minimum of 100 V above the tune value.

2.3.1 The quantification and confirmation ions used in the analysis of selected PAH are listed in Table 1. If possible ion groups should be selected so that no more than 20 ions are monitored in a single group. It should be noted that as the number of ions scanned per group increases and the individual dwell times decrease, sensitivity will also decrease. Each ion in a group should have identical dwell times to ensure that correct ion ratios are preserved. Total group dwell time should not exceed 400 ms, individual dwell times should be a minimum of 20 ms.

2.3.2 Prior to the first analysis of analytical standards and/or samples in SIM, a 10 ng/μL analytical standard and reference sample (e.g. North Slope Crude Oil) should be analyzed in full-scan mode, under the conditions presented in Section 2.2. The full-scan total ion chromatograms (TIC's) from these analyses should be used to determine the proper group start and stop times in the SIM acquisition method.

TABLE 1. SELECTED PAH QUANTIFICATION AND CONFIRMATION IONS FOR GC/MS FULL-SCAN AND SIM ANALYSIS

Analyte	Quantification Ions (AMU)	Recommended Confirmation Ions (AMU) [% of Base Peak][a]
d_8-naphthalene[b]	136	134 [15]
naphthalene	128	127 [15]
C_1-naphthalenes	142	141 [80]
2-methylnaphthalene	142	141 [80]
1-methylnaphthalene	142	141 [80]
C_2-naphthalenes	156	141
2,6-dimethylnaphthalene	156	141
C_3-naphthalenes	170	155
2,3,5-trimethylnaphthalene	170	155
C_4-naphthalenes	184	169, 141
d_{10}-acenaphthene[b]	164	162 [95]
acenaphthylene	152	153 [15]
acenaphthene	154	153 [98]
biphenyl	154	152 [30]
d_{10}-biphenyl[b]	164	162
d_{10}-fluorene[b]	176	174 [85]
dibenzofuran	168	169 [20]
fluorene	166	165 [95]
C_1-fluorenes	180	165 [100]
C_2-fluorenes	194	179 [25]
C_3-fluorenes	208	193
d_{10}-phenanthrene[b]	188	184
phenanthrene	178	176 [20]
anthracene	178	176 [20]
C_1-phenanthrenes/anthracenes	192	191 [60]
1-methylphenanthrene	192	191 [60]
C_2-phenanthrenes/anthracenes	206	191
C_3-phenanthrenes/anthracenes	220	205
C_4-phenanthrenes/anthracenes	234	219, 191
dibenzothiophene	184	152 [15] 139
C_1-dibenzothiophenes	198	184 [25] 197
C_2-dibenzothiophenes	212	197
C_3-dibenzothiophenes	226	211
fluoranthene	202	101 [15]
pyrene	202	101 [15]
C_1-fluoranthene/pyrenes	216	215 [60]
C_2-fluoranthene/pyrenes	230	215
C_3-fluoranthene/pyrenes	244	229, 215
d_{12}-chrysene[b]	240	236
benz[a]anthracene	228	226 [20]
chrysene	228	226 [30]
C_1-benz[a]anthracenes/chrysenes	242	241
C_2-benz[a]anthracenes/chrysenes	256	241
C_3-benz[a]anthracenes/chrysenes	270	255
C_4-benz[a]anthracenes/chrysenes	284	269, 241
d_{12}-benzo[e]pyrene[b]	264	260 [20]
d_{12}-benzo[a]pyrene[b]	264	260 [20]

TABLE 1 Continued. SELECTED PAH QUANTIFICATION AND CONFIRMATION IONS FOR GC/MS FULL-SCAN AND SIM ANALYSIS

Analyte	Quantification Ions (AMU)	Recommended Confirmation Ions (AMU) [~% of Base Peak][a]
benzo[b]fluoranthene	252	253 [30], 125 [10]
benzo[k]fluoranthene	252	253 [30], 125 [10]
benzo[e]pyrene	252	253 [20]
benzo[a]pyrene	252	253 [30], 125 [10]
perylene	252	253 [20]
indeno[1,2,3-c,d]pyrene	276	277 [25], 138 [30]
dibenz[a,h]anthracene	278	279 [25], 139 [20]
benzo[g,h,i]perylene	276	277 [25], 138 [20]
OTP	230	198
Biomarkers:		
5b-cholane[b]	217	
C30a,b-hopane	191	
C30b,b-hopane	191	
LAB's:		
1-phenylonane[b]	91	92, 105
1-phenyldecane(s)	91	92, 105
1-phenylundecane(s)	91	92, 105
1-phenyldodecane(s)	91	92, 105
1-phenyltridecane(s)	91	92, 105
1-phenyltetradecane(s)	91	92, 105
Phenols:		
d_5-phenol[b]	99	98, 71
phenol	94	66
C_1-phenols	108	107
C_2-phenols	122	107
C_3-phenols	136	107, 77
C_4-phenols	150	135, 107
C_5-phenols	164	149, 121
C_6-phenols	178	163, 135
C_7-phenols	192	177
C_8-phenols	206	107
C_9-phenols	220	107, 135
Phthalates:		
d_4-bis(2-ethylhexyl)phthalate[b]	153	171, 283
bis(2-ethylhexyl)phthalate	149	167, 279
dimethyl phthalate	163	164, 194
diethyl phthalate	149	150, 177
di-n-butyl phthalate	149	150, 104
butyl benzyl phthalate	149	91, 206
di-n-octyl phthalate	149	150, 279

TABLE 1 Continued. SELECTED PAH QUANTIFICATION AND CONFIRMATION IONS FOR GC/MS FULL-SCAN AND SIM ANALYSIS

Analyte	Quantification Ions (AMU)	Recommended Confirmation Ions (AMU) [% of Base Peak][a]
Other:		
decalin	138	
C_1-decalins	152	
C_2-decalins	166	
C_3-decalins	180	
C_4-decalins	194	
benzothiophene	134	
C_1-benzothiophenes	148	
C_2-benzothiophenes	162	
C_3-benzothiophenes	176	
C_4-benzothiophenes	190	
naphthacene	228	226 [30]
chloronaphthalenes	162	127, 126

[a]Relative abundance of ions within any given isomer group will vary considerably, depending on isomer of interest. Relative abundances should be determined from analysis of crude oil solution. [b]Representative spiking compound.

2.3.3 In the event that a section of the analytical column is removed, or the analytical column is replaced, a 10 ng/μL analytical standard and reference sample should be analyzed in SIM mode under the conditions presented in Section 2.3. The extracted ion profiles (EIP's) from these analyses should be used to reassign the proper group start and stop times of the SIM acquisition method.

3.0 PROCEDURES

3.1 CALIBRATION

Demonstration of a linear initial calibration is required prior to the analysis of samples. A continuing calibration is required at the beginning and end of each 12 hour period during which analyses are performed (approximately every eight injections). Sample data may be used if bracketed by a passing continuing calibration. Initial calibrations from previous sequences may be used if the continuing calibrations still show acceptable linearity and no major instrument maintenance has been performed (i.e. source cleaning, column change, etc.).

3.1.1 INITIAL CALIBRATION

Analyze a minimum of five analytical standards that will represent sample concentration range, unless otherwise specified in the project protocol.

3.1.1.1 The concentration of the low standard should be 2 to 3 times the detection limit. The medium concentration standard should be near the concentration range expected in the samples. The high concentration standard should be at least 1 order of magnitude more concentrated than the low concentration standard.

3.1.1.2 For each level of calibration, calculate the response factors for each analyte of interest using the software supplied with the GC/MS data system (see SOP 3-092). For each analyte, calculate the average response factor (RF) and the percent relative standard deviation (RSD). For a valid initial calibration, the percent RSD for each analyte should be less than 25 percent, unless specifically stated otherwise in project protocols (see Section 4.1 for calculation of RF and RSD). The average RSD for all targets should be ≤15%.

3.1.2 CONTINUING CALIBRATION

A continuing calibration will be performed using the following procedure at the beginning and end of each 12 hour period in which samples are analyzed.

3.1.2.1 Analyze a medium concentration analytical standard under the same analytical conditions used in the initial calibration. Calculate response factors for each analyte of interest and compare them to the average response factor calculated in Section 3.1.1.2, above.

3.1.2.2 The percent difference between the average response factor and the continuing calibration response factor is less than 25 percent, the initial calibration is still valid and sample analyses may continue (see Section 4.2 for calculation of percent difference). If the percent difference of a target RF exceeds 25 percent, remedial action should be taken and the medium concentration standard reanalyzed. If the continuing calibration fails again, then sample analyses should be terminated, maintenance performed, and a new initial calibration analyzed, unless specifically stated otherwise in project protocols. In addition the average percent difference of all the targets should be ≤15%. Analysts should be visually monitoring the retention time (RT) deviation (in minutes) and percent area deviation recorded on the continuing calibration reports. Ideally, these values should not exceed 0.5 minutes for RT, and <50% or >200% area deviation.

3.2 SAMPLE ANALYSIS

Samples are analyzed under the same analytical conditions used in the analysis of the analytical standards. A quantification internal standard (QIS, also known as RIS - recovery internal standard) is added to the samples immediately preceding analysis. The type and amount of QIS added is project specific, consult the project protocols.

The criteria presented in Sections 3.2.1 through 3.2.7 must be satisfied to verify identification of an analyte in a sample. Analyte peaks that do not satisfy these criteria in the preliminary quant are deleted from the final quantification reports/files. Original working copies of these reports are maintained with the project files as "QDel Reports" stored in unused data (See SOP No. 3-092).

3.2.1 RELATIVE RETENTION TIME

The sample component relative retention time (RRT) should be within ±0.1 min (6 sec) of the standard component. The standard must have been run within 12 hours of the sample.

3.2.2 SAMPLE SPECTRUM

For each analyte suspected to be present in the sample, the corresponding ions listed in Table 1 must be present and the relative intensities must agree to within ±20 percent of the relative intensities found in spectra of the standard component. This criteria may be modified for trace level analysis and is left to the project manager's/analyst's discretion. Modifications to sample spectrum criteria will be documented. If the intensity of the quantification ion in a sample spectrum is greater than the intensity of the same ion in the high concentration analytical standard the sample should be diluted and reanalyzed.

3.2.3 SIGNAL TO NOISE RATIO

A quantifiable analyte peak *should* (but is not limited to) exhibit a signal:noise ratio of 3:1 or greater unless specified otherwise in the project protocol.

3.2.4 PEAK SYMMETRY

Suspected peaks should be examined for proper shape and symmetry.

3.2.5 PATTERNS

Suspected sample components should display established patterns. See Attachment 1.

3.2.6 MINIMUM AREA

It is recommended that each of the sample components have a minimum area of 1/2 the abundance count of the component in the low standard. Quantitation of a component is not limited to this criteria however. For instance, a component may have an area abundance slightly less than 1/2 the low standard area abundance but exhibit a signal to noise ratio of 5:1. This component would be considered for quantitation.

3.2.7 ALYKL HOMOLOGUES

Certain projects require identification and quantification of the alkyl homologues of selected PAH. This will be detailed in the project-specific protocol. Alkyl homologues are identified by the procedures in Sections 3.2.8. Quantification of alkyl homologues is covered in Section 3.2.10, below (adopting parent response factors is discussed in SOP No. 3-092).

3.2.8 TENTATIVE IDENTIFICATION OF UNKNOWNS AND ALKYL HOMOLOGUES

For components not contained in the analytical standards, a library search may be performed on the sample spectrum for the purpose of tentative identification (Registry of Mass Spectral Data Library). Computer-generated search algorithms should not employ unusual normalization, tilting, or smoothing of the unknown spectrum.

3.2.8.1 Major ions (ions >10 percent of the primary ion) in the reference spectrum should be present in the sample spectrum.

3.2.8.2 Relative intensities of the ions in the sample spectrum should agree with the reference spectrum to within ±20 percent.

3.2.8.3 Quantification of tentatively identified compounds is covered in Section 3.2.11.

3.2.9 QUANTIFICATION OF ANALYTES

Quantification of analytes identified in samples will be performed by the internal standard method, using the average response factor from the initial calibration, unless otherwise specified in the project protocols. See Section 4.3 for additional information regarding calculations used for the determination of target analyte concentrations in samples.

3.2.10 QUANTIFICATION OF ALKYL HOMOLOGUES

Alkyl homologue groups are quantified by the internal standard method. The molecular ion of the alkyl homologue should be extracted, and the areas of the individual isomers summed by a straight line, baseline integration.

3.2.10.1 The response factor used to quantify a specific homologous series (e.g. C_1-naphthalenes) should be the response factor of parent compound (e.g. naphthalene) unless specified otherwise in the project protocols.

3.2.11 QUANTIFICATION OF TENTATIVELY IDENTIFIED COMPOUNDS

Tentatively-identified compounds (see Section 3.2.8) should be quantified by the internal standard method assigning a response factor of 1. The data report should indicate that these compounds are uncorrected for response.

4.0 CALCULATIONS

4.1 INITIAL CALIBRATION

The average response factors from the initial calibration are calculated using the following equation:

$$RF = (A_a/A_b) \times (C_b/C_a)$$

where:

A_a = Area of quantification ion for target analyte in the standard
A_b = Area of quantification ion for internal standard in the standard
C_a = Concentration of target analyte in the standard
C_b = Concentration of internal standard in the standard

The percent relative standard deviation (RSD) is evaluated with following equation:

$$RSD = (SD/\overline{RF}) \times 100$$

where:

SD = standard deviation of the mean RF, calculated as n-1.

See Section 3.1.1.2 for details regarding initial calibration acceptability criteria.

4.2 CONTINUING CALIBRATION

The response factors determined from the continuing calibration are checked against those determined from the initial calibration. The percent difference is calculated using the following

$$\text{Percent Difference} = [(\overline{RF}_i - RF_c)/\overline{RF}_i] \times 100$$

where:

RF_i = Average response factor from initial calibration
RF_c = Response factor from continuing calibration

See Section 3.1.2.2 for continuing calibration acceptability criteria.

4.3 QUANTIFICATION OF SAMPLES

Samples are quantified as detailed in Section 3.2. The concentration of target analytes is determined using the following equation:

$$C_a = [(A_a/A_i) \times (Amt_i/RF_i) \times D]/V_a$$

where:

C_a = Concentration target analyte
A_a = Area quantification ion for target analyte
A_i = Area quantification ion for internal standard
Amt_i = Amount internal standard added to sample
RF_i = Average RF for analyte determined from initial calibration
D = Dilution factor if applicable
V_a = Sample size

Sample size may refer to sample volume or sample dry/wet weight. The project protocol will specify reporting criteria.

5.0 QUALITY CONTROL

The GC/MS Facility is operated and maintained in accordance with SOP No. 3-092 and all GC/MS Facility operations are documented according to SOP No. 6-011. Hardcopies of all initial and continuing calibrations are maintained with the project data. The number of procedural blanks, spiked blanks, matrix spikes, and other types of quality control samples will be specified in individual project protocols. Acceptability criteria and reporting limits will also be outlined in the project-specific protocols. The following quality control procedures have been adapted from relevant procedures in U.S. Environmental Protection Agency (EPA) Method 8270, Gas Chromatography/Mass Spectrometry for Semivolatile Organics: Capillary Column Technique (1986).

5.1 Each day that analysis is performed, the daily calibration standard should be evaluated to determine if the chromatographic system is operating properly.

5.1.1 Peak shape should be evaluated for proper peak shape and symmetry.

5.1.2 The instrumental response should be comparable to previous calibrations.

5.1.3 The system must be recalibrated if the analytical column is replaced.

5.2 There must be an initial calibration and calibration verification of the instrument as specified in Section 3.1.1 and 3.1.2.

6.0 TRAINING

6.1 The operator must be experienced with the operation and maintenance of the GC/MS system, and must have passed the training requirements presented in SOP No. 3-092.

6.2 The trainee must read and fully understand this SOP. The trainee will then be given a demonstration of all aspects of the SOP.

6.3 Individuals may work independently once they have satisfactorily performed the following training test.

6.3.1 The trainee will perform an initial calibration and analyze one sample of known concentration, such as a North Slope Crude check sample.

6.3.2 An experienced operator will then review the same sample results. Successful completion will consist of a valid initial calibration and sample results within 25 percent from historical (certified) values.

6.4 When training is completed, the trainee will be issued a Certificate of Training (Attachment 2). The original completed training certificate and all supporting documentation will be sent to the Quality Assurance Office.

7.0 SAFETY

All GC/MS operators must be experienced with the operation and maintenance of the GC/MS system, and must have passed the training requirements presented in this SOP and SOP No. 3-092 (see additional safety comments in SOP No. 3-092).

7.1 Protective clothing should be worn when appropriate.

7.2 Proper care must be exercised when using syringes.

7.3 Certain areas of the GC/MS system are heated zones and bodily contact with these zones should be avoided.

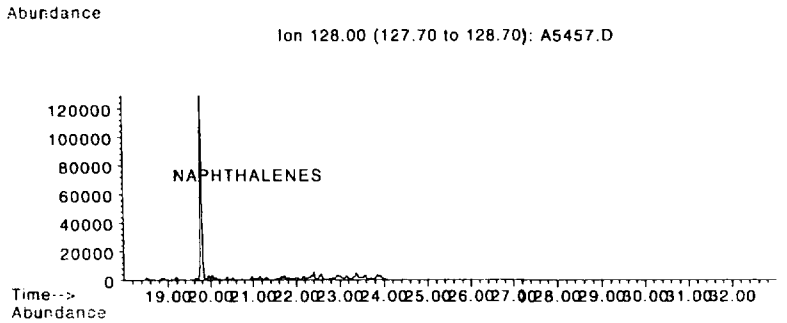
7.4 High voltages exist in certain marked areas of the GC/MS system and bodily contact with these areas is to be avoided.

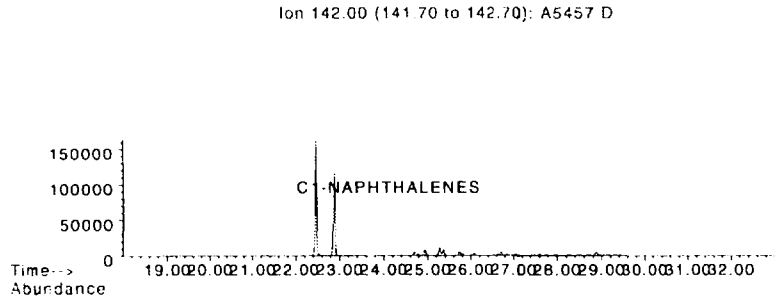
8.0 ATTACHMENTS

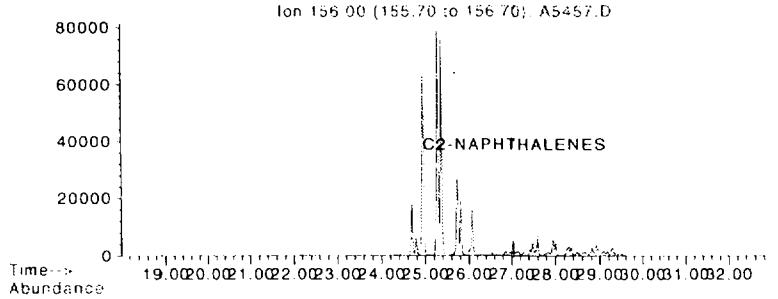
Attachment 1. Alkyl Homologue Patterns
Attachment 2. Certificate of Training

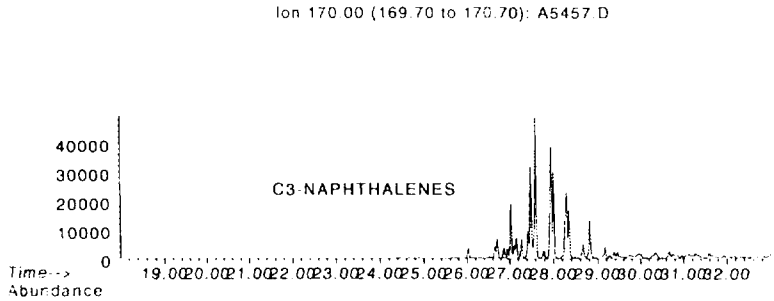
Author	Daniel P. Barton for JAF	8-10-99
Laboratory Manager	Charles S. Ler for RL	8/10/99
QA Coordinator	Karen B. Burke	8-10-99
Environmental Chemistry Section Manager	ASUH for GSDurell	8-10-99
	Name	**Date**

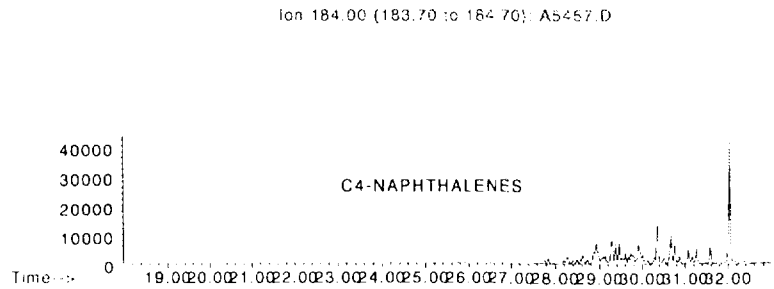
ATTACHMENT 1











ATTACHMENT 1 CONTINUED

Abundance

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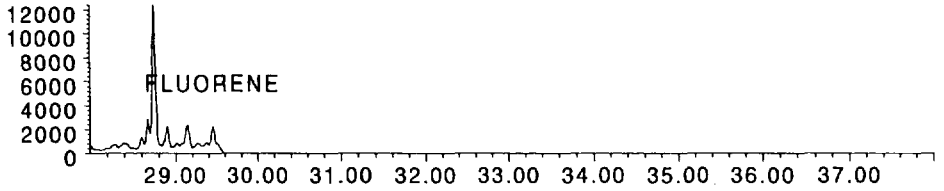

Time-->
Abundance

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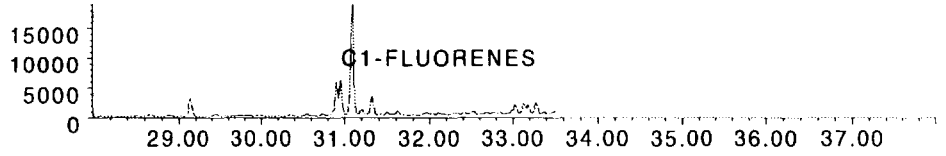

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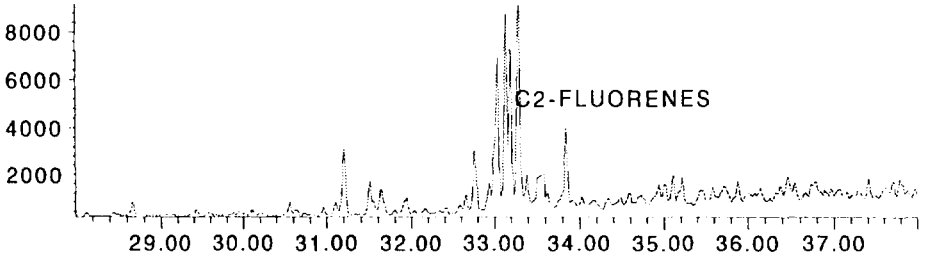

Time-->
Abundance

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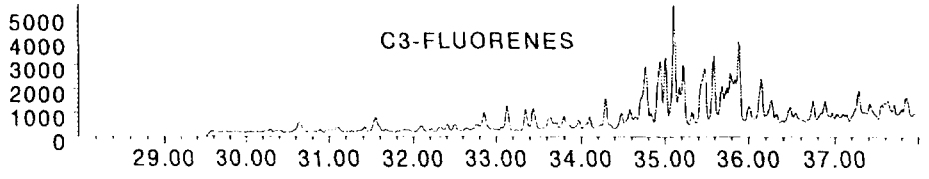

Time-->

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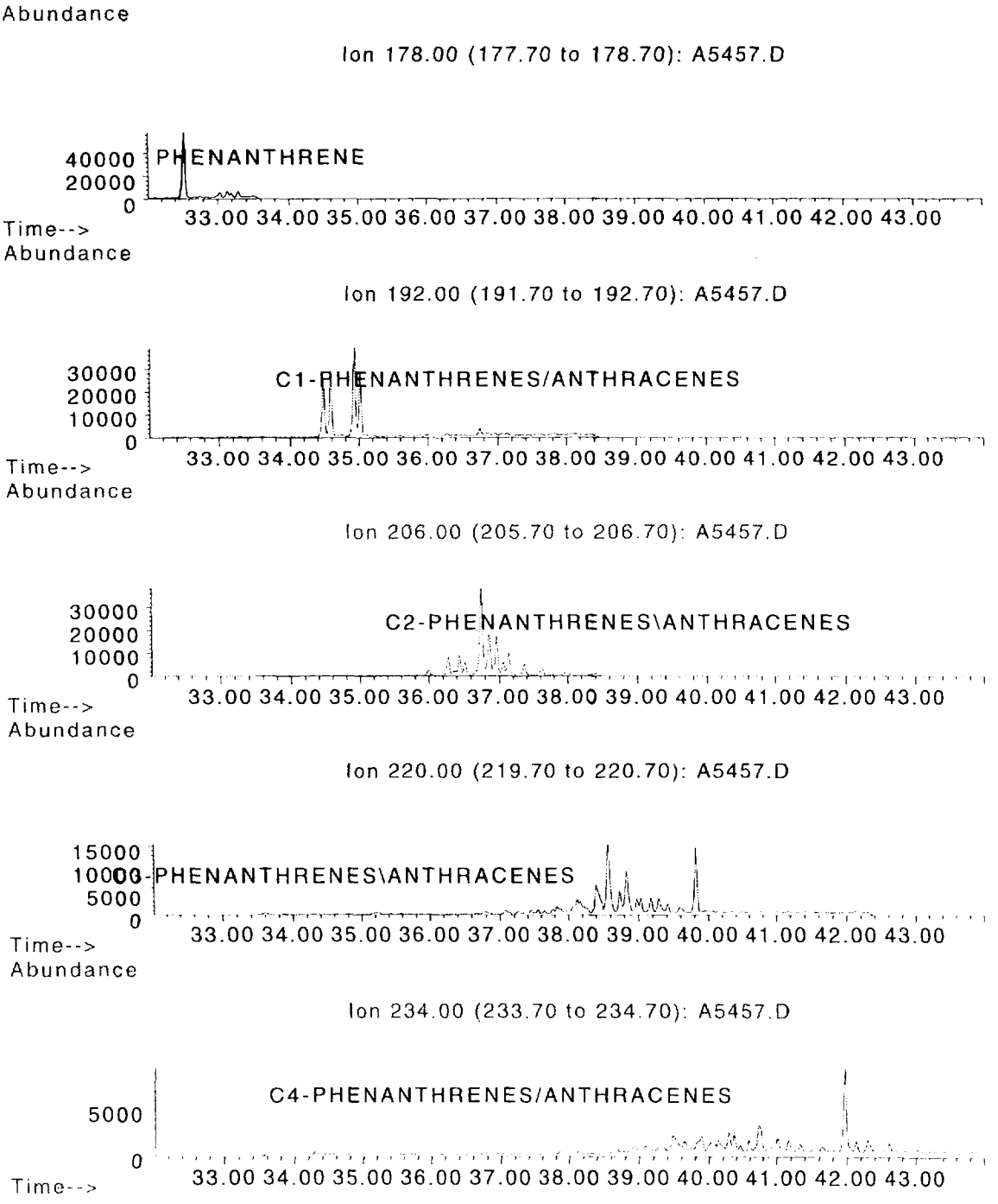

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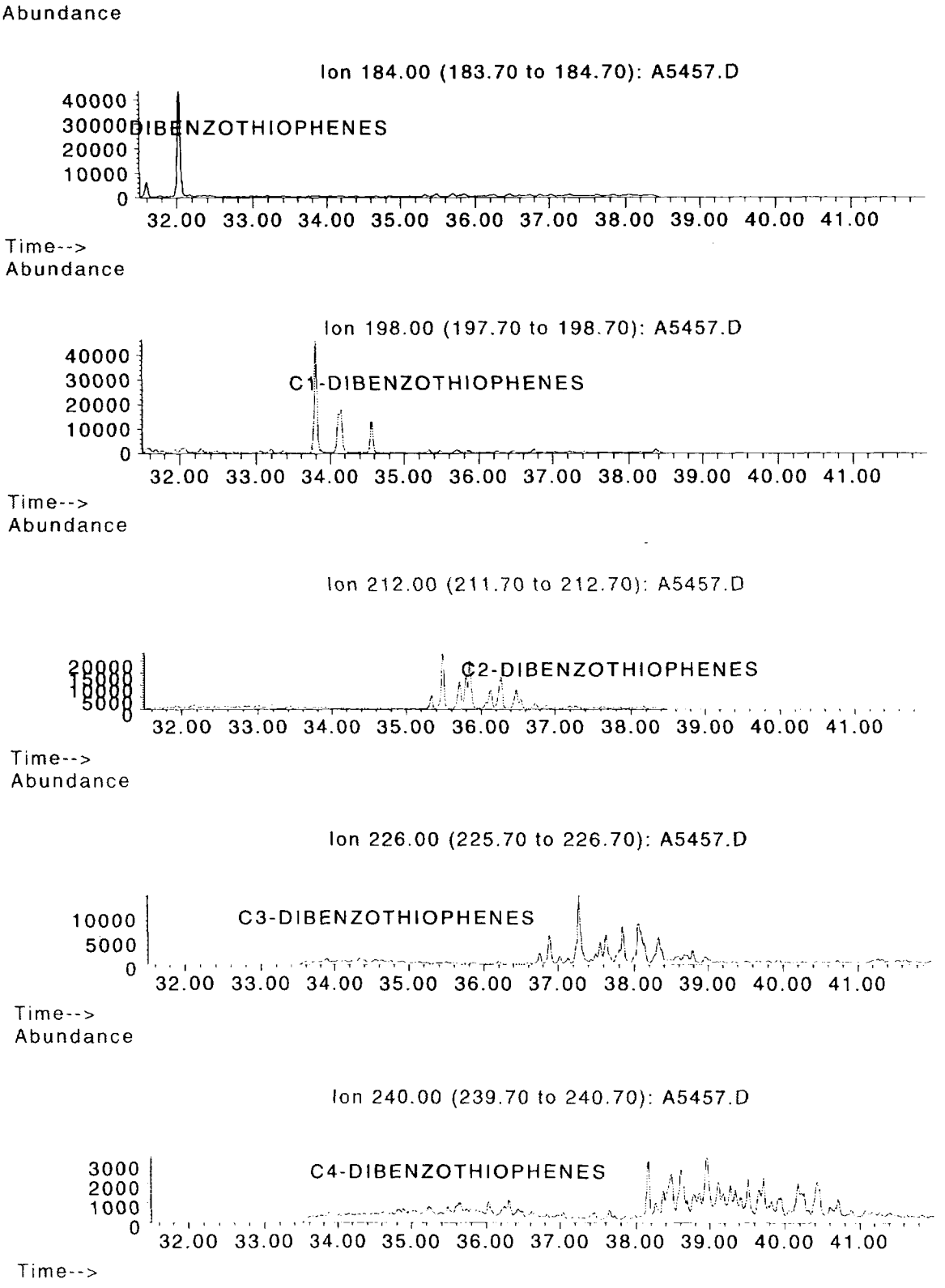

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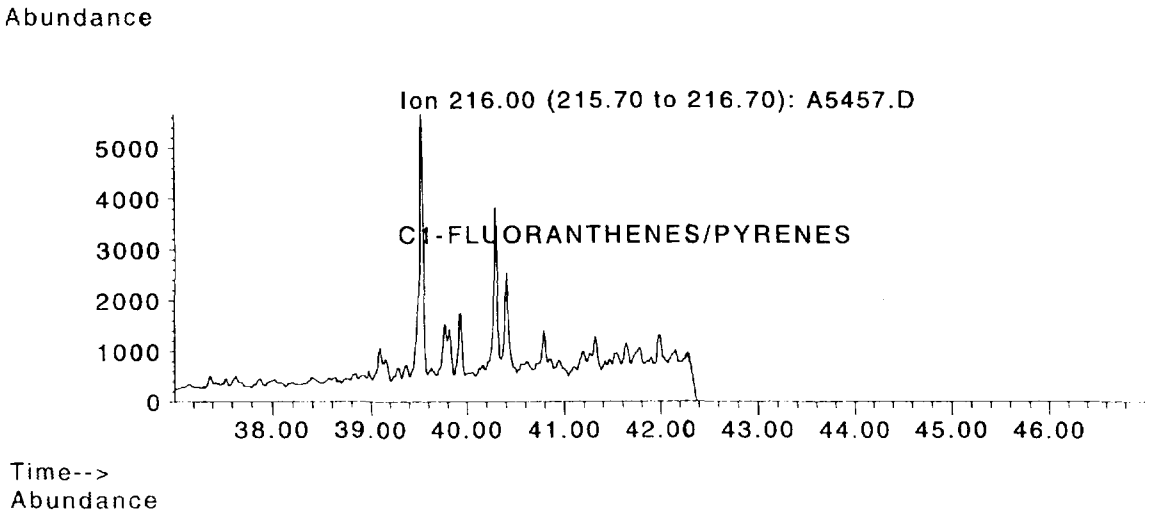

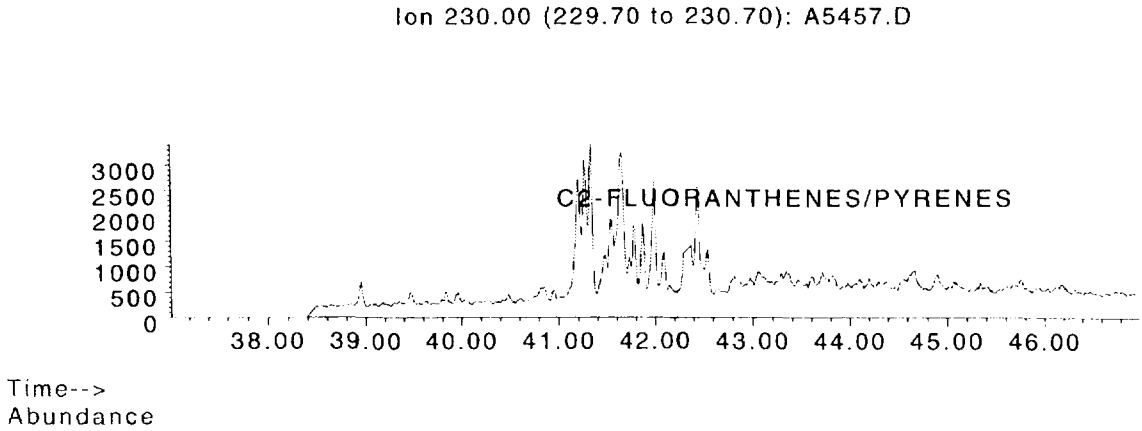

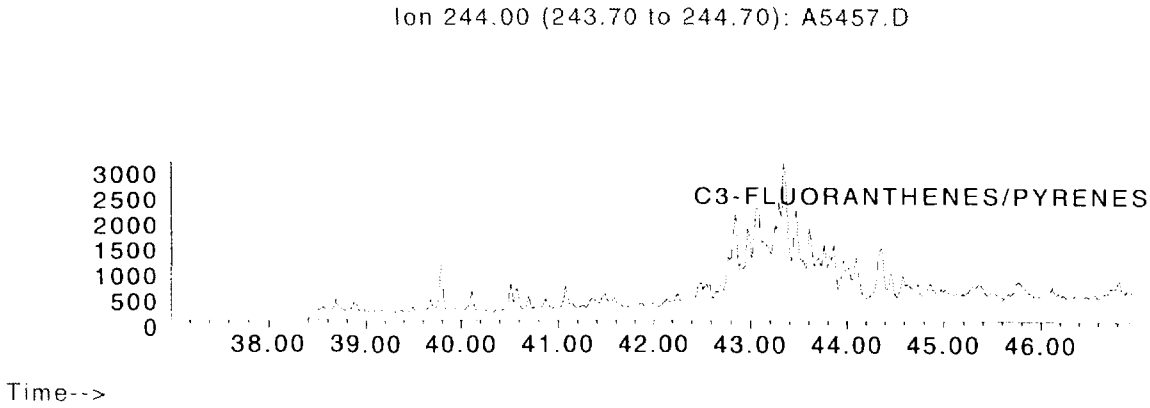

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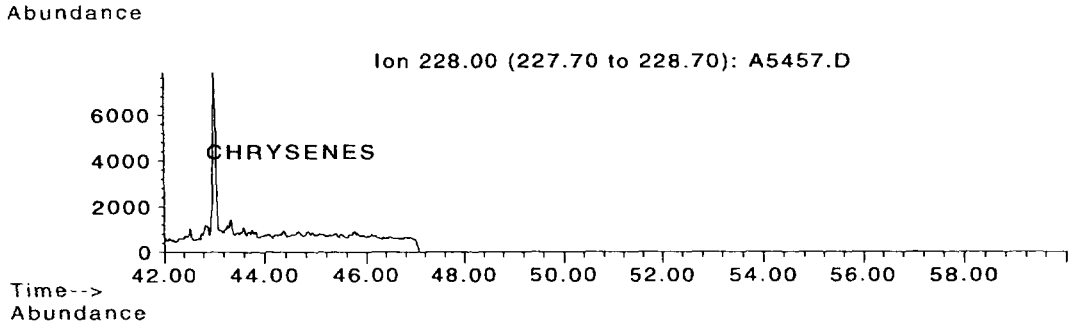

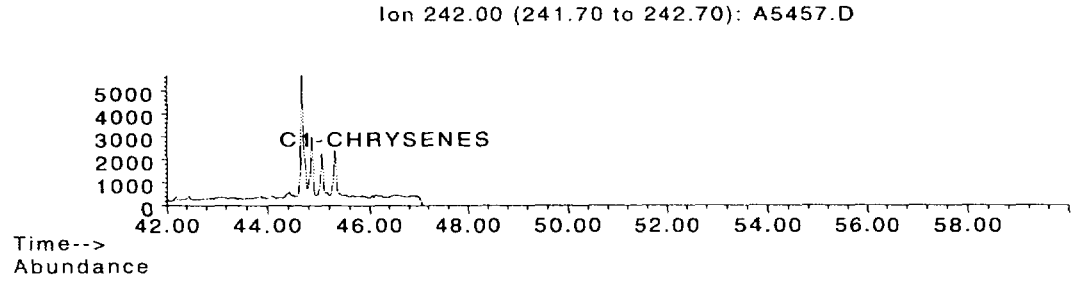

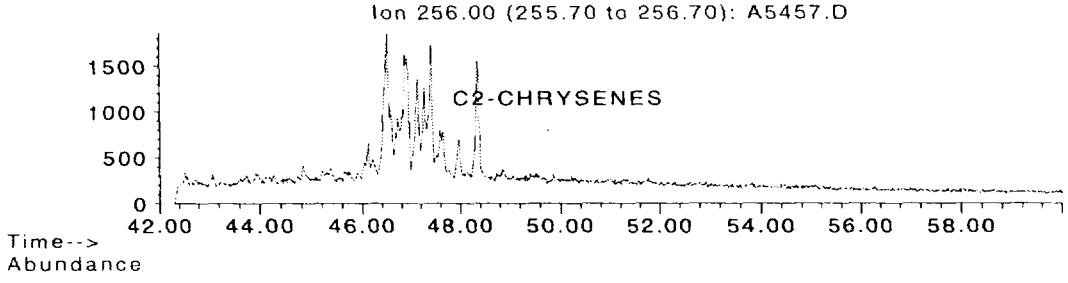

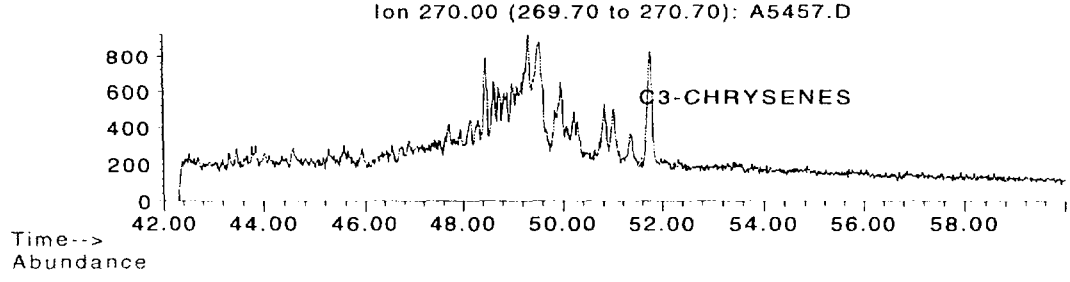

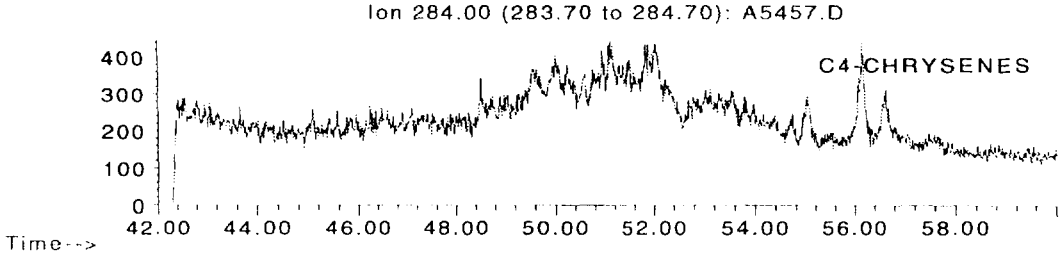

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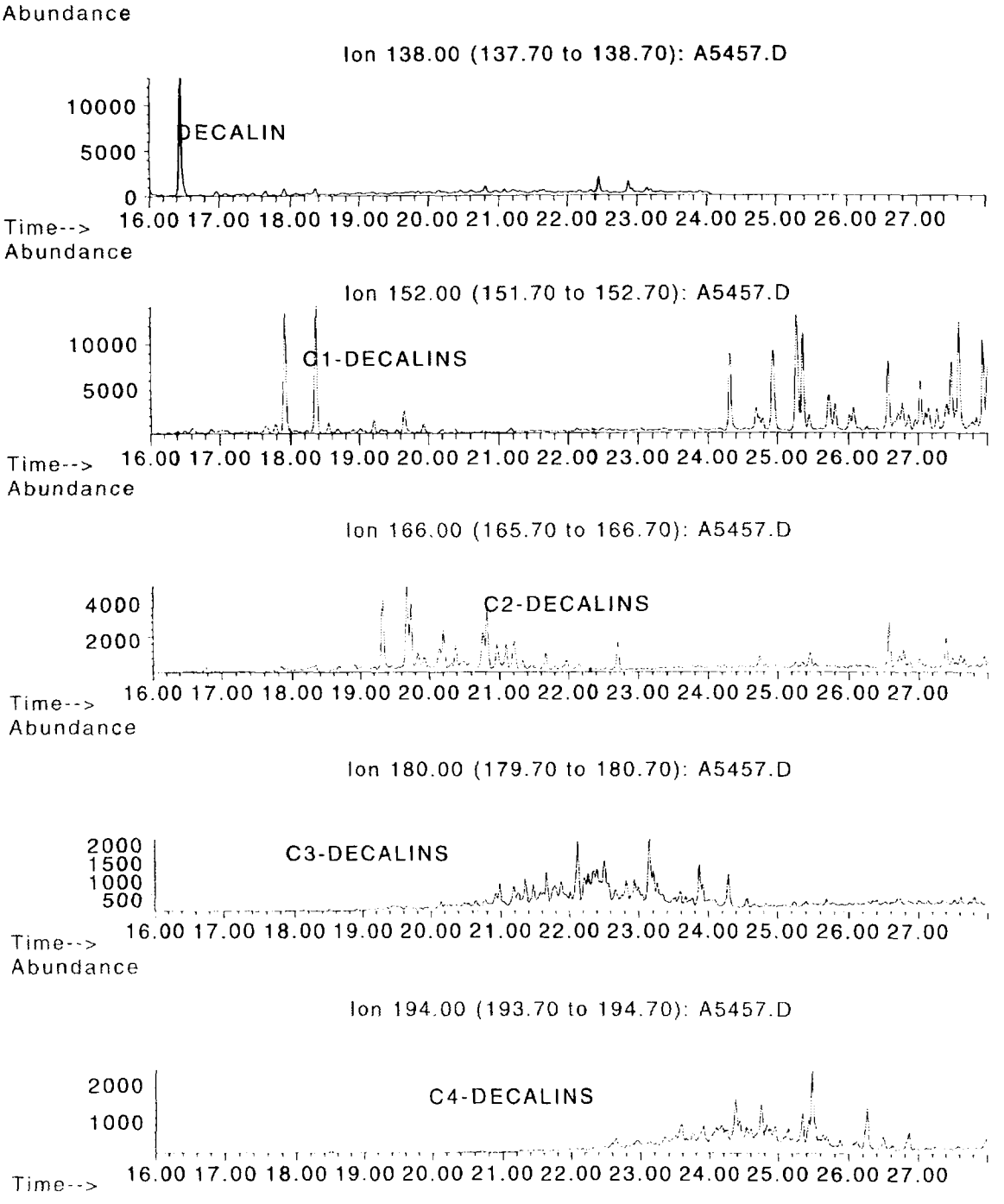


ATTACHMENT 2
BATTELLE DUXBURY OPERATIONS
CERTIFICATE OF TRAINING

FOR

SOP No. _____

Title _____

_____

_____

_____

_____

Trainee: _____

Instructor: _____

Date of Completion
of Proficiency Test _____

_____

TEST RESULTS

APPROVED BY: _____ **DATE:** _____

ORIGINAL

Battelle Duxbury Operations
Standard Operating Procedures

for

TISSUE EXTRACTION FOR TRACE LEVEL SEMI-VOLATILE ORGANIC CONTAMINANT ANALYSIS

Summary of changes in this version: This SOP is the tissue component of what was the combined tissue/sediment extraction SOP. The SOP includes clarifications of the alumina preparation and cleanup steps, and the organization of the SOP has been improved.

1.0 OBJECTIVE

The objective of this document is to define standard procedures for extracting trace levels of semi-volatile organic analytes from tissue matrices for analysis by gas chromatography. The extraction procedures are suitable for analysis of semi-volatile organic pollutants including those listed in EPA Methods 608, 610, 625, 8081, 8082, and 8270. The method may be suitable for other analytes once acceptable extraction efficiency has been demonstrated. Generally, this procedure will be used to prepare extracts that will be further cleaned up by liquid chromatography procedures before analysis.

2.0 PREPARATION

2.1 APPARATUS AND MATERIALS

Apparatus for homogenizing tissue

- Tekmar Tissuemizer with probes, or equivalent
- Teflon jar or centrifuge bottle, 250-mL capacity
- 500-mL Erlenmeyer flask

Apparatus for determining wet weight and dry weight (~~tissue and sediment~~)

- Top-loading balance capable of weighing to 0.01 g (SOP No. 3-160)
- Aluminum weighing pan, stored in an aluminum foil "package" at 105°C
- Stainless steel spatula
- Drying oven maintained at 105-120 °C, Blue M Model SW-17TA or equivalent

Apparatus for determining lipid weight:

- Class A volumetric pipette or 10 mL syringe
- Aluminum weighing pan

Centrifuge

Balance accurate to 0.01 g

Glass wool heated to 400°C for at least 4 h, then stored in a covered glass container at 105°C, or thoroughly solvent cleaned prior to use if wool is temperature sensitive and becomes brittle at 400°C.

Glass fiber filters heated to 400°C for at least 4 h, then stored in aluminum foil "package" at 105°C or thoroughly solvent cleaned prior to use if filters are temperature sensitive and become brittle at 400°C.

19/21-mm chromatography column with ~200 mL reservoir and Teflon stopcock

Kuderna-Danish (K-D) apparatus (listed below) or Turbovap concentration units and tubes

- Reservoir, 500 mL
- Snyder column, three-ball macro
- Concentrator tube, 10- or 20-mL

Hot water bath capable of reaching 100 °C, located in fume hood.

Boiling chips, solvent rinsed

Nitrogen evaporation apparatus, N-Evap or equivalent, with heated water bath maintained at about 25 °C

Glass funnels

Glass graduated cylinders, 100- and 500-mL

Erlenmeyer flasks, 250- and 500-mL

Microliter syringes

2.2 REAGENTS

Dichloromethane (DCM), pesticide grade or equivalent

Milli-Q water

Sodium sulfate--anhydrous, reagent grade, heated to 400°C for at least 4 h, then cooled and stored in a tightly-sealed glass container at room temperature.

Alumina, F-20 (Aldrich Chemical. CAS #B44-28-1. 80-200 mesh).
Activate the alumina by heating in a shallow dish to 400 °C for at least 4 hr. Allow the alumina to cool in an oven (do not keep in open lab atmosphere for extended periods). Store the alumina in a covered glass container (with an activation date label) at 105 °C, and use within 1 week of activation.

Deactivate the alumina prior to use with 2% water. Prepare the 2% deactivated alumina in batches by adding 5 mL of Milli-Q water (accurately measured with a syringe or volumetric pipette) to 250 g of alumina in a 1,000 mL round bottom glass flask. Seal with a glass or Teflon stopper, shake vigorously by hand for 1 min to begin the process. Vent the flask, put the stopper back on, seal with Teflon tape, and allow to *vigorously* mix on a shaker table for 2 hr (± 15 min). Pack columns within 2 hr of completing the deactivation and use the same day. Unused deactivated alumina should be discarded.

Surrogate Internal Standards (SIS) and Recovery Internal Standards (RIS) are project specific and are defined in the project work plan.

2.3 LABWARE PREPARATION

Unless otherwise specified in the project specific work plan, all glassware contacting samples must be cleaned according to laboratory protocols defined in SOP No. 5-216.

2.4 RECORD KEEPING

Samples will be assigned unique identification numbers and logged in on the Chemistry Laboratory Sample Identification Log according to laboratory protocols (SOP No. 6-007).

Analytical data will be reported on appropriate data forms included in the sample batch books. Information to be recorded includes wet and dry weight information, dates of extraction/processing procedures, initials of laboratory personnel who perform the procedures, types and amount of internal standards added to samples, and, if necessary, comments regarding individual samples.

3.0 PROCEDURES

3.1 GENERAL

Samples should be extracted in batches of 20 or fewer field samples unless otherwise stated in the project specific work plan. Quality control samples accompanying each batch may include a procedural blank, laboratory control sample, matrix spike, reference material and/or field sample duplicate. (See Section 5.0 for more detail).

Each sample, including QC samples, should be spiked with SIS. Generally, the amount of SIS is such that the concentration of SIS compounds in the final extract should be approximately 1 µg/mL for GC/MS analysis, and/or 0.05 µg/mL for GC/ECD analysis.

The tissue samples should be ***thoroughly*** homogenized prior to any aliquotting for chemical or physical characterization, to ensure that a representative sub-sample is taken.

3.2 PERCENT MOISTURE DETERMINATION

Weigh approximately 5 g of well mixed tissue homogenate into a pre-weighed, pre-baked, aluminum weighing pan and record to the nearest 0.01 g. Place the sample in a drying oven and dry overnight at ca. 105 °C. After approximately 24 h, allow the sample to cool at room temperature for at least 30 min. Record dry weight to the nearest 0.01 g in sample batch book. Calculate the percent dry weight and/or percent moisture as in Section 4.0.

3.3 EXTRACTION, CONCENTRATION, AND CLEANUP

Tissue Sample Extraction

- Weigh 20-30 g of well mixed tissue into a 250 mL centrifuge jar (Teflon), weighing the sample to the nearest 0.01 g. Record sample weight.

- Add approximately 50 g sodium sulfate, mix well with spatula. Add more sodium sulfate if lumpy — some sodium sulfate should move freely in the solvent indicating that no more water is available.

- Add appropriate SIS and 75 mL DCM. Macerate/extract the sample with Tissumizer (or equivalent) at high speed for 2 min. Avoid spattering the sample.

- Centrifuge the sample for 5 min. at between 1500 and 2500 rpm. Longer or faster (if below 2000 rpm) centrifugation may be needed for samples containing fine particulates or suspended matter. *Carefully* decant the extract into an Erlenmeyer flask and cap the flask with aluminum foil.

- Repeat the maceration/extraction once more with an additional 75 mL DCM. Centrifuge as above and decant extract into Erlenmeyer, combining the sample extract.

- Add 50 mL DCM to the sample, seal centrifuge bottle and shake on a shaker table for about 30 min. Centrifuge as above, then decant solvent into the Erlenmeyer with the rest of the sample extract.

- Determine the lipid weight (DCM extractable) as follows:

  Gently swirl the Erlenmeyer to mix the extract. Mark the meniscus on the outside of the Erlenmeyer. With a clean 10 mL class "A" volumetric pipette or 10 mL syringe, remove 10 mL of the extract and place in a pre-weighed aluminum weighing pan. Cover weighing pan with foil, and allow extract to air dry overnight or until DCM is no longer visible. After drying, weigh pan and record weight. Record the total extract volume (see under Extract Concentration below) and calculate the lipid content as described in Section 4.

- Add approximately 20-50 g sodium sulfate to the Erlenmeyer and swirl. Add more sodium sulfate if sample is lumpy — some sodium sulfate should move freely in the solvent indicating that no additional water is available. Wait approximately 1 hr.

Note: *The final combined extract should not be stored in the Erlenmeyer flask for more than 1 day. If longer storage is needed before completing the sample preparation, the extract should be concentrated to several milliliters (see below), transferred to a glass vial, securely capped, and stored in darkness in a refrigerator or freezer.*

Extract Concentration — K-D Technique

- Transfer the sample extract to a K-D flask and receiver tube. If there is any evidence of particulates the extract must be filtered, using a glass funnel containing a pre-cleaned glass fiber filter, or pre-cleaned glass wool, topped with sodium sulfate. Rinse the Erlenmeyer twice with about 10 mL of DCM and add to K-D.

- Add 3-5 boiling chips to the K-D receiver and insert a Snyder column. Pre-wet the condenser column with approximately 5-mL DCM. Place the K-D apparatus in a hot water bath maintained at 60-65 ° C (monitored by a mercury thermometer), such that the concentrator tube is partially immersed in hot water and the entire lower rounded surface of the flask is bathed in hot water vapor. At the proper rate of evaporation, the balls of the Snyder column will actively chatter, but will not flood with condensed solvent. Continue concentration until the sample volume is reduced to approximately 10 mL.

- Notify the Lab Manager for instructions on how to proceed if the rate of concentration significantly slows down.

- Remove the K-D apparatus and allow it to drain and cool for at least 10 min. Transfer the concentrator tube to the N-Evap unit and concentrate the sample until the volume is 1-2 mL, maintaining a water bath temperature of approximately 25 °C.

- *Lipid weight extract volume determination*: After extract is poured from the original Erlenmeyer, rinse flask and remove sodium sulfate. Add tap water to the meniscus line drawn prior to removal of aliquot for lipid weight determination, and determine, and record, the original volume by measuring it with a 250 mL graduated cylinder. Calculate the lipid weight according to formulae presented in Section 4.0. Also, determine the lipid weight correction factor so the final data can be corrected for the proportion of extract removed for the lipid determination.

Note 1: The water bath for the K-D is maintained at 60-65 ° C for concentrating DCM, 70-75 ° C for concentrating acetone, and a boiling bath is used for concentrating hexane or toluene.

Note 2: Adjust the flow of nitrogen on the N-Evap to a ***gentle*** *stream — do not allow the sample to bubble or splatter, or have a large "dimple" on the surface, as this will result in the loss of sample. A small dimple is expected on the surface of the solvent.*

Extract Concentration — TurboVap Technique (optional)

If specified in the work plan, TurboVap may be used for concentrating the extracts rather than using K-D.

- Transfer sample extract to a 200 mL TurboVap tube with a 1 mL collection reservoir. TurboVap waterbath temperature should be set at approximately 25 °C; nitrogen pressure should be approximately 4 to 6 psi.

- Follow manufacturers instructions for operation and maintenance. After initial TurboVap concentration the extract should be quantitatively transferred to a 4 mL vial (with DCM rinses), and concentrated until the volume is 1-2 mL.

Extract Cleanup — Alumina

Alumina cleanup is routinely performed on tissue extracts. This may be the only cleanup, but is typically a cleanup step that is performed prior to HPLC/GPC cleanup. Sample extracts must be in DCM when loading onto the column, so solvent exchange may be needed if another extraction solvent is used.

Note: Phenol, and C1-C4 phenols, are retained by the alumina column and this cleanup step cannot be used if these compounds are target analytes.

Column Preparation

- Prepare the 2% deactivated F-20 alumina as described in Section 2.2.
- Pack the tip of the 19/21-mm ("fat") chromatography column with a small piece of glass wool. Add approximately 10 mL DCM and tap glasswool with clean glass rod to remove bubbles. Drain solvent into waste cup.
- Add approximately 25 mL DCM to the column. Weigh 40-g, 2% deactivated alumina into a beaker. Add ~50 mL DCM and swirl to remove bubbles. Slowly pour alumina slurry into the column while rinsing beaker with DCM
- Place approximately 1 g of sodium sulfate on top of the alumina. Tap the column to remove bubbles. Drain column to top of packing and discard solvent. Column is now ready for use; columns must be used the day they are prepared.

Column Elution

- Put a clean glass collection flask under the column (e.g., Erlenmeyer flask, K-D apparatus, or TurboVap tube).
- Load the 1 to 2 mL sample extract (must be in DCM) onto the column.
- Slowly drain and stop at the top of the alumina packing. Rinse the sample vial with about 1 mL DCM and load onto column. Slowly drain and stop at the top of the packing. Repeat vial rinse one time.
- Load with 150 mL of DCM.

Note: 150 mL of DCM will effectively elute our standard analytes (PAH, PCB, chlorinated pesticides, phthalates, and LABs). However, endrin aldehyde ***typically*** *elutes with the rest of the pesticides but sometimes has unpredictable alumina retention and may require 250-275 mL DCM to elute. Additionally, coprostanol and androstanol require 225 mL to elute. The project work plan will specify if more than 150 mL should be used to elute the column.*

- Drain the column slowly (about 2 mL/min), collecting the column eluent containing the target analytes, and stop at the top of the column — do not drain the column dry into the collection vial.
- Open up and drain the column dry into a solvent waste jar.

Extract Cleanup — GPC

The sample is now ready for further cleanup and processing. Size exclusion HPLC/GPC cleanup (described in SOP No. 5-191) should be performed unless the work plan specifies to omit this step.

- The DCM extract volume should be adjusted to approximately 900 µL and centrifuged, if necessary, to settle out any particulates. The volume is measured with a gas tight syringe (to within 5 µL), the volume recorded, and the sample transferred to a 2-mL GC vial.

- Process the samples as described in SOP No. 5-191.

Final Preparation

If no further processing is required, the samples should be adjusted to the desired final extract volume (specified in work plan) and prepared for instrumental analysis.

- Adjust the final extract volume using N-Evap concentration. Adjust the flow of nitrogen on the N-Evap to a gentle stream — do not allow the sample to bubble or splatter, or have a large "dimple" on the surface, as this will result in the loss of sample. A small dimple is expected on the surface of the solvent.

Note: *Sample extracts at larger volumes (5 mL or more) and in larger vials (e.g., K-D receiver tubes or GPC/HPLC eluent collection tubes) should be concentrated on the N-Evap with the vials in the water bath at a temperature of 20-25°C (room temperature or thermostatically set at 25°C). Once the sample extracts are at lower volumes and in smaller vials (e.g., 4 mL vials), the N-Evap concentration should occur with the vials out of the water bath.*

- Spike the final extract with the appropriate recovery internal standards (RIS), as listed in the work plan, and split the extract as necessary, transferring the samples to GC vials. The GC/ECD split is solvent exchanged with hexane if this analysis is required, and the sample(s) submitted to the instrument analyst(s) for analysis.

4.0 CALCULATIONS

Calculate percent dry weight with the following equation:

$$\%\ dry\ weight = \frac{aliquot\ dry\ wt.^{a}}{aliquot\ wet\ wt.^{a}} * 100$$

Calculate percent moisture with the following equation:

$$\%\ moisture = \frac{aliquot\ wet\ wt.^{a} - aliquot\ dry\ wt.^{a}}{aliquot\ wet\ wt.^{a}} * 100$$

Calculate sample lipid weight with the following equations:

$$Total\ lipid\ weight\ (mg) = \frac{volume\ of\ sample\ extract\ (mL)}{aliquot\ vol(mL)} * aliquot\ dry\ wt.\ (mg)^{a}$$

[a] corrected for pan weight

Calculate sample dry weight with the following equation:

$$Sample\ dry\ weight\ (g) = \%\ dry\ wt. * sample\ wet\ wt.\ (g)$$

5.0 QUALITY CONTROL

The QC program for each project is defined in the project work plan, which will define the type and amount of internal standards/spiking solutions to be added to the samples, the specific QC samples to be processed, any modifications to the standard QC acceptance criteria, and the corrective action required if QC results do not meet those acceptance criteria.

Quality control methods for this procedure may consist of analysis of procedural blanks (working through procedure without sample; includes extraction solvent, sodium sulfate, copper and SIS), laboratory control samples (like procedural blank but also fortified with target analytes), matrix spikes (tissue fortified with SIS and target analytes; an aliquot of the matrix material will be analyzed separately to determine background levels of contamination), reference materials (SRM, CRM), or field sample duplicates. SOP 7-029 describes the default reporting format and data quality objectives for quality control data.

6.0 TRAINING

Analysts performing the procedure must initially work under the supervision of the Laboratory Manager, task leader, or other designated (trained) individual. Individuals may work independently once they have satisfactorily completed extraction on a set of triplicate laboratory control samples (spiked with a representative suite of analytes), and procedural blank. These samples may be analyzed directly by GC without further processing. Analyses will be considered satisfactory if the recovery of all spiked analytes fall between 70 and 110 percent of the true values and the procedural blank contains less than or equal to

the method detection limit of all analytes of interest, and/or if analytical results demonstrate proficiency as determined and documented by the Lab Manager in the training files.

Upon successful completion of training, a training certificate (Attachment 1) will be issued. The original will be kept on file in the Quality Assurance Office.

7.0 SAFETY

As part of the above training program, the analyst will be made aware of the particular safety concerns of this procedure, including:

- Use of protective eyewear and clothing
- Proper use of fume hoods
- Location and use of laboratory safety devices; eyewashes, emergency showers, fire extinguishers, fire blankets, and first aid kits, as well as MSDS sheets.

8.0 ATTACHMENTS

Attachment 1. Example of Battelle Certificate of Training.

9.0 APPROVALS

APPROVALS

Author	Name	Date 12·9·99
Laboratory Manager	Name	Date 12·9·99
QA Coordinator	Name	Date 12-9-99
Environment Chemistry Section Manager	Name	Date 12/9/99

ATTACHMENT 1

Battelle Duxbury Operations

CERTIFICATE OF TRAINING

Title: **TISSUE EXTRACTION FOR TRACE LEVEL SEMI-VOLATILE ORGANIC CONTAMINANTS**

SOP#: **5-190-06**

Trainee: _____

Instructor: _____

Date SOP was read: _____ (*To be completed by trainee*)

Date training was completed: _____ (*To be completed by instructor*)

APPROVED _____, _____
Laboratory Manager Date

ORIGINAL

Date Effective: Sept. 17, 1998

Battelle Duxbury Operations
Standard Operating procedure

for

HPLC CLEANUP OF SAMPLE EXTRACTS FOR SEMIVOLATILE ORGANIC POLLUTANTS

Summary of Changes in this Version: Several sections have been updated with clarifications and new information. These include the reference to new equipment and modifications to the fractionation time determinations.

1.0 OBJECTIVE

The objective of this document is to describe the cleanup of sample extracts, and other sample solutions, by High Performance Liquid Chromatography (HPLC) prior to analysis for semi-volatile organic compounds. This document describes the operation of the HPLC as it relates to sample cleanup as well as sample preparation procedures associated with HPLC cleanup.

The HPLC system uses a pump which provides a constant isocratic or gradient flow (depending on the application), a UV detector, an autosampler, a programmable fraction collector, a recorder or integrator for monitoring the UV response, and appropriate HPLC column(s) for the particular cleanup/fractionation to be performed.

This SOP focuses on sample cleanup using gel permeation chromatography (GPC) and silica columns, although other columns and instrumental conditions may be used for particular applications once acceptable performance has been demonstrated.

2.0 PREPARATION

2.1 APPARATUS AND MATERIALS

1. A liquid chromatography pump capable of providing a constant flow of 5 or 10 mL/min, depending on the application at hand (Thermo-Separations P1000, Spectra-Physics 8800, or equivalent).

2. HPLC columns:
   (1) **GPC**. A 22.5 × 300 mm, 100 Å pore size, Phenogel GPC/size exclusion column, and one 7.8 × 50 mm Phenogel precolumn (Phenomenex, Rancho Palos Verde, CA), or equivalent. Columns are arranged so that the precolumn can be backflushed periodically. Other column dimensions, and columns connected in series, may be used if acceptable performance is demonstrated. The GPC is commonly used for separating PCB, chlorinated pesticide, PAH, and other semivolatile compounds from other tissue and sediment matrix components, thus purifying the extract before instrumental analysis.

(2) **Silica**. A 25 × 100 mm, 10 μm particle size, 125 Å pore size, μPorasil silica column, and a 25 × 10 mm μPorasil precolumn (Waters Chromatography, Milford, MA), or equivalent. Columns are arranged so that they can automatically be backflushed after each sample (e.g. with an electronic Valco valve). Other column dimensions may be used if acceptable performance is demonstrated. The silica column is commonly used for separating/fractionating hydrocarbons [e.g., traditional F_1 (saturated HCs), F_2 (polyaromatic HCs), and F_3 (polar HCs) fractionations], but can also be used for other compound or class specific sample cleanup (e.g., to isolate PCBs from complex matrices).

(3) **Other**. Other columns may be appropriate for other applications. For instance, a cyano column may be suitable to isolate highly polar compounds from other sample components. The suitability of a column and associated chromatographic conditions for the compounds of interest will be verified and documented before being used on actual project samples.

3. Auto sampler capable of injecting 500 to 600 μL of sample with minimum sample loss in transfer lines, loop flushing, or elsewhere (Spectra-Physics AS3000, Gilson 231/401, or equivalent). A 1000 or 2000 μL sample loop is used to assure that none of the 500 to 600 μL sample is flushed to "waste" during sample loading.

4. Programmable fraction collector which is synchronized with the injector (Foxy 200, Gilson 201, or equivalent).

5. Recorder or integrator (H-P 3396, or equivalent) which is synchronized with the injector and records the signal from a UV detector (Spectra-Physics 8450, or equivalent) fixed at a suitable wavelength for the target compounds (typically 254 nm).

6. Microliter syringes

7. Glass vials with Teflon lined caps
   1.8 mL HPLC autosampler vials, with Teflon/Silicone/Teflon septa
   60 mL fraction collector vials/tubes

2.2 REAGENTS

1. Dichloromethane, hexane, and/or methanol, as applicable (standard laboratory grade). These are the three solvents routinely used with the system. The actual solvent delivery programs vary with application, and the most commonly used programs are discussed in Attachment 5. Non-routine applications and solvent programs will be described in the QAPP.

2. HPLC calibration solution compounds. In general, the first and last eluting compounds should be in the calibration solution [e.g., 4,4'-dibromooctafluorobiphenyl (DBOFB) and perylene or benzo(g,h,i)perylene; d_8-naphthalene or naphthalene and perylene or benzo(g,h,i)perylene; Cl_1(1) and Cl_{10}(209)]. Potential interferants/contaminants from which the samples are to be separated and cleaned (e.g., corn oil and sulfur) may also be added.

2.3 LABWARE PREPARATION

Unless otherwise specified in the QAPP, glass materials contacting samples must be washed, baked and solvent rinsed according to SOP 3-019.

2.4 RECORD KEEPING

Samples and standards processed by HPLC cleanup are recorded on HPLC Sample Fractionation Log Forms (Attachment 1) which are kept in a 3-ring binder along with the sample chromatograms. If re-fractionation of samples is necessary, this will be indicated for the affected samples on the original form and a new form will be completed for the samples to be refractionated. Refractionated samples are assigned the suffix "-R" to distinguish them from the original sample. The sample ID recorded must agree with that used in the Sample Preparation Records. The laboratory sample ID (SOP No. 6-007) will be used to track samples through the laboratory, unless otherwise specified in the QAPP.

The HPLC Sample Fractionation Log Form will be completed for each set of fractionations/analyses. Column type, injection volume, flow rate, mobile phase, any automatic valve switching times, and results of the calibration [i.e., retention times of calibration analytes and fractionation window(s)] are also recorded on this form. After completing the HPLC processing the sample IDs will be marked on the UV chromatograms and the chromatograms filed with the Sample Fractionation Form. System maintenance (e.g., column replacement, replacement of tubing, unclogging of system plugging) will be recorded on the appropriate forms in the HPLC System's Log Book.

3.0 PROCEDURE

3.1 GENERAL

Samples are extracted, or otherwise prepared, according to procedures specified in project-specific plans, run through a rapid clean-up, filtering, and/or centrifugation procedure, as needed, concentrated, and submitted for HPLC cleanup. The retention time window is determined and the instrument calibrated using a calibration standard. A portion of the sample is injected on the HPLC, the UV response monitored, and the analyte fraction(s) collected, concentrated and submitted for further instrumental analysis. The QAPP will identify the specific cleanup procedure (e.g., standard GPC column, F_2-hydrocarbons by silica column). Conditions for non-standard HPLC fractionations will be described in the QAPP.

3.2 STANDARDS PREPARATION

Prepare an HPLC fractionation time standard appropriate for the cleanup/fractionation to be performed. The following are examples. HPLC calibration standards are for qualitative determination of retention times, and can therefore be assigned an expiration date of 3 years.

GPC Column – NS&T type PAH, PCB/pesticide analytes. Prepare a standard of approximately 20 µg/mL DBOFB, 20 µg/mL perylene or 10 µg/mL benzo[g,h,i]perylene, 20 mg/mL corn oil, and 40 µg/mL sulfur in dichloromethane. This standard will be used as a retention time (RT) marker

since DBOFB elutes towards the beginning and perylene/benzo[g,h,i]perylene towards the end of the analytes of interest in these analyses, and corn oil and sulfur represent potential interferences. Perylene elutes just before benzo[g,h,i]perylene. Project-specific RT marker compounds can be used, or project-specific fractionation windows determined, and will then be specified in the QAPP.

Silica Column - PAH (F_2) analytes. Prepare a standard of approximately 20 µg/mL d_8-naphthalene or naphthalene and 10 µg/mL perylene or benzo[g,h,i]perylene in approximately the same solvent composition that will be used for the samples. A mixture of approximately 1:1 hexane/dichloromethane is appropriate for most $F_1/F_2/F_3$ hydrocarbon work. The compound d_8-naphthalene elutes just before naphthalene and perylene elutes just before benzo[g,h,i]perylene. These are the first and last eluting PAH of interest in most of our F_2 analyte analyses. F_1 and F_3 target analytes may not have UV detector response (n-alkanes, for instance, do not), but fractionation windows for such target analytes can be established/calibrated relative to the F_2 calibration analytes by collecting multiple fractions of a standard containing all target analytes, along with the HPLC analysis of the F_2 calibration standard, and analyzing the fractions for the target compounds. Should the standard RT marker compounds not be the first and last eluting analytes of interest for a new analysis, the retention time marker analytes will have to be changed accordingly, and will be specified in the QAPP.

Silica or GPC Column - PCB analytes. Prepare a standard of approximately 20 µg/mL each of $Cl_1(1)$ and $Cl_{10}(209)$ — on the silica column the $Cl_{10}(209)$ is the first eluting peak and $Cl_1(1)$ elutes last. Prepare the standard in the solvent that the samples are to be loaded onto the column in (always hexane for the silica column).

3.3 PRE-HPLC SAMPLE PREPARATION

1. Pre-HPLC sample cleanup and/or filtration may or may not be necessary, depending on the lipid content and amount of particulates in the extracts. Specific cleanup/filtration techniques to be used will be determine on a project by project basis by the project team, and documented in the sample preparation records.

   The following is an example of sample cleanup procedure using alumina that may be suitable to remove excess lipid material from some extracts:

   > Prepare a preliminary cleanup/filtering column for each sample to be processed by pouring 20 g of 2% deactivated alumina into a 19 mm ID chromatography column with a glass wool plug at the bottom. Alumina and glass wool are cleaned and baked out as for standard column chromatography. Fill column with dichloromethane and drain to the top of the column packing.
   >
   > Pour the sample extract through the column and collect the eluent. For pre-concentrated extracts pour an additional 80 mL of dichloromethane through the column and collect with the sample eluent, and for unconcentrated extracts pour 50 mL through and collect with the sample eluent (these elution volumes must be doubled if endrin aldehyde is a target analyte).

   Sample extract centrifugation and filtration may be appropriate to remove particulates, and will be determined on a project by project basis by the Sample Prep Task Leader in consultation with the Project Manager or Laboratory Manager, and will be documented in the sample preparation records.

2. Extracts are concentrated to approximately 900 to 1000 µL using at first a Kuderna-Danish apparatus and then nitrogen evaporation. Transfer the sample to a 1.8 mL HPLC autosampler vial. If particulate matter is present in the sample it may be necessary to filter the sample. Alternatively, the sample may be separated from the particulates by centrifugation and carefully removing the extract with a pipette or syringe, leaving the particulates, and a minimum amount of sample, behind and then dispensing the sample into a 1.8 mL HPLC autosampler vial. It is ***imperative*** that all samples be totally free of particulate matter and suspended solids. The presence of particulates and any project specific sample filtration/separation procedures will be documented in the sample preparation records.

   Measure the sample volume to the nearest 10 µL and record the volume on the HPLC Split Documentation Form (Attachment 2). Place the samples in a refrigerator until shortly before HPLC analysis, but do not store for more than 1 day because evaporation may alter the measured volume.

3.4 HPLC FRACTIONATION TIME DETERMINATION

1. Check the frit and distribution disk on the in-line filter, and replace if needed.

2. Turn on the power for HPLC pump, autosampler, fraction collector, detector, and integrator. Allow at least 15 min for the detector to warm up before use.

3. Rinse out and put fresh solvent in all solvent-holding bottles at least monthly. As needed, de-aerate the mobile phase and autosampler rinse solvent bottle (S-P AS300 only) solvent by bubbling a gentle stream of clean nitrogen or helium through them for at least 1 hour (this can be done while running calibration standards).

4. Purge air out of mobile phase and auto sampler lines by opening the bypass valve and allowing the solvent to be purged into a beaker. If necessary, prime the pump prior to purging.

5. Purge air out of syringe/injector rinse solvent lines and flush several milliliters through the dilutor and sample syringes. *No air bubbles should be visible at top of syringe or elsewhere in sample delivery lines.* Minor bubbles in the solvent deliverey line are generally acceptable.

6. Make sure the waste bottle can hold the volume of mobile phase that will be used.

7. **GPC Column.** Before each day of HPLC analysis with the GPC column, backflush the precolumn with DCM at a flow rate of 2–5 mL/min for 10 to 20 min with the backflushing valve in the RINSE position. Return the valve to the RUN position before analyzing any standards or samples. Alternatively, use an electronic switching valve and program it to backflush the precolumn for 2-4 min at the end of each sample run.

   Note: *Never* backflush the main GPC column. The flow through this column must always be in the forward direction.

   Silica Column. The silica column may need to be backflushed periodically with methanol to remove accumulations of polar compounds, if this is not part of the sample fractionation program. The need, and frequency, of the methanol rinse will depend on the samples analyzed and will be determined by the Project Manager.

8. Program the autosampler (Attachment 3), fraction collector (Attachment 4), HPLC pump (Attachment 5), and integrator for analysis of the HPLC fractionation time standard and the samples to be fractionated. Attachment 5 includes examples of typical solvent delivery conditions for common applications (PAH/PCB/pesticide cleanup by GPC; $F_1/F_2/F_3$ fractionation by silica; PCB cleanup by silica). Attachment 6 summarizes examples of general chromatographic and detector conditions.

   Detailed information on operation and maintenance of the HPLC pump, autosampler, fraction collector, and detector can be found in the respective instrument manuals, which are kept with the instruments.

9. Start the pump. Record the pressure once it has equilibrated. Should the pressure be unacceptably high (over 700 PSI for the GPC column and over 500 for the silica column) it may be necessary to replace the in-line filter, and possibly the precolumn. Other possible causes of elevated pressure are clogged tubing at a fitting (reverse flow through tubing) and accumulations in the detector cell (clean cell with 5-10% nitric acid, as per description in detector manual). Should the pressure be low (under 100 PSI) it may be necessary to replace one of the HPLC pump piston seals.

10. Start the auto sampler program for analysis of HPLC fractionation time standards. Analyze the HPLC fractionation time standard at least twice. If the RT differences between runs is greater than 0.1 min (GPC) to 0.2 min (silica) reanalyze until acceptable RTs are obtained from two consecutive runs. Average the RTs from the reproducible HPLC runs for the retention time marker compounds separately. Pressure readings are recorded on the HPLC Sample Fractionation Log Form at the start of each set of fractionations. Documentation of corrective action and maintenance needed to correct RT drift is kept with the HPLC.

11. Determine the analyte fractionation window, and program the fraction collector accordingly. The programmed fractionation time will be recorded on the HPLC Sample Fractionation Log Form, and the fractionation time programming will be witnessed by another qualified operator, if available. If no-one is available to witness the programming at the beginning of the run then someone should witness what was programmed in at the end of the run before samples are removed from the system.

    The fractionation window depends on the analysis to be performed. The following examples are approximate and actual windows will depend on the column dimensions, analytes, flow rates, solvent composition etc. Other project-specific fractionation times will be documented in the QAPP, or in project records with Project Manager or Laboratory Manager approval.

    GPC. The following table provides general guidelines for establishing a fractionation window for a project. These assume that the commonly used GPC conditions (e.g., single 300 mm long column, pre-column, 5 mL/min flow rate of 100% DCM) are used.

    Attachment 7 lists GPC elution patterns for selected analytes, and this, along with the above table, should be used by Project Managers and other staff to establish project RT windows. If, for instance, the analysis if for PAH only, the fractionation window can be optimized for these compounds.

Example HPLC/GPC Fractionation Windows for Selected Analytes
(Based on Instrument Configuration April, 1998 — applies to both Instruments #1 and #2)

Analytes	Start Time Relative to DBOFB RT	End Time Relative to Perylene RT
PAHs	+0.5 min	+1.5 min
Phenols (C0-C4)	+0.8 min	+0.8 min
PCBs	+0.5 min	+0.5 min
DDTs	–0.5 min	±0 min
LABs	–1.8 min	±0 min
NS&T Pesticides	–0.3 min	+0.5 min
608/8081 Pesticides (without methoxychlor)	–0.8 min	+0.5 min
608/8081 Pesticides (with methoxychlor)	–1.2 min	+0.5 min
NS&T Organic List (PAH/PCB/Pesticides)	–0.5 min	+1.5 min
USACE Organic List (PAH/PCB/Pesticides)[a]	–0.8 min	+1.5 min

[a] Assumes 608/8081 pesticides ***without*** methoxychlor. Would need to start 0.4 min earlier to capture methoxychlor.

The data in the above table and Attachment 7 are for both HPLC System #1 and #2 and are valid on 04/01/98. These values will change with system changes, and should therefore be updated/checked when appropriate, as indicated in Section 5.0.

Silica. Silica-column fractionation windows are highly dependent on HPLC conditions but also on the solvent composition of the sample. Ideally, the standards and the samples will both be of the same solvent composition being delivered by the HPLC pump at the beginning of the run (t0) — this will give the best chromatography, but is not always practical. Because the silica column solvent delivery program generally begins with 100% hexane, amounts of more polar solvents in the sample (e.g., DCM) can significantly affect the retention times of the analytes. It is therefore best if the RT calibration solutions have the same solvent composition as the samples, but this too is not always practical because the chromatography may suffer and it may be difficult to read the calibration chromatogram. It may therefore be appropriate to run combinations of standards dissolved in hexane and the sample solvent (if different) to obtain an assessment of the column retention.

For most standard $F_1/F_2/F_3$ fractionation work samples and standards will be in a solvent composition of approximately 1:1 hexane:dichloromethane. Standard F_2 fraction collection can be performed by collecting from approximately 0.5 min before the RT of d_8-naphthalene or naphthalene to approximately 1.5 min after the RT of perylene or benzo[g,h,i]perylene, if standards and samples are in 1:1 hexane:dichloromethane. However, if the calibration solution is primarily hexane and the samples are primarily DCM the fractionation window may need to be from 3.0 before to 2.0 min after the RTs of the RT analytes, based on a calibration solution that is in 100% hexane. The Project Manager and/or the Lab Manager should be consulted if the solvent composition of the sample and standard are not the same or where questions regarding RT windows occur.

PCB isolation/fractionation with the silica column will need to be equally carefully calibrated. During standard PCB fractionation (100% hexane mobile phase from the beginning to several minutes past the elution of PCBs) the congeners elute between approximately 5 and 8 minutes, and the collection window should begin approximately 1.5 min before the RT of Cl_{10}(209) and end approximately 2.0 minutes after the RT of Cl_1(1).

Fractionation windows may be modified by the Project Manager depending on analytes, matrix, and other project specific considerations.

3.5 SAMPLE FRACTIONATION/CLEANUP

1. Place a rack with the appropriate number of 50 mL fraction collection tubes on the fraction collector, and place the HPLC samples on the auto sampler. Complete the HPLC Sample Fractionation Log Form. Confirm the order and labeling of the HPLC samples (on the autosampler) and fraction collection tubes (on the fraction collector). Include an HPLC fractionation time standard for analysis approximately every 10–12 samples.

2. Check that the HPLC pump, autosampler, fraction collector, and integrator programs are correct.

3. Start the HPLC sample fractionation/cleanup run by starting the autosampler program. It is important that the HPLC pump runs continuously after the running of the retention time standards used for fractionation window determination, because if the pump is shut off and restarted it may equilibrate at a different pressure and RTs may shift.

3.6 POST HPLC SAMPLE PREPARATION FOR INSTRUMENTAL ANALYSIS

The collected fractions are removed from the fraction collector and concentrated by nitrogen evaporation or TurboVap. Concentrate the samples to the appropriate project-specific final volume, transfer to an autosampler vial, add the internal standard, cap, vortex, and submit for instrumental analysis.

Visually (with a reference vial) check the volumes remaining in the autosampler vials as soon as the analysis sequence is completed. If the volume appears to be significantly different from the expected, measure and record all volumes and adjust the HPLC Injection Volume form, as appropriate. Identify the injection problem and resolve.

3.7 REFRACTIONATION OF SAMPLES

Should the fractionated sample not be usable for final instrumental analysis (e.g., pump shut off in the middle of a run), the unfractionated portion of the original extract can be used for HPLC processing. The unfractionated portion is then brought to a volume of approximately 900 to 1000µL with the appropriate solvent, the exact volume indicated on an HPLC Split Documentation Form, and the sample analyzed/fractionated as described in previous sections. Unusable sample fractionations will be so indicated on the HPLC Sample Fractionation Form, explained, dated, and initialed. Refractionations will be indicated as such on the new HPLC Sample Fractionation Form that is prepared, and the number of the refractionation will also be documented (e.g., "-R": first refractionation; "-R2": the second time the samples are refractionated).

4.0 CALCULATIONS

Determination of fractionation window is the only calculation required as part of this procedure, and was discussed in Section 3.4 - items #9 and #10. The RT window may be modified by the Project Manager to what is appropriate for the particular project analytes, HPLC conditions, and sample matrices. The portion of the sample processed through the HPLC is calculated and recorded on the HPLC Split Documentation Form using the following equation.

First Fractionation: $[(IV_1)/(EV_1)] \times 100\%$

Re-fractionation: $[1-((IV_1+AV)/EV_1)] \times [(IV_R)/(EV_R)] \times 100\%$

IV_1 = Sample injection volume (µL) in original/first fractionation
EV_1 = Pre-HPLC sample extract volume (µL) in original/first fractionation
AV = Autosampler volume difference between aspiration and dispension (generally 0 µL with the S-P AS3000 and 20 µL with the Gilson 231/401).
IV_R = Sample injection volume (µL) in re-fractionation
EV_R = Pre-HPLC sample extract volume (µL) in re-fractionation

5.0 QUALITY CONTROL

1. Quality control methods for this procedure will consist of establishing the RTs of the fractionation window calibration analytes before each batch of samples are run by multiple injections of an HPLC fractionation time standard until two analyses result in RT differences of less than 0.1 min (0.2 min for silica column). The average RTs from these analyses will be used to program the fraction collector. An HPLC fractionation time check standard will be analyzed every 10–12 samples to check the RTs of the calibration compounds, which should be ±0.5 min of the RTs used to program the fraction collector.

2. After significant HPLC maintenance (e.g., new columns installed or major system"replumbing"), or when performance dictates, the performance will be checked against recently achieve good performance. This will be done qualitatively/semiquantitatively by comparing the chromatography obtained with the calibration standard (e.g., peak shape and absolute and relative retention times of the first and last eluting target analytes are reviewed). From these data it can be determined if the system is performing well (i.e., good peak shape, approximately the same retention times of the peaks, and approximately the same separation between the peaks).

   If the system appears to be performing poorly or differently from before, this should be investigated and resolved before proceeding with sample analysis. If new chromatography/performance appears to be correct, the procedure described under item #3 below should be performed to check/establish retention time windows. If "atypical" system performance is accepted without additional quantitative check (e.g., item #3 below) this should be documented and approved by the Laboratory Manager or Project Manager before proceeding with sample analysis.

3. After major HPLC system changes (e.g., column(s) from different manufacturer, new column dimensions installed, new dimension or length of lines/tubing), or when performance dictates,

calibrations will be done to determine the size of the fractionation window needed to collect >95% of, at a minimum, the first and last eluting target analytes. This calibration will be done by fractionating an injection of the HPLC fractionation time standard and collecting small fractions (e.g., 0.5 min) from several minutes before to several minutes after the HPLC RTs of these compounds (a single larger window can be collected between the two RT marker analytes) and analyzing the fractions by the appropriate instrumental method.

Alternatively, the recoveries of all analytes will be determined by running an analyte standard mixture through the HPLC fractionation procedure and analyzing for the analytes by the applicable instrumental method. This recovery check will always be carried out if the HPLC cleanup procedure is to be used in the analysis of analytes for which the procedure has previously not been used (i.e., first and last eluting compound is not known). Attachment 7 list recent data from such a calibration.

From these data it can be determined how long before to how long after the RTs of the RT marker compounds fraction collection must be performed (see table on page 7; Section 3.4)

The quality control procedure described under item #1 above will be carried out for every batch of samples. The procedure under item #2 when column replacement, or other significant maintenance has been performed. The procedure described under items #3 will be performed when major HPLC system changes have been performed, when performance dictates, when procedures or analytes have changed, and at the discretion of the Laboratory Manager. Additionally, relevant sample and analysis specific information will be recorded on the HPLC Sample Fractionation Log Form. Other project specific quality control programs will be addressed as specified in the QAPP

6.0 MAINTENANCE

This procedural SOP is not intended to include detail on system maintenance. Maintenance considerations and procedures are presented in the various instrument manuals. However, a few specific items that need be considered are listed below. Non-routine maintenance (e.g., pump seal, column or other hardware replacement, or dislodging of a system clog) should be recorded in the HPLC System's Log Book. Routine daily maintenance, such as pre-column backflushing and filter and frit changes, need not be recorded.

Column/Precolumn Cleanup/Conditioning. Before each day of HPLC analysis with the GPC column, backflush the precolumn with DCM at a flow rate of 2–5 mL/min for 10 to 20 min with thebackflushing valve in the RINSE position. Return the valve to the RUN position before analyzing any standards or samples. Alternatively, a 2-4 min automatic backflush may be programmed in to the end of each sample run, if an electronic switching valve is used. In a 30 min GPC run the backflushing would, typically, be from 26 to 29 min, which allows for a 1 min re-equilibration in the normal flow direction.

With time the backpressure due to accumulations in the precolumn (see below) will become unacceptably high, and the precolumn will need to be replaced. It is important to replace it before the main column begins deteriorating. The main column could last several years if the system is maintained well.

The silica column may need to be backflushed periodically with methanol to remove accumulations of polar compounds, if this is not part of the sample fractionation program. The need, and frequency, of

the methanol rinse will depend on the samples analyzed and will be determined by the Project Manager or Laboratory Manager. This is not performed routinely. The use of methanol and other polar mobile phases, as well as samples with much polar material, will shorten the life of the silica column and precolumn. The precolumn should be replaced every 1 to 2 months (~100 samples), depending on the amount of use, types of samples processed, and mobile phases used. The main column should last 6 to 12 months (~500 samples), but this too will depend on use and types of analyses performed. Column deterioration will be evident by shorter retention times, and worsening chromatography. If column performance is suspected of having worsened it should be replaced or checked with target analytes (Section 5), to determine if adequate separation/cleanup is achieved (e.g., adequate separation between F_1 and F_2 analytes).

High/Low Pressure. The system pressure is checked each day before samples are analyzed. Should the pressure be unacceptably high (over 700 PSI for the GPC column and over 400 PSI for the silica column) it may be necessary to clean or replace the in-line filter and/or pre-column. Accumulations in the main column and detector (clean cell with 5-10% nitric acid, as per description in detector manual) may also cause elevated pressure. Other possible causes of elevated pressure are clogged tubing at a fitting (reverse flow through tubing) and accumulations in the detector cell. Should the pressure be low (under 100 PSI) there may be a leak or it may be necessary to replace one of the HPLC pump piston seals.

7.0 TRAINING

An analyst performing the procedures described in this document must initially work under the supervision of Laboratory Manager, task leader, or another individual designated by the Laboratory Manager. Individuals may work independently once they have satisfactorily completed supervised training. Once training has been completed a Certificate of Training (Attachment 8) will be completed and filed in the Quality Assurance Unit (training files).

8.0 SAFETY

As part of the above-mentioned training program, the analyst will be made aware of the safety concerns of this procedure, including:

Use of protective eyeware and clothing

Location and use of laboratory safety devices - eyewashes, emergency showers, fire extinguishers, fire blankets, and first aid kits.

9.0 ATTACHMENTS

Attachment 1. HPLC Sample Fractionation Log Form

Attachment 2. HPLC Split Documentation Form

Attachment 3. Example Program for the HPLC Auto Sampler

Attachment 4. Example Programs for the Fraction Collector

Attachment 5. Example Programs for the HPLC Pump

Attachment 6. Example HPLC Chromatographic and Detector Conditions

Attachment 7. Example of Approximate GPC Elution Patterns for Selected Analytes

Attachment 8. Certificate of Training

Attachment 9. HPLC Units

APPROVALS

Author: Richard L Motta, 9/11/98

Laboratory Manager: _____, 9-11-98

QA Coordinator: _____, 9-16-98

Environmental Chemistry Section Manager: _____, 9/17/98
Name **Date**

Battelle Duxbury Operations

HPLC SAMPLE FRACTIONATION LOG FORM

Project Name: _____
Project Number: _____ Page _____ of _____

HPLC System ID: _____ Column Type: _____ Inject Volume (µL): _____
Mobile Phase: _____ Flow (mL/min): _____
First Fractionation (check): _____ Refractionation (and #): _____
Operator Name: _____ Date: _____
Program Witnessed by: _____ Date: _____

Sample ID	Report Number	Time	Pressure (PSI)	Comments/Mobile Phase Program[a]

[a] Record (1) Calibration standard RT marker components, retention times, and fractionation window, (2) pump mobile phase program (if not isocratic), (3) valve switching times (if programmed in), and (4) sample-specific and otherwise noteworthy, occurrences and observations.

ATTACHMENT 2

BATTELLE - DUXBURY OPERATIONS

HPLC DILUTION DOCUMENTATION

Project Title

Project Number, Batch ID, Matrix

[illegible]	[illegible]	[illegible]	[illegible]
UN01PB			
UN02BS			
UN03MS			
UN04MSD			
Z0001			
Z0002			
Z0003			
Z0004			
Z0005			
Z0006			
Z0007			
Z0008			
Z0009			
Z0010			
Z0011			
Z0012			
Z0013			
Z0014			
Z0015			
Z0016			
Z0017			
Z0018			
Z0019			
Z0020			
Z0021			
Z0022			
Z0023			
Z0024			
Z0025			
Z0026			
Z0027			
Z0028			
Z0029			
Z0030			
Z0031			

HPLC System ID:_____ Date/Initials:_____

Comments:

EXAMPLE PROGRAM FOR THE HPLC AUTO SAMPLER

Gilson 231/401 Autosampler

LINE #	ENTRY	EXPLANATION
1	RINSE	Moves needle to rinse position
2	DIS 0/5000/9	Rinses needle with 5000 µL solvent
3	TUBE 0/0	Moves needle to injector
4	INJECT 0	Puts injection valve in LOAD position
5	DIS 0/3000/5	Rinses injection port and sample loop with 3000 µL solvent
6	INJECT 1	Puts injection valve into INJECT position
7	RACK CODE 0	Identifies sample rack as type 0
8	FOR B = 1/X	X = the number of rows of sample vials
9	FOR A = 1/Y	Y = the number of sample vials in each row
10	HEIGHT	Lifts needle into air
11	ASPIR 0/50/1	Draws 50 µl of air into sample transfer line
12	WAIT /7/0	Checks to see if pump is running - will not proceed if pump off
13	TUBE A/B	Moves needle to sample vial
14*	ASPIR 0/620/2	Draws 620 µl of sample into sample transfer line *
15	TUBE 0/0	Moves needle to injector
16	INJECT 0	Puts injection valve into LOAD position
17	DIS 0/600/3	Dispenses 600 µl of sample
18	WAIT 5	Waits 0.05 min for pressure to equalize
19	INJECT 1	Injects sample
20	AUXIL 4/1	Sends a start signal to the integrator
21	WAIT 2	Sends a start signal to the integrator
22	AUXIL 4/0	Sends a start signal to the integrator
23	AUXIL 5/1	Sends a start signal to fraction collector
24	WAIT 2	Sends a start signal to fraction collector
25	AUX 5/0	Sends a start signal to fraction collector
26	AUX 6/1	Sends start signal to HPLC pump

27	WAIT 2	Sends start signal to HPLC pump
28	AUX 6/0	Sends start signal to HPLC pump
29	DIS 0/2000/5	Rinses injection port with 2000 µL solvent
30	RINSE	Moves needle to rinse position
31	DIS 0/2000/9	Rinses needle with 2000 µL solvent
32	TUBE 1/5	Moves needle to 1/5 position
33	ASPIR 0/1000/5	Rinses needle with 1000 µL from rinse vial
34	DISP 0/1000/5	Rinses needle with 1000 µL from rinse vial
35**	WAIT 3000	Waits 30 min before continuing **
36	NEXT A	Sends loop back to line #9 of program
37	NEXT B	Sends loop back to line #8 of program
38	TUBE 16/5	Moves needle to row 5 position 16 (position of RT standard)
39	ASPIR 0/520/3	Draws 520 µl of standard into sample transfer line
40	TUBE 0/0	Moves needle to injector
41	INJECT 0	Puts injector in LOAD position
42	DIS 0/500/3	Dispenses 500 µl of standard
43	WAIT 5	Waits 0.05 min for pressure to equalize
44	INJECT 1	Injects sample
45	AUXIL 4/1	Sends start signal to integrator
46	WAIT 2	Sends start signal to integrator
47	AUX 4/0	Sends start signal to integrator
48	AUX 6/1	Sends start signal to HPLC pump
49	WAIT 2	Sends start signal to HPLC pump
50	AUXIL 6/0	Sends start signal to HPLC pump
51	DIS 0/2000/5	Rinses injection port with 2000 µL DCM
52	RINSE	Moves needle to rinse position
53	DIS 0/2000/9	Rinses needle with 2000 µL DCM
54	HOME	Moves needle to "home" position

* Enter ASPIR 0/620/1 for slow aspiration should there be a chance of particulates being present and settling in the sample vial.

** Use approximately 2800-3200 for tissues and approximately 3000-3400 for sediments.

If the electronic backflush valve is used the following lines may be inserted before line 36 of the original program (last page) for backflushing as part of the fractionation program. This would make line 36 in the original program line 40 in a program with the automatic backflushing valve:

LINE #	ENTRY	EXPLANATION
35	WAIT 1300	Enter initial time/duration of flow in forward direction (1300=13 min approximately equal to 14.3 min into the run because of the post-injection valve and syringe rinse routines). This line replaces line 35 in original program
36	AUX 7/1	Sends signal to backflush valve to switch to reverse flow
37	WAIT 1500	Enter time/duration of flow in reverse direction (1500=15 min)
38	AUX 7/0	Sends signal to backflush valve to switch to forward flow
39	WAIT 1100	Enter time/duration of flow in forward direction (1100=11 min) — time remaining of program

EXAMPLE PROGRAM FOR THE HPLC AUTO SAMPLER (continued)

Spectra-Physics AS300 Autosampler

The programming of this autosampler is more straight forward than for the Gilson 231/401 autosampler. The injection conditions are programmed into a method at prompted lines, and the following are the specific considerations that must be addresses and programmed:

- Check and add, as needed, solvent to the solvent rise bottle (same solvent as mobile phase).
- Purge the rinse solvent with helium or nitrogen, and keep a gentle gas steam flowing.
- Flush Prep syringe and Sample syringe thoroughly, in that order. Typically, 5 mL or more is required for each syringe. Make sure there is no air in the syringes or transfer lines — the transfer lines from the rinse bottle are long, so make sure they are completely filled with air bubble-free solvent.
- Check and/or set Injection Volume (typically 600 μL) and Cycle Time (typically 30 min).
- Timed events can be used to automatically backflush the column (typically from 26 min to 29 min in a 30 min GPC run — see Section 3.4).
- Use the "Rainbow" Sample Prep Program (a canned program built into the autosampler) to rinse the syringe between samples and minimize carry-over.
- Use "Viscous" as the sample viscosity and "Normal" as the prep viscosity, and a Flush Volume of 1,000 μL.
- Use the "Pull" injection mode, with an injection range that matches the sample/injection syringe (the syringe on the right when you look at it from the front of the autosampler).
- Use only sample vial crimp caps that have been demonstrated/approved for use with this particular autosampler.

EXAMPLE PROGRAMS FOR THE FRACTION COLLECTOR

Foxy 200 Fraction Collector

PARAMETER SELECT BUTTON	MENU DISPLAYED
Press POWER ON button :	RUN A , EDIT A , LOAD , QUICK REVIEW
Press EDIT A button :	TIME/DROP/VOLUME , PEAK , DELAY , PAGE 2
Press TIME/DROP/VOLUME :	TIME , DROP , VOLUME , 4 COLUMN
Press TIME button :	Will you use time windows yes or no ?
Press YES button :	Programming for : RACK 1 or RACK 2
Press RACK 1 button :	Tube size 25/28. Tubes/Rack=X (enter # of tubes for X)
Press ENTER :	Peak collection options should be set to Peak = Rack 1 or Rack 1&2 (if more than 36 tubes needed), Nonpeak = Valve
Press ENTER :	Peak fraction size should be set to 20:00 min
Press ENTER :	TIME/DROP/VOLUME , PEAK , DELAY , PAGE 2
Press PEAK button :	MODE : Time windows only
Press ENTER :	(set time #1 for start of fraction window according to retention time standards) *
Press ENTER :	(set time #2 for end of fraction window according to retention time standards) *
	Add times for subsequent windows if >1 fraction is to be collected
Press EXIT :	TIME/DROP/VOLUME , PEAK , DELAY , PAGE 2
Press PAGE 2 button :	PAGE 1 , RESTART , INIT. WASTE , PAGE 3
Press RESTART button :	MOMENTARY , LEVEL , TIMED , NONE
Press MOMENTARY :	# of collections before advancing to fresh tubes should be set to 1 (1 is usually blinking)
Press ENTER :	NEXT TUBE , NEXT ROW , SKIP TUBE
Press NEXT TUBE :	PAGE 1 , RESTART , INIT, WASTE , PAGE 3
Press PAGE 3 :	PAGE 2 , OUTPUTS , SERIAL , TECHNICAL
Press TECHNICAL :	EDIT-MENU , DROP-WAIT , PATTERN
Press PATTERN :	STANDARD , ALWAYS LEFT TO RIGHT
Press ALWAYS LEFT TO RIGHT :	EDIT-MENU , DROP-WAIT , PATTERN
Press RUN button :	RUN A , SAVE AS , EDIT A , QUICK REVIEW
Press SAVE AS :	A FULL , B OPEN , C OPEN , D OPEN
Press A FULL button :	READY A , SAVE AS , EDIT A , QUICK REVIEW (PROGRAM IS NOW SAVED)
Press READY A button :	RUN A , EDIT A , LOAD , QUICK REVIEW
Press RUN A or RUN button :	Fraction collector is now waiting for an external start signal

* Times are in minutes and seconds, not in minutes and tenths or hundreds of a minute as with the Gilson autosampler, Gilson fraction collector, and Spectra-Physics pump.

If flashing parameter is desired, ENTER may be pressed as an alternative to the corresponding parameter button.

HITTING STOP TWICE WILL ABORT RUN SHOULD THIS BE NECESSARY!

EXAMPLE PROGRAMS FOR THE HPLC PUMP

The below programs are primarily for the S-P 8800 gradient HPLC pump, but the S-P 1000 can also be used for the isocratic program conditions described.

FRACTIONATION PROGRAM

With the power turned on push the EDIT key for entering your program. Hit ENTER after each entry of a value. The following are example pump programs - other programs may be used, as determined by the Project Manager and specified in the project files:

GPC Column - Isocratic Fractionation/Cleanup

File	1	
MAX PSI	1200	
MIN PSI	100	
TIME 0	%A 100	FLOW 5.0
TIME 30.0*	%A 100	FLOW 5.0

* Slightly longer or shorter run times can be set as needed for any particular sample matrix.

Silica Column - Gradient Fractionation (standard F_1, F_2, F_3 fractionation)

File	4			
MAX PSI	800			
MIN PSI	50			
TIME 0	%A 0*	%B 100*	%C 0*	FLOW 10.0
TIME 1	%A 0.5	%B 99.5	%C 0	FLOW 10.0
TIME 2	%A 2	%B 98	%C 0	FLOW 10.0
TIME 3	%A 4.5	%B 95.5	%C 0	FLOW 10.0
TIME 4	%A 8	%B 92	%C 0	FLOW 10.0
TIME 8	%A 32	%B 68	%C 0	FLOW 10.0
TIME 10	%A 50	%B 50	%C 0	FLOW 10.0
TIME 14	%A 50	%B 50	%C 0	FLOW 10.0
TIME 14.1	%A 100	%B 0	%C 0	FLOW 10.0
TIME 29	%A 100	%B 0	%C 0	FLOW 10.0
TIME 29.1	%A 0	%B 100	%C 0	FLOW 10.0
TIME 40	%A 0	%B 100	%C 0	FLOW 10.0

* Where A=DCM, B=hexane, C=methanol
Flow direction switched at 14 and 29 minutes, typically.

If a methanol column rinse is needed the following may be an appropriate program modifications from after 14.1 minutes

TIME 20	%A 100	%B 0	%C 0	FLOW 10.0
TIME 20.1	%A 0	%B 0	%C 100	FLOW 10.0
TIME 25	%A 0	%B 0	%C 100	FLOW 10.0
TIME 25.1	%A 100	%B 0	%C 0	FLOW 10.0
TIME 35	%A 100	%B 0	%C 0	FLOW 10.0
TIME 35.1	%A 0	%B 100	%C 0	FLOW 10.0
TIME 50	%A 0	%B 100	%C 0	FLOW 10.0

Flow direction switched at 14 and 35 minutes, typically.

Silica Column - PCB Isolation/Fractionation

File	4			
MAX PSI	800			
MIN PSI	50			
TIME 0	%A 0*	%B 100*	%C 0*	FLOW 10.0
TIME 15	%A 0	%B 100	%C 0	FLOW 10.0
TIME 15.1	%A 100	%B 0	%C 0	FLOW 10.0
TIME 20	%A 100	%B 0	%C 0	FLOW 10.0
TIME 20.1	%A 0	%B 0	%C 100	FLOW 10.0
TIME 25	%A 0	%B 0	%C 100	FLOW 10.0
TIME 25.1	%A 100	%B 0	%C 0	FLOW 10.0
TIME 30	%A 100	%B 0	%C 0	FLOW 10.0
TIME 30.1	%A 0	%B 100	%C 0	FLOW 10.0
TIME 45 (or 50)	%A 0	%B 100	%C 0	FLOW 10.0

* Where A=DCM, B=hexane, C=methanol

Flow direction switched at 15 and 30 minutes, typically.

If the PCBs all elute before 10 minutes all times above can be 5 minutes earlier (i.e., end the 100% hexane time at 10.0 minutes instead of 15.0 minutes), thereby shortening the run by 5 minutes. The flow direction switching times would then have to be adjusted accordingly.

GPC COLUMN MANUAL BACKFLUSH PROGRAM

The GPC precolumn will be backflushed each day before any standards or samples are run on the HPLC. The backflushing is done by switching the Rheodyne valve to the "backflush" position and pumping mobile phase through it at a flow rate of 2 mL/min for 15 to 20 min. HPLC pump file 2 may be used for backflushing. Push the EDIT key for entering your program. Hit ENTER after each entry of a value. The following is an example program for isocratic cleanup runs:

File	2	
MAX PSI	500	
MIN PSI	50	
TIME 0	%A 100	FLOW 2.0

CLEANUP PROGRAM

A CLEANUP file program will always be programmed into the S-P 8800 HPLC pump along with the sample fractionation program. This program is used primarily to shut the pump off at the end of an analysis/fractionation sequence, and to a lesser degree to clean up the column. This program will automatically begin when a specified time has elapsed (see below), after the completion of a run, without receiving a new injection. Use File 9 as the cleanup file. Again, enter EDIT and then the program. An example program is:

File	9	
MAX PSI	500	
MIN PSI	20	
TIME 0	%A 100*	FLOW 2
TIME 10	%A 100	FLOW 2
TIME 10.1	%A 100	FLOW 0
TIME 10.2	%A 100	FLOW 0

* Enter the solvent composition used at the beginning of the run (generally 100% DCM for GPC column and 100% hexane for the silica column).
After 10 min of rinsing the column at 2 mL/min the pump will shut off. It is essential that the last two lines in the cleanup program have a flow of 0 for the pump to stop.

To specify that a cleanup program is in use, and the time the pump will pump for after completing a run before switching to the cleanup file (unless it receives an injection before this time has elapsed), enter the following keys and values:

TEST	
CLEANUP	
ENTER	
TIME 30	FILE 9

STARTING THE PUMP FOR ANALYZING RETENTION TIME STANDARDS AND FRACTIONATION OF SAMPLES

A. Make sure the above programming is completed. Changes in the programs will not be accepted unless the pump is stopped and re-INITIALIZED, and stopping and restarting the pump will usually result in slightly different column pressure and retention times, thus rendering any established fractionation window invalid.

B. Make sure all mobile phase and autosampler/dilutor lines are purged. This may require priming the pump before purging.

C. Press the INITIALIZE key, then the number for the Program File desired, the press ENTER. It is critical to enter the number of the desired file to be used, not simply pressing the enter key even if the correct number shows up in parenthesis (default) on the screen - the pump will not load the new edited file unless the ENTER key is pressed.

Once the ENTER key has been pressed the pump will start at the TIME 0 conditions entered in the specified file.

D. At first the status of the pump will read INIT, but when the pressure has equilibrated the status will change to RDY and the red ready light will be on.

E. Allow the pump to pump for a few minutes before beginning to run the first analyses of the day (generally the HPLC fractionation time standards).

F. Should the pump need to be stopped for any reason simply press the STOP key.

EXAMPLE HPLC CHROMATOGRAPHIC AND DETECTOR CONDITIONS

Columns:	**GPC**	One 7.8 × 50 mm Phenogel 100 Å, precolum. One 22.5 × 300 mm Phenogel 100 Å column (or two 22.5 × 250 mm Phenogel 100 Å columns, in series).
	Silica	One 25 × 10 mm µPorasil silica, precolumn. One 25 × 100 mm µPorasil silica column.
Injection Loop Volume:		1000 or 2000 µL
Injection Volume:		500 to 600 µL
Mobile Phase:		A = 100% dichloromethane B = 100% hexane C = 100% methanol
Flow Rate:		5-10 mL/min, depending on the application (generally 5 mL/min for the GPC column and 10 mL/min for the silica column).
Temperature:		ambient
Column Pressure:		700 PSI maximum for GPC column, but for continuous operation the pressure should ideally be kept below 500 PSI. 600 PSI maximum for silica column, but for continuous operation the pressure should ideally be kept below 400 PSI.
Detector:		254 nm, UV.
Detector Range:		0.10 AUFS
Detector Response Time:		1 sec
Detector Mode:		AU (absorbance units)

The chromatographic conditions may change depending on the specific application. Any changes will be specified by the Project Manager and documented in the Project Files.

GPC SYSTEM CALIBRATION

Approximate % of Compounds Eluting in the Different Fractions During Instrument Calibration
(Calibration on Both Instruments #1 and #2 in March, 1998)

	Elution Fraction #										
	1	2	3	4	5	6	7	8	9	10	11
GC/ECD Analytes											
Dursban/Methoxychlor	0	50	50	0	0	0	0	0	0	0	0
Endosulfan sulfate	0	<5	70	25	0	0	0	0	0	0	0
Endisulfan I/Endrin aldehyde/Endrin ketone	0	0	20	55	25	0	0	0	0	0	0
Heptachlor epoxide	0	0	5	70	20	5	0	0	0	0	0
Oxychlordane	0	0	0	55	40	5	0	0	0	0	0
DDT compounds	0	0	10	40	40	10	0	0	0	0	0
Chlorobenzenes/chloroaliphatics	0	0	0	0	5	70	20	5	0	0	0
Other NS&T/608 pesticides	0	0	0	10	35	35	20	0	0	0	0
PCBs	0	0	0	0	15	80	5	0	0	0	0
GC/MS Analytes											
Di-N-octylphathalate	85	15	0	0	0	0	0	0	0	0	0
Bis-(2-ethylhexyl)phathalate	80	20	<5	0	0	0	0	0	0	0	0
Butylbenzylphthalate	80	20	<5	0	0	0	0	0	0	0	0
Di-N-butylphathalate	75	20	5	0	0	0	0	0	0	0	0
Diethylphathalate	5	85	10	0	0	0	0	0	0	0	0
Dimethylphathalate	0	35	65	0	0	0	0	0	0	0	0
Isophorone	0	10	80	10	0	0	0	0	0	0	0
Phenols (C0-C4)	0	0	0	0	0	60	40	0	0	0	0
Chloronaphthalenes	0	0	0	0	0	75	25	0	0	0	0
"Standard" PAHs (except those listed below)	0	0	0	0	5	80	15	0	0	0	0
Perylene/Benzo(e)pyrene/Benzo(a)pyrene	0	0	0	0	0	30	45	25	0	0	0
Benzo(g,h,i)perylene	0	0	0	0	0	5	40	30	20	0	0
GC/HPLC Analytes											
"Standard" SJRWMD Chlorophenolics	0	0	0	0	10	80	10	0	0	0	0
3,4,6-trichlorocatechol/Tetrachlorocatechol	Do not elute										

Fraction #1 −1.5 min of RT of DBOFB to −1.0 min of RT of DBOFB
Fraction #2 1.0 min of RT of DBOFB to −0.5 min of RT of DBOFB
Fraction #3 −0.5 min of RT of DBOFB to ±0.0 min of RT of DBOFB
Fraction #4 ±0.0 min of RT of DBOFB to +0.5 min of RT of DBOFB
Fraction #5 +0.5 min of RT of DBOFB to +1.0 min of RT of DBOFB
Fraction #6 +1.0 min of RT of DBOFB to ±0.0 min of RT of Perylene
Fraction #7 ±0.0 min of RT of Perylene to +0.5 min of RT of Perylene
Fraction #8 +0.5 min of RT of Perylene to +1.0 min of RT of Perylene
Fraction #9 +1.0 min of RT of Perylene to +1.5 min of RT of Perylene
Fraction #10 +1.5 min of RT of Perylene to +2.0 min of RT of Perylene
Fraction #11 +2.0 min of RT of Perylene to +2.5 min of RT of Perylene

Work performed on both HPLC Units #1 and #2 with 300×21.2mm main column, 50×7.8mm pre-column, 5 mL/min DCM. Results apply to system configurations on April 1, 1998.

ATTACHMENT 8

Battelle Duxbury Operations

CERTIFICATE OF TRAINING

FOR

SOP NO. 5-191-06

TITLE: HPLC CLEANUP OF SAMPLE EXTRACTS FOR SEMIVOLATILE ORGANIC POLLUTANTS

TRAINEE: _____

INSTRUCTOR: _____

DATE OF COMPLETION OF TRAINING: _____

APPROVED: _____ _____

Name Date

ATTACHMENT 9

Defined Units:

Unit #1: Gilson Model 231 Autosampler (this removes an extra 20 uL during injection)
Thermoseperations P1000 Pump
Spectraphysics SP8450 UV Detector
Foxy 200 fraction Collector
HP integrator

Unit #2: Thermoseperations AS3000 Autosampler
Spectrophysics 8800 Pump
Alltech Model 450 UV detector
Foxy 200 fraction Collector
HP integrator

Battelle Duxbury Operations
Standard Operating Procedures

for

SOIL/SEDIMENT EXTRACTION FOR TRACE LEVEL SEMI-VOLATILE ORGANIC CONTAMINANT ANALYSIS

Summary of changes in this version: This SOP is the soil/sediment component of what was the combined tissue/soil/sediment SOP. The SOP includes clarifications of the alumina and activated copper preparation and cleanup steps, and the organization of the SOP has been improved.

1.0 OBJECTIVE

The objective of this document is to define standard procedures for extracting trace levels of semi-volatile organic analytes from soil/sediment matrices for analysis by gas chromatography. The extraction procedures are suitable for analysis of semi-volatile organic pollutants including those listed in EPA Methods 608, 610, 625, 8081, 8082, and 8270. The method may be suitable for other analytes once acceptable extraction efficiency has been demonstrated. Generally, this procedure will be used to prepare extracts that will be further cleaned up by liquid chromatography procedures before analysis.

Note: Soils originate on land (terrestrial environments) and are typically dry mixtures of geological and organic materials, while sediments originate from the bottom of open aqueous environments (e.g., coast, rivers, lakes) and are typically wet mixtures; both will be treated the same in this SOP.

2.0 PREPARATION

2.1 APPARATUS AND MATERIALS

Apparatus for soil/sediment sample extraction

- Orbital shaker table (Lab-Line Instruments Inc. Model 3520 or equivalent) or roller extractor
- Teflon jar or centrifuge bottle; 250-mL capacity
- Optional: Disposable glass jar with Teflon lined cap; 250-mL capacity
- 500-mL Erlenmeyer flask

Apparatus for determining wet weight and dry weight

- Top-loading balance capable of weighing to 0.01 g (SOP No. 3-160)
- Aluminum weighing pans, stored in an aluminum foil "package" at 105°C
- Stainless steel spatula
- Drying oven maintained at 105-120 °C, Blue M Model SW-17TA or equivalent

Centrifuge

Balance accurate to 0.01 g

Glass wool heated to 400°C for at least 4 h, then stored in a covered glass container at 105°C, or thoroughly solvent cleaned prior to use if wool is temperature sensitive and becomes brittle at 400°C.

Glass fiber filters heated to 400°C for at least 4 h, then stored in aluminum foil "package" at 105°C or thoroughly solvent cleaned prior to use if filters are temperature sensitive and become brittle at 400°C.

19/21-mm chromatography column with ~200 mL reservoir and Teflon stopcock

Kuderna-Danish (K-D) apparatus (listed below) or Turbovap concentration units and tubes

- Reservoir, 500 mL
- Snyder column, three-ball macro
- Concentrator tube, 10- or 20-mL

Hot water bath capable of reaching 100 °C, located in fume hood.

Boiling chips, solvent rinsed

Nitrogen evaporation apparatus, N-Evap or equivalent, with water bath maintained at about 25 °C

Glass funnels

Glass graduated cylinders, 100- and 500-mL

Erlenmeyer flasks, 250- and 500-mL

Microliter syringes

2.2 REAGENTS

Dichloromethane (DCM), pesticide grade or equivalent

Acetone, pesticide grade or equivalent

Milli-Q and de-ionized (DI) water

HCl, 12N

Sodium sulfate--anhydrous, reagent grade, heated to 400°C for at least 4 h, then cooled and stored in a tightly-sealed glass container at room temperature.

Alumina, F-20 (Aldrich Chemical. CAS #B44-28-1. 80-200 mesh).
Activate the alumina by heating in a shallow dish to 400 °C for at least 4 hr. Allow the alumina to cool in an oven (do not keep in open lab atmosphere for extended periods). Store the alumina in a covered glass container (with an activation date label) at 105 °C, and use within 1 week of activation.

Deactivate the alumina prior to use with 2% water. Prepare the 2% deactivated alumina in batches by adding 5 mL of Milli-Q water (accurately measured with a syringe or volumetric pipette) to 250 g of alumina in a 1,000 mL round bottom glass flask. Seal with a glass or Teflon stopper, shake vigorously by hand for 1 min to begin the process. Vent the flask, put the stopper back on, seal with Teflon tape, and allow to *vigorously* mix on a shaker table for 2 hr (± 15 min). Pack columns within 2 hr of completing the deactivation and use the same day. Unused deactivated alumina should be discarded.

Granular Copper, 99% pure (copper J.T. Baker CAS #7440-50-8 or equivalent), activated.
To activate copper: add 20-25 g copper to a beaker (should be no more than 1 inch "height" of copper). Add Milli-Q or DI water to beaker, then add an equal volume of 12N HCl. Stir the mixture for 2-3 min (until uniformly bright copper colored) with a spatula, and decant to acid waste. Rinse with Milli-Q or DI water until there is no evidence of acid (typically at least 8-10 times), rinse with acetone until there is no evidence of water (typically at least 3-5 times), and rinse with DCM until there is no evidence of acetone (typically at least 3-5 times). If the DCM appears cloudy after the first addition, there is still water present in the copper; repeat process from the addition of acetone. Activate copper no more than one hour before use. Unused activated copper should be discarded.

Surrogate Internal Standards (SIS), Recovery Internal Standards (RIS) and target compound spiking solutions are project specific and are defined in the project work plan.

2.3 LABWARE PREPARATION

Unless otherwise specified in the project specific work plan, all glassware contacting samples must be cleaned according to laboratory protocols defined in SOP No. 5-216.

2.4 RECORD KEEPING

Samples will be assigned unique identification numbers and logged in on the Chemistry Laboratory Sample Identification Log according to laboratory protocols (SOP No. 6-007).

Analytical data will be reported on appropriate data forms included in the sample batch books. Information to be recorded includes wet and dry weight information, dates of extraction/processing procedures, initials of laboratory personnel who perform the procedures, types and amount of internal standards added to samples, and, if necessary, comments regarding individual samples.

3.0 PROCEDURES

3.1 GENERAL

Samples should be extracted in batches of 20 or fewer field samples unless otherwise stated in the project specific work plan. Quality control samples accompanying each batch may include a procedural blank, laboratory control sample, matrix spike, reference material and/or field sample duplicate. (See Section 5.0 for more detail).

Each sample, including QC samples, should be spiked with SIS. Generally, the amount of SIS is such that the concentration of SIS compounds in the final extract should be approximately 1 µg/mL for GC/MS analysis, and/or 0.05 µg/mL for GC/ECD analysis.

The soil/sediment samples should be thoroughly homogenized prior to any aliquotting for chemical or physical characterization. Any overlying water on samples should be decanted prior to stirring and subsampling unless otherwise specified in the project work plan.

3.2 PERCENT MOISTURE DETERMINATION

Weigh approximately 10 g of well mixed soil/sediment, into a pre-weighed, pre-baked, aluminum weighing pan and record to the nearest 0.01 g. Place the sample in a drying oven and dry overnight at ca. 105 °C. After approximately 24 h, allow the sample to cool at room temperature for at least 30 min. Record dry weight to the nearest 0.01 g in sample batch book. Calculate the percent moisture as described in Section 4.

3.3 EXTRACTION, CONCENTRATION, AND CLEANUP

Soil/Sediment Sample Extraction

- Weigh 30 to 40 g (defined in work plan) of wet, well mixed, soil/sediment into a 250 mL Teflon jar or Teflon centrifuge bottle (or glass jar with Teflon lined cap) to the nearest 0.01 g. Record the weight.

- Add approximately 60 g sodium sulfate, mix well with spatula. Add appropriate SIS and 100 mL DCM. Add more sodium sulfate if sample is lumpy — some sodium sulfate should move freely in the solvent indicating that no additional water is available. Hardened sodium sulfate should be broken up periodically to ensure optimum extraction and efficient solvent rinsing of the sample.

- Cap bottle and shake by hand until contents are loose. Shake sample on a shaker table for at least 12 hr (overnight), checking the bottles after 30-60 min and "breaking up" the sample, if needed.

- Centrifuge the sample for 5 min. at 1500-2000 rpm if in Teflon jar or centrifuge bottle. Longer centrifugation may be needed for samples with fine particulates. If a glass jar is used for the extraction, allow to settle for at least 10 min on the lab bench. *Carefully* decant the extract into an Erlenmeyer flask and cap with aluminum foil.

- Repeat the extraction with an additional 100 mL DCM for approximately 4 hr, after first "breaking up" the sample, if needed (i.e., ensure that sample is free-flowing when swirled). Centrifuge (or allow to settle) and combine the extracts in the Erlenmeyer flask.

- Repeat the extraction a third time with 100 mL DCM for ca. 0.5 hr, after first "breaking up" the sample, if needed. Centrifuge sample (or allow to settle) and decant and combine the extracts in the Erlenmeyer flask. Break up, if needed, and perform a final rinse of the extracted solid sample mixture with 25 mL of DCM, combining it with the rest of the extract in the Erlenmeyer flask.

- Add approximately 50 g of sodium sulfate to the combined extract, swirl, and allow to sit for at least 15 min. Add more sodium sulfate if it all becomes lumpy — some sodium sulfate should move freely in the solvent indicating that no additional water is available.

Note 1: *One of the first two extractions must last for approximately 12 hr, or more. The other extraction must last 4 hr, or longer. The third extraction is essentially a rinse; a shaking period of approximately 0.5 hr should be performed.*

Note 2: *The final combined extract should not be stored in the Erlenmeyer flask for more than 1 day. If longer storage is needed before completing the sample preparation, the extract should be concentrated to several milliliters (see below), transferred to a glass vial, securely capped, and stored in darkness in a refrigerator or freezer.*

Extract Concentration — K-D Technique

- Transfer sample extract to a K-D flask and receiver tube. If there is any evidence of particulates the extract must be filtered, using a glass funnel containing a pre-cleaned glass fiber filter, or pre-cleaned glass wool, topped with sodium sulfate. Rinse the Erlenmeyer twice with about 10 mL of DCM and add to K-D.

- Add 3-5 boiling chips to the K-D receiver and insert a Snyder column. Pre-wet the condenser column with approximately 5-mL DCM. Place the K-D apparatus in a hot water bath maintained at 60-65 ° C (monitored by a mercury thermometer), such that the concentrator tube is partially immersed in hot water and the entire lower rounded surface of the flask is bathed in hot water vapor. At the proper rate of evaporation, the balls of the Snyder column will actively chatter, but will not flood with condensed solvent. Continue concentration until the sample volume is reduced to approximately 10 mL.

- Notify the Lab Manager for instructions on how to proceed if the rate of concentration slows down significantly.

- Remove the K-D apparatus and allow it to drain and cool for at least 10 min. Transfer the concentrator tube to the N-Evap unit and concentrate the sample until the volume is 1-2 mL, maintaining a water bath temperature of approximately 25 °C.

Note 1: *The water bath for the K-D is maintained at 60-65 ° C for concentrating DCM, 70-75 ° C for concentrating acetone, and a boiling bath is used for concentrating hexane or toluene.*

Note 2: *Adjust the flow of nitrogen on the N-Evap to a* ***gentle*** *stream — do not allow the sample to bubble or splatter, or have a large "dimple" on the surface, as this will result in the loss of sample. A small dimple is expected on the surface of the solvent.*

Extract Concentration — TurboVap Technique (optional)

If specified in the work plan, TurboVap may be used for concentrating the extracts rather than using K-D.

- Transfer sample extract to a 200 mL TurboVap tube with a 1 mL collection reservoir. TurboVap waterbath temperature should be set at approximately 25 °C; nitrogen pressure should be approximately 4 to 6 psi.

- Follow manufacturers instructions for operation and maintenance. After initial TurboVap concentration the extract should be quantitatively transferred to a 4 mL vial (with DCM rinses), and concentrated until the volume is 1-2 mL.

Extract Cleanup — Alumina

Alumina cleanup is routinely performed on soil/sediment extracts. This may be the only cleanup, but is typically a cleanup step that is performed prior to HPLC/GPC cleanup. Sample extracts must be in DCM when loading onto the column, so solvent exchange may be needed if another extraction solvent is used.

Note: Phenol, and C1-C4 phenols, are retained by the alumina column and this cleanup step cannot be used if these compounds are target analytes.

Column Preparation

- Prepare the 2% deactivated F-20 alumina as described in Section 2.2.

- Pack the tip of the 19/21-mm ("fat") chromatography column with a small piece of glass wool. Add approximately 10 mL DCM and tap glasswool with clean glass rod to remove bubbles. Drain to waste.

- Add approximately 25 mL DCM to the column. Weigh 20-g, 2% deactivated alumina into a beaker. Add ~30 mL DCM and swirl to remove bubbles. Slowly pour alumina slurry into the column while rinsing beaker with DCM

- Place approximately 1 g of sodium sulfate on top of the alumina. Tap the column to remove bubbles. Drain column to top of packing and discard solvent. Column is now ready for use; columns must be used the day they are prepared.

Column Elution

- Put a clean glass collection flask under the column (e.g., Erlenmeyer flask, K-D apparatus, or TurboVap tube).

- Load the 1 to 2 mL sample extract (must be in DCM) onto the column.

- Slowly drain and stop at the top of the alumina packing. Rinse the sample vial with about 1 mL DCM and load onto column. Slowly drain and stop at the top of the packing. Repeat vial rinse one time.

- Load with 100 mL of DCM.

Note: 100 mL of DCM will effectively elute our standard analytes (PAH, PCB, chlorinated pesticides, phthalates, and LABs). However, endrin aldehyde ***typically*** *elutes with the rest of the pesticides but sometimes has unpredictable alumina retention and may require 175-200 mL DCM to elute. Additionally, coprostanol and androstanol require 150 mL to elute. The project work plan will specify if more than 100 mL should be used to elute the column.*

- Drain the column slowly (about 2 mL/min), collecting the column eluent containing the target analytes, and stop at the top of the column — do not drain the column dry into the collection vial.

- Open up and drain the column dry into a solvent waste jar.

Extract Cleanup — Copper Treatment

Activated granular copper (prepared as described in Section 2.2) is added to the extract to remove sulfur, which interferes with GC analyses.

- Concentrate the alumina purified extract by K-D or TuboVap (described earlier) to a volume of approximately 10 mL.

- Add a "scoop" (about 1-2 g) of activated granular copper to the extract using a clean stainless steel spatula. Allow to react for at least 10 min and observe. Add additional activated copper if the copper in the extract has turned black color (sulfur causes the copper to become black), and observe.

- Repeat the activated copper addition step until the lastly added copper remains unchanged in color. The extracts should remain in the vial with copper for at least 1 hour, but not over night (extended contact with copper is not good for some less stable compounds).

Extract Cleanup — GPC

The sample is now ready for further cleanup and processing. Size exclusion HPLC/GPC cleanup (described in SOP No. 5-191) should be performed unless the work plan specifies to omit this step.

- The DCM extract volume should be adjusted to approximately 900 µL and centrifuged, if necessary, to settle out any particulates. The volume is measured with a gas tight syringe (to within 5 µL), the volume recorded, and the sample transferred to a 2-mL GC vial.

- Process the samples as described in SOP No. 5-191.

Final Preparation

If no further processing is required, the samples should be adjusted to the desired final extract volume (specified in work plan) and prepared for instrumental analysis.

- Adjust the final extract volume using N-Evap concentration. Adjust the flow of nitrogen on the N-Evap to a gentle stream — do not allow the sample to bubble or splatter, or have a large "dimple" on the surface, as this will result in the loss of sample. A small dimple is expected on the surface of the solvent.

Note: *Sample extracts at larger volumes (5 mL or more) and in larger vials (e.g., K-D receiver tubes or GPC/HPLC eluent collection tubes) should be concentrated on the N-Evap with the vials in the water bath at a temperature of 20-25°C (room temperature or thermostatically set at 25°C). Once the sample extracts are at lower volumes and in smaller vials (e.g., 4 mL vials), the N-Evap concentration should occur with the vials out of the water bath.*

- Spike the final extract with the appropriate recovery internal standards (RIS), as listed in the work plan, and split the extract as necessary, transferring the samples to GC vials. The GC/ECD split is solvent exchanged with hexane if this analysis is required, and the sample(s) submitted to the instrument analyst(s) for analysis.

4.0 CALCULATIONS

Calculate percent dry weight with the following equation:

$$\%\ dry\ weight = \frac{aliquot\ dry\ wt.^{a}}{aliquot\ wet\ wt.^{a}} * 100$$

Calculate percent moisture with the following equation:

$$\%\ moisture = \frac{aliquot\ wet\ wt.^{a} - aliquot\ dry\ wt.^{a}}{aliquot\ wet\ wt.^{a}} * 100$$

[a] corrected for pan weight

Calculate sample dry weight with the following equation:

$$Sample\ dry\ weight\ (g) = \%\ dry\ wt. * sample\ wet\ wt.\ (g)$$

5.0 QUALITY CONTROL

The QC program for each project is defined in the project work plan, which will define the type and amount of internal standards/spiking solutions to be added to the samples, the specific QC samples to be processed, any modifications to the standard QC acceptance criteria, and the corrective action required if QC results do not meet those acceptance criteria.

Quality control methods for this procedure may consist of analysis of procedural blanks (working through procedure without sample; includes extraction solvent, sodium sulfate, and SIS), laboratory control samples (like procedural blank but also fortified with target analytes), matrix spikes (soil/sediment fortified with SIS and target analytes; an aliquot of the matrix material will be analyzed separately to determine background levels of contamination), reference materials (SRM, CRM), or field sample duplicates. SOP 7-029 describes the default reporting format and data quality objectives for quality control data.

6.0 TRAINING

Analysts performing the procedure must initially work under the supervision of the Laboratory Manager, task leader, or other designated (trained) individual. Individuals may work independently once they have satisfactorily completed extraction on a set of triplicate laboratory control samples (spiked with a representative suite of analytes), and procedural blank. These samples may be analyzed directly by GC without further processing. Analyses will be considered satisfactory if the recovery of all spiked analytes fall between 70 and 110 percent of the true values and the procedural blank contains less than or equal to the method detection limit of all analytes of interest, and/or if analytical results demonstrate proficiency as determined and documented by the Lab Manager in the training files.

Upon successful completion of training, a training certificate (Attachment 1) will be issued. The original will be kept on file in the Quality Assurance Office.

7.0 SAFETY

As part of the above training program, the analyst will be made aware of the particular safety concerns of this procedure, including:

- Use of protective eyewear and clothing
- Proper use of fume hoods
- Location and use of laboratory safety devices; eyewashes, emergency showers, fire extinguishers, fire blankets, and first aid kits, as well as MSDS sheets.

8.0 ATTACHMENTS

Attachment 1. Example of Battelle Certificate of Training.

9.0 APPROVALS

APPROVALS Author	Name	Date 12-9-99
Laboratory Manager	Name	Date 12-9-99
QA Coordinator	Name	Date 12-9-99
Environment Chemistry Section Manager	Name	Date 12/9/99

ATTACHMENT 1

Battelle Duxbury Operations

CERTIFICATE OF TRAINING

Title: **SOIL/SEDIMENT EXTRACTION FOR TRACE LEVEL SEMI-VOLATILE ORGANIC CONTAMINANTS**

SOP#: **5-192-04**

Trainee: _____

Instructor: _____

Date SOP was read: _____ (*To be completed by trainee*)

Date training was completed: _____ (*To be completed by instructor*)

APPROVED _____, _____
Laboratory Manager Date

Battelle Duxbury Operations
Standard Operating Procedures

for

WATER EXTRACTION FOR TRACE LEVEL SEMI-VOLATILE ORGANIC CONTAMINANT ANALYSIS

Summary of changes in this version: This SOP includes clarifications of the alumina and activated copper preparation and cleanup steps, and the organization of the SOP has been improved.

1.0 OBJECTIVE

This document describes an application and adoption of EPA SW846 Method 3510 and Method 3611, and also incorporates Method 608 and Method 625, for isolation/extraction of semivolatile organic compounds (SVOCs), including petroleum hydrocarbon compounds, from water and an extract cleanup procedure. The method may be used together with Battelle's instrumental analysis SOPs for the analysis of a variety of SVOC compounds, including PAH, saturated and total hydrocarbons (TPH), PCB, and pesticides.

In this procedure, 1 to 2.5-L water samples are spiked with the appropriate surrogate internal standard (SIS) compounds and serially extracted with methylene chloride using a separatory funnel (larger sample volumes may be extracted using the same procedure to improve detection limits). The extract is dried with sodium sulfate, concentrated, and optionally split into aliquots for instrumental analysis.

2.0 PREPARATION

2.1 APPARATUS AND MATERIALS

The following supplies are needed to perform the water extraction and optional alumina sample cleanup procedures:

Separatory funnel: 1, 2, or 3-L, with Teflon stopcock

Balance accurate to 0.01 g

Glass wool heated to 400°C for at least 4 h, then stored in a covered glass container at 105°C, or thoroughly solvent cleaned prior to use if wool is temperature sensitive and becomes brittle at 400°C.

Glass fiber filters heated to 400°C for at least 4 h, then stored in aluminum foil "package" at 105°C or thoroughly solvent cleaned prior to use if filters are temperature sensitive and become brittle at 400°C.

11/13-mm chromatography column with reservoir and Teflon stopcock

Kuderna-Danish (K-D) apparatus (listed below) or Turbovap concentration units and tubes

- Reservoir, 500 mL
- Snyder column, three-ball macro
- Concentrator tube, 10- or 20-mL

Hot water bath capable of reaching 100 °C, located in fume hood.

Boiling chips, solvent rinsed

Nitrogen evaporation apparatus, N-Evap or equivalent, with heated water bath at about 25 °C

Glass funnels

Graduated cylinder: 1 to 2-L

Erlenmeyer flasks, 250- and 500-mL

Microliter syringes

2.2 REAGENTS

The following solvents and reagents are needed to perform the water extraction and cleanup procedure:

Reagent Water: Reagent water is defined as water in which an interferant is not observed at the method detection limit of the compounds of interest (typically pre-extracted Milli-Q water).

Solvents: Acetone (for rinsing)

Dichloromethane (DCM), pesticide grade or equivalent)

Hexane, pesticide grade or equivalent

HCl, 6 to 12N

Sodium sulfate--anhydrous, reagent grade, heated to 400°C for at least 4 h, then cooled and stored in a tightly-sealed glass container at room temperature.

Alumina, F-20 (Aldrich Chemical. CAS #B44-28-1. 80-200 mesh).
Activate the alumina by heating in a shallow dish to 400 °C for at least 4 hr. Allow the alumina to cool in an oven (do not keep in open lab atmosphere for extended periods). Store the alumina in a covered glass container (with an activation date label) at 105 °C, and use within 1 week of activation.

Deactivate the alumina prior to use with 2% water. Prepare the 2% deactivated alumina in batches by adding 5 mL of Milli-Q water (accurately measured with a syringe or volumetric pipette) to 250 g of alumina in a 1,000 mL round bottom glass flask. Seal with a glass or Teflon stopper, shake vigorously by hand for 1 min to begin the process. Vent the flask, put the stopper back on, seal with Teflon tape, and allow to vigorously mix on a shaker table for 2 hr (± 15 min). Pack columns within 2 hr of completing the deactivation and use the same day. Unused deactivated alumina should be discarded.

Granular Copper, 99% pure (copper J.T. Baker CAS #7440-50-8 or equivalent), activated.
To activate copper: add 20-25 g copper to a beaker (should be no more than 1 inch "height" of copper). Add Milli-Q or DI water to beaker, then add an equal volume of 12N HCl. Stir the mixture for 2-3 min (until uniformly bright copper colored) with a spatula, and decant to acid waste. Rinse with Milli-Q or DI water until there is no evidence of acid (typically at least 8-10 times), rinse with acetone until there is no evidence of water (typically at least 3-5 times), and rinse with DCM until there is no evidence of acetone (typically at least 3-5 times). If the DCM appears cloudy after the first addition, there is still water present in the copper; repeat process from the addition of acetone. Activate copper no more than one hour before use. Unused activated copper should be discarded.

Surrogate Internal Standards (SIS), Recovery Internal Standards (RIS) and target compound spiking solutions are project specific and are defined in the project work plan.

2.3 LABWARE PREPARATION

Unless otherwise specified in the project specific work plan, all glassware contacting samples must be cleaned according to laboratory protocols defined in SOP No. 5-216.

2.4 RECORD KEEPING

Samples will be assigned unique identification numbers and logged in on the Chemistry Laboratory Sample Identification Log according to laboratory protocols (SOP No. 6-007).

Analytical data will be reported on appropriate data forms included in the sample batch books. Information to be recorded includes wet and dry weight information, dates of extraction/processing procedures, initials of laboratory personnel who perform the procedures, types and amount of internal standards added to samples, and, if necessary, comments regarding individual samples.

3.0 PROCEDURES

3.1 SAMPLE COLLECTION, PRESERVATION, AND HOLDING

Aqueous samples are typically collected in 1 to 2.5-L pre-cleaned amber bottles. The samples are stored at 4 °C until laboratory processing can begin. Samples should be extracted within 7 days of verified sample collection date. The extract holding time is 40 days.

3.2 GENERAL

Samples should be extracted in batches of 20 or fewer field samples unless otherwise stated in the project specific work plan. Quality control samples accompanying each batch may include a procedural blank, laboratory control sample, matrix spike, reference material and/or field sample duplicate. (See Section 5).

Each sample, including QC samples, should be spiked with SIS. Generally, the amount of SIS is such that the concentration of SIS compounds in the final extract should be approximately 1 µg/mL for GC/MS analysis, and/or 0.05 µg/mL for GC/ECD analysis.

The samples should be thoroughly mixed prior to any aliquotting for chemical characterization.

3.3 EXTRACTION, CONCENTRATION, AND CLEANUP

Note 1: *The sample will be extracted without pH adjustment, because neutral PAH, saturated hydrocarbons, PCB, and most persistent chlorinated pesticides will be extracted efficiently from water regardless over a wide range of pH. If phenols or other pH-sensitive compounds are to be analyzed, the sample will first be extracted once with no pH adjustment, the pH adjusted as appropriate (pH<2 with HCl for phenols — other pH adjustments are project specific and will be noted in the work plan) and exrtracted two more times.*

Note 2: *Extractions for specific classes of compounds can be performed with compounds-optimized solvents. For instance, if only PCBs are to be analyzed the extraction is best performed using hexane as the extraction solvent.*

- Using a 1- or 2-L graduated cylinder, measure the well mixed sample and transfer it to the separatory funnel. Record the volume.
- Add the appropriate amount of SIS.
- Rinse the sample bottle with 60 mL methylene chloride (or 120 mL if sample volumes are > 1-L). Add the bottle extract to the sample in the separatory funnel.
- Seal and shake the separatory funnel vigorously for 2-3 minutes, with periodic venting to release excess pressure.
- Allow the organic layer to separate from the water phase for a minimum of 10 min. If the emulsion interface between layers is more than 1/3 the size of the solvent layer, refer to EPA Method 3510 for possible mechanical techniques to break emulsion.
- Collect the extract in an Erlenmeyer flask.
- Repeat the extraction two more times using fresh portions of methylene chloride (60 mL for 1-L samples and 120 mL for >1-L samples).

See Note 1 above for sample acidification and extraction if polar compounds are to be determined (e.g., phenols); acidification is performed (using 6 N HCl) between the first and second solvent extraction, if this is required, and will then be specified in the work plan.

- The three methylene chloride extracts are combined in the Erlenmeyer flask and anhydrous sodium sulfate is added. The amount of sodium sulfate required is highly sample-specific. Add more sodium sulfate if sample is lumpy — some sodium sulfate should move freely in the solvent indicating that no additional water is available. Allow the extract to sit over sodium sulfate to sit for 1/2 h.

Extract Concentration — K-D Technique

- Transfer sample extract to a K-D flask and receiver tube, filtering the extract using a glass funnel containing a pre-cleaned glass fiber filter, or pre-cleaned glass wool, topped with approximately 50 g of sodium sulfate. Rinse the Erlenmeyer twice with about 10 mL of DCM and add to K-D.

- Add 3-5 boiling chips to the K-D receiver and insert a Snyder column. Pre-wet the condenser column with approximately 5-mL DCM. Place the K-D apparatus in a hot water bath maintained at 60-65 ° C (monitored by a mercury thermometer), such that the concentrator tube is partially immersed in hot water and the entire lower rounded surface of the flask is bathed in hot water vapor. At the proper rate of evaporation, the balls of the Snyder column will actively chatter, but will not flood with condensed solvent. Continue concentration until the sample volume is reduced to approximately 10 mL.

- Notify the Lab Manager for instructions on how to proceed if the rate of concentration slows down significantly.

- Remove the K-D apparatus and allow it to drain and cool for at least 10 min. Transfer the concentrator tube to the N-Evap unit and concentrate the sample until the volume is 1-2 mL, maintaining a water bath temperature of approximately 25 °C.

Note 1: The water bath for the K-D is maintained at 60-65 ° C for concentrating DCM, 70-75 ° C for concentrating acetone, and a boiling bath is used for concentrating hexane or toluene.

Note 2: Adjust the flow of nitrogen on the N-Evap to a gentle stream — do not allow the sample to bubble or splatter, or have a large "dimple" on the surface, as this will result in the loss of sample. A small dimple is expected on the surface of the solvent.

Extract Concentration — TurboVap Technique (optional)

If specified in the work plan, TurboVap may be used for concentrating the extract rather than using K-D.

- Transfer sample extract to a 200 mL TurboVap tube with a 1 mL collection reservoir. TurboVap waterbath temperature should be set at approximately 25 °C; nitrogen pressure should be approximately 4 to 6 psi.

- Follow manufacturers instructions for operation and maintenance. After initial TurboVap concentration the extract should be quantitatively transferred to a 4 mL vial (with DCM rinses), and concentrated until the volume is 1-2 mL.

Extract Cleanup — Alumina

Alumina cleanup is routinely performed on water extracts, unless otherwise specified in the project plan. This may be the only cleanup, but for most complex environmental water matrices it is typically a cleanup step that is performed prior to HPLC/GPC cleanup. Sample extracts must be in DCM when loading onto the column, so solvent exchange may be needed if another extraction solvent is used.

Note: Phenol, and C1-C4 phenols, are retained by the alumina column and this cleanup step cannot be used if these compounds are target analytes.

Column Preparation

- Prepare the 2% deactivated F-20 alumina as described in Section 2.2.

- Pack the tip of the 11/13-mm ("skinny") chromatography column with a small piece of glass wool. Add approximately 10 mL DCM and tap glasswool with clean glass rod to remove bubbles. Drain solvent into waste cup.

- Add approximately 25 mL DCM to the column. Weigh 10-g, 2% deactivated alumina into a beaker. Add ~20 mL DCM and swirl to remove bubbles. Slowly pour alumina slurry into the column while rinsing beaker with DCM

- Place approximately 1 g of sodium sulfate on top of the alumina. Tap the column to remove bubbles. Drain column to top of packing and discard solvent. Column is now ready for use; columns must be used the day they are prepared.

Column Elution

- Put a clean glass collection flask under the column (e.g., Erlenmeyer flask, K-D apparatus, or TurboVap tube).

- Load the 1 to 2 mL sample extract (must be in DCM) onto the column.

- Slowly drain and stop at the top of the alumina packing. Rinse the sample vial with about 1 mL DCM and load onto column. Slowly drain and stop at the top of the packing. Repeat vial rinse one time.

- Load with 60 mL of DCM.

Note: 60 mL of DCM will effectively elute our standard analytes (PAH, PCB, chlorinated pesticides, phthalates, and LABs). However, endrin aldehyde typically elutes with the rest of the pesticides but sometimes has unpredictable alumina retention and may require 100-120 mL DCM to elute. Additionally, coprostanol and androstanol require 90 mL to elute. The project work plan will specify if more than 60 mL should be used to elute the column.

- Drain the column slowly (about 2 mL/min), collecting the column eluent containing the target analytes, and stop at the top of the column — do not drain the column dry into the collection vial.

- Open up and drain the column dry into a solvent waste jar.

Extract Cleanup — Copper Treatment (optional)

This is an *optional* cleanup step for water samples and will *not* be routinely performed, but should be included (and specified in the work plan) for samples where high levels of sulfur may be present (e.g., wastewater, and elutriate or pore water from anoxic sediments). Activated granular copper (prepared as described in Section 2.2) is added to the extract to remove sulfur, which interferes with GC analyses.

- Concentrate the alumina purified extract by K-D or TurboVap (described earlier) to a volume of approximately 10 mL.

- Add a "scoop" (about 1-2 g) of activated granular copper to the extract using a clean stainless steel spatula. Allow to react for at least 10 min and observe. Add additional activated copper if the copper in the extract has turned black color (sulfur causes the copper to become black), and observe.

- Repeat the activated copper addition step until the lastly added copper remains unchanged in color. The extracts should remain in the vial with copper for at least 1 hour, but not over night (extended contact with copper is not good for some less stable compounds).

Extract Cleanup — GPC

The sample is now ready for further cleanup and processing. Size exclusion HPLC/GPC cleanup (described in SOP No. 5-191) should be performed unless the work plan specifies to omit this step.

- The DCM extract volume should be adjusted to approximately 900 µL and centrifuged, if necessary, to settle out any particulates. The volume is measured with a gas tight syringe (to within 5 µL), the volume recorded, and the sample transferred to a 2-mL GC vial.

- Process the samples as described in SOP No. 5-191.

Final Preparation

If no further processing is required, the samples should be adjusted to the desired final extract volume (specified in work plan) and prepared for instrumental analysis.

- Adjust the final extract volume using N-Evap concentration. Adjust the flow of nitrogen on the N-Evap to a gentle stream — do not allow the sample to bubble or splatter, or have a large "dimple" on the surface, as this will result in the loss of sample. A small dimple is expected on the surface of the solvent.

Note: Sample extracts at larger volumes (5 mL or more) and in larger vials (e.g., K-D receiver tubes or GPC/HPLC eluent collection tubes) should be concentrated on the N-Evap with the vials in the water bath at a temperature of 20-25°C (room temperature or thermostatically set at 25°C). Once the sample extracts are at lower volumes and in smaller vials (e.g., 4 mL vials), the N-Evap concentration should occur with the vials out of the water bath.

- Spike the final extract with the appropriate recovery internal standards (RIS), as listed in the work plan, and split the extract as necessary, transferring the samples to GC vials. The GC/ECD split is solvent exchanged with hexane if this analysis is required, and the sample(s) submitted to the instrument analyst(s) for analysis.

4.0 CALCULATIONS

No calculations are required as part of this SOP.

5.0 QUALITY CONTROL

The QC program for each project is defined in the project work plan, which will define the type and amount of internal standards/spiking solutions to be added to the samples, the specific QC samples to be processed, any modifications to the standard QC acceptance criteria, and the corrective action required if QC results do not meet those acceptance criteria.

Quality control methods for this procedure may consist of analysis of procedural blanks (working through procedure without sample; includes extraction solvent, sodium sulfate, SIS, alumina, and/or copper, as appropriate), laboratory control samples (like procedural blank but also fortified with target analytes), matrix spikes (field water sample fortified with SIS and target analytes; an aliquot of the matrix material will be analyzed separately to determine background levels of contamination), or field sample duplicates. SOP 7-029 describes the default reporting format and data quality objectives for quality control data.

6.0 TRAINING

Technicians performing the procedure must initially work under the supervision of the Laboratory Manager, task leader, or other designated (trained) individual. Individuals may work independently once they have satisfactorily completed extraction on a set of triplicate laboratory control samples (spiked with a representative suite of analytes), and procedural blank. These samples may be analyzed directly by GC without further processing. Analyses will be considered satisfactory if the recovery of all spiked analytes fall between 70 and 110 percent of the true values and the procedural blank contains less than or equal to the method detection limit of all analytes of interest, and/or if analytical results demonstrate proficiency as determined and documented by the Lab Manager in the training files.

Upon successful completion of training, a training certificate (Attachment 1) will be issued. The original will be kept on file in the Quality Assurance Office.

7.0 SAFETY

As part of the above training program, the analyst will be made aware of the particular safety concerns of this procedure, including:

- Use of protective eyewear and clothing
- Proper use of fume hoods
- Location and use of laboratory safety devices; eyewashes, emergency showers, fire extinguishers, fire blankets, and first aid kits, as well as MSDS sheets.

8.0 ATTACHMENTS

Attachment 1. Example of Battelle Duxbury Operations Certificate of Training

9.0 APPROVALS

APPROVALS

Author	Name	12·9·99 Date
Laboratory Manager	Name	12·9·99 Date
QA Coordinator	Name	Date
Environmental Chemistry Section Manager	Name	12/9/99 Date

ATTACHMENT 1

Battelle Duxbury Operations

CERTIFICATE OF TRAINING

Title: **WATER EXTRACTION FOR TRACE LEVEL SEMI-VOLATILE ORGANIC CONTAMINANTS**

SOP#: **5-200-03**

Trainee: _____

Instructor: _____

Date SOP was read: _____ (*To be completed by trainee*)

Date training was completed: _____ (*To be completed by instructor*)

APPROVED _____, _____
Laboratory Manager Date

Battelle Duxbury Operations
Standard Operating Procedure

for

CLEANING OF ORGANIC CHEMISTRY LABWARE

Summary of changes in this version: Minor edits and clarification of the conical cleaning procedures.

1.0 OBJECTIVE

Proper cleaning of glassware and non-glass labware for organic residue analysis is of central importance to the reliability of data for all projects. These procedures must be standardized and documented to assure reproducible results and responsible communications between Battelle and its contractors.

2.0 PREPARATION

2.1 EQUIPMENT

This procedure requires the following equipment:

- Deionized water system,
- Forma Scientific Fury 2000 Washer Model 8825,
- Drying ovens (operating at approximately 150° C),
- Ignition furnaces (operating at approximately 400° C),
- Ultrasonic cleaner bath (200 watt or greater).

2.2 SUPPLIES

This procedure requires the following supplies:

- Micro® Liquid Laboratory Cleaner,
- Forma Scientific Laboratory Cleaning Detergent,
- Acetone, pesticide residue grade,
- Dichloromethane (DCM), pesticide residue grades.

2.3 CLEANING AREAS

The sinks and sink counters are designated for handwashing labware. All other washing occurs in the laboratory glassware preparation room.

Solvent rinsing: All solvent rinsing occurs in exhaust hood areas. No solvent rinsing, solvent transfer of any kind, or storage of solvent bottles of any kind may occur in or near a laboratory sink. All solvent must be evaporated before glassware may enter the washing procedure. Solvent-rinsed glassware should air dry in the exhaust hood until visibly dry.

2.4 GENERAL PREPARATION

Stopcocks and labels are physically removed before placing labware in cleaning area. Stopcock plugs are reassembled to avoid losing pieces. All labware is brought to designated cleaning areas by the technician or analyst. Do not allow dirty labware to remain in work areas.

3.0 PROCEDURES

The cleaning procedure generally consists of washing, rinsing (with water and acetone), drying, and baking (at 400° C). **All glassware should be rinsed with acetone prior to drying in the drying oven.** Open ends of labware are generally covered with aluminum foil during the baking process. The aluminum foil remains on the labware during storage. Labware is generally given a final solvent rinse before use.

Labware may either be washed by hand, or with the automatic washer. Numerous types of labware may not be dried or baked in an oven. Depending on the nature of the labware, a solvent prerinse may also be required.

Separation of labware by project is not required unless specified in the project plan.

Any labware with a visible residue should be either discarded or rinsed thoroughly with solvent by the laboratory technician before it enters the formal cleaning procedure. The Lab Manager (or designee) must be consulted regarding the disposal of any labware. Labware that has not been solvent rinsed should not be accepted by the dishwasher or lab helper for cleaning.

3.1 HANDCLEANING

The following labware should be washed by hand, dried in the drying oven until all traces of solvent or water are removed, and baked in the ignition furnace at ca. 400° C for a minimum of 30 min.

- Beakers of all sizes,
- Snyder columns,
- Glass stoppers.

The following labware should be washed by hand, but not be placed in the drying oven or ignition furnace:

- Volumetric glassware (may be dried but not baked in ignition furnace),
- Homogenizer probes and other homogenizer parts,
- Plastic or Teflon forceps, stoppers, stopcocks, bottles, or caps,
- Metal spatula forceps.

Conical vials are not disposable and as a result they must be scrupulously cleaned prior to reuse. **Avoid cleaning large quantities of conicals together in one beaker. Conicals should be split up into groups of 50 or less for cleaning.** Conical vials are cleaned by first rinsing several times with DCM inside and out. Physically remove any residue that is not removed after the DCM rinses. Next, conicals are sonicated for 15 minutes in acetone and 15 minutes in DCM (2x). Once the solvent sonication steps are

complete the conicals should be dried in the drying oven for approximately 30 minutes and baked at 400° C for a minimum of 30 min..

3.1.1 Handwashing

Handwashing for Snyder columns consists of plain hot tap water rinse (no soap), Deionized water rinse, acetone rinse, and oven drying, in that order.

Handwashing for beakers, graduated cylinders, spatulas, forceps, stoppers, and stopcocks consists of brushing thoroughly with hot, soapy water, rinsing thoroughly with warm tap water until all traces of detergent are removed, and then rinsing with Deionized water. Labware is then moved to the exhaust hood where all inside and outside surfaces are rinsed thoroughly with acetone and air-dried. All homogenizer probes and parts are acetone-rinsed, dried, and stored. They are solvent-rinsed before use. Stopcocks are not placed in the drying oven.

The volumetric flasks and pipettes are rinsed thoroughly with warm tap water and then rinsed thoroughly with Deionized water. The volumetric glassware is then moved to the exhaust hood where all inside and outside surfaces are rinsed thoroughly with acetone and air-dried. Volumetric glassware is sonicated for 15 minutes in acetone, DCM, and hexane. Volumetric glassware is dried in the drying oven, but may not be baked in the ignition furnace.

3.1.2 Drying

When the acetone has evaporated, appropriate labware is placed into the drying oven for at least 30 minutes or until dry.

All labware (with the exceptions mentioned above) must be dried in the drying oven located in the laboratory preparation room and/or labware preparation room. Both ovens have a upper limit button that must be depressed to re-set the heater if the oven temperature has exceeded the upper (pre-set) temperature limit.

3.1.3 Baking

After drying, the glassware may then be prepared for baking in the ignition furnace located in the laboratory preparation room and/or the labware preparation room.

Glass to be baked must be Pyrex or equivalent, and cleaned and dried according to above procedures. All openings must be loosely covered with aluminum foil. Place in furnace leaving spaces between pieces.

Set temperature to 400° C and close door. Refer to the manual (section on operation), located in the labware preparation room. The oven takes approximately 2 h to reach this temperature. Glassware is baked for a minimum of 30 min., unless otherwise specified in the project plan.

3.2 MACHINE WASHING

All other labware (either Teflon or glass) may be washed in the automatic washer located in the labware preparation room. No plastic labware should be washed in this machine because of the possibility of contaminating the washer.

Any visible solid or powdery residue on labware should be removed by brushing in soapy water. Do not place labware with solid or powdery residue into the automatic washer.

Teflon bottles must be washed at a lower temperature than glass; thus the washer must be set at "plastic". Refer to the Forma Scientific Furry 2000 logbook for instructions on the operation and maintenance of the automatic washer.

Machine-washed labware are dried and baked according to procedures presented above.

3.3 FINAL SOLVENT RINSING

After labware has been baked and cooled, most projects require that it be solvent rinsed before use. All surfaces that will be in contact with the sample must be well rinsed with solvent. Using aspirator bottles, rinse inside surfaces with DCM, unless otherwise specified in the project QAPP or SOP. In general, glassware prepared for butyltin analyses will be rinsed with 10% hydrochloric acid, acetone (2x), DCM (2x), and hexane (2x), in that order. The acid rinse procedure is used on all glassware pre-derivitization. Hexane rinses are all that is needed post-derivitization.

This procedure is carried out in exhaust hoods. All rinse solvent is collected and discarded as Liquid Hazardous Waste according to SOP No. 5-114.

4.0 CALCULATIONS

Calculations are not required.

5.0 QUALITY CONTROL

The quality of the cleaning procedures is verified by the analysis of procedural blanks associated with the sample batch. If contamination of samples is apparent and adversely affects the quality of the sample data, then a search for the source commences. If the source is found to be the labware, then all associated labware cleaned following the suspect batch must be recleaned following the procedures outlined in this SOP.

6.0 TRAINING

The Lab Manager (or designee) is responsible for overseeing all glassware cleaning and preparation. The trainee must first read this SOP. The Lab Manager (or designee) then demonstrates proper glassware cleaning procedures, instruct the trainee regarding proper use and handling of solvents, and relay information contained on appropriate Material Safety Data Sheets (MSDS). Once the trainee demonstrates proficiency in the cleaning procedures listed above, a Certificate of Training is issued (Attachment 1). The original of the certificate is sent the Quality Assurance Unit.

7.0 SAFETY

Use of safety glasses and lab coats are required for all procedures.

Use of Nitrile gloves may be necessary. Please consult with the Lab Manager or Safety Officer on the use of gloves.

Instruction in the proper use and disposal of solvents is necessary.

Solvent skin contact. Remove contaminated clothing immediately. Skin should be rinsed immediately with cold water. Pay strict attention in thoroughly rinsing under nails. Eyewashes are available if solvent contacts the eye. Wash eyes immediately with large amounts of water, occasionally lifting upper and lower lids, until no evidence of solvent remains (15 minutes or longer). In case of eye contact, medical attention is warranted.

Notify the Lab Manager and Safety Officer immediately in case of solvent eye contact.

8.0 ATTACHMENTS

1. Example of Battelle Duxbury Operations Certificate of Training.

APPROVALS

Author		8·6·00
Laboratory Manager		8·6·00
Quality Systems Manager		8-7-00
Environmental Chemistry Resource Manager	Name	8/7/2000 Date

CERTIFICATE OF TRAINING
For
SOP No. 5-216-07, Cleaning of Organic Chemistry Labware

The above mentioned SOP is relevant to your work. Your signature below signifies that you have read and understood the requirements associated with this procedure.

Trainee_____

Instructor_____

Date SOP read and understood_____

Comments:

Approval and Date_____

Battelle Duxbury Operations
Standard Operating Procedures

for

CHEMISTRY LABORATORY SAMPLE IDENTIFICATION

Summary of changes in this version: Nomenclature has been updated to reflect current practice.

1.0 OBJECTIVE

Projects performed by the Chemistry Section laboratory at Battelle require a sample tracking system by which uniquely identified samples can be traced from the time of sample collection or receipt to final analysis. This Standard Operating Procedure (SOP) describes the procedure by which the Chemistry Section assigns a unique alphanumeric code (Laboratory Identification Number) to each sample for use within the laboratory. At the discretion of the project manager, field or client identification (ID) numbers can be used in the lab but a Laboratory Identification Number should still be assigned to each sample upon receipt. The use of alternate IDs will be specified in the project work plan.

2.0 PROCEDURE

2.1 FIELD SAMPLES

Samples admitted to the laboratory will be received and logged in by the Laboratory Sample Custodian or the Alternate Laboratory Sample Custodian (SOP 6-010). At the time of receipt, each sample is assigned a Laboratory Identification Number that is indelibly marked on each sample container and cross referenced to the sample's original identification.

- An individual container that is uniquely identified as a specific sample by the client is assigned a separate sample ID number.
- Multiple containers that are uniquely identified as a specific sample by the client and targeted for the same analysis are assigned the same sample ID number.
- Multiple containers that are uniquely identified as a specific sample by the client and targeted for different analyses are assigned unique sample ID numbers.

Laboratory Identification Numbers are assigned sequentially to samples in the following alphanumeric format:

Xnnnn

where: X = a single letter (Z...A)
nnnn = 0001.....9999

2.2 QUALITY CONTROL SAMPLES

Quality control (QC) samples generated within the Chemistry Laboratory are treated identically except that the Laboratory Identification Numbers are assigned by hand by the person who is preparing the sample (Technician, Researcher) and the number sequence can be in the format stated above but is usually in the following format:

XXnn

where: XX = AA,AB,AC,.....ZX,ZY,ZZ
nn = 01,02,03,.....97,98,99

If a sample is split for separate analysis within Battelle (e.g. FID, PAH) both samples will retain the original Laboratory Identification Number. Similarly, if a sample is split for analysis as part of the QC program, the original Laboratory Identification Number will be retained, but will be appended with a suffix to indicate the type of replicate. The following is a list of suffixes:

- 1,-2 etc.: Reextraction number (e.g. first reextraction Z0001-1)
- R: Indicates that sample has been refractionated by HPLC (e.g., Z0001-R, Z0001-R2)
- NF: Indicates that the non-fractionated aliquot from HPLC will be analyzed
- D1 etc.: Indicates that sample has been diluted (e.g., Z0001-D1, Z0001-D2)
- LCS: Laboratory Control Sample (e.g. AB01LCS)
- LCSD: Laboratory Control Sample Duplicate (e.g. AB01LCSD)
- MS: Matrix Spike (e.g. AB01MS or Z0001MS)
- MSD: Matrix Spike Duplicate (e.g. AB01MSD or Z0001MSD)
- PB: Procedural Blank (e.g. AB01PB)
- DUP: Indicates that a second extraction of a sample has been performed (e.g., Z0001DUP)
- TRIP: Indicates that a third extraction of a sample has been performed (e.g., Z0001TRIP)
- REP#: Replicated samples (e.g. Triplicate Analysis: Z0001-Rep1, Z0001-Rep2, Z0001-Rep3)
- F#: Column fractionation (e.g. F1, F2, F3)
- SRM: Standard Reference Material (e.g., AB01SRM)
- GC/MS etc. Indicates that sample has been split for separate analyses (e.g., Z0001-GC/MS. Other analyses may include: GC/ECD, GC/VOA, HPLC)
- -MS-DI: Indicates that a specific split of a sample has been diluted (e.g., Z0001-MS-DI)

3.0 DOCUMENTATION

Every sample received in the Chemistry Section will be logged into the Chemistry Laboratory Sample Receipt Database and assigned a Laboratory ID number (Attachment 1). Information entered at log-in includes collection information, client IDs, matrix, storage location, and sample specific comments.

Records of Laboratory Identification Numbers and their corresponding original field identifications are the responsibility of the Laboratory Sample Custodian. Hard copies of custody forms are stored in a 3-ring binder in the access-controlled custody room. The bound Sample ID Log (QC samples) is kept in the laboratory (Attachment 2).

4.0 TRAINING

All Chemistry Section personnel who log field or quality control samples into the Chemistry Laboratory Sample Receipt Database or Sample Receipt Log are required to read this SOP. Individuals are considered trained once they have read this SOP and signed the associated training certificate (Attachment 3). The original certificate is filed in the Quality Assurance Unit office.

ATTACHMENTS

1. Example of Chemistry Laboratory Sample Receipt Database Laboratory ID Log
2. Example of Sample Identification Log (QC Samples)
3. Example of Training Certificate

APPROVALS

Author		7·10·00
Laboratory Manager		7·10·00
Quality Systems Manager		8-4-00
Environmental Chemistry Section Manager		8/4/2000
	Signature	Date

Attachment 1

Battelle Duxbury Operations

Example of Chemistry Laboratory Sample Receipt Database Laboratory ID Log

BATTELLE Laboratory Sample Login Report

Project # :

Client : EARTH TECH INC.

Receive Date : 08/04/2000 10:30:00 AM

Logged in By : MEARAM

Collection Date	*Login Date*	*Lab ID*	*Client Sample ID*	*#Containers*	*Matrix*	*Preservative*	*Storage Location*	*Login Comments*
08/03/2000 2:15:00 P	08/04/2000 11:20:31	X7222	EA00185HS	1	SED	NONE	WALK IN FRIDGE	
08/03/2000 3:00:00 P	08/04/2000 11:20:51	X7223	EA00186HS	1	SED	NONE	WALK IN FRIDGE	
08/03/2000 3:20:00 P	08/04/2000 11:21:12	X7224	EA00187HS	1	SED	NONE	WALK IN FRIDGE	
08/03/2000 3:25:00 P	08/04/2000 11:21:35	X7225	EA00188HS	1	SED	NONE	WALK IN FRIDGE	
08/03/2000 3:30:00 P	08/04/2000 11:21:57	X7226	EA00189HS	1	SED	NONE	WALK IN FRIDGE	
08/03/2000 3:35:00 P	08/04/2000 11:22:20	X7227	EA00190HS	1	SED	NONE	WALK IN FRIDGE	
08/03/2000 3:45:00 P	08/04/2000 11:22:40	X7228	EA00191HS	1	SED	NONE	WALK IN FRIDGE	
08/03/2000 3:55:00 P	08/04/2000 11:22:59	X7229	EA00192HS	1	SED	NONE	WALK IN FRIDGE	
08/03/2000 4:05:00 P	08/04/2000 11:23:26	X7230	EA00193HS	1	SED	NONE	WALK IN FRIDGE	
08/03/2000 4:10:00 P	08/04/2000 11:23:54	X7231	EA00194HS	1	SED	NONE	WALK IN FRIDGE	

Attachment 2

Example Sample Identification Log (QC Samples)

Sample ID	Log-in Date/ Initials	Client Name/ Field Sample ID	Sample Type[1]	Comments[2]
OO01				
OO02				
OO03				
OO04				
OO05				
OO06				
OO07				
OO08				
OO09				
OO10				
OO11				
OO12				
OO13				
OO14				
OO15				
OO16				
OO17				
OO18				
OO19				
OO20				
OO21				
OO22				
OO23				
OO24				
OO25				

[1] Sample Type - Matrix, QC Sample Description
[2] Comments - Sample volume (Waters), Number of containers.

Attachment 3

RECORD OF TRAINING
for
SOP No. 6-007, *Chemistry Laboratory Sample Identification*

The above mentioned SOP is relevant to your work. Your signature below signifies that you have read this SOP and understand the requirements associated with this procedure.

Name (printed)	Name (Signature)	Date

Battelle Duxbury Operations
Standard Operating Procedures

for

SAMPLE RECEIPT, CUSTODY, AND HANDLING

Summary of changes in this version: Additions about VOC refrigerator blanks, delivery of samples outside of normal hours, and other minor edits.

1.0 OBJECTIVE

Sample control is a vital aspect of any environmental monitoring program which generates data that may be used for regulatory purposes or as evidence in a court of law. Additionally, the complexity of many environmental sampling programs, which may involve the collection and analysis of samples of various media from different sites to be analyzed for several parameters, makes a sample control system essential. This standard operating procedure (SOP) defines the procedures, organizational responsibilities, and documentation requirements associated with the Laboratory sample control system.

The routine flow of samples through the laboratory is illustrated in Attachment 1. Additional sample control procedures may be required to meet the needs of specific projects. These procedures will be defined in the project plan.

2.0 DEFINITIONS

Custody Records — The administrative records associated with the possession history of each sample from the time of collection, through analysis, to final disposal.

Chain-of-Custody Records — The administrative records associated with the physical possession and/or storage history of each individual sample from the purchase and preparation of each sample container and sampling apparatus to the final analytical result and sample disposal.

Legal or Evidentiary Chain of Custody (COC) – A special type of sample custody which requires that the physical possession, transport and storage of a sample be documented in writing. The records must account for all periods of time from sample container acquisition through sample disposal.

Sample control — The formal system designed to provide sufficient information to reconstruct the history of each sample, including collection, shipment, receipt and distribution within the laboratory, analysis, storage or disposal, and data reporting.

Sample custody — Samples are considered to be in a person's custody if

- The samples are in a person's actual possession
- The samples are in a person's view after being in that person's possession

- The samples were in a person's possession and then were locked or sealed up to prevent tampering
- The samples are in a secure area

3.0 RESPONSIBILITIES

Sample Collector — The person collecting the samples is responsible for

- Collecting and preserving samples in accordance with approved procedures, as specified in the project-specific plan and SOPs
- Adjusting the pH to < 2 if the sample is intended for volatile organics analysis (and also adding sodium thiosulfate if total residual chlorine is present)
- Assigning a number or code at the time of collection that uniquely identifies that sample
- Labeling each sample container with the sample number, project identification, date of collection, collector's initials, and storage requirements (room temperature, frozen, chilled)
- Documenting sample collection and preservation
- Packaging samples for shipment in a manner that minimizes the risk of breaks and leaks and to ensure that the samples are maintained at the appropriate temperature
- Completing and signing the chain-of-custody records accurately and legibly
- Ensuring integrity of the samples by sealing or locking the shipping container(s) and applying custody tape (if required)
- Arranging timely transportation of samples to the laboratory, including identifying on the shipping label the name of the person to whom the samples should be delivered

Laboratory Sample Custodian — The responsibilities of the Laboratory Sample Custodian include:

- Receiving samples (for details see Section 4.1).
- Maintaining records of sample receipt, movement in and out of storage, and release, archival, and disposal
- Distributing completed custody forms according to Section 4.1.5.
- Returning the shipping cooler to the client or shipper.
- Communicating sample custody problems to the project manager and implementing corrective action as directed (Section 4.8).

Alternate Sample Custodian — The Alternate Sample Custodian is responsible for assisting the Laboratory Sample Custodian and for performing the above tasks in the absence of the Laboratory Sample Custodian.

Project Manager — The Project Manager is responsible for communicating

- expected receipt dates and project-specific receipt requirements to the Sample Custodian
- the potential presence of total residual chlorine to the Sample Custodian
- sample custody-related problems to the client
- corrective action to the sample custodian and laboratory manager

Laboratory Manager — The Laboratory Manager is responsible for designating the Laboratory Sample Custodian and the Alternate Sample Custodian and for ensuring that these individuals are trained to perform the tasks specified in this SOP.

4.0 PROCEDURES

4.1 SAMPLE RECEIPT

4.1.1 Hours

Samples that are admitted to the laboratory during normal business hours are either delivered to the front desk or to a designated area in the Chemistry North Building. The Sample Custodian is notified immediately.

It is Battelle policy that samples are not received outside of regular business hours unless the project manager has made specific arrangements with the laboratory manager and the sample custodian in advance. Samples that are delivered outside of regular business hours should be treated equally as samples received during the week. Receipt temperatures should be recorded and the proper storage of the samples should occur. The project manager should coordinate deliveries with the lab manager and the custodian assigned to receive the samples *before such deliveries are scheduled.* The project manager should also make every effort to be available (be able to be reached), in case unexpected problems occur.

- If samples are transported from the field to the laboratory by Battelle sampling personnel, the samples should be placed in a pre-arranged, secure location until they can be formally relinquished to the Laboratory Sample Custodian.

In either case, on the next business day, the Laboratory Sample Custodian logs in the samples. (Note that the receipt form allows for separate entries of receipt and log-in date).

Upon receipt of the samples, the Laboratory Sample Custodian will move the shipping containers to the sample custody room.

4.1.2 Sample Handling

The shipping container should only be opened under the vented hood. The sample custodian must determine whether the sample condition upon receipt is acceptable. That is, that the sample temperatures, pH, and containers, are appropriate for the intended analysis; and that the samples have been received within the required holding times. Attachment 2 defines acceptable sample handling and holding times. If sample containers, preservation, or timely delivery do not meet the criteria in Attachment 2 and section 4.1.3, then the sample custodian must notify the project manager who in turn must notify the client (section 4.8).

The sample custodian must review and document the following for proper receipt of the samples:

1. Method of delivery (*i.e.* commercial carrier, hand delivered) and presence/absence of chains-of-custodies.

2. Inspect the s ipping container(s) for the presence/absence and condition of custody seals.

3. Inspect each sample for the presence/absence and condition of samples and custody seals.

4. Inspect each sample for breaks or leaks (see Section 5.0 for safety instructions).

5. Review the accompanying records for completeness and accuracy of sample labels and sample transmittal forms.

6. Measure and record the temperature of each container to document whether or not the samples were maintained at the appropriate temperature (frozen, cool, or room temperature) during shipment. The temperature of a cooler blank (if available), melt water, or the external temperature of the sample containers should be measured and documented. (Thermometers or probes are never inserted into a sample container).

7. Measure and record initial pH of water samples unless otherwise directed by the laboratory manager, project manager, or QAPP (see Section 4.2.1).

8. If the project manager indicates that TRC may be present, measure samples for total residual chlorine (TRC) and treat samples with sodium thiosulfate (Section 4.2.2).

9. Inspect VOC vials for bubbles of sizes greater than 1% of the vial volume. If present, notify the Project Manager immediately.
   *A refrigerator blank must be made for all VOA samples received. This blank should travel with the samples and be included with the sample analysis.

10. Upon completion of the sample inspection, the Laboratory Sample Custodian formally acknowledges receipt of the samples by signing, dating, and noting the current time on the sample transmittal form(s).

11. Log-in and assign unique laboratory identification numbers to each sample (see Section 4.3).

12. Storage of samples in the appropriate storage location until samples are ready to be further processed. VOC samples to be stored in a separate storage location than samples for other organic analyses. This includes releasing samples to the laboratory and to outside contractors.

13. Communicate sample custody problems to the project manager and implement corrective action as directed.

14. Distribute completed custody forms according to Section 4.1.5.

15. Return the shipping coolers to the client or shipper, if necessary.

4.1.3 Sample Acceptance/Rejection Criteria

Under some circumstances Battelle will place itself at risk by accepting samples for analysis because data that are generated from samples that do not meet chain of custody or handling requirements (Section 4.1.1) may be rejected by EPA. Battelle may currently analyze samples for the following regulatory programs:

- Resource Conservation and Recovery Act (RCRA)
- Comprehensive Environmental Response, Compensation and Liability Act (Superfund) (CERCLA)
- Clean Water Act (CWA)

It is the responsibility of the sample custodian to ensure that the following conditions are recorded on the Sample Receipt Form. The Laboratory Sample Custodian will notify the Project Manager and Laboratory Manager in writing (See Section 4.8 and Attachment 3) of sample receipt, condition, and problems (e.g., breakage, leakage, missing samples, excessive temperatures). Upon completion of sample inspection, the Laboratory Sample Custodian formally acknowledges receipt of the samples by signing, dating, and noting the current time on the sample transmittal form(s).

It is the responsibility of the project manager to specify in the QAPP that project samples are being analyzed for compliance monitoring. In these cases samples could be rejected if:

- The integrity of the samples is compromised (leaks, cracks, grossly contaminated container exteriors or shipping cooler interiors, obvious odors, etc.)
- The identity of the container cannot be verified
- The proper preservation of the container cannot be established
- VOC vials contain bubbles of sizes greater than 1% of the vial volume
- Incomplete sample custody forms: the sample collector is not documented or the custody forms are not signed and dated by the person who relinquished the samples
- The sample collector did not relinquish the samples.
- Samples are designated for VOA analysis but no VOA trip blank is provided.

If the sample custodian identifies any of the above conditions the project manager must be notified (Section 4.8).

4.1.4 Documentation

Documentation of sample receipt includes the original sample custody forms (or copies if the originals are returned to the shipper), any additional records of transmittal, the shipper's air bill (if applicable), and the Sample Receipt form. Sample custody records are filed by date in the Custody Logbook which is kept in the access controlled sample custody room. A record of Battelle Laboratory ID numbers, including cross-reference to original field IDs, is entered into the custody database and hard copies are stored in the custody room.

The condition of the samples, integrity of the custody seals, discrepancies between sample labels and transmittal forms, and unusual events or deviations from the project work plan or SOPs are documented in detail on a Sample Receipt Form (Attachment 4). Any problems are also recorded on the original custody forms, if present.

Occasionally, samples are received with only a letter of transmittal or no transmittal forms at all. In these cases the Sample Custodian should complete the sample log-in procedures (Section 4.3) and attach a printout from the Chemistry Laboratory Sample Receipt Database to the Sample Receipt form to provide a record in the Custody Log of the samples received.

4.1.5 Distribution

The sample custodian will provide the Project Manager, the QAU, and Lab Manager with a copy of all documentation that accompanied the samples.

The custodian should make the following distribution of custody forms:

- Copies of the sample receipt and custody forms are provided to the project manager (2 copies), laboratory manager, and the quality assurance officer.
- If the custody forms that are received with the samples are multi-copied then the copies should be distributed as indicated on each copy (e.g., laboratory, customer (client), shipper).
- If the custody forms that are received with the samples are not multi-copied then the custodian should consult the project manager to determine if a copy of the custody records should be returned to the client and/or shipper.

4.2 SAMPLE PRESERVATION ADJUSTMENTS

4.2.1 pH Measurement and Adjustment

If water samples will not be extracted within 72 hours then the pH is typically adjusted according to the preservation requirements in Attachment 2. To measure pH, withdraw a small volume of water (0.1 mL) from the sample container using a baked Pasteur pipette. Place 1 drop on a narrow range pH paper strip and follow the instructions included with the pH paper to read the results. Record the pH on the Sample Receipt form (Attachment 4). If water sample pH is NOT between pH 5 and 9 consult the project and/or laboratory manager and make pH adjustments as directed by these managers. The decision to adjust sample pH is based on the target analyte list and is therefore made on a case-by-case basis.

To adjust the sample pH, sulfuric or hydrochloric acid (1+1) or 10 N NaOH is added until the sample pH is between 5 and 9. (Note that NaOH solutions must be pre-extracted prior to use to prevent sample contamination). The final pH and the volume of solution added in making the pH adjustment is recorded on the Sample Receipt form.

Samples intended for VOA analysis should NOT be opened. pH adjustments must be made in the field.

4.2.2 Total Residual Chlorine Measurement and Treatment.

If the project manager indicates that samples may contain total residual chlorine (TRC) then the sample must be treated with sodium thiosulfate according to Attachment 2 (PCB/Pest, PAH). The sample custodian must work directly with the project manager in these cases. Ideally, the sample is treated for chlorine in the field. However, the sample custodian should verify the absence of TRC using a commercial test kit. If TRC is detected then sodium thiosulfate is added at the ratio of 80 mg/L sample. This treatment is documented on the sample receipt form.

Samples intended for VOA analysis should NOT be opened. Treatment for TRC must be performed in the field.

4.3 SAMPLE LOG-IN

The receipt of all samples received by the Chemistry Laboratory will be recorded in the Chemistry Laboratory Sample Receipt Database (SOP 6-007). Each incoming sample is assigned a unique ID number, which is clearly and indelibly marked on each sample container and the custody form. Samples that contain more than one jar for the same analysis will be labeled with the same Lab ID and the jar number (e.g. 1 of 2 and 2 of 2). Alternatively, a separate ID number can be assigned to each container. The full ID including the jar number will be called out in the preparatory records. Upon completion of log-in procedures, samples are placed in a limited-access area at the appropriate temperature. The storage area is documented on the Sample Receipt form.

4.4 SAMPLE STORAGE

Upon completion of sample log-in procedures, samples are transferred to a secure location for storage. This location may be a room, refrigerator, or freezer, depending on the storage requirements of the samples, but must be an area that can be locked from the outside. The initial storage location is documented on the Sample Receipt form. Only the sample custodian and the facilities manager have keys to these controlled-access areas.

The following storage requirements are applied to samples received at Battelle unless otherwise specified in the QAPP: tissue and sediment samples: ≤20°C; water samples: 4±2°C. Samples collected for compliance monitoring according to EPA regulatory methods are stored according to the conditions specified in Attachment 2 and should be specified in the QAPP.

Samples that are to be analyzed for volatile organic compounds must be stored in a separate storage location from the samples being analyzed for semi-volatile organic compounds.

4.5 SAMPLE TRACKING

Sample custody is transferred from the Sample Custodian to the sample prep Task Leader when sample preparation is initiated. The transfer of custody to laboratory personnel and all sample movement within the laboratory is documented on Daily Sample Tracking forms (Attachment 5) that are maintained with the prep records. Each technician is responsible for the care and appropriate storage of the samples in his/her custody, and for documenting the conditions under which the samples are maintained. Labs/areas which house samples in-progress must be controlled-access (locked) during non-working hours.

4.6 SAMPLE SPLITTING

The aliquotting of samples for multiple analyses is documented on Sample Split and Transfer Logs (Attachment 6). Split samples retain their original Laboratory Sample identification number. Sample Split Logs are maintained with the original sample custody records. Lab Ids are distinguished by the analyses for which the sample was split.

4.7 SHIPMENT OF SAMPLES

Distribution of samples that are aliquotted at Battelle and shipped to external laboratories for analyses is normally documented on the Battelle Sample Split and Transfer Log. If the samples were collected or generated by Battelle, sample custody is tracked on Battelle Custody forms (Attachment 7) that are shipped with the samples. In either case the person who has custody of the samples is responsible for

- packing the samples for shipment such that temperatures requirements are maintained and samples are protected from breaks or leaks
- arranging for transportation.

A *copy* of the transfer or custody form should be retained with the original custody records for tracking purposes. The custodian at the receiving laboratory documents sample receipt and condition on the form and retains the yellow copy. The white (original) and pink copies are returned to Battelle. The original is maintained with the original custody records and the pink copy is sent to the client, if requested. The preparation of field kits, custody of sample containers, and sample packing procedures are defined in SOP 5-210.

4.8 CLIENT NOTIFICATION

The client must be notified immediately if problems are noted during sample receipt and log-in so that corrective action may be initiated. The sample custodian may communicate directly with the client custodian or representative if discrepancies between sample labels and custody forms are noted or if samples are missing. The project manager should communicate other problems (e.g., holding time exceedences, preservation issues, incomplete or improper custody records – see Section 4.1.3). This notification and the clients directions for corrective action is documented on the Corrective Action form (Attachment 3). It must be specifically documented if the client approves analysis of the samples. All corrective action is communicated to the sample custodian or laboratory manager in writing.

Specific samples may include other client notification requirements (e.g., if permit thresh hold limits are exceeded the client must be notified within 24 hours of verified sample data). The project manager should define requirements these in the QAPP.

4.9 SAMPLE ARCHIVAL AND DISPOSAL

Sample extracts and unextracted field samples are returned to the custody of the sample custodian. Once sample analysis is considered final the samples can be archived.

The decision to archive samples should be made by the client and the Project Manager when the project is initiated. Sample disposition and the length of storage should be defined in the project plan. In the absence of other directives, unexpended samples are archived for six months after the delivery of the final data. Unless otherwise specified by the client, the samples will be discarded in the proper waste stream after this period. The project manager will be notified prior to the disposal of samples.

Sample extracts are held for one month after delivery of the final data. Unless otherwise specified by the client the extracts will be discarded in the proper waste stream after this period. The project manager is not notified of extract disposal. It is the responsibility of the project manager to include sample disposition requirements in the QAPP and to communicate them to the sample custodian. The following documentation is required:

1. Samples or extracts for archival are boxed by batch.
2. The project numbers, title/clients, batch numbers, and extract fractions are labeled on the box.
3. A copy of the label is filed in the Sample Archival logbook and the archive location of the box (box number and freezer number) is documented in the logbook.

Sample disposal is documented in the Sample Archival Log Book by documenting "Discarded" with the date and initials of the custodian directly on the label copy. The appropriate handling and disposal procedures for sample and sample extract are discussed in SOP 5-114.

5.0 SAFETY

Sample handling must always assume that samples are potentially "contaminated." Therefore, sample shipping containers are always opened in a vented fume hood, and personnel protective equipment is worn when unpacking samples (safety glasses, lab coat, and gloves).

Occasionally, samples are received broken. Because the potential hazard may be unknown all spills must be treated as if the material is hazardous. Clean-up materials should be maintained in the sample custody room, or easily accessable. These consist of

- absorbent (e.g., speedi-dry)
- dust pan and brush
- glass disposal container
- heavy-duty gloves
- paper towels
- plastic bags
- solid waste stream container

The hazardous waste coordinator should be contacted to determine the proper disposal procedures for spilled sample. In general, water samples are absorbed into chemical absorbent; sediment, soil, or tissues are placed in heavy-duty plastic bags. These are both disposed of in the laboratory's solid waste stream. Broken glass containers are placed in the glass disposal container.

6.0 TRAINING

A person who is being trained as a sample custodian must first read this SOP. The person may then perform specific tasks under the supervision of a qualified instructor (Laboratory Sample Custodian or Alternate). Tasks performed by the trainee are reviewed and co-signed by the Laboratory Sample Custodian or Alternate until it has been established that the trainee is able to perform these tasks without supervision. A certificate of training (Attachment 8) is issued upon completion of training and provided to the Quality Assurance Unit.

ATTACHMENTS

1. Sample Flowchart
2. Sample Handling Requirements
3. Sample Custody Corrective Action Form

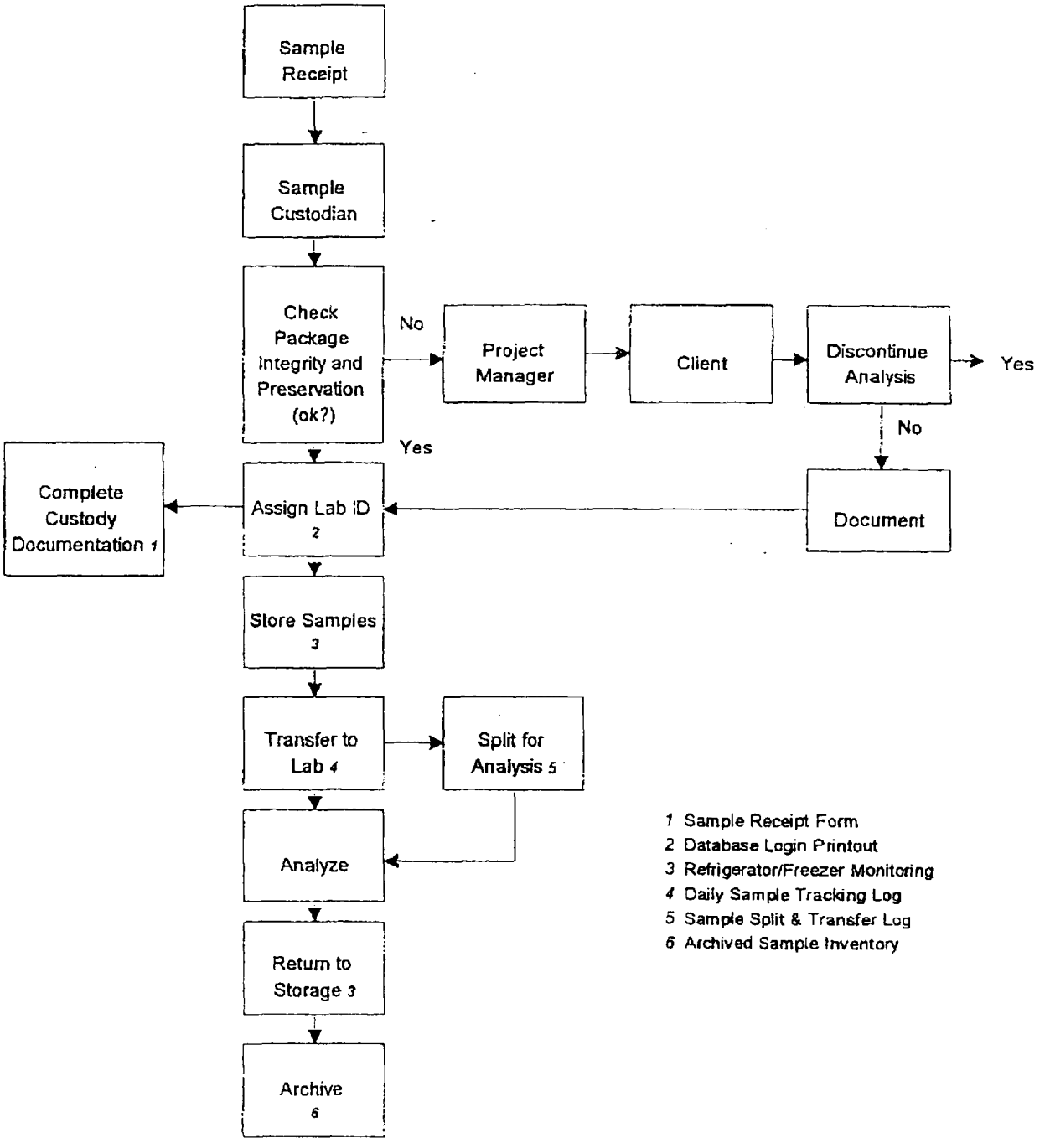
4a. Sample Receipt Form
4b. Sample Receipt Auxiliary Form

5. Daily Sample Tracking Form
6. Sample Split and Transfer Log
7. Battelle Chain-of- Custody Record
8. Certificate of Training

APPROVALS

Author		7·10·00
Laboratory Manager		7·10·00
Quality Systems Manager		8-4-00
Environmental Chemistry Section Manager		8/7/2000
	Name	Date

ATTACHMENT 1
Battelle Duxbury Operations
Sample Flowchart



ATTACHMENT 2
Sample Handling Requirements

WATER

Compound Class	Containers	Preservation		Holding Time
		Temperature	Other	
PESTICIDES[1]	Glass with Teflon lined caps	Cool 4°C ±2°C	pH 5-9 if held longer than 72 hours Store in dark	7 days until extraction, 40 days after extraction
PCBs[1]				
PAH[1]			Store in dark	
Other SVOA • Haloethers • Phthalate Esters • Nitroaromatics • Isophorones • Nitrosamines				
TPH or FINGERPRINT				
VOA[1]			pH<2 Headspace ≤1% of sample	14 days
TBT	Polycarbonate	Freeze ≤20°C		90 days

[1]If Residual Chlorine is present in the sample it must be treated with sodium thiosulfate.

SEDIMENT/SOIL

Compound Class	Containers	Preservation		Holding Time
		Temperature	Other	
PESTICIDES **PCBS**	Glass with Teflon lined caps	Cool 4°C ± 2°C		14 days until extraction, 40 days after extraction
PAH **SVOA**		Freeze ≤20°C		1 Year
VOA		Cool 4°C ± 2°C		14 days
TPH or FINGERPRINT		Cool 4°C ± 2°C		14 days until extraction, 40 days after extraction
TBT		Freeze ≤20°C		1 Year

All tissue samples are stored frozen (≤20°C).

ATTACHMENT 3
Battelle Duxbury Operations
Sample Custody Corrective Action Form

Project Number _____ Client _____

Description of Problem (continue on back, if needed):

The sample custodian must contact the project manager on the day that problems are identified. If the project manager is not in the office the laboratory manager must be notified.

Documentation of project manager notification:

Sample Custodian: _____ _____
Signature Date

Project Manager _____ _____
Signature Date

Documentation of client notification (to be completed by project manager):

On _____ I contacted _____ at _____
Date Name of client contact Name of client organization

Results of communication with client (Describe any corrective action directed by the client):

RETURN THIS ORIGINAL TO THE SAMPLE CUSTODIAN. THE SAMPLE CUSTODIAN WILL PROVIDE COPIES TO THOSE ON THE ORIGINAL SAMPLE CUSTODY DISTRIBUTION LIST.

Date that this form was received by the custodian:_____

ATTACHMENT 4a
Battelle Duxbury Operations
Sample Receipt Form

Project Number _____ Client _____
No. of Shipping Containers _____ Date/Time Received _____

SHIPMENT
Method of Delivery: _____ Commercial Carrier (Air bill No. _____)
_____ Hand Delivered
_____ US Mail (RPS No. _____)
COC Forms: _____ Shipped with samples _____No forms
Cooler(s)\Box(es) were sealed with: _____ Tape _____ Custody Seals_____(Other specify)
Were the seals intact for each shipping container_____Yes_____No
If seals were broken (list impacted samples):

SAMPLES

Sample Labels: _____ Sample labels agree with COC forms _____ Discrepancies (see COC forms)

Container Seals: _____ Tape _____ Custody Seals_____(Other specify)
_____Seals intact for each shipping container
_____Seal broken (list impacted samples):

Condition of Samples:_____ Sample containers intact
_____ Sample containers broken/leaking (see COC forms)

Temperature upon receipt (°C): _____ Temperature blank used _____Yes____No
(Note: If temperature upon receipt differs from required conditions, list impacted samples):

Initial pH 5 - 9? (Y/N):____ *If no, individual sample adjustments on the Auxiliary Sample Receipt Form.*

Total Residual Chlorine Present?(water) (Y/N): _____
If yes, individual sample adjustments on the Auxiliary Sample Receipt Form.

Head Space <1% in samples for VOC analysis _____Yes_____No
Individual sample deviations listed below.

Sample Containers:
Samples returned in PC-grade sampling jars (Yes/No). _____ Lot No. _____
All but the following samples were returned in Battelle-prepped bottles:

Storage Location: _____ **BDO IDs Assigned**: _____
Additional Comments:

Samples logged in by: _____ Date/Time: _____

ATTACHMENT 4b
Battelle Duxbury Operations
Auxiliary Sample Receipt Form

Sample ID	pH initial	pH Final	Vol. Added Acid/Base	Initial TRC	Final TRC	Vol. Added Sodium Thiosulfate

Work Perform by/Entered by _____ **Date**_____

ATTACHMENT 5
Battelle Duxbury Operations
Example Daily Sample Tracking Form[1]

Samples Relinquished by Custodian :	Date :	Time:
Location from which retrieved :	Date :	Time:
Samples received for sample prep by :	Date :	Time:
Storage until prep initiated :	Date :	Time:
Samples Returned to Custodian :	Date :	Time:
Location Stored :	Date :	Time:

Date Extracts Removed :	Initials:	Location removed from:
Date Extracts Returned :	Initials:	Storage Location:
Date Extracts Removed :	Initials:	Location removed from:
Date Extracts Returned :	Initials:	Storage Location:
Date Extracts Removed :	Initials:	Location removed from:
Date Extracts Returned :	Initials:	Storage Location:
Date Extracts Removed :	Initials:	Location removed from:
Date Extracts Returned :	Initials:	Storage Location:
Date Extracts Removed :	Initials:	Location removed from:
Date Extracts Returned :	Initials:	Storage Location:
Date Extracts Removed :	Initials:	Location removed from:
Date Extracts Returned :	Initials:	Storage Location:
Date Extracts Removed :	Initials:	Location removed from:
Date Extracts Returned :	Initials:	Storage Location:
Date Extracts Removed :	Initials:	Location removed from:
Date Extracts Returned :	Initials:	Storage Location:
Date Extracts Removed :	Initials:	Location removed from:
Date Extracts Returned :	Initials:	Storage Location:
Date Extracts Removed :	Initials:	Location removed from:
Date Extracts Returned :	Initials:	Storage Location:

Comments:

VALIDATION CHECKLIST

Check sample custody records to verify client Ids	
Verify all samples in batch are reported and all samples reported are in the prep batch	
Complete surrogate and internal standard spikng forms	
Check sample dilution, grav weights, and correction factors	
Check HPLC tables 100%	
Check dry weight and lipid weight tables	
Ensure sample transfer and documented by proper sign-off	

[1] Separate forms should be initiated for each sample prep batch and for each analysis. If sample is not consumed, document the storage of the remainder.

ATTACHMENT 6
Battelle Duxbury Operation
Sample Split and Transfer Log

Battelle
. . . Putting Technology To Work

Sample Split and Transfer Log

Project Number _____ Date of Work _____

Project Title _____

Analysis Type(s) _____

Splitting Procedure _____

_____

(include description of amount or weight of split, packaging, storage)

Name _____ Date _____

Sample ID	Sample ID	Sample ID	Sample ID	Sample ID	Sample ID

Released	Received
Signature/Date:	Signature/Date:
Storage Location/Conditions:	Storage Location/Conditions:
Released	**Received**
Signature/Date:	Signature/Date:
Storage Location/Conditions:	Storage Location/Conditions:

ATTACHMENT 7
Battelle Duxbury Operations
Chain-of-custody Record

Battelle
. . . Putting Technology To Work

Chain of Custody

Proj. No	Proj. Name

SAMPLERS: Signature

ANALYSIS REQUESTED → "NUMBER OF CONTAINERS"

DATE	TIME	BATTELLE ID	CLIENT ID	SAMPLE DESCRIPTION	FEST	PCB	TPH FINGERPRINT	PAH	VOA	TBT	METALS	OTHER	ACIDIFIED	PRESERVED	Total Number of Containers

Relinquished by:	Date/Time	Received by:	Date/Time
Relinquished by:	Date/Time	Received by:	Date/Time

Comments:

ORIGINAL

ATTACHMENT 8
Battelle Duxbury Operations
Certificate of Training

SOP No. 6-010

SOP Title: Chemistry Laboratory Sample Control

Trainee_____

Instructor_____

SOP Read:_____ _____
Signature Date

Date Training Completed:_____

The above mentioned trainee has satisfactorily completed the training requirements associated with this SOP. Supporting documentation (if applicable) is attached.

Comments:

Approved By/Date: _____

BATTELLE DUXBURY OPERATIONS
STANDARD OPERATING PROCEDURES

FOR

DOCUMENTATION PROCEDURES IN THE GAS CHROMATOGRAPHY/ MASS SPECTROMETRY (GC/MS) FACILITY

Summary of changes in this version: Data archival procedures are removed from this SOP. Archival of GC/MS data will be conducted according to SOP 6-036. Reference to a GC/MS tracking logbook is included.

1.0 OBJECTIVE

This SOP is a guide for completing and compiling all the forms and printouts necessary in each logbook. All documentation relating to the operation and maintenance of the GC/MS Facility is compiled and contained in the following logbooks:

1. GC/MS Sample Tracking Logbook
2. GC/MS Logbook
3. GC/MS Tune Logbook

2.0 PROCEDURES

2.1 GC/MS SAMPLE TRACKING LOGBOOK

The GC/MS sample tracking logbook is used to document the type, quantity, and the date on which a batch of samples are received into the GC/MS facility. The logbook documents the date on which GC/MS acquisition of the batch of samples begins and records which instrument is used for GC/MS data acquisition (see Attachment 1).

2.2 GC/MS LOGBOOK

The GC/MS Logbook is divided into five sections:

1. Sample Acquisition Records
2. Maintenance Records
3. Record of Service Calls
4. Service Contracts

2.2.1 All SOPs, which relate to the operation and maintenance of the GC/MS systems, will be kept in separate QA manuals located in designated site areas.

2.2.2 Each Sample Acquisition Record consists of an Acquisition Conditions Form (Attachment 2) and a hardcopy of a sequence file listing (Attachment 3).

2.2.2.1 The Acquisition Conditions Forms are numbered sequentially starting at A001, for GC/MS letter A, B001 for GC/MS letter B, C001 for GC/MS letter C, and V001 for GC/MS/VOC. All pertinent information should be filled out completely and inapplicable information should be noted as NA or dashed (-).

2.2.2.2 Each sample analyzed will be assigned a sequential data file name and the file range will be noted in the comments section of the Acquisition Conditions Form equal to sequence file and data files listed. Naming criteria for data files are as follows:

>xnnnn

where x is the instrument designator "A" for GC/MS letter A, "B" for GC/MS letter B, "C" for GC/MS letter C, "V" for GC/MS/VOC, and nnnn is a sequential number starting at 0001. At the turnover event (ex. >A9999), the numeric portion will be reset to 0001 again with appropriate notation in the comments section.

2.2.2.3 Sequence file listings may contain the following pertinent information: sample name, corresponding data file name, acquisition method used, injection volume, vial location, operator, data path runstrings, and project number/name comments. Sequence files are named as follows:

Sqxnnn

Where "x" denotes GC/MS letter and "nnn" is a sequential number starting at 001. At the turnover event (ex. >SQA999), the numeric portion will be reset to SQx001 again with appropriate notation in the comments section.

2.2.2.4 In the event that a sequence does not run to completion or the data from that sequence will not be used, it will be noted in the comments section of the Acquisition Conditions Form. The data file names allocated to the unused sequence will not be used again and the next sequence will continue sequentially.

2.2.3 Maintenance is performed on the instruments according to SOP 3-092. A record of the routine and non-routine maintenance performed will be kept on a GC/MS Maintenance Log (Attachment 4). The operator must date and initial each form.

2.2.4 Service calls placed for a GC/MS system will be recorded on a Record of Service Calls and Requests Form (Attachment 5).

2.2.4.1 Each form will include the date of the service call, the nature of the problem, and the service call number. The service call number is obtained from Hewlett-Packard support personnel upon placing the call.

2.2.4.2 When the problem with the GC/MS system is resolved, the date and nature of the corrective action taken will be recorded on the Record of Service Calls and Requests Form.

2.2.5 Hardcopies of relevant service contracts are stored in the GC/MS Logbook. Service contracts should be referenced when there are hardware problems. Hewlett-Packard requires model identification and serial numbers upon placing a service call.

2.3 GC/MS TUNE LOGBOOK

The GC/MS Tune Logbook contains two sections for each GC/MS system.

1. PFTBA Tune Hardcopies
2. DFTPP Tune Hardcopies

2.3.1 Prior to the start of a new sequence (or after non-routine maintenance) GC/MS systems are tuned with perfluorotributylamine (PFTBA) according to the procedures in SOP 3-092. Hardcopies of the reports

generated by the tuning programs AUTOTUNE, MANUAL TUNE, or TARGET TUNE will be kept in the GC/MS Tune Logbook.

2.3.2 The GC/MS systems can be tuned with decafluorotriphenylphosphine (DFTPP) according to the procedures in SOP 3-092. Hardcopies of all tune reports will be kept in the GC/MS Tune Logbook.

3.0 RESPONSIBILITY

3.1 Persons operating the Hewlett-Packard GC/MS systems are responsible for following the documentation procedures outlined in this document.

3.2 The GC/MS Logbook will be audited periodically by the system manager and Laboratory Manager to assure that entries are made according to this SOP.

4.0 TRAINING

The trainee must read, understand, and be able to perform all the functions listed in this SOP. Once this has been demonstrated a certificate of training (Attachment 6) will be issued and filed in the Quality Assurance Officer training files.

5.0 ATTACHMENTS

ATTACHMENT 1. GC/MS THROUGHPUT MONITORING SHEET
ATTACHMENT 2. GC/MS DATA ACQUISITION FORM
ATTACHMENT 3. SAMPLE SEQUENCE FILE LISTING
ATTACHMENT 4. GC/MS MAINTENANCE LOG
ATTACHMENT 5. RECORD OF SERVICE CALLS AND REQUESTS
ATTACHMENT 6. CERTIFICATE OF TRAINING

APPROVALS:

Author:	Julie A. Seavey	Dec 11, 1998
Laboratory Manager:	Robert H. J.	12-11-98
QA Coordinator:	Dawn Burke	12-11-98
Environmental Chemistry Section Manager:	Gregory Dull	12/11/98
	Name	**Date**

ATTACHMENT 1

BATTELLE – DUXBURY
GC/MS THROUGHPUT MONITORING SHEET

PROJECT NAME	BATCH ID	MATRIX	No. SAMPLES	DATE RECEIVED	DATE SETUP	INSTR.

ATTACHMENT 2

BATTELLE - DUXBURY
GC/MS DATA ACQUISITION FORM

Page _____

Project No:_____ Project Title:_____
Batch ID:_____ Matrix(Circle): Soil H2O Tissue Oil Other
Date: _____ Analyst: _____
Sequence. File: _____ Quantification Method: _____

METHOD FILES: Acq. File _____SIM /Full (Low m/z____ High m/z _____)

InjectionVolume (Circle One) 1 or 2 µL

GC CONDITIONS: Column DB-5 (60m) Carrier Gas: Helium

GC TEMP PRGM: Injector 300 ºC Trans Line 280 ºC Initial Temp 40 ºC

Initial Hold 1.0 min Ramp Rate 1 6 ºC/min Final Temp 290 ºC

Final Time _____min

EPC SETTINGS: Vacuum Compensation ON

Pressure Program (Circle One): Constant or Ramp

Initial Pressure: 30 psi Hold time: 1.0 min

MS ACQ. CONDITIONS: MSD Relative Abundance + ~100 EV above ATUNE.U

TUNING: Tuning Date (s)_____ Circle One: PFTBA DFTPP

MSD Vacuum (Ion Gauge) @ Tune (EPC @ 1 ml/min) _____x 10^{-5} Torr

MSD Vacuum (Tune report) _____ m Torr

Electron Multiplier @ Tune_____volts

Circle one: Autotune Manual Tune saved as: ATUNE.U

Files > _____ through >_____

COMMENTS: _____

TAPE ARCHIVE: CD#: _____

Notes: A hardcopy of the sequence file listing should be inserted in the logbook immediately following this page.

ATTACHMENT 3

SAMPLE SEQUENCE FILE LIST

Sequence Name: C:\HPCHEM\1\SEQUENCE\SQA493.S
Comment: ASE Tests B#98-352/98-352/98-364/98-357
Operator: DPB
Data Path: D:\SQA493\
Pre-Seq Cmd:
Post-Seq Cmd:

Method Sections To Run
(X) Full Method
( ) Reprocessing Only

On A Barcode Mismatch
(X) Inject Anyway
( ) Don't Inject

DPB 12-5-98

Line	Type	Vial	DataFile	Method	Sample Name
1	Sample	1	A5533	MWRATHIA	ET55
2	Sample	2	A5534	MWRATHIA	WA19PB
3	Sample	3	A5535	MWRATHIA	WA20PB
4	Sample	4	A5536	MWRATHIA	WA21PB
5	Sample	5	A5537	MWRATHIA	WA22PB
6	Sample	6	A5538	MWRATHIA	WA23PB
7	Sample	7	A5539	MWRATHIA	WA24PB
8	Sample	8	A5540	MWRATHIA	WA73PB
9	Sample	9	A5541	MWRATHIA	WA74PB
10	Sample	10	A5542	MWRATHIA	WA75PB
11	Sample	11	A5543	MWRATHIA	ET55
12	Sample	12	A5544	MWRATHIA	WA76PB
13	Sample	13	A5545	MWRATHIA	WA77PB
14	Sample	14	A5546	MWRATHIA	WA78PB
15	Sample	15	A5547	MWRATHIA	WA25MDL
16	Sample	16	A5548	MWRATHIA	WA26MDL
17	Sample	17	A5549	MWRATHIA	WA27MDL
18	Sample	18	A5550	MWRATHIA	WA29MDL
19	Sample	19	A5551	MWRATHIA	WA30MDL
20	Sample	20	A5552	MWRATHIA	WA31MDL
21	Sample	21	A5553	MWRATHIA	ET55
22	Sample	22	A5554	MWRATHIA	WA79PB
23	Sample	23	A5555	MWRATHIA	WA80SRM
24	Sample	24	A5556	MWRATHIA	VZ23COMP
25	Sample	25	A5557	MWRATHIA	VZ29COMP
26	Sample	26	A5558	MWRATHIA	VZ31COMP
27	Sample	27	A5559	MWRATHIA	ET55

ATTACHMENT 4

BATTELLE - DUXBURY
GC/MS
MAINTENANCE LOG

Date:_____ **Operator:**_____ **Initials:**_____

Flows checked (ml/min) (no) _____ (yes) _____ column_____ purge_____ split_____
Oring changed _____ Seal replaced _____
Column cut (~cm) _____ Washer replaced _____
Column position (~cm) _____ Merlin microseal chged _____
Glass wool position (~cm) _____ Syringe barrel cleaned _____
Injection port cleaned _____ Column changed (date) _____
Source Cleaned (date) _____ Other _____

Date:_____ **Operator:**_____ **Initials:**_____

Flows checked (ml/min) (no) _____ (yes) _____ column_____ purge_____ split_____
Oring changed _____ Seal replaced _____
Column cut (~cm) _____ Washer replaced _____
Column position (~cm) _____ Merlin microseal chged _____
Glass wool position (~cm) _____ Syringe barrel cleaned _____
Injection port cleaned _____ Column changed (date) _____
Source Cleaned (date) _____ Other _____

Date:_____ **Operator:**_____ **Initials:**_____

Flows checked (ml/min) (no) _____ (yes) _____ column_____ purge_____ split_____
Oring changed _____ Seal replaced _____
Column cut (~cm) _____ Washer replaced _____
Column position (~cm) _____ Merlin microseal chged _____
Glass wool position (~cm) _____ Syringe barrel cleaned _____
Injection port cleaned _____ Column changed (date) _____
Source Cleaned (date) _____ Other _____

Date:_____ **Operator:**_____ **Initials:**_____

Flows checked (ml/min) (no) _____ (yes) _____ column_____ purge_____ split_____
Oring changed _____ Seal replaced _____
Column cut (~cm) _____ Washer replaced _____
Column position (~cm) _____ Merlin microseal chged _____
Glass wool position (~cm) _____ Syringe barrel cleaned _____
Injection port cleaned _____ Column changed (date) _____
Source Cleaned (date) _____ Other _____

ATTACHMENT 5

BATTELLE - DUXBURY
GC/MS
RECORD OF SERVICE CALLS
AND REQUESTS

Date of Call: _____ **Initials:**_____**Service Call Number:**_____
Description of Problem:_____

Corrective Action Taken or Recommended: _____

Date Corrected/Repaired:_____ **Repaired By:**_____
Description of How Problem Was Fixed: _____

Date of Call: _____ **Initials:**_____**Service Call Number:**_____
Description of Problem:_____

Corrective Action Taken or Recommended: _____

Date Corrected/Repaired:_____ **Repaired By:**_____
Description of How Problem Was Fixed: _____

ATTACHMENT 6

BATTELLE DUXBURY OPERATIONS
CERTIFICATE OF TRAINING

SOP No. _____

TITLE _____

TRAINEE _____

INSTRUCTOR _____

DATE OF
COMPLETION
OF TRAINING_____

APPROVED _____ _____
Name Date

ORIGINAL

Battelle Ocean Sciences
Standard Operating Procedures

for

DATA RECORDING

Summary of changes in this version: Minor modifications are made throughout the document to reflect current practice: the definition list updated, the use of LRBs de-emphasized, and the requirement for documenting formulas emphasized. Adds one error code. This SOP does not address the specific requirements of EPA Good Automated Laboratory Practices (EPA 2185 8/10/95).

1.0 OBJECTIVE

Data generated during the course of projects must be capable of withstanding challenges to its validity, accuracy, and legibility. Therefore, data must be recorded in standardized formats and in accordance with prescribed procedures. This standard operating procedure (SOP) describes the procedures and personnel responsibilities associated with recording field and laboratory data.

2.0 DEFINITIONS

Raw data are defined as any worksheets, records, memoranda, or notes that result from original observations and activities of a project and that are necessary for the reconstruction and evaluation of the project. Raw data may include, but are not limited to, information recorded in

Field survey logs

Battelle Laboratory Record Books (LRB)

Instrument maintenance logs and calibration records

Sample chain-of-custody forms

Standard or stock solution preparation records

Laboratory data sheets (e.g., sample preparation or spiking sheets, miscellaneous documentation forms)

Project-specific data forms

Taxonomic Species ID forms

Raw data may also include photographs, maps, microfilm or microfiche copies, computer printouts, magnetic media such as dictated observations, recorded data from automated instruments, and correspondence related to planning, conduct, and interpretation of a project.

3.0 PROCEDURES

3.1 GENERAL

Raw data should be recorded on standardized data forms contained in bound notebooks (e.g., three-ring binder), or in Battelle LRBs. Data should be entered directly onto the appropriate form; entries should not be recorded on intermediate materials (e.g., scrap paper) and then transcribed to page. If data must be collected and cannot be entered directly onto the appropriate form the data should be recorded in a coherent and organized manner and attached permanently in the project notebook or LRB.

Procedures for recording data in LRBs are detailed in SOP 6-003. If standard forms are used to record data, the forms must contain enough information to ensure traceability. The minimum information required on each form is the project number, project phase or task (if necessary to distinguish it from other work conducted for that project), descriptive title identifying the type of data to be recorded (e.g., sample weights), date the work was performed, and the name or initials of the person(s) performing the work and recording the data. If the recorded data includes measurements, units must be included. Unused or non-applicable areas of the forms should be deleted or marked "NA".

All entries must be made in ink, preferable black. Felt-tipped pens should be avoided because many of the inks are soluble in water or organic solvents. The use of pencil to record data is not acceptable, except in rare circumstances (e.g., inclement weather conditions in the field). If pencil must be used, the data must be photocopied, stamped or marked as a verified copy, and signed and dated. The photocopies should be maintained with the original data in the project files.

All data must be recorded promptly and legibly. Entries must be signed or initialed by the person performing the work. If another individual recorded the data, that person must also sign or initial. Entries must be dated on the day of entry; times should be recorded for activities for which time intervals are critical.

3.2 DATA CORRECTIONS

Corrections to data should be made by drawing a single line through the original entry and replacing it with the correct value. Original data should not be obliterated or written over. All corrections and changes must be initialed, dated, and justified. Write-overs are considered data changes and must be treated in the same manner as other changes, i.e., the written-over value must be deleted with a single line and replaced with the correct value, and the correction initialed, dated, and justified. Justifications for changes should be clear and concise; vague explanations such as Wrong number" should be avoided. Suggested error codes for the more common changes are provided in Attachment 1. If a code other than the ones listed in Attachment 1 is used, an explanation of the code must be included in the project files.

3.3 DATA TRANSCRIPTIONS

Data that are transcribed from other sources must be traceable to their original source (i.e., either the specific location of the original data must be identified or the data transfer process must be described in an SOP or the project plan). Data entered in spreadsheets are assumed to be transcribed unless clearly marked as direct-entry data. If data are transcribed by hand, vs. instrument transfer, the name of the person transcribing the data and

the date of transcription must be recorded. If the transcription was verified, the name of the verifier and the date of verification should be documented.

3.4 CALCULATIONS

Calculations should be thoroughly documented so that the calculation can be duplicated by a person other than the originator. If the formula applied to data is not documented in an SOP then it must be documented with the data (as miscellaneous documentation, a footnote, etc.). All data sources, methods of calculation, and assumptions should be documented or identified. If data relevant to the calculation have been recorded elsewhere, the location of that data should be specified.

3.5 COMPUTER-DRIVEN DATA COLLECTION AND ANALYSIS

In computer-driven data systems, the individual responsible for entering the data and the date of entry must be identified. All printouts must be initialed and dated by the person responsible. Changes in computer entries must be traceable to the original entry, and the reason for the change, the date of the change, and the name of the individual responsible for the change must be documented.

3.6 EXACT COPIES

For studies conducted under GLPs (EPA FIFRA and TSCA Good Laboratory Practices), exact copies are required for original instrument data recorded on heat-sensitive paper, owing to the tendency of that paper to fade within a relatively short period of time. These photocopies must be stamped or labeled Verified Copy," initialed and dated by the person responsible, and maintained with the original data.

4.0 PERSONNEL RESPONSIBILITIES AND TRAINING

It is the responsibility of the technical managers to ensure that all staff performing the procedures described in this SOP are properly trained and that documentation of training exists prior to the performance of those procedures. Individuals whose responsibilities include data recording are responsible for reading and understanding this SOP and for performing the procedures in accordance with the stated requirements.

Individuals are considered trained once they have read this SOP and signed the associated training certificate (Attachment 2). The original certificate is filed in the Quality Assurance Unit office.

ATTACHMENTS

1. Error Code List
2. Example of Record of Training

APPROVALS

Author	Dana F Bahl	5-21-96
QA Coordinator	Dana F Bahl	5-21-96
Vice President, Battelle Ocean Sciences	Anthony Aiello	5-22-96
	Name	Date

Attachment 1

ERROR CODE LIST

Error codes should be written near the correction and must be accompanied by the initials of the person making the change and the date of the change.

WL Inadvertently recorded in the wrong location (e.g., row, column, page)

CC Changed for greater clarity

WO Write over

SE Spelling error

IR Inadvertently not recorded at the time of initial observation

CE Calculation error

TE Transcription error

RE Rounding error

EI Entry not initialed, dated, and/or justified at the time of entry

TI Incorrect time

DA Incorrect date

UN Incorrect units

ID Incorrect sample ID

MD See miscellaneous documentation form (refer to page or Misc Doc #)

WP Peak misidentification (gas chromatography only; analyst judged peak to be incorrectly identified by instrument software)

S/B Should be; clarifies correct data

Attachment 2

RECORD OF TRAINING
for
SOP No. 6-017-03, *Data Recording*

The above mentioned SOP is relevant to your work. Your signature below attests to the fact that you have read this SOP and understand the requirements associated with this procedure.

Name (Printed)	Signature	Date

Battelle Duxbury Operations

Standard Operating Procedures

for

LABORATORY VERIFICATION AND VALIDATION OF ANALYTICAL DATA

Summary of changes in this version: All references to "validation" have been changed to "verification" to reflect the currently accepted definitions of these terms. A separate section on laboratory validation is added. Specific verification and validation procedures required for certain Navy programs are addressed.

1.0 INTRODUCTION

Analytical data must be verified by he analyst for accuracy, completeness, compliance with the Quality Assurance Project Plan (QAPP), standard operating procedures (SOPs). Data validation is performed by the project manager by assessing quality control data vs. the project data quality objectives, and by reviewing the data for technical reasonableness prior to submission to the Quality Assurance Office. This SOP describes the procedures and responsibilities for data verification and validation.

2.0 PREPARATION

Prior to data verification and validation, the data package should be completed with the documentation necessary to support the reported analyses. (The contents of a data packages are defined in SOP 6-032). The following items will be required to complete data verification and validation:

Completed data package
QAPP (also called work plan or QA Plan)
Sample prep records
Standard prep forms
Instrument calibration records
Raw data files
Final report tables

3.0 VERIFICATION

Verification is defined as *Confirmation by examination and provision of evidence that specified requirements have been met (*National Environmental Laboratory Accreditation Conference 1999).

3.1 PROCEDURES AND DOCUMENTATION

Data verification proceeds from raw data or the last verified step to the data being reviewed (from the known to the unknown). The Data Verification Checklist (Attachment 1) provides guidance on the types of entries and level of detail to be verified. Manual entries and hand-calculations are 100% verified. At least one spreadsheet calculation per sample is reproduced by hand, verifying the formula components from raw or verified data and then reproducing the reported result. Formulas that are reported with the data are also confirmed in this process. Data for a variety of analytes should be verified during this process, and particular attention should be focused on samples that required individual processing procedures (splits, dilution, additional spiking, or re-extraction).

Data verification is documented on the checklist with a check (✓) and the dated initials of the person who performed the verification. The date of spreadsheet preparation should reflect the most recent version. If a spreadsheet is updated to correct errors then this date should be updated. Validation of the new spreadsheet should consist of verification that the most recent version of the spreadsheet was corrected, that the errors were corrected as anticipated, and that unaffected data are unchanged. Old spreadsheets should either be discarded or filed in the "unused data" section of the data package. In the latter case the spreadsheet should be crossed out with the routine documentation of date, initials, and justification.

4.0 VALIDATION

Validation is defined as *The process of substantiating specified performance criteria* (EPA-QAD). Data validation is performed by the laboratory and project managers unless otherwise specified in the QAPP. This validation consists of a review of analytical quality control results (QC samples and instrument QC) vs. the project data quality objectives and the requirements of the SOPs to determine whether the analytical system was in control for the analyses. If the system is in control then the data meet performance criteria. The signature of the laboratory and project managers on the title page of the data package signify that this validation has been performed.

5.0 RESPONSIBILITIES

In general, verification may be performed by the person who originally performed the work or by another trained analyst. If calculations are unusual or complicated it is advisable to ask for an independent review.

Sample Custodian:

The sample custodian is responsible for comparing

- the sample container IDs vs. the sample custody logs
- the completed Sample Identification Logbook vs the Battelle Duxbury Operations (BDO) and client IDs on the containers

The Sample Receipt Log-in file is verified 100% vs. the chain of custody forms by an independent reviewer. This review is documented directly on the hardcopy Log form.

Sample Prep Task Leader:

The sample prep task leader is responsible for validating

- Project name and project number
- Standard expiration dates
- Sample IDs vs. the custody records
- Assignment of sample suffixes for re-extractions, dilutions, etc.
- All prep summary tables (dry and lipid weights, gravimetric data, etc.)
- Documentation (complete and correct), including deviations from the QAPP and SOPs

Any changes made to the prep records after distribution to the analysts must be reported to the analysts.

Analysis Task Leaders:

The analysis Task Leaders are responsible for verifying the following quantification information:

- Internal standard amounts vs. approved standard prep forms
- Sample multipliers and sample weights
- Project name and number
- The standard amounts of all new calibration standards and methods
- Units
- Documentation (complete and correct)

In addition, the analytical task leader is responsible for report table and spreadsheet verification. The following information should be verified:

- All information in the table headings
- Sample IDs vs. the validated prep records
- All manual entries
- One analyte and one surrogate recovery; at least one value in every column should be checked.
- Qualifiers
- MDLs (including appropriateness - same standards, processing procedures, etc)
- The analyses vs. the QAPP, with all deviations documented.

The task leader documents this verification by signing the title page of the data package.

Laboratory Manager:

The laboratory manager reviews the data package for completeness and reviews the quality control results. This verification is documented by signing the title page of the data package.

Project Manager:

The project manager is responsible for the final review of the data. This review should consist of the following assessment:

- The data are clearly presented, in a manner that facilitates client review. Depending on the purpose of the study, this may include reporting samples from the same station, species, or survey together. The reporting format should be specified in the QAPP.

- The scope of the analyses was completed according to the contract and QAPP

- All documentation (miscellaneous documentation forms and documentation of deviations) in the data package is reviewed and approved.

- The quality control results are acceptable or that corrective action has been performed and documented.

Quality Assurance Auditor:

The QA auditor is responsible for verifying that data have been verified in the laboratory. The independent au lit of data may not proceed on unvalidated data without the direct approval of the projec

or laboratory manager. Otherwise, if there is no evidence that a package submitted for audit has been validated then the package will be returned to the analytical task leader.

Unless specific exceptions are approved by the Resource Manager, all data packages are audited by the Quality Assurance Unit. These audits are defined in SOP 4-015. This review provides an independent verification of the reported data and the completeness of the data package.

Certain U.S. Navy programs require that defined staff conduct specific review procedures: peer, supervisory, management, and QA. The responsibilities are defined in Attachment 2. The implementation of the 4-tiered review process must be defined in the QAPP.

6.0 TRAINING

All laboratory personnel must understand their responsibilities for data validation and must read this SOP. This is documented on the Documentation of Completion form (Attachment 3) which is signed and dated once this SOP is read. The original is forwarded to the QA Office.

Attachment 1. Validation Checklist
Attachment 2. Verification and Validation Requirements for Certain Navy Programs
Attachment 3. Documentation of Completion Form

APPROVALS

Author	Bonnie Buhl	8-2-99
Laboratory Manager	[illegible]	8·2·99
Quality Systems Manager	Bonnie Buhl	8-3-99
Environmental Chemistry Section Manager	[illegible] Name	8/3/99 Date

Attachment 1
Data Verification Checklist for Data Packages

SAMPLE PREP REVIEW

- Check sample custody records to verify client IDs
- Verify that all samples in batch are reported and that all samples reported are in the prep batch
- Complete surrogate and internal standard spiking forms
- Check sample dilution, gravimetric weights, and correction factors; complete form if more than 1 applies to a sample.
- Check HPLC tables 100%
- Check the dry weight and lipid weight data
- Ensure sample transfers are documented by proper sign-off

CALIBRATIONS

- Spot check the response factors for the initial calibration (1/column and 1 mean) and verify that the standard concentrations are correct and that calibration meets the SOP or work plan criteria.
- Check that mid-check standards bracket all reported samples, and that results meet the SOP or work plan criteria (frequency and acceptance level).
- Verify that the correct method file is used to quantify each sample.
- Check that Q-deletes have been deleted correctly.

DATA TABLES

- Check transcription of weight data onto the report tables
- Check any standard amounts and dilutions entered on the quant reports
- Verify the report table reporting units and header information.
- Check that reported analytes are those called for by the Work Plan.
- Spot-check at least one data point for each sample. Recalculate from scratch using all calibration and prep factors
- Check qualifiers and MDLs vs work plan.
- Spot-check surrogate recoveries, focusing on samples with differing dilutions, etc. and check vs work plan criteria
- In required, verify that reported PCBs have been confirmed.
- Calculate QC data (BS, MS) and check qualifiers vs work plan.

DOCUMENTATION

- Flag questions, documentation ambiguities and errors in the data package
- Review the work plan to ensure that all work plan specifications have been met.

Verification completed by: _____ Date: _____

Attachment 2
Verification and Validation Requirements for Certain Navy Programs[1]

PEER REVIEW
An analyst that is qualified to perform the subject analytical method must perform peer reviews. Peer reviews will include, as appropriate:

- 100% of manual entries and manual calculations;
- spot checks of computer calculations to verify program validity;
- check for compliance with method and project-specific QC requirements;
- check for completeness of raw data or supporting materials;
- confirm spectral assignments and identification of TICs;
- check descriptions of deviations from method or requirements;
- check for appropriate use of significant figures and rounding;
- check reported values for dilutions; and
- evaluate reasonableness of results.

SUPERVISOR REVIEW
Technical reviews by the Project Manager or designated alternate must be performed on 100% of reported data. The supervisory review may be less detailed than the peer review, but must include the following:

- check for compliance with Method and project-specific requirements;
- check the completeness of the reported information;
- check the information for the report narrative, and
- evaluate the reasonableness of results.

MANAGEMENT APPROVAL
The Laboratory Manager must perform a 100% check of all data. All data must receive management approval prior to release. Authority to release data may be delegated to a technical supervisor or other party, if the term of the delegated authority is documented in the QA program file.

QA REVIEW
The QAU audits all reported data prior to submission to the client. Among the review procedures the following will be verified:

- check for compliance with required QC practices;
- check for compliance with approved SOPs; and
- check for compliance with Method and project requirements.

[1] From: Navy Installation Restoration Laboratory Quality Assurance Guide (February 1996).

Attachment 3
Record of laboratory staff who have read SOP 6-027

SOP Title: Laboratory Verification And Validation Of Analytical Data

The above mentioned SOP is relevant to your work. Your signature below attests that you have read this SOP and understand the requirements associated with this procedure, and that a copy of the SOP is available to you at your place of work during performance of this procedure.

Name (printed)	Name (Signature)	Date

ORIGINAL

Battelle Duxbury Operations
Standard Operating Procedure

for

PREPARATION OF ANALYTICAL CONTROL CHARTS

Summary of changes in this version: Separation of matrix and method for control charts has been added.

1.0 SCOPE

Control charts are used in the laboratory to monitor and control the accuracy and precision of an analytical method. Control charts provide a graphic representation of on-going laboratory quality through chronological plotting of selected data quality characteristics (e.g., recovery). This standard operating procedure (SOP) defines the laboratory requirements for maintenance of control charts in the organics and metals laboratories. It ensures that the quality of every analytical batch is monitored against statistical levels of confidence. Though requirements for occasional analyses (e.g., DO, chlorophyll) are not defined in this SOP, the same principles apply. EPA-regulated programs require the use of approved analytical methods (e.g., Method 608, 625, and the SW846 series) that describe a rigorous control chart program. There is no attempt to adopt these requirements in this SOP because Battelle does not routinely perform analyses for these programs. Specific project work plans must identify additional requirements for control charts that will be performed in addition to, but not in place of, these requirements.

2.0 PREPARATION

Laboratory performance is monitored though the incorporation of quality control samples into every analytical batch. Quality control (QC) samples are prepared and analyzed according to approved SOPs and are treated identically to authentic field samples. A Laboratory Control Sample (LCS) prepared and analyzed with each batch of samples, will be plotted on control charts:

Separate control charts are maintained by matrix and method type:

PAH	Aqueous
PCBs/pesticides	Sediment/Soil
VOA	Tissue
Alkanes	
Butyltins	

Attachment 1 defines the QC parameter that will be monitored for each analysis. When new or modified procedures are instituted in the laboratory the procedure will incorporate an appropriate quality control sample which will be used to generate baseline data for establishment of control chart statistics.

3.0 PROCEDURES

3.1 Initial Development of Control Charts

Beginning in 1999, all control charts were developed using Control Chart!Pro@Plus. See the software manual for further details about the use of the software. Control charts are prepared to chart performance of routine analytical procedures. The quality control samples defined in Section 2.0 should be analyzed with successive analytical batches according to approved SOPs. Once data are validated, the mean and standard deviation (n-1) of the quality control sample results are determined. Generally, a minimum of 20 data points is needed to establish the control chart limits. However, as few as five values can be used in cases where the analysis is infrequent and less than 20 points have been accumulated over a 6-month period. Additionally, the Laboratory Manager may direct the preparation of interim control charts before 20 data points are generated in order to monitor laboratory performance of a new or infrequent procedure.

A master control chart for each method type (Section 2.0) is prepared at the end of the year using the quality control sample results generated from the last 20 batches of data analyzed during the year. (Or if 20 points are not available, then the values generated during the previous six months will be used). Note that the master charts for 2000 will use available data from 1999. The Laboratory Manager or designee prepares these charts. At a minimum, the QC results [percent recovery] are plotted on the Y-axis and the batch ID is plotted on the X-axis (Attachment 2). Separate charts are prepared for each analyte, matrix, and method combination.

The charts will contain

- A centerline: the calculated QC value average
- Upper and lower warning limits: $\pm 2\sigma$ of the calculated average
- Upper and lower control limits: $\pm 3\sigma$ of the calculated average
  where σ = standard deviation

All standard deviations are calculated for the population (n-1).

The preparation of a typical master control chart is described in the following example.

Example: Twenty blank spike samples (Attachment 1) are analyzed for PAHs in sediments. The summary statistics calculated for the percent recovery of acenaphthylene are

$\bar{x}$ = 107%
SD (σ) = 7.33%
n = 20

Center Line is 107%

Warning Limits (WL) are calculated as the average X2σ

$\bar{x}$ X2(7.33) or 107X14.66, therefore

Upper Warning Limit (UWL) = 107 + 14.66 = 121.66 %
Lower Warning Limit (LWL) = 107 - 14.66 = 92.34 %

Control Limits are calculated as the average 3Xσ

$\bar{X}$ X3(7.33) or 107 ±21.99, therefore

Upper Control Limits (UCL) = 107 + 21.99 = 128.99%
Lower Control Limits (LCL) = 107 - 21.99 = 85.01%

Once established for an analysis, these limits will not be recalculated within a calendar year unless

- A significant drift over time indicates a shift in laboratory or instrument performance. In either case, if corrective action requires changes in procedures or significant instrument maintenance, new control chart values may need to be established.
- It appears that differences exist between instruments or processes which should be segregated and plotted separately.
- The laboratory changes sample processing procedures or new instrument conditions are established for analysis. Separate control charts will not be plotted for each instrument or analyst unless specified by the client.

The control chart limits will be reestablished each year using the last 20 QC values from the previous year.

3.2 Routine Maintenance of Control Charts

Once recovery data are generated and validated, the analyst delivers the electronic data to the Laboratory Manager or designee. The recovery value is formatted to fit the CCPRO+ software. The control chart can then be generated and the data reviewed for trends that could identify an out-of-control process.

3.3 Control Chart Administration and Reporting

The Laboratory Manager or his/her designee reviews control charts weekly to assess data trends. This review is documented by initialing the control chart Logbook. The Logbook is kept with the Laboratory Manager and the electronic control charts are stored on the hard drive of the PC of the generator of the charts. Over 150 control charts are maintained electronically. Control charts will be printed quarterly and as needed for out-of control events.

4.0 QUALITY CONTROL

4.1 Out-of-Control Events

A laboratory procedure is considered out-of-control whenever, at a minimum, if any one of the following conditions is demonstrated by a control chart.

1. any one point is outside of the control limits;
2. any three consecutive points are outside of the warning limits;
3. any eight consecutive points are on the same side of the centerline;
4. any six consecutive points are such that all the points are larger or smaller than their immediate predecessor; and
5. any obvious cyclic pattern is seen in the points.

4.2 Corrective Actions

When any one of the conditions listed in Section 4.1 is identified it is the responsibility of the analyst to notify the Laboratory Manager immediately. Initially, a review of the out-of-control data is performed by the Laboratory Manager and the analyst. They will determine the appropriate corrective action, complete a Corrective Action form (Attachment 3), and notify the QA officer of the out-of-control event by submitting a copy of the corrective action form with a copy of the control chart.

- In some cases corrective action investigations may identify a mathematical error or an isolated incident in the laboratory. If the cause of the out-of-control event is identified (contaminated glassware, degraded standards), then the affected control chart points are circled on the chart with an explanation. These circled points are not included in future Section 4.1 analyses. Calculation errors will be corrected on the chart with appropriate documentation.

- If no apparent cause is found and the out-of-control event(s) appear to be isolated to a specific period, the data generated during that period may be unreliable. In these cases, the quality control data generated with the sample batch should be reviewed.

  (a) If the data for QC samples meet the SOP and work plan criteria then the data are considered acceptable, but corrective action for the analytical system should be initiated and documented in the instrument log.

  (b) If QC data do not meet the SOP or work plan criteria then the reported data associated with the QC check should be flagged as suspect (s). Re-analysis of project data is left to the discretion of the Project Manager. However, the project files must include documentation that the analytical system may have been out of control by documenting the deviation, with justification and approval by the project manager, and a copy of the control chart.

- Generally, data generated in the same batch as a data point which exceeds the Control Limits is considered unreliable. Reanalysis will occur after corrective action has been taken and the procedure is determined to be back in control. If the project manager determines that reanalysis will not occur, the project files must document this deviation, with justification and approval by the project manager, and a copy of the control chart.

- If a trend is observed that appears to be permanent and still within acceptable laboratory operating limits, then it is probably necessary to re-calculate the control limits at this point. The Quality Systems Manager must be consulted prior to this action.

- If routine plotting reveals distinct trends for a particular matrix, instrument, or analyst then these data will be plotted as a separate control chart.

5.0 RESPONSIBILITIES

Each analyst is responsible for

- immediately supplying the results of QC sample analysis to the designated generator of the control charts.

The Laboratory Manager or designate are responsible for

- preparing the master control charts for each analysis type,
- reviewing the control charts weekly,
- reviewing all out-of-control events and determining, with the analyst the appropriate corrective action,
- ensuring that the appropriate corrective action is implemented and documented if warning or control limits are breached, and
- ensuring that any data generated during out-of-control periods is reviewed, the project manager notified, and any affected samples are reanalyzed as directed by the project manager.
- notifying the Quality Systems Manager that an out-of-control event has occurred, and
- collecting, distributing, and filing the control charts.

The Quality Systems Manager is responsible for

- reviewing the results of control charts quarterly,
- working with the Laboratory Manager to determine appropriate corrective action or determining whether new control limits should be established,
- ensuring that out-of-control events and the resulting corrective action are adequately documented, and
- ensuring that the quality control criteria for any reported data generated during out-of-control events are acceptable or flagged appropriately.

6.0 DOCUMENTATION

Current control charts are maintained electronically for immediate update and review. A full set of control charts will be printed on a quarterly basis and kept in the Control Chart Logbook. All historical control charts, with all appropriate documentation of corrective action, will be maintained by the Laboratory Manager or designee in Control Chart Logbook.

7.0 TRAINING

Any individual who will prepare control charts must first read this SOP. Next the analyst must become familiar with Control Chart Pro+. The Laboratory Manager will review data plotting and documentation

procedures with his/her designee about generating control charts. Once these training steps have been completed a certificate of training (Attachment 4) will be completed. The original Certificate of Training will be provided to the QAU.

ATTACHMENTS

Attachment 1. Organics Analyses Control Charts
Attachment 2. Example Control Chart
Attachment 3. Corrective Action Log form
Attachment 4. Certificate of Training

APPROVALS:

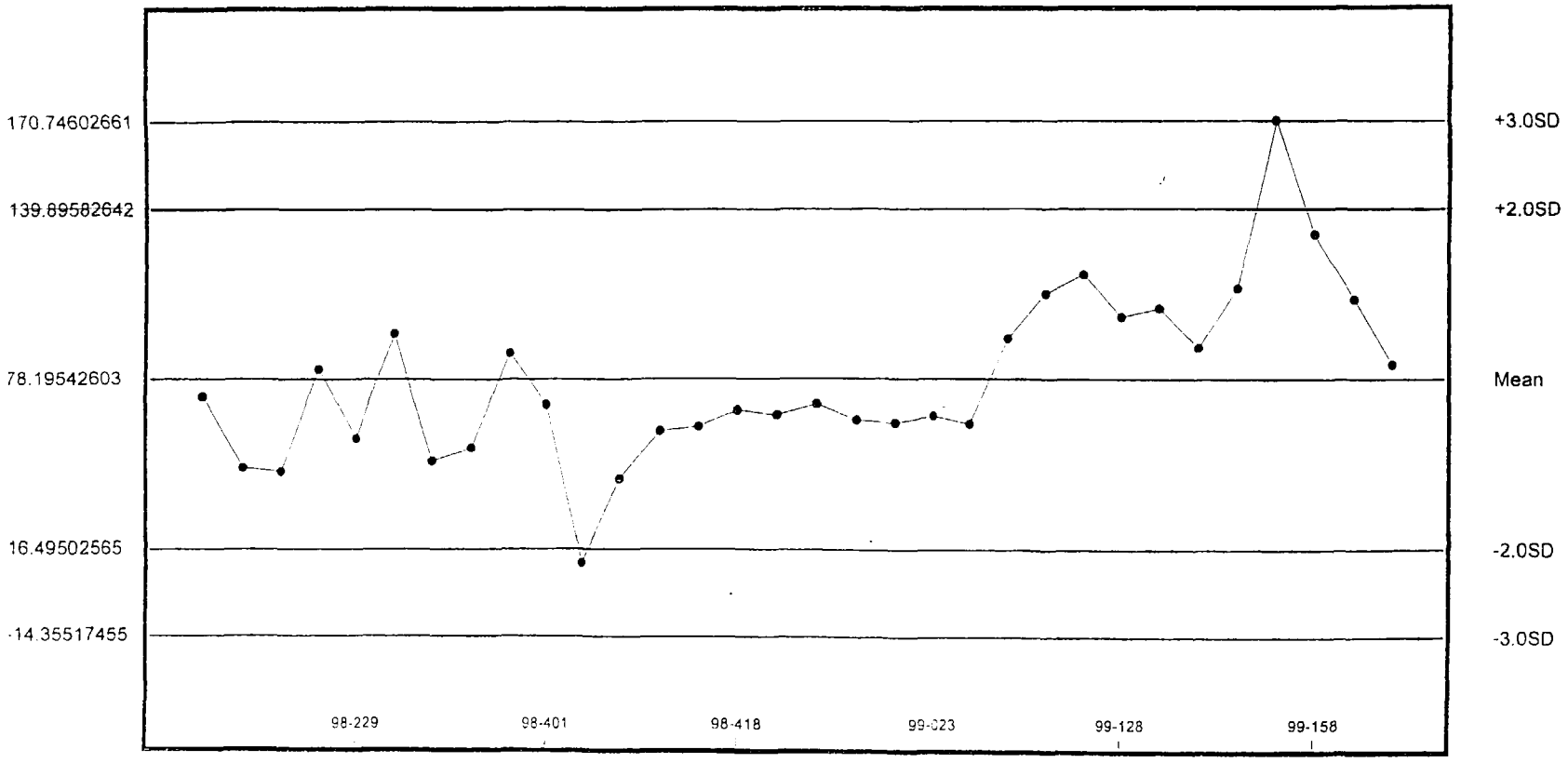
Author		12·10·99
Laboratory Manager		12·10·99
Quality Systems Manager		12-10-99
Environmental Chemistry Section Manager	Signature	12/10/99 Date

Attachment 1. Organics Analyses Control Charts[1]

Analysis Type	QC Sample	QC Statistic
PAH	Laboratory Control Sample	Recovery
Phenols	Laboratory Control Sample	Recovery
Pesticide/PCB	Laboratory Control Sample	Recovery
Alkanes	Laboratory Control Sample	Recovery
Butyltins	Laboratory Control Sample	Recovery
VOAs	Laboratory Control Sample	Recovery

[1]Control charts will track the recovery of all analyzed compounds in LSC samples. All recoveries will be reported vs. the recovery internal standard (RIS). Master Control Chart limits are based on 20 data points, if possible.

Attachment 2
Example Control Chart



n= 32 mean= 78.19542694 SD= 30.85020065 %CV= 39.45% min= 11.16000000 max= 170.94710710

e: D:\Active\Control Charts\TBT 1999\TBT1999.ccp
olumn DBT

JPB 9-20-99

Attachment 3
Corrective Action Log

Battelle Duxbury Operations
Corrective Action Log

Project No. _____

Analyst_____ Analysis Type_____

Date_____

Description of Problem:

Affected Samples:

Corrective Action Plan:

RESOLUTION

Results of corrective action plan:

Was data quality affected?

Was the client contacted?

APPROVAL

Laboratory Manager_____ **Date**_____

Project Manager_____ **Date**_____

QA Officer _____ **Date**_____

Attachment 4

Battelle Duxbury Operations
Certificate of Training

SOP No. 7-028

SOP Title **PREPARATION OF ANALYTICAL CONTROL CHARTS**

SOP read and understood _____ _____
Name Date

Description of Training;

The above mentioned trainee has satisfactorily completed the training requirements associated with this SOP.

Approval _____ Date _____

ORIGINAL

Battelle Duxbury Operations
Standard Operating Procedure

for

PREPARATION, ANALYSIS,AND REPORTING QUALITY CONTROL DATA IN THE CHEMISTRY LABORATORY

Revisions to the SOP: The content is expanded and the title changed to reflect added scope. Sections for sample preparation and analysis are added. Definitions and frequencies of QC samples are provided. Examples of corrective action are added. The discussion of method detection limits and reporting limits was moved to SOP 5-291. Definitions and criteria for several QC samples are added or modified.

1.0 OBJECTIVES

The purpose of this SOP is to describe the preparation, analysis, and standard reporting requirements for quality control (QC) samples. At a minimum, one suite of quality control samples including a procedural blank, a measure of accuracy, and a measure of precision are required with every set of 20 or fewer field samples. Description of common QC samples are described in Attachment 1. The project manager will define the required QC samples in the QAPP.

2.0 PREPARATION

Attachment 1 defines the preparation and frequency of quality control samples that are required routinely. All field and quality control samples are spiked with the same surrogate internal standards prior to processing and with recovery internal standards prior to analysis.

3.0 ANALYSIS

Quality control samples are analyzed using the same calibration, instrument settings, and procedures used for field samples. No extraordinary means may be used in analysis or quantification.

The method blank that was prepared with the processing batch should be analyzed with each analytical sequence (run).

4.0 REPORTING

Project reporting requirements are highly variable. However, the following conventions are applied unless otherwise specified in the QAPP.

4.1 SAMPLE MATRIX

The sample matrix reported with quality control (QC) samples such as the procedural blank (PB) or laboratory control sample (LCS) should be reflective of the associated field samples. For example, the matrix reported for the PB associated with a sediment batch should be "sediment".

4.2 UNITS

Unless otherwise specified in the QAPP, the PB will be reported in the same concentration units as the associated field samples. The LCS sample will be reported in amount units, reflective of the amount of target analyte(s) added to the sample. As a quantitation of analytical accuracy, the percent recovery of each analyte will also be reported.

4.3 SAMPLE SIZE

Unless defined otherwise in the QAPP, the weight/volume of PB samples will be reported as the *approximate* average weight/volume of the field samples in the associated sample batch. This value, an integer, will be determined by the sample preparation Task Leader (or appropriate designee), and documented in the sample preparation records. Sample size will be reported for QC samples as follows:

PB	average field sample weight (described above)
LCS	NA
MS	actual weight

4.4 ANALYTES REPORTED

Only those analytes required by the client, defined in the QAPP, specified in the Method, or otherwise requested and documented by the Project Manager will be reported for QC samples. Any additional analytes quantified or included in the SRM or spike solution will not be reported on the final data tables reported to the client. This applies to PB and LCS samples as well as standard reference materials (SRM) and matrix spike (MS) samples. Exception: In the case of PCBs, if samples are analyzed for Aroclors, and congeners are quantified in the QC samples, the congener data will be reported.

4.5 SRM DATA

The default format for SRM data tables should include the target analytes in concentration units, the certified values or ranges, and the percent difference between the concentration detected and the certified values or range. If the certified value is defined as a range, then the PD based on either the upper or lower concentration limit defined in the Certificate of Analysis, whichever is appropriate. (The appropriate range value is the one closest to the detector value). Values within the range have a PD of 0%.

Unless otherwise requested by the client, only concentrations >10 times MDL will be qualified if they are outside the range stated as the DQO.

4.6 DATA QUALITY CRITERIA AND DATA QUALIFIERS

Default Data Quality Criteria (DQC) for QC samples are presented in Attachment 2. These values/criteria will serve as the DQCs for all projects unless otherwise defined by the client, defined in a method-specific SOP, or unless they do not adequately serve a particular project or matrix. The DQCs are defined in the QAPP. QC sample results that exceed acceptance criteria should be investigated and the appropriate corrective action implemented. Examples of corrective action for quality control samples are provided in Attachment 3.

The use of data qualifiers is often project-specific and defined in the QAPP. In the absence of other specifications the following data qualifiers.

Qualifier	Use
J	Analyte detected below the MDL/RL.
B	Analyte detected at a level greater than 3X MDL/RL in the PB. The qualifier is entered for the PB and affected field samples.
ME	Estimated value; significant Matrix Interference.
U	Analyte not detected. "ND" will be reported in the value column
&	QC value outside the accuracy or precision DQC.

As a default reporting convention, analytes not detected will be reported as "ND" and qualified with a "U." Alternative qualifiers/MDL/RL values will be inserted on a project-specific basis. "NA" is only applicable when a value or descriptor is not available or not applicable (e.g., sampling time is required on report table but is not included, or the Client ID for an internal QC sample).

4.7 SIGNIFICANT FIGURES

Unless specified in the QAPP, sample concentration data are reported to two decimal places. An attempt should be made to limit significant figures to no greater than four. Additional significant figures should be removed after all data manipulations have been completed. All QC data reported as recovery (e.g., surrogate recoveries, analyte recoveries in LSC, MS/MSD samples) should be reported as whole numbers. All QC data reported as a measure of precision (RPD between duplicates; percent difference for SRMs) should be reported to one decimal place.

5.0 CALCULATIONS

5.1 PERCENT RECOVERY

The percent recovery is a measurement of accuracy, where one value is compared with a known/certified value. The formula for calculating this value is:

$$Percent\ \ Recovery = \frac{amount\ \ detected}{amount\ \ expected} x\ 100$$

5.2 PERCENT DIFFERENCE

The percent difference (PD) is a measurement of precision as an indication of how a measured value is difference from a "real" value. It is used when one value is known or certified, and the other is measured. The formula for calculating PD is:

$$Percent\ \ Difference = \frac{X_2 - X_1}{X_1} x\ 100$$

where: X_1 = known value (e.g., SRM certified value)
X_2 = determined value (e.g., SRM concentration determined by analyst)

5.3 RELATIVE PERCENT DIFFERENCE

The relative percent difference (RPD) is a measurement of ***precision***; it is a comparison of two similar samples (matrix spike/matrix spike duplicate pair, field sample duplicates). The formula for calculating RPD is:

$$RPD = \left| \frac{2\ x\ (X_1 - X_2)}{(X_1 + X_2)} \right| x\ 100$$

where: X_1 is concentration or percent recovery in sample 1
X_2 is concentration or percent recovery in sample 2

Note: Report the absolute value of the result -- the RPD is always positive.

5.3.1 Field Sample Duplicates

The RPD between field sample duplicates is a quantitative measure of the variability between duplicates using the native concentrations of target analytes as comparison values. The assumption is that the two samples are exactly alike, and that the concentrations of analytes in the duplicates are expected to be identical.

Step 1: Calculate the concentration of the analytes in the two samples.

Step 2: Substitute the concentrations for X_1 and X_2 in the RPD formula. (An example calculation is presented in Attachment 4).

5.3.2 Matrix Spike/Matrix Spike Duplicate Samples

With MS and MSD duplicate samples there are several variables that must be considered when performing the RPD calculation, including sample size, background concentrations, and amount of

analyte(s) spiked into the samples. To calculate the RPD:

Step 1: Calculate analyte concentration in the MS, MSD, and unspiked background sample (units: weight/weight, e.g. ng/g).

Step 2: Subtract background **concentration** of each analyte detected in the background sample from the MS and MSD. This will, theoretically, leave us with the concentration due to the spike alone. (units: weight/weight, e.g. ng/g).

Step 3: Determine the **amount** of each analyte detected in the MS and MSD samples. To do this, multiply the concentration value of each analyte AFTER background subtraction, by the sample weight. (units: weight, e.g. ng, µg).

Step 4: Calculate percent recoveries of the analytes in the MS/MSD samples:

$$\frac{Amount\ detected\ in\ MS\ (or\ MSD)}{Amount\ expected} x\ 100$$

where the amount expected is the amount of the analyte spiked into the sample prior to extraction.

Step 5: Calculate the RPD between the MS and MSD samples. This should be based on the percent recoveries of the analytes detected in each sample. These recoveries will be inserted into the RPD equation as X_1 and X_2. An example calculation is displayed in Attachment 5.

The reason recoveries are calculated based on the **amount** of analyte recovered in the spiked MS/MSD samples rather than the concentration is because the concentrations are not comparable unless the samples are <u>exactly</u> the same size. For example, assume sample A weighs 30 g and sample B weighs 40 g. If they were each spiked with 100 ng acenaphthene prior to extraction, the amount of analyte detected should be the same,

$$Concentration\ in\ A = \frac{100\ \ ng\ acenaphthene}{30\ \ g} = 3.3\ \ ng/g$$

however the concentrations would differ because of the sample size:

$$Concentration\ in\ B = \frac{100\ \ ng\ acenaphthene}{40\ \ g} = 2.5\ \ ng/g$$

5.4 RELATIVE STANDARD DEVIATION

The relative percent difference (RSD) is a measurement of ***precision***; it is a comparison of three or more similar samples (e.g., field sample triplicates, initial calibration, MDLs). The formula for calculating RSD is:

$$\%RSD = \frac{\textit{Standard Deviation of All Samples}}{\textit{Average of All Samples}} \times 100$$

ATTACHMENTS

1. Definitions of Typical Laboratory Quality Control Samples and Frequency
2. Data Quality Criteria
3. Probably Sources and Expected Corrective Actions
4. Field Sample Duplicate Calculations
5. MS/MSD Sample Calculations

APPROVALS:

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Attachment 1
Definitions of Typical Laboratory Quality Control Samples and Frequency

QC Sample	Definition	Frequency
Method or Procedural Blank (PB)	A combination of solvents, surrogates, and all reagents used during sample processing, processed concurrently with the field samples;. Monitors purity of reagents and laboratory contamination. Matrices: Water (MilliQ); soil or sediment (Ottawa sand or sodium sulfate); tissue (sodium sulfate)	1/sample set [a] For some projects the PB is analyzed with each instrument run.
Trip Blank (TB)	A container of MilliQ water that accompanies sample bottles to the field, during collection, and returned with the field samples. Used to assess contamination that may accumulate during shipment of sample containers and samples. Processed in the lab as a routine field sample.	1/ site location if required by the QAPP
Equipment Blank (EB)	A field sample that consists of MilliQ water that is poured over, around, or into field sampling equipment after routine decontamination procedures to assess the effectiveness of the cleaning and to measure carry-over contamination from the sampling equipment. Processed in the lab as a routine field sample.	Highly variable and specified in the QAPP.
Field Blank (FB)	An empty sample container that is transported to the field and treated as a sampling container but actually left capped throughout the sampling effort. Processed in the lab by adding the appropriate volume of solvent to the container and "extracting" the container. The extracts are added to collection flasks and processed as a routine field sample.	Highly variable and specified in the QAPP.
MDL Verification Spikes	A field sample spiked with the analytes of interest for verification of MDLs and processed concurrently with the field samples; monitors effectiveness of method on sample matrix; performed in duplicate for tissues and soils.	1/sample set if required by the QAPP
Laboratory Control Sample (LCS)	A LCS sample is a matrix-specific sample that is prepared with each processing batch. It is spiked with the analytes of interest and processed identically to the field samples. Matrices are the same as those used for the procedural blank.	1/sample set
Matrix Spike (MS)	A field sample spiked with the analytes of interest at 10 X the MDL, processed concurrently with the field samples; monitors effectiveness of method on sample matrix; performed in duplicate for tissues and soils.	1/sample set

Attachment 1 (continued)
Definitions of Typical Laboratory Quality Control Samples and Frequency

QC Sample/Parameter	Definition	Frequency[a]
Duplicate Sample (DUP) or Matrix Spike Duplicate (MSD)	Second aliquot of a field sample processed and analyzed to monitor precision; each samples set should contain a duplicate. The duplicate may be a second matrix spike sample.	1/batch
Recovery Internal Standards (RIS)	All field and QC samples are spiked with recovery internal standards just prior to analysis; used to quantify surrogates to monitor extraction efficiency on a per sample basis.	Each sample
Surrogate Internal Standards (SIS)	All field and QC samples are spiked with a known amount of surrogates just prior to extraction; recoveries are calculated to quantify extraction efficiency.	Each sample
Standard Reference Material (SRM)	An external reference sample which contain a certified level of target analytes; act as a monitor of accuracy. Extracted and analyzed with samples of a like matrix	1/batch if required by the QAPP
Performance Evaluation (PE) or Performance Test (PT)	Blind sample of unknown composition that is analyzed as a routine sample. The PE is provided by either a government or commercial agency. Results are submitted to the supplier who determines whether the results fall within a statistically acceptable range.	2/year
Independent Instrument Check (IC)	Direct spike of target analytes into solvent where the spike source is independent of that used to prepare the calibration standards to assess instrument performance.	1/analytical run if an independent source is available.
System Performance Check Compounds (SPCC)	System performance check to ensure that minimum RFs are met before calibration curve is used.	1/initial calibration if required by the QAPP
Calibration check compound (CCC)	Check of the integrity of the calibration system.	1/batch if required by the QAPP
Reference Oil	Analysis of an oil standard to assess measurement results against historical data.	1/batch if required by QAPP
Solvent Blank	An injection of straight solvent to assess sample carry-over in GCs (not GC/MSs).	1/every 10 samples
Reagent or Solvent Purity Checks	All reagents are lot-checked prior to use.	Per lot purchase

[a] A batch is defined as 20 field samples processed simultaneously and sharing the same QC samples.

Attachment 2
Data Quality Criteria

QC Sample Type/ QC Parameter	QC Criteria	Comments
PB	<3 x MDL/RL	Or the associated sample analytes are 10 X the PB
LSC	70 to 130% recovery vs. SIS 40 to 120% recovery vs. RIS	
MS/MSD RPD	70 to 130% recovery vs. SIS 40 to 120% recovery vs. RIS <30%	For at least 80% of analytes Spike level must be 5-10 times the background level to be used for MS/MSD assessment.
DUP	<30% RPD [b]	Analyte concentration must be 10 x MDL to be used for DQA
TRIP	<30% RSD [b]	Analyte concentration must be 10 x MDL to be used for DQA
SIS	40 - 120% recovery	
SRM	Within 30% on average and not to exceed 35% for more than 30% of analytes Measured from the upper or lower 95% confidence interval (from certifying agency) as applicable.	Analyte concentration must be 10 x MDL to be used for DQA
PE/PT	NA	Acceptance defined by provider
IC	≤15% PD	
SPCC	Reference SW846 for criteria	
CCC	Reference SW846 for criteria	
Reference Oil	PAH, alkane, THC: PD<25% [b]	
Solvent Blank	No distinguishable signal	
Reagent or solvent purity checks	No distinguishable signal	See SOP 5-015

Attachment 3
Probably Sources and Expected Corrective Actions[1]

1. **BLANKS**
   a. Sources and expected review procedures:
      1. Contaminated reagents – verify reagent sources.
      2. Environmental Contamination (all sample collection, sample and analysis conditions) – review sampling handling protocols.
      3. Improper or incomplete laboratory and/or field decontamination/cleaning procedures – review cleaning protocols.
      4. Contaminated sample containers – verify source and storage conditions.
   b. Expected Corrective Actions:
      1. Review data with respect to reported contamination levels. If sample concentrations are near the reported blanks levels, reprocess (reextract or digest) associated samples or resample. If sample concentrations or the reporting levels are significantly higher than blanks, or contaminants are not detected in the samples, report the sample data and concentrations in blank.
      2. Take measure to eliminate future problems: discard reagents, revise protocols, perform preventative maintenance on system, adjust use of interfering chemicals (solvents, fuels, etc.).

2. **CALIBRATIONS**
   a. Sources and expected review procedures:
      1. Improperly prepared or outdated standards – review preparation logs for calculation/dilution errors and use of expired sources.
      2. Improperly prepared or outdated check standard – verify check standard.
      3. Poor instrument response – determine if preventative maintenance is required.
      4. Incorrect calculations – review and verify all calculations.
      5. Contamination problems (see blanks above).
   b. Expected Corrective Actions:
      1. Recalculate calibration curve.
      2. Prepare fresh standards.
      3. Recalibrate instrument.
      4. Perform preventative maintenance.
      5. Perform mass calibration and retune.
      6. Reanalyze all samples bracketing those from previous ACCEPTABLE QC check through next acceptable QC check.
      7. Take measures to eliminate sources of contamination.

3. **SYSTEM PERFORMANCE CHECKS**
   a. Sources and expected review procedures:
      1. Pesticides:
         Poor column performance – replace/repack column.

[1] From Department of Environmental Protection Standard Operating Procedures for Laboratory Operations and Sample Collection Activities (DEP - QA-001/92 Section 11.0).

Attachment 3 (Continued)
Probably Sources and Expected Corrective Actions

2. Standard Reference Materials and QC Check Samples:
   a. Improper sample preparation or analysis – review all protocols associated with sample preparation and analysis.
   b. Incorrect dilutions or calculations – recheck all calculations.
   c. Contamination (see blanks above).

b. Expected Corrective Actions:
   1. Reanalyze all samples bracketing those from previous ACCEPTABLE QC check through next acceptable QC check.
   2. Reprocess all samples associated with QC check sample or standard reference material (unless the problem is unique to processing of the check sample).
   3. Take measures to eliminate sources of contamination.

4. SPIKES

a. Sources and expected review procedures:
   1. Error in calculation – review/recheck all calculations.
   2. Error in preparing or using spike solutions – review all preparation and/or analytical logs (including sample preparation) for proper dilutions, solvents, buffers, etc.
   3. Outdated standards – review expiration dates and standard preparation logs.
   4. Contamination problems (see blanks above).
   5. Poor instrument response – determine if preventative maintenance is required.

b. Expected Corrective Actions:
   1. Take measures to eliminate contamination problems, reprocess if necessary.
   2. Perform required maintenance and revise pm schedules.
   3. Review preparation, calculation and record keeping to determine if additional training or more stringent protocols are needed.
   4. If the laboratory has no historical data to show that the sample matrix produces consistently unacceptable (out of control) recoveries, and none of the sources discussed above are responsible for the problem, the sample must be reprocessed and reanalyzed. If reanalysis produces the same result, associated samples should be reported with qualified results. If results are different, all associated samples must be reprocessed for analysis.

5. DUPLICATES

a. Sources and expected review procedures:
   1. Nonrepresentative sample – review sample collection and/or sample processing protocols.
   2. Error in calculations – recheck calculations.
   3. Contamination problems (see blanks above).
   4. See matrix spikes above.

b. Expected Corrective Actions:
   1. Report data with qualifiers and explanation.
   2. Revise sample collection/sample processing protocols to assure a representative sample.
   3. Take measures to eliminate contamination problems.
   4. Reprocess and reanalyze sample set (if laboratory generated replicate).

Attachment 3 (Continued)
Probably Sources and Expected Corrective Actions

6. SURROGATE SPIKES

a. Sources and expected review procedures:
   1. See 4.a above.

b. Expected Corrective Actions:
   1. See 4.b above.

7. INTERNAL STANDARDS

a. Sources and expected review procedures:
   1. See 4.a above.

b. Expected Corrective Actions:
   1. See 4.b above.
   2. Reanalyze samples from last acceptable QC check to next acceptable QC check.

8. SPLIT SAMPLES

a. Sources and expected review procedures:
   1. See 5.a above.

b. Expected Corrective Actions:
   1. See 5.b above.

Attachment 4.
Field Sample Duplicate Calculations

Two samples, Tissue A and Tissue B are duplicates of the same field sample. The following table summarizes the characteristics of these samples:

Sample	Dry Weight (g)	Amount of Compound X Detected (ng)
Tissue A	3.6	51.98
Tissue B	3.3	45.01

The concentration of Compound X in Tissue A is 14.44 ng/g
The concentration of Compound X in Tissue B is 13.64 ng/g

Substituting these concentration values into the RPD equation:

$$RPD = \left| \frac{2 \, x \, (14.44 - 13.64)}{14.44 + 13.64} \right| x \, 100 = 5.70$$

The relative percent difference between the concentrations of Compound X in Tissue A and Tissue B is 5.7%.

Attachment 5
MS/MSD Sample Calculations

MS and MSD samples are spiked with equal amounts of Compound X. The following table presents the information required to calculate percent recoveries, and the RPD between the results:

Sample	Sample Dry Wt. (g)	Amount of Compound X (ng)	Amount of Compound X Spiked (ng)
Background	3.7	62.0	0
MS	3.5	714.0	825
MSD	3.7	667.0	825

Step 1. Calculate concentration

$$Concentration\ in\ background\ sample = \frac{62.0\ ng}{3.7\ g} = 16.76\ ng/g$$

$$Concentration\ in\ MS = \frac{714.0\ ng}{3.5\ g} = 204.0\ ng/g$$

$$Concentration\ in\ MSD = \frac{667\ ng}{3.7\ g} = 180.27\ ng/g$$

Step 2: Subtract background concentration from MS and MSD samples

$$MS\ background\ corrected = 204.0\ ng/g - 16.76\ ng/g = 187.24\ ng/g$$

$$MSD\ background\ corrected = 180.3\ ng/g - 16.76\ ng/g = 163.54\ ng/g$$

Step 3. Re-calculate amount of analyte in MS and MSD based on corrected concentrations

$$Background\ corrected\ amount\ of\ Compound\ X\ in\ MS = 187.24\ ng/g \times 3.5\ g = 655.34\ ng$$

$$Background\ corrected\ amount\ of\ Compound\ X\ in\ MSD = 163.54\ ng/g \times 3.7\ g = 605.10\ ng$$

Step 4. Calculate analyte recovery

$$\%\ Recovery\ of\ Compound\ X\ in\ MS = \frac{655.34\ ng}{825\ ng} \times 100 = 79\%$$

$$\%\ Recovery\ of\ Compound\ X\ in\ MSD = \frac{605.0\ ng}{825\ ng} \times 100 = 73\%$$

Step 5. Calculate RPD

$$RPD = \frac{2 \times (79 - 73)}{79 + 73} \times 100 = 7.9\%$$

BATTELLE COLUMBUS SOPs

OCT 30 2000

STANDARD OPERATING PROCEDURE (SOP) FOR THE ANALYSIS OF POLYCHLORINATED DIBENZO-P-DIOXIN/POLYCHLORINATED DIBENZOFURAN (PCDD/PCDF) USING HIGH RESOLUTION GAS CHROMATOGRAPHY/HIGH RESOLUTION MASS SPECTROMETRY (HRGC/HRMS) USING MODIFIED METHODS 8290 AND 1613

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Approved by: Mary Schnoll Date: 10/26/00
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I./II. Scope/Purpose

This SOP describes routine procedures for HRGC/ HRMS analysis of samples for PCDD/PCDF. These analyses follow general guidelines described in EPA Methods 8290 and 1613, with some minor modifications/improvements. The purpose of this SOP is to provide a description of PCDD/PCDF sample analysis activities using modified Methods 8290 and 1613 procedures and covers the following:

- Chromatographic/mass spectrometric conditions and data acquisition parameters
- Calibration
- Analysis
- Calculations
- System performance criteria.

III. References

A. SW-846, Method 8290. Polychlorinated Dibenzodioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) by High Resolution Gas Chromatography/High Resolution Mass Spectrometry (HRGC/HRMS), Revision 0, 1994.
B. EPA Method 1613: Tetra-Through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, Revision B, 1994, EPA 821-B-94-005.
C. VG Opusquan 2.0 Reference Manual, Issue 4, March 1995
D. Private communication from John Bill, Fisons Instruments, 04-20-95

IV. Definitions

VG, Fisons, and Micromass all refer to the same company, and may be used interchangeably.

V. Procedures

A. Personnel Qualifications
Personnel assigned to laboratory activities meet the educational, work experience, and training requirements for their positions. Records on personnel qualifications and training are maintained in personnel files accessible for review during audit activities. Training is conducted in accordance with standard operating procedures and is available to all laboratory personnel. Employees must demonstrate proficiency at specific tasks and this capability is documented and kept in a central file.

B. Apparatus and Materials
All calibration, column performance, window defining, recovery standards, internal standards spiking solutions, PARs, SRMs, etc. are obtained from commercial sources such as Cambridge Isotope Labs.

C. Instrument or Method Calibration

The GC/HRMS instrumentation is calibrated according to Method 1613, Section 10.0 (see Appendix A), with the following exceptions: the calibration solution concentrations are those specified in Method 1613, Table 4 (see Appendix A) with one additional calibration standard at concentrations equivalent to ½ the level of Method 1613's lowest calibration point.

Using the option in Method 1613, Section 10.2 (see Appendix A), only 1 µl of calibration solution or sample extract is injected per run. The samples are injected on-column.

A mid-level standard from a secondary source such as Wellington EPA-1613CS3 (or equivalent) shall be run immediately following each initial calibration curve as a verification check for the concentrations of the calibration standard solutions.

For DB-5 continuing calibration analyses, a combination solution made by Cambridge Isotope Labs, composed of calibration solution 3, window defining mixture, and tetra dioxin GC column performance mixture, is injected at the beginning and end of each 12 hour run period. This allows determination of calibration and column performance in a single run. Check the continuing calibration results against EPA Method 1613, Table 6 "VER" requirements (see Appendix A). Flag any analyte which fails this criteria. Corrective action, which may include re-tuning and re-analysis of the calibration solution or analysis of a new initial calibration curve or use of the continuing calibration response factor in calculating sample concentrations, will be taken. All corrective actions taken will be fully documented and addressed in the sample report.

D. Sample Preparation and Analysis

1. Sample Preparation

For sample preparation procedures, see SOP ASAT.II-002, *SOP for Polychlorinated Dibenzo-p-dioxin/Polychlorinated Dibenzofuran (PCDD/PCDF) Sample Preparation Using Modified Methods 8290 and 1613.*

2. Sample Analysis

The GC/MS parameters listed in Method 1613, Section 10.1 (see Appendix A) are followed with the following exceptions. The GC column listed in Method 1613, Section 6.9.1 (see Appendix A) is used, but the temperature program has a different initial temperature (140°C) to allow the solvent peak to elute slowly enough to not trip the source ion gauge. The upper temperature of the ramp is held to 320°C rather than 330°C to accommodate the upper temperature limit of the column.

The ions monitored are those listed in Appendix B. A 1µL injection volume is used.

E. Data Acquisition, Calculations, and Data Reduction
Calculations are carried out using Opusquan, a software program designed for dioxin/furan analysis by VG/Micromass Co. Ltd. These calculations are the same as specified in Method 1613, Sections 17.1 to 17.4 (see Appendix A).

Estimated detection limit is calculated by measuring the sum of the heights of a native peak at the predicted retention time, times 2.5, divided by the total area of its internal standard ions, using the equation:

$$EDL = (F * Ni * Si * A/H * Qs) / (RRF * As * S)$$

Where
F = the user factor (dl_factor) in the form "fullrun" which will be set equal to 2.5 by the analyst.
Ni = the sum of the noise level of the analyte ions
Si = the sum of the "min_sig_to_noise" keyword value for each of the analyte ions
A/H = the mean area: height ratio of all ions of this analyte's internal standard
Qs = the internal standard amount
RRF = the mean relative response factor of the analyte
As = the total area of all internal standard ion peaks
S = the weight of the sample

A method blank is analyzed and processed using Opusquan in the "blank" mode. The noise factor for the natives in this blank run is then subtracted from subsequent runs, which are processed in the "quantitation" mode to obtain an accurate detection limit for each analyte in each run.

F. Computer Hardware and Software
Calculations will be carried out using Opusquan, a software program designed for dioxin/furan analysis by VG/Micromass Co. Ltd.

G. Quality Control and Quality Assurance (System Performance Criteria)
A combination calibration solution 3/window defining mixture/column performance mixture is injected at the beginning and end of each 12-hour period. This is to ensure adequate resolution of the isomeric peaks, to ascertain that the windows are set correctly to see all the isomers in each congener group, and to verify that the HRMS is adequately tuned. A PFK resolution check is also hard-copied at the beginning and end of each GC/HRMS analysis batch for non-regulatory work and every 12 hours for regulatory work to verify mass resolution. The analyst will provide an initial review/assessment of the data generated, according to SOP ASAT.II-010. A QC check sample (either CIL EDF-2526 or CIL EDF-2513) will be analyzed at least quarterly and the results maintained in the QC check sample binder.

VI. Revision History

1. This SOP was reformatted from SOP0802-01-01.
2. Added Appendices of referenced methods.
3. Version –02 adds criteria for second source calibration standard analyzed with the initial calibration, analysis of a QC check sample quarterly, and clarification on the frequency of PFK resolution checks. Many Method 8290 references were replaced with equivalent references from Method 1613.

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APPENDIX A

Method 1613 References

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

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

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

6.8.2 Kuderna-Danish (K-D) Concentrator

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

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

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

6.8.2.4 Boiling chips

6.8.2.4.1 Glass or silicon carbide—Approximately 10/40 mesh, extracted with methylene chloride and baked at 450°C for one hour minimum.

6.8.2.4.2 Fluoropolymer (optional)—Extracted with methylene chloride.

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

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

6.8.4 Sample vials

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

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

6.9 Gas Chromatograph—Shall have splitless or on-column injection port for capillary column, temperature program with isothermal hold, and shall meet all of the performance specifications in Section 10.

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

9.8 Depending on specific program requirements, field replicates may be collected to determine the precision of the sampling technique, and spiked samples may be required to determine the accuracy of the analysis when the internal standard method is used.

10.0 Calibration

10.1 Establish the operating conditions necessary to meet the minimum retention times for the internal standards in Section 10.2.4 and the relative retention times for the CDDs/CDFs in Table 2.

10.1.1 Suggested GC operating conditions:

Injector temperature:	270°C
Interface temperature:	290°C
Initial temperature:	200°C
Initial time:	Two minutes
Temperature program:	200-220°C, at 5°C/minute
	220°C for 16 minutes
	220-235°C, at 5°C/minute
	235°C for seven minutes
	235-330°C, at 5°C/minute

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

Optimize GC conditions for compound separation and sensitivity. Once optimized, the same GC conditions must be used for the analysis of all standards, blanks, IPR and OPR aliquots, and samples.

10.1.2 Mass spectrometer (MS) resolution—Obtain a selected ion current profile (SICP) of each analyte in Table 3 at the two exact m/z's specified in Table 8 and at ≥10,000 resolving power by injecting an authentic standard of the CDDs/CDFs either singly or as part of a mixture in which there is no interference between closely eluted components.

10.1.2.1 The analysis time for CDDs/CDFs may exceed the long-term mass stability of the mass spectrometer. Because the instrument is operated in the high-resolution mode, mass drifts of a few ppm (e.g., 5 ppm in mass) can have serious adverse effects on instrument performance. Therefore, a mass-drift correction is mandatory and a lock-mass m/z from PFK is used for drift correction. The lock-mass m/z is dependent on the exact m/z's monitored within each descriptor, as shown in Table 8. The level of PFK metered into the HRMS during analyses should be adjusted so that the amplitude of the most intense selected lock-mass m/z signal (regardless of the descriptor number) does not exceed 10% of the full-scale deflection for a given set of detector parameters. Under those conditions, sensitivity changes that might occur during the analysis can be more effectively monitored.

NOTE: Excessive PFK (or any other reference substance) may cause noise problems and contamination of the ion source necessitating increased frequency of source cleaning.

10.1.2.2 If the HRMS has the capability to monitor resolution during the analysis, it is acceptable to terminate the analysis when the resolution falls below 10,000 to save reanalysis time.

10.1.2.3 Using a PFK molecular leak, tune the instrument to meet the minimum required resolving power of 10,000 (10% valley) at m/z 304.9824 (PFK) or any other reference signal close to m/z 304 (from TCDF). For each descriptor (Table 8), monitor and record the resolution and exact m/z's of three to five reference peaks covering the mass range of the descriptor. The resolution must be greater than or equal to 10,000, and the deviation between the exact m/z and the theoretical m/z (Table 8) for each exact m/z monitored must be less than 5 ppm.

10.2 Ion Abundance Ratios, Minimum Levels, Signal-to-Noise Ratios, and Absolute Retention Times—Choose an injection volume of either 1 µL or 2 µL, consistent with the capability of the HRGC/HRMS instrument. Inject a 1 µL or 2 µL aliquot of the CS1 calibration solution (Table 4) using the GC conditions from Section 10.1.1. If only 2,3,7,8-TCDD and 2,3,7,8-TCDF are to be determined, the operating conditions and specifications below apply to analysis of those compounds only.

10.2.1 Measure the SICP areas for each analyte, and compute the ion abundance ratios at the exact m/z's specified in Table 8. Compare the computed ratio to the theoretical ratio given in Table 9.

10.2.1.1 The exact m/z's to be monitored in each descriptor are shown in Table 8. Each group or descriptor shall be monitored in succession as a function of GC retention time to ensure that all CDDs/CDFs are detected. Additional m/z's may be monitored in each descriptor, and the m/z's may be divided among more than the five descriptors listed in Table 8, provided that the laboratory is able to monitor the m/z's of all the CDDs/CDFs that may elute from the GC in a given retention-time window. If only 2,3,7,8-TCDD and 2,3,7,8-TCDF are to be determined, the descriptors may be modified to include only the exact m/z's for the tetra- and penta-isomers, the diphenyl ethers, and the lock m/z's.

10.2.1.2 The mass spectrometer shall be operated in a mass-drift correction mode, using perfluorokerosene (PFK) to provide lock m/z's. The lock-mass for each group of m/z's is shown in Table 8. Each lock mass shall be monitored and shall not vary by more than ±20% throughout its respective retention time window. Variations of the lock mass by more than 20% indicate the presence of coeluting interferences that may significantly reduce the sensitivity of the mass spectrometer. Reinjection of another aliquot of the sample extract will not resolve the problem. Additional cleanup of the extract may be required to remove the interferences.

10.2.2 All CDDs/CDFs and labeled compounds in the CS1 standard shall be within the QC limits in Table 9 for their respective ion abundance ratios; otherwise, the mass spectrometer shall be adjusted and this test repeated until the m/z ratios fall within the limits specified. If the adjustment alters the resolution of the mass spectrometer, resolution shall be verified (Section 10.1.2) prior to repeat of the test.

10.2.3 Verify that the HRGC/HRMS instrument meets the minimum levels in Table 2. The peaks representing the CDDs/CDFs and labeled compounds in the CS1 calibration standard must have signal-to-noise ratios (S/N) greater than or equal to 10.0. Otherwise, the mass spectrometer shall be adjusted and this test repeated until the minimum levels in Table 2 are met.

10.2.4 The absolute retention time of $^{13}C_{12}$-1,2,3,4-TCDD (Section 7.12) shall exceed 25.0 minutes on the DB-5 column, and the retention time of $^{13}C_{12}$-1,2,3,4-TCDD shall exceed 15.0 minutes on the DB-225 column; otherwise, the GC temperature program shall be adjusted and this test repeated until the above-stated minimum retention time criteria are met.

10.3 Retention-Time Windows—Analyze the window defining mixtures (Section 7.15) using the optimized temperature program in Section 10.1. Table 5 gives the elution order (first/last) of the window-defining compounds. If 2,3,7,8-TCDD and 2,3,7,8-TCDF only are to be analyzed, this test is not required.

10.4 Isomer Specificity

10.4.1 Analyze the isomer specificity test standards (Section 7.15) using the procedure in Section 14 and the optimized conditions for sample analysis (Section 10.1.1).

10.4.2 Compute the percent valley between the GC peaks that elute most closely to the 2,3,7,8-TCDD and TCDF isomers, on their respective columns, per Figures 6 and 7.

10.4.3 Verify that the height of the valley between the most closely eluted isomers and the 2,3,7,8-substituted isomers is less than 25% (computed as 100 x/y in Figures 6 and 7). If the valley exceeds 25%, adjust the analytical conditions and repeat the test or replace the GC column and recalibrate (Sections 10.1.2 through 10.7).

10.5 Calibration by Isotope Dilution—Isotope dilution calibration is used for the 15 2,3,7,8-substituted CDDs/CDFs for which labeled compounds are added to samples prior to extraction. The reference compound for each CDD/CDF compound is shown in Table 2.

10.5.1 A calibration curve encompassing the concentration range is prepared for each compound to be determined. The relative response (RR) (labeled to native) vs. concentration in standard solutions is plotted or computed using a linear regression. Relative response is determined according to the procedures described below. Five calibration points are employed.

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

$$RR = \frac{(A1_n + A2_n)\ C_l}{(A1_l + A2_l)\ C_n}$$

where,

$A1_n$ and $A2_n$ = The areas of the primary and secondary m/z's for the CDD/CDF.

$A1_l$ and $A2_l$ = The areas of the primary and secondary m/z's for the labeled compound.

C_l = The concentration of the labeled compound in the calibration standard (Table 4).

C_n = The concentration of the native compound in the calibration standard (Table 4).

10.5.3 To calibrate the analytical system by isotope dilution, inject a volume of calibration standards CS1 through CS5 (Section 7.13 and Table 4) identical to the volume chosen in Section 10.2, using the procedure in Section 14 and the conditions in Section 10.1.1 and Table 2. Compute the relative response (RR) at each concentration.

10.5.4 Linearity—If the relative response for any compound is constant (less than 20% coefficient of variation) over the five-point calibration range, an averaged relative response may be used for that compound; otherwise, the complete calibration curve for that compound shall be used over the five-point calibration range.

10.6 Calibration by Internal Standard—The internal standard method is applied to determination of 1,2,3,7,8,9-HxCDD (Section 17.1.2), OCDF (Section 17.1.1), the non-2,3,7,8-substituted compounds, and to the determination of labeled compounds for intralaboratory statistics (Sections 9.4 and 15.5.4).

10.6.1 Response factors—Calibration requires the determination of response factors (RF) defined by the following equation:

$$RF = \frac{(A1_s + A2_s)\ C_{is}}{(A1_{is} + A2_{is})\ C_s}$$

where,

$A1_s$ and $A2_s$ = The areas of the primary and secondary m/z's for the CDD/CDF.

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

C_{is} = The concentration of the internal standard (Table 4).

C_s = The concentration of the compound in the calibration standard (Table 4).

NOTE: There is only one m/z for $^{37}Cl_4$-2,3,7,8-TCDD. See Table 8.

10.6.2 To calibrate the analytical system by internal standard, inject 1.0 µL or 2.0 µL of calibration standards CS1 through CS5 (Section 7.13 and Table 4) using the procedure in Section 14 and the conditions in Section 10.1.1 and Table 2. Compute the response factor (RF) at each concentration.

10.6.3 Linearity—If the response factor (RF) for any compound is constant (less than 35% coefficient of variation) over the five-point calibration range, an averaged response factor may be used for that compound; otherwise, the complete calibration curve for that compound shall be used over the five-point range.

10.7 Combined Calibration—By using calibration solutions (Section 7.13 and Table 4) containing the CDDs/CDFs and labeled compounds and the internal standards, a single set of analyses can be used to produce calibration curves for the isotope dilution and internal standard methods. These curves are verified each shift (Section 15.3) by analyzing the calibration verification standard (VER, Table 4). Recalibration is required if any of the calibration verification criteria (Section 15.3) cannot be met.

10.8 Data Storage—MS data shall be collected, recorded, and stored.

10.8.1 Data acquisition—The signal at each exact m/z shall be collected repetitively throughout the monitoring period and stored on a mass storage device.

10.8.2 Response factors and multipoint calibrations—The data system shall be used to record and maintain lists of response factors (response ratios for isotope dilution) and multipoint calibration curves. Computations of relative standard deviation (coefficient of variation) shall be used to test calibration linearity. Statistics on initial performance (Section 9.2) and ongoing performance (Section 15.5) should be computed and maintained, either on the instrument data system, or on a separate computer system.

11.0 Sample Preparation

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

For samples known or expected to contain high levels of the CDDs/CDFs, the smallest sample size representative of the entire sample should be used (see Section 17.5).

For all samples, the blank and IPR/OPR aliquots must be processed through the same steps as the sample to check for contamination and losses in the preparation processes.

11.1.1 For samples that contain particles, percent solids and particle size are determined using the procedures in Sections 11.2 and 11.3, respectively.

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

11.1.2.1 Aqueous samples visibly absent particles are prepared per Section 11.4 and extracted directly using the separatory funnel or SPE techniques in Sections 12.1 or 12.2, respectively.

16.3 The ratio of the integrated areas of the two exact m/z's specified in Table 8 must be within the limit in Table 9, or within ±10% of the ratio in the midpoint (CS3) calibration or calibration verification (VER), whichever is most recent.

16.4 The relative retention time of the peak for a 2,3,7,8-substituted CDD or CDF must be within the limit in Table 2. The retention time of peaks representing non-2,3,7,8-substituted CDDs/CDFs must be within the retention time windows established in Section 10.3.

16.5 Confirmatory Analysis—Isomer specificity for 2,3,7,8-TCDF cannot be achieved on the DB-5 column. Therefore, any sample in which 2,3,7,8-TCDF is identified by analysis on a DB-5 column must have a confirmatory analysis performed on a DB-225, SP-2330, or equivalent GC column. The operating conditions in Section 10.1.1 may be adjusted to optimize the analysis on the second GC column, but the GC/MS must meet the mass resolution and calibration specifications in Section 10.

16.6 If the criteria for identification in Sections 16.1 through16.5 are not met, the CDD or CDF has not been identified and the results may not be reported for regulatory compliance purposes. If interferences preclude identification, a new aliquot of sample must be extracted, further cleaned up, and analyzed.

17.0 Quantitative Determination

17.1 Isotope Dilution Quantitation—By adding a known amount of a labeled compound to every sample prior to extraction, correction for recovery of the CDD/CDF can be made because the CDD/CDF and its labeled analog exhibit similar effects upon extraction, concentration, and gas chromatography. Relative response (RR) values are used in conjunction with the initial calibration data described in Section 10.5 to determine concentrations directly, so long as labeled compound spiking levels are constant, using the following equation:

$$C_{ex}\ (ng/mL) = \frac{(A1_n + A2_n)\ C_l}{(A1_l + A2_l)\ RR}$$

where,

C_{ex} = The concentration of the CDD/CDF in the extract, and the other terms are as defined in Section 10.5.2.

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

17.1.2 Because $^{13}C_{12}$-1,2,3,7,8,9-HxCDD is used as an instrument internal standard (i.e., not added before extraction of the sample), it cannot be used to quantitate the 1,2,3,7,8,9-HxCDD by strict isotope dilution procedures. Therefore, 1,2,3,7,8,9-HxCDD is quantitated using the averaged response of the labeled analogs of the other two 2,3,7,8-substituted HxCDD's: 1,2,3,4,7,8-HxCDD and 1,2,3,6,7,8-HxCDD. As a result, the concentration of 1,2,3,7,8,9-HxCDD is corrected for the average recovery of the other two HxCDD's.

17.1.3 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 2,3,7,8-isomers at the same level of chlorination.

17.2 Internal Standard Quantitation and Labeled Compound Recovery

17.2.1 Compute the concentrations of 1,2,3,7,8,9-HxCDD, OCDF, the ^{13}C-labeled analogs and the ^{37}C-labeled cleanup standard in the extract using the response factors determined from the initial calibration data (Section 10.6) and the following equation:

$$C_{ex}\ (ng/mL) = \frac{(A1_s + A2_s)\ C_{is}}{(A1_{is} + A2_{is})\ RF}$$

where,

C_{ex} = The concentration of the CDD/CDF in the extract, and the other terms are as defined in Section 10.6.1.

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

17.2.2 Using the concentration in the extract determined above, compute the percent recovery of the ^{13}C-labeled compounds and the ^{37}C-labeled cleanup standard using the following equation:

$$Recovery\ (\%) = \frac{Concentration\ found\ (\mu g/mL)}{Concentration\ spiked\ (\mu g/mL)} \times 100$$

17.3 The concentration of a CDD/CDF in the solid phase of the sample is computed using the concentration of the compound in the extract and the weight of the solids (Section 11.5.1), as follows:

$$Concentration\ in\ solid\ (ng/kg) = \frac{(C_{ex} \times V_{ex})}{W_s}$$

where,

C_{ex} = The concentration of the compound in the extract.
V_{ex} = The extract volume in mL.
W_s = The sample weight (dry weight) in kg.

17.4 The concentration of a CDD/CDF 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 11.4 or 11.5), as follows:

$$\textit{Concentration in aqueous phase (pg/L)} = \frac{(C_{ex} \times V_{ex})}{V_s}$$

where,

C_{ex} = The concentration of the compound in the extract.
V_{ex} = The extract volume in mL.
V_s = The sample volume in liters.

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

17.5.1 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 per Sections 11 through 14.

17.5.2 For samples containing greater than 1% solids, extract an amount of sample equal to 1/10, 1/100, etc., of the amount used in Section 11.5.1. Re-prepare, extract, clean up, and analyze per Sections 11 through 14.

17.5.3 If a smaller sample size will not be representative of the entire sample, dilute the sample extract by a factor of 10, adjust the concentration of the instrument internal standard to 100 pg/µL in the extract, and analyze an aliquot of this diluted extract by the internal standard method.

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

17.6.1 Reporting units and levels

17.6.1.1 Aqueous samples—Report results in pg/L (parts-per-quadrillion).

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

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

17.6.1.4 Reporting level

Appendix A

TABLE 2. RETENTION TIME REFERENCES, QUANTITATION REFERENCES, RELATIVE RETENTION TIMES, AND MINIMUM LEVELS FOR CDDS AND CDFS

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

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TABLE 2. RETENTION TIME REFERENCES, QUANTITATION REFERENCES, RELATIVE RETENTION TIMES, AND MINIMUM LEVELS FOR CDDS AND CDFS

CDD/CDF	Retention time and quantitation reference	Relative retention time	Minimum level[1] Water (pg/L; ppq)	Solid (ng/kg; ppt)	Extract (pg/µL; ppb)
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	1.057–1.151			
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	1.086–1.110			
$^{13}C_{12}$-OCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	1.032–1.311			

[1]The Minimum Level (ML) for each analyte is defined as the level at which the entire analytical system must give a recognizable signal and acceptable calibration point. It is equivalent to the concentration of the lowest calibration standard, assuming that all method-specified sample weights, volumes, and cleanup procedures have been employed.

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

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TABLE 4. CONCENTRATION OF CDDS/CDFS IN CALIBRATION AND CALIBRATION VERIFICATION SOLUTIONS[1] (section 15.3)

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

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TABLE 6. ACCEPTANCE CRITERIA FOR PERFORMANCE TESTS WHEN ALL CDDS/CDFS ARE TESTED[1]

CDD/CDF	Test Conc. (ng/mL)	IPR[2,3] s (ng/mL)	IPR[2,3] X (ng/mL)	OPR (ng/mL)	VER (ng/mL)
2,3,7,8-TCDD	10	2.8	8.3–12.9	6.7–15.8	7.8–12.9
2,3,7,8-TCDF	10	2.0	8.7–13.7	7.5–15.8	8.4–12.0
1,2,3,7,8-PeCDD	50	7.5	38–66	35–71	39–65
1,2,3,7,8-PeCDF	50	7.5	43–62	40–67	41–60
2,3,4,7,8-PeCDF	50	8.6	36–75	34–80	41–61
1,2,3,4,7,8-HxCDD	50	9.4	39–76	35–82	39–64
1,2,3,6,7,8-HxCDD	50	7.7	42–62	38–67	39–64
1,2,3,7,8,9-HxCDD	50	11.1	37–71	32–81	41–61
1,2,3,4,7,8-HxCDF	50	8.7	41–59	36–67	45–56
1,2,3,6,7,8-HxCDF	50	6.7	46–60	42–65	44–57
1,2,3,7,8,9-HxCDF	50	6.4	42–61	39–65	45–56
2,3,4,6,7,8-HxCDF	50	7.4	37–74	35–78	44–57
1,2,3,4,6,7,8-HpCDD	50	7.7	38–65	35–70	43–58
1,2,3,4,6,7,8-HpCDF	50	6.3	45–56	41–61	45–55
1,2,3,4,7,8,9-HpCDF	50	8.1	43–63	39–69	43–58
OCDD	100	19	89–127	78–144	79–126
OCDF	100	27	74–146	63–170	63–159
$^{13}C_{12}$-2,3,7,8-TCDD	100	37	28–134	20–175	82–121
$^{13}C_{12}$-2,3,7,8-TCDF	100	35	31–113	22–152	71–140
$^{13}C_{12}$-1,2,3,7,8-PeCDD	100	39	27–184	21–227	62–160
$^{13}C_{12}$-1,2,3,7,8-PeCDF	100	34	27–156	21–192	76–130
$^{13}C_{12}$-2,3,4,7,8-PeCDF	100	38	16–279	13–328	77–130
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	100	41	29–147	21–193	85–117
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	100	38	34–122	25–163	85–118
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	100	43	27–152	19–202	76–131
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	100	35	30–122	21–159	70–143
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	100	40	24–157	17–205	74–135
$^{13}C_{12}$-2,3,4,6,7,8,-HxCDF	100	37	29–136	22–176	73–137
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	100	35	34–129	26–166	72–138
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	100	41	32–110	21–158	78–129
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	100	40	28–141	20–186	77–129
$^{13}C_{12}$-OCDD	200	95	41–276	26–397	96–415
$^{37}Cl_4$-2,3,7,8-TCDD	10	3.6	3.9–15.4	3.1–19.1	7.9–12.7

[1] All specifications are given as concentration in the final extract, assuming a 20 µL volume.
[2] s = standard deviation of the concentration.
[3] X = average concentration.

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TABLE 7A. LABELED COMPOUND RECOVERY IN SAMPLES WHEN ONLY TETRA COMPOUNDS ARE TESTED

Compound	Test Conc. (ng/mL)	Labeled compound recovery (ng/mL) [1]	Labeled compound recovery (%)
$^{13}C_{12}$-2,3,7,8-TCDD	100	31-137	31-137
$^{13}C_{12}$-2,3,7,8-TCDF	100	29-140	29-140
$^{37}Cl_4$-2,3,7,8-TCDD	10	4.2–16.4	42–164

[1] Specification given as concentration in the final extract, assuming a 20 µL volume.

TABLE 8. DESCRIPTORS, EXACT M/Z's, M/Z TYPES, AND ELEMENTAL COMPOSITIONS OF THE CDDs AND CDFs

Descriptor	Exact M/Z [1]	M/Z Type	Elemental Composition	Substance [2]
1	292.9825	Lock	$C_7 F_{11}$	PFK
	303.9016	M	$C_{12} H_4 {}^{35}Cl_4 O$	TCDF
	305.8987	M+2	$C_{12} H_4 {}^{35}Cl_3 {}^{37}Cl O$	TCDF
	315.9419	M	${}^{13}C_{12} H_4 {}^{35}Cl_4 O$	TCDF[3]
	317.9389	M+2	${}^{13}C_{12} H_4 {}^{35}Cl_3 {}^{37}Cl O$	TCDF[3]
	319.8965	M	$C_{12} H_4 {}^{35}Cl_4 O_2$	TCDD
	321.8936	M+2	$C_{12} H_4 {}^{35}Cl_3 {}^{37}Cl O_2$	TCDD
	327.8847	M	$C_{12} H_4 {}^{37}Cl_4 O_2$	TCDD[4]
	330.9792	QC	$C_7 F_{13}$	PFK
	331.9368	M	${}^{13}C_{12} H_4 {}^{35}Cl_4 O_2$	TCDD[3]
	333.9339	M+2	${}^{13}C_{12} H_4 {}^{35}Cl_3 {}^{37}Cl O_2$	TCDD[3]
	375.8364	M+2	$C_{12} H_4 {}^{35}Cl_5 {}^{37}Cl O$	HxCDPE
2	339.8597	M+2	$C_{12} H_3 {}^{35}Cl_4 {}^{37}Cl O$	PeCDF
	341.8567	M+4	$C_{12} H_3 {}^{35}Cl_3 {}^{37}Cl_2 O$	PeCDF
	351.9000	M+2	${}^{13}C_{12} H_3 {}^{35}Cl_4 {}^{37}Cl O$	PeCDF
	353.8970	M+4	${}^{13}C_{12} H_3 {}^{35}Cl_3 {}^{37}Cl_2 O$	PeCDF [3]
	354.9792	Lock	$C_9 F_{13}$	PFK
	355.8546	M+2	$C_{12} H_3 {}^{35}Cl_4 {}^{37}Cl O_2$	PeCDD
	357.8516	M+4	$C_{12} H_3 {}^{35}Cl_3 {}^{37}Cl_2 O_2$	PeCDD
	367.8949	M+2	${}^{13}C_{12} H_3 {}^{35}Cl_4 {}^{37}Cl O_2$	PeCDD [3]
	369.8919	M+4	${}^{13}C_{12} H_3 {}^{35}Cl_3 {}^{37}Cl_2 O_2$	PeCDD [3]
	409.7974	M+2	$C_{12} H_3 {}^{35}Cl_6 {}^{37}Cl O$	HpCDPE
3	373.8208	M+2	$C_{12} H_2 {}^{35}Cl_5 {}^{37}Cl O$	HxCDF
	375.8178	M+4	$C_{12} H_2 {}^{35}Cl_4 {}^{37}Cl_2 O$	HxCDF
	383.8639	M	${}^{13}C_{12} H_2 {}^{35}Cl_6 O$	HxCDF [3]
	385.8610	M+2	${}^{13}C_{12} H_2 {}^{35}Cl_5 {}^{37}Cl O$	HxCDF [3]
	389.8157	M+2	$C_{12} H_2 {}^{35}Cl_5 {}^{37}Cl O_2$	HxCDD
	391.8127	M+4	$C_{12} H_2 {}^{35}Cl_4 {}^{37}Cl_2 O_2$	HxCDD

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TABLE 8. DESCRIPTORS, EXACT M/Z's, M/Z TYPES, AND ELEMENTAL COMPOSITIONS OF THE CDDs AND CDFs

Descriptor	Exact M/Z [1]	M/Z Type	Elemental Composition	Substance [2]
	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]
	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	$C_{12} {}^{35}Cl_6 {}^{37}Cl_2 O$	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).

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TABLE 9. THEORETICAL ION ABUNDANCE RATIOS AND QC LIMITS

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

[1] QC limits represent ±15% windows around the theoretical ion abundance ratios.
[2] Does not apply to $^{37}Cl_4$-2,3,7,8-TCDD (cleanup standard).
[3] Used for $^{13}C_{12}$-HxCDF only.
[4] Used for $^{13}C_{12}$-HpCDF only.