Supporting Information for:

Statistical survey of persistent organic pollutants: Risk estimations to humans and wildlife though consumption of fish from U.S. rivers

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Supporting Information

SI Document 1 – **Microsoft word document, 52 pages**

Figures S1-S3

Standard Operating Procedures for both the instrumental analysis method and the fish tissue sample extraction method.

SI Document 2 – **Excel Spreadsheet, 6 sheet tabs**

Tables S1-S4, which contain individual sample concentration information for all 50 analytes, including the PCBs (Table S1), PBDEs (Table S2), and pesticides (Table S3). Table S4 includes significance of difference (p values) between mean concentrations of organic compounds in fish tissue between ecoregions and site types.

Figure S-1. Weighted Mean PCB Congener Concentrations in Fish Tissue from U.S. River Sites, Non-Urban and Urban

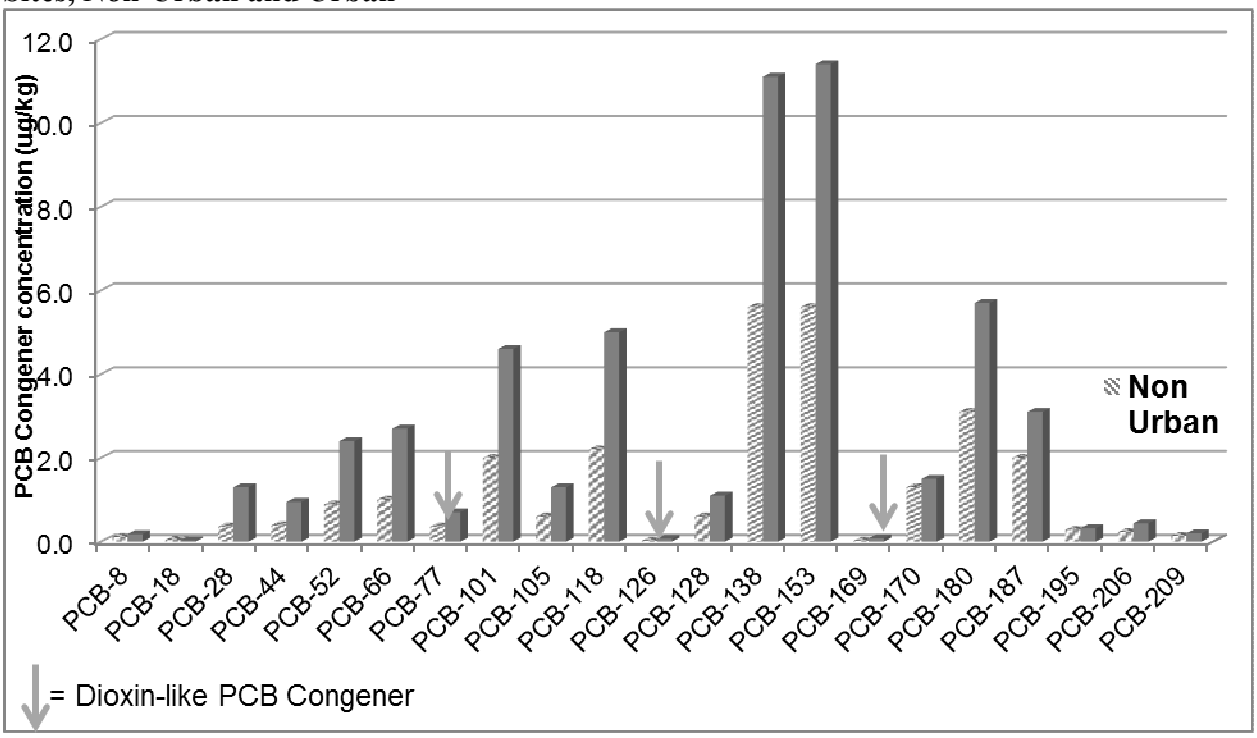


Figure S-2. Percentile distribution of Summed PBDE congener concentrations in Fish Tissue from U.S. River Sites, Non-Urban and Urban and by Eco-region

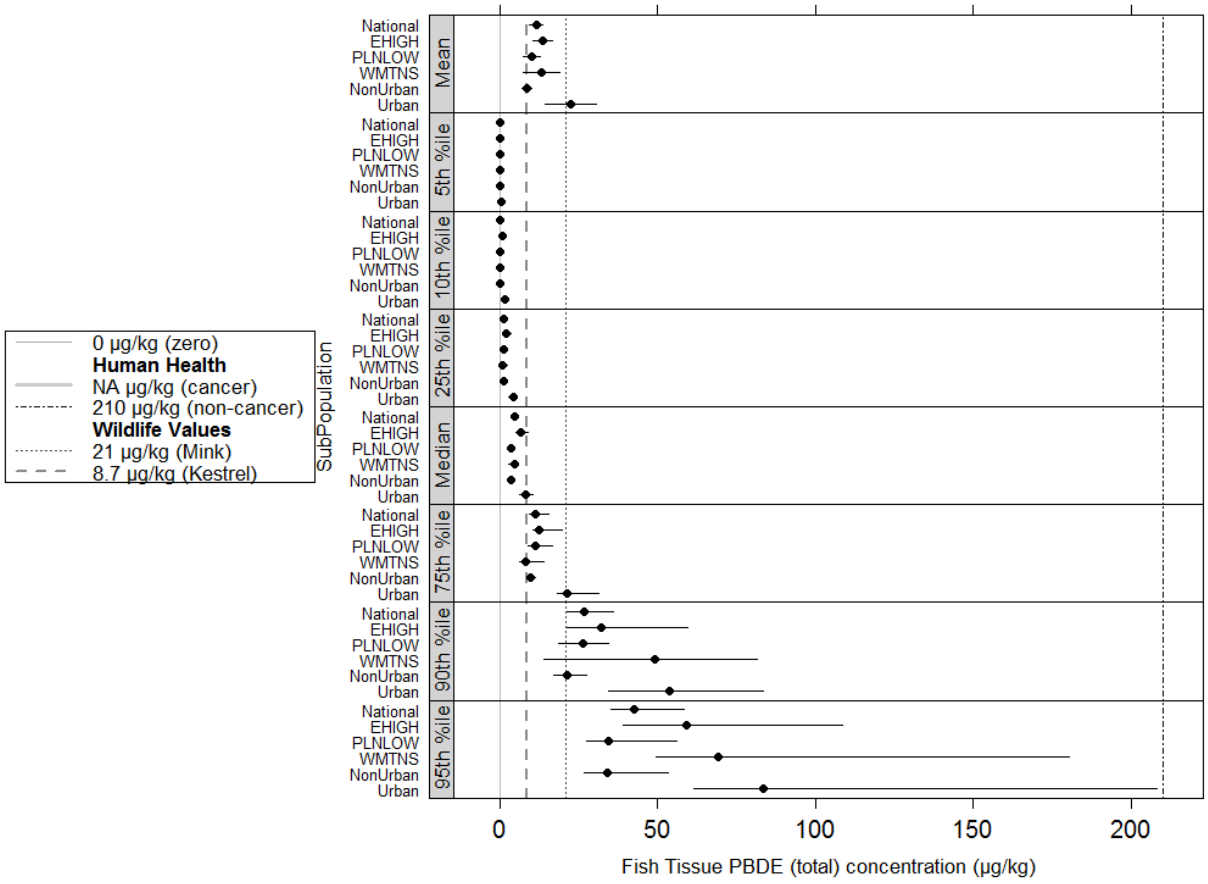


Figure S-3. Weighted Mean PBDE Compound Concentrations in Fish Tissue from U.S. River Sites, Non-Urban and Urban

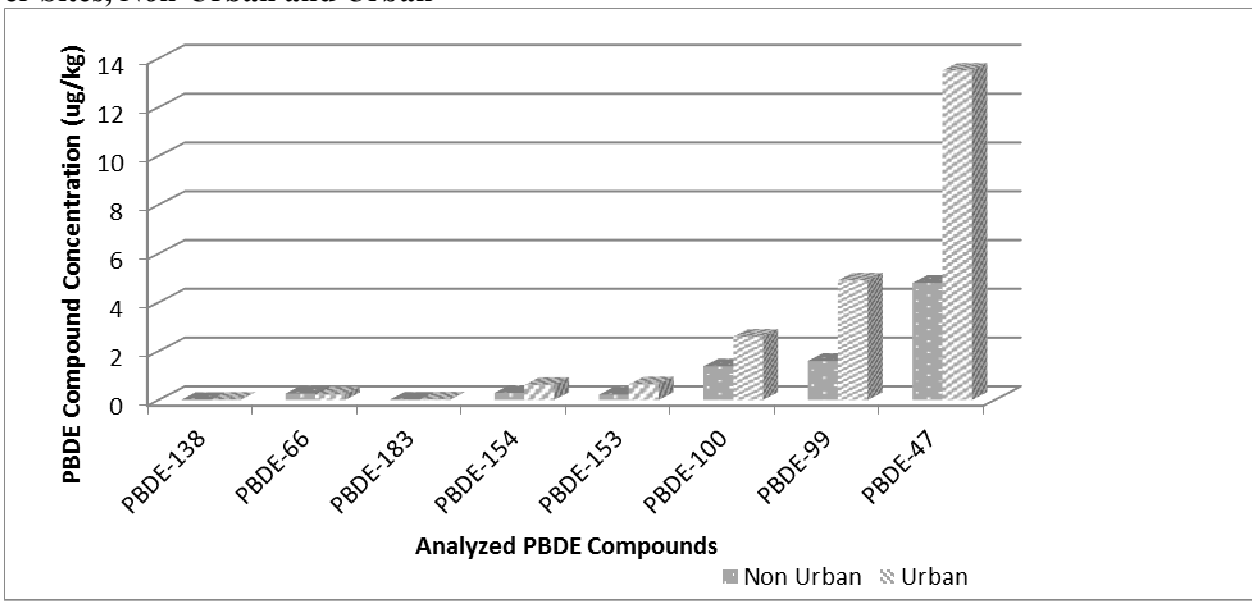
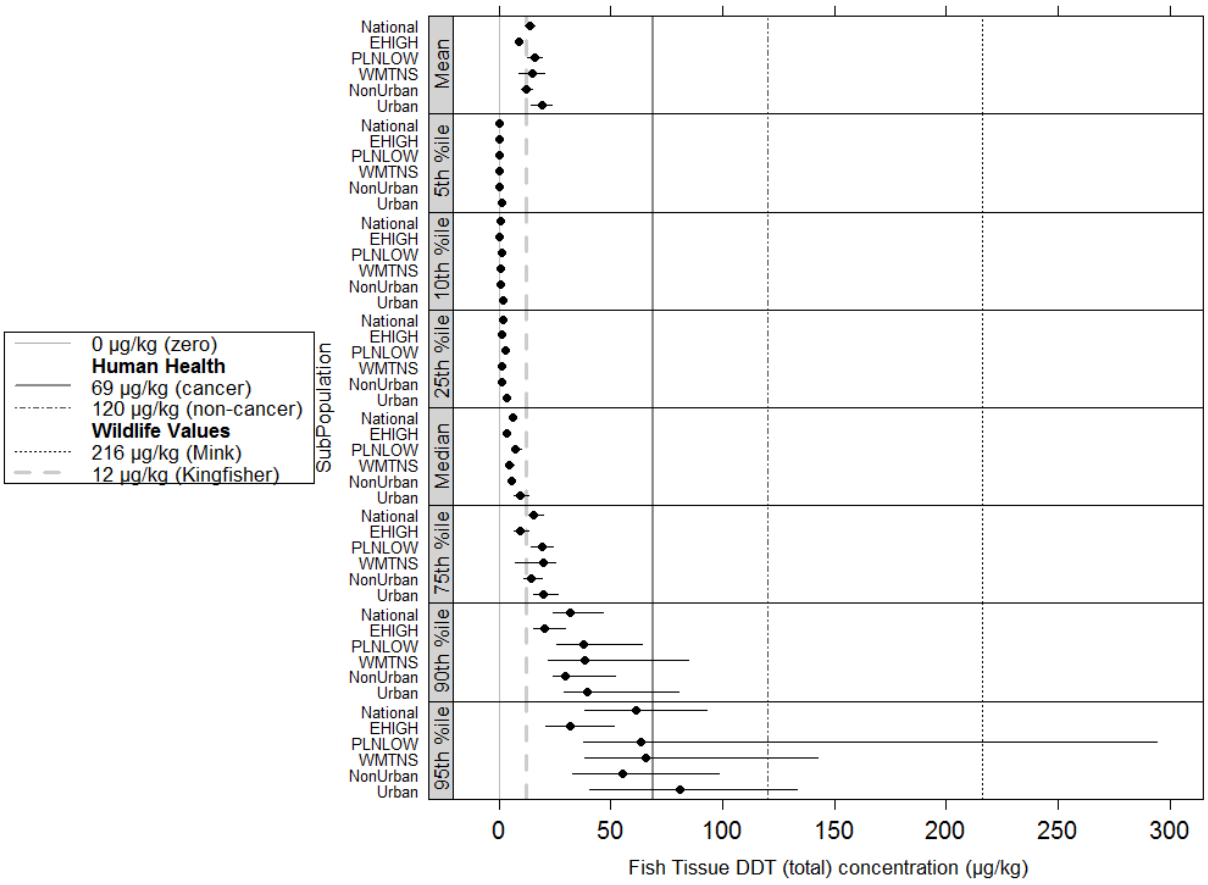


Figure S-4. Percentile distribution of Total DDT concentrations in Fish Tissue from U.S. River Sites, Non-Urban and Urban and by Eco-region



This portion of the **Supporting Information** contains two Standard Operating Procedures (SOPs), which describe the methods used in the manuscript in full detail, including:

1. GC ANALYSIS OF ORGANICS (Organic GC Analysis of Wet Tissue and Wet Sediment Samples) - which describes the analytical tissue analysis procedure using gas chromatography with electron capture detection
2. ASE EXTRACTION AND CLEANUP OF WET TISSUE, SEDIMENT, AND BENTHIC MATERIALS FOR TRACE ORGANICS – which describes the tissue extraction and extract cleanup procedure using Accelerated Solvent Extraction

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STANDARD OPERATING PROCEDURE

Title: **GC ANALYSIS OF ORGANICS (Organic GC Analysis of Wet Tissue and Wet Sediment Samples)**

Number: MIRB-046.2E	Effective Date:

SOP was Developed ☐ In-house ☒ Extramural

Alternative Identification:

SOP Steward

Name: Daniel F. Bender, Organic Chemist, EERD/MIRB

Signature: Date:

Approval

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Signature: Date:

Concurrence*

Name:
Title:

Signature: Date:

STANDARD OPERATING PROCEDURES

For

GC ANALYSIS OF ORGANICS

(Organic GC Analysis of Wet Tissue and Wet Sediment Samples)

Written by:
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Organic GC Analysis of Wet Tissue and Wet Sediment Samples

1. Scope and Application

1.1. This SOP is applicable to the analysis of extracted and cleaned frozen fish or other animal tissue and sediment or other similar matrixes to determine the concentrations of chlorinated pesticides and herbicides, polychlorinated biphenyls (PCB) as individual congeners and polybrominated diphenyl ethers (PBDE) as individual congeners by capillary gas chromatography**.**

1.2. This SOP provides procedures for using gas chromatography with a micro-cell ^{63}Ni electron capture detector (μECD) and a split/splitless inlet in a dual column analysis to analyze the target analytes listed in Table1. Other analytes can be added provided they are carried through an initial demonstration of capability and meet current QC acceptance limits. In dual column analysis each extracted and cleaned sample is analyzed on two different columns that have different phases. Performing dual column analysis allows the laboratory to confirm the presence of the analyte and can guarantee that at least one of the two columns will provide baseline resolution for all analytes.

1.3. The lower reporting limit is 0.625ng/g for tissue samples and the lower reporting limit for sediment samples is 0.333ng/g. The upper range may be extended using sample dilutions. The method detection limits (MDL) are determined with each project or at a minimum of once per year and saved with the project files. The MDL and reporting limits are based on 8g of tissue and 15g sediment sample weights.

2. Method Summary

2.1. A measured mass of tissue or sediment is extracted using pressurized solvent extraction and cleaned up following SOP MIRB045.2E.

2.2. Once the extract is brought to a final volume it is injected into a gas chromatograph equipped with pressure pulsed splitless injection, a narrow bore column and micro ECD detector.

2.3. Standard preparation, calibration procedures, analytical QC measures, and data analysis and validation are described in this SOP.

3. Definitions

3.1. CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to

analyte concentration.

3.2. LABORATORY REAGENT BLANK (LRB) -- An aliquot of the blank matrix, typically sodium sulfate or hydromatrix, that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates, and sample preservatives that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.3. LABORATORY FORTIFIED BLANK (LFB) -- An aliquot of the blank matrix, typically sodium sulfate or hydromatrix, to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, including the use of sample preservatives, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.4. LABORATORY FORTIFIED SAMPLE MATRIX (LFM) and DUPLICATE (LFMD)-- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results.

3.5. INTERNAL STANDARD (ISTD) -- A pure analyte(s) added to a sample, extract, or standard solution in known amount(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same solution. The internal standard must be an analyte that is not a sample component.

3.6. SURROGATE ANALYTE (SUR) -- A pure analyte, which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in a known amount before extraction or other processing, and is measured with the same procedures used to measure other sample components. The purpose of the SUR is to monitor method performance with each sample throughout the entire extraction and analysis technique.

3.7. CONTINUING CALIBRATION VERIFICATION (CCV) -- A calibration standard containing one or more method analytes. The CCV is analyzed every ten samples to verify the accuracy of the existing calibration for those analytes.

3.8. METHOD DETECTION LIMIT (MDL) – The statistically calculated minimum amount of an analyte that can be measured with 99% confidence that the reported value is greater than zero.

3.9. DEGRADATION CHECK – A solution of 4,4’-DDT and endrin prepared at a concentration in the mid point of the calibration curve and injected prior to calibration.

The degradation check solution checks the GC system for reactivity by measuring the percent breakdown of each of the injected compounds.

4. Health and Safety Warnings

4.1. The toxicity for the chemicals used in this SOP have not been fully defined therefore the chemicals used should be treated as a potential health hazard. To prevent adverse health effects, minimize exposure to all chemicals and use the correct personal protection equipment.

4.2. The ECD detector utilizes radioactive isotope ^{63}Ni. Care should be used when handling the ECD cell to prevent the ingestion or inhalation of the isotope. Under no condition should the cell be dismantled. Regular radioactive leak testing must by performed and ECD effluent must be vented outside the lab.

4.3. Protective eyewear, lab coat, and nitrile gloves are required safety precautions for this procedure.

5. Target Compound

5.1. All target compounds are listed in Table 1. Additional analytes may be added provided an initial demonstration of capability and MDL analysis are performed per analyte following quality control requirements.

Table 1. List of Target Organic Compounds, Internal Standards (ISTD), and Surrogates (Sur).

Compound	CAS Registry No.	IUPAC #
Aldrin	309-00-2	N/A
Alachlor	015972-60-8	N/A
α-Chlordane (cis)	5103-71-9	N/A
α-BHC	319-84-6	N/A
γ-Chlordane (trans)	5103-74-2	N/A
γ-BHC (Lindane)	58-89-9	N/A
Chlorpyrifos	2921-88-2	N/A
Cyanizine	21725-46-2	N/A
2,4'-DDD	53-19-0	N/A
4,4'-DDD	72-54-8	N/A
2,4'-DDE	3424-82-6	N/A
4,4'-DDE	72-55-9	N/A
2,4'-DDT	789-02-6	N/A
4,4'-DDT	55-29-3	N/A
Dacthal	1861-32-1	N/A
Dieldrin	60-57-1	N/A
Endosulfan I	959-98-8	N/A
Endosulfan II	33213-65-9	N/A
Endosulfan Sulfate	1031-07-8	N/A
Endrin	72-20-8	N/A
Endrin aldehyde	7421-93-4	N/A

Compound	CAS Registry No.	IUPAC #
Endrin ketone	53494-70-5	N/A
Heptachlor	76-44-8	N/A
Heptachlor epoxide	1024-57-3	N/A
Hexachlorobenzene	118-74-1	N/A
Metolachlor	51218-45-2	N/A
Mirex	2385-85-5	N/A
cis-Nonachlor	5103-73-1	N/A
trans-Nonachlor	39765-80-5	N/A
Oxychlordane	27304-13-8	N/A
Propachlor	1918-16-7	N/A
Terbacil	5902-51-2	N/A
Trifluralin	1582-09-8	N/A
4,4-Dibromobiphenyl	92-86-4	N/A (Sur)
4-Chloro-3-nitrobenzotrifluoride	121-17-5	N/A (Sur)
Tetrachloro-m-xylene	877-9-8	N/A (Sur)
4,4'-Dibromooctafluorobiphenyl	10386-84-2	N/A (Sur)
4,4-Dichlorobiphenyl	2050-68-2	15 (Sur)
Pentachloronitrobenzene	82-68-8	N/A (ISTD)
1-Bromo-2-nitrobenzene	577-19-5	N/A (ISTD)
2,4'-Dichlorobiphenyl	34883-43-7	8
2,2',5-Trichlorbiphenyl	37680-65-2	18
2,4,4'-Trichlorobiphenyl	7012-37-5	28
2',3,5-Trichlorobiphenyl	37680-68-5	34 (Sur)
2,2',3,5'-Tetrachlorobiphenyl	41464-39-5	44
2,2',5,5'-Tetrachlorobiphenyl	35693-99-3	52
2,3',4,4'-Tetrachlorobiphenyl	32598-10-0	66
3,3',4,4'-Tetrachlorobiphenyl	32598-13-3	77
3,4,4',5-Tetrachlorobiphenyl	70362-50-4	81
2,2',3,6,6'-Pentachlorobiphenyl	73575-54-9	96 (ISTD)
2,2',4,5,5'-Pentachlorobiphenyl	37680-73-2	101
2,2'4,5',6-Pentachlorobiphenyl	60145-21-3	103 (ISTD)
2,2',4,6,6'-Pentachlorobiphenyl	56558-16-8	104 (Sur)
2,3,3',4,4'-Pentachlorobiphenyl	32598-14-4	105
2,3,3',4',6-Pentachlorobiphenyl	38380-03-9	110
2,3,3',5,6-Pentachlorobiphenyl	74472-36-9	112 (Sur)
2,3',4,4',5-Pentachlorobiphenyl	31508-00-6	118
3,3',4,4',5-Pentachlorobiphenyl	57465-28-8	126
2,2',3,3',4,4'-Hexachlorobiphenyl	38380-07-03	128
2,2',3,4,4',5-Hexachlorobiphenyl	35065-28-2	138
2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1	153
2,3,4,4',5,6-Hexachlorobiphenyl	41411-63-6	166 (ISTD)
3,3',4,4',5,5'-Hexachlorobiphenyl	32774-16-6	169
2,2',3,3',4,4',5-Heptachlorobiphenyl	35065-30-6	170
2,2',3,4,4',5,5'-Heptachlorobiphenyl	35065-29-3	180
2,2',3,4',5,5',6-Heptachlorobiphenyl	52663-68-0	187
2,2',3,3',4,4',5,6-Octachlorobiphenyl	52663-78-2	195
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	40186-72-9	206
Decachlorobiphenyl	2051-24-3	209
2,4,4'-Tribromodiphenyl ether	41318-75-6	28
2,2',4,4'-Tetrabromodiphenyl ether	5436-43-1	47
2,3',4,4'-Tetrabromodiphenyl ether	189084-61-5	66
2,2'3,4,4'-Pentabromodiphenyl ether	182346-21-0	85
2,2',4,4',5-Pentabromodiphenyl ether	60348-60-9	99

Compound	CAS Registry No.	IUPAC #
2,2',4,4',6-Pentabromodiphenyl ether	189084-64-8	100
2,2',4,4',5,5'-Hexabromodiphenyl ether	68631-49-2	153
2,2',4,4',5,6'-Hexabromodiphenyl ether	207122-15-4	154
2,2',3,4,4',5',6-Heptabromodiphenyl ether	207122-16-5	183

6. Interferences

6.1. Interferences fall into three general descriptions: contamination from reagents, solvents, glassware or hardware; contamination from GC carrier gases, inlets, column or detector surfaces; and from compounds co-extracted from sample matrix which will vary depending on the individual matrix.

6.1.1. Interferences from reagents, solvents, and glassware.

6.1.1.1. Contamination from reagents and solvents can be minimized by using ultra-pure solvents muffling reagents at 400°C for four hours.

6.1.1.2. Contamination from phthalate esters can be reduced by avoiding using plastic materials and by utilizing a sulfuric acid cleanup.

6.1.1.3. Scrupulous cleaning of all glassware and hardware can significantly reduce sample contamination. Glassware should be treated to soap washing followed by DI and acetone rinsing.

6.1.2. Interferences from GC sources.

6.1.2.1. Contamination of GC parts can generally by minimized with the use of ultra-pure carrier and detector gases with appropriate traps, use of guard columns and regular scheduled maintenance.

6.1.2.2. Routine maintenance consists of replacement of septa and injector liners, trimming the guard and or injector side of the analytical column, baking of column and cleaning of the metallic inlet surfaces.

6.1.3. Interferences co-extracted from sample matrix.

6.1.3.1. The best way to reduce interferences co-extracted from the sample matrix is to utilize adequate cleanup method that separates interferences from the target analytes. SOP MIRB -007.0E should reduce most common interferences.

6.1.3.2. The occurrence of elevated baselines and "hump-o-grams" is an indication of excessive contamination. These occurrences will effect quantification of data. Generally elevated baselines and hump-o-grams also indicate that the GC

hardware will need to undergo thorough maintenance to remove the contamination from the GC system.

7. Personnel Qualifications

7.1. Personnel performing this procedure are required to have training in GC using capillary columns and ChemStation data acquisition software.

8. Equipment, Supplies and Reagents

8.1. Instrumentation-Gas Chromatograph (GC) with μECD detector and split/splitless inlet - Agilent 6890 or equivalent

8.2. GC Columns

8.2.1. Primary Column: Agilent HP-5 capillary column: 30 m length, 0.25mm diameter and 0.25 μm film thickness

8.2.2. Confirmatory Column; J&W DB - XLB column, 30m length, 0.25mm diameter and 0.25μm film thickness

8.3. GC Gasses

8.3.1. Carrier gas: Hydrogen gas - grade 5.0 or equivalent

8.3.2. Detector make-up gas: Argon methane 5% gas - UHP grade or equivalent

8.4. GC Operating Conditions -- Slight variations in GC programs are acceptable to account for changes within the column due to trimming, contamination or age.

8.4.1. Primary Column: RTX-5MS-All compounds

Oven Program	Inlet Program	Column Program	Detector Program
Initial temp: 60°C	Mode: Pulsed splitless	Mode: constant flow	Temperature: 350°C
Initial time: 2.00 min	Initial temp: 275°C	Initial flow: 1.2 ml/min	Mode: constant column + makeup flow
Equilibration time: 0.5 min	Pressure: 6.53 psi	Average velocity: 23 cm/sec	Combined flow: 30.0 ml/min
Maximum temp: 325°C	Pulse pressure: 30.0 psi		Makeup gas type: Argon methane 5%
Ramp:	Pulse time: 0.60 min		

Oven Program	Inlet Program	Column Program	Detector Program
50°C/min until 120°C, hold 8.00 min 20°C/min until 180°C, hold 8.00 min 1.5°C/min until 210°C, hold 0.00 min 5°C/min until 300°C, hold 2.00 min			
Post temp: 60°C	Purge flow: 55.1 ml/ml		
Run time: 62.20 min	Purge time 0.50 min		
	Total flow 59.3 ml/min		

8.4.2. Confirmatory Column: DB-XLB or similar-All compounds

Oven Program	Inlet Program	Column Program	Detector Program
Initial temp: 120°C	Mode: Pulsed splitless	Mode: constant flow	Temperature: 350°C
Initial time: 1.00 min	Initial temp: 290°C	Initial flow: 2.2 ml/min	Mode: constant column + makeup flow
Equilibration time: 0.5 min	Pressure: 13.28 psi	Average velocity: 40 cm/sec	Combined flow: 30.0 ml/min
Maximum temp: 350°C	Pulse pressure: 30.0 psi		Makeup gas type: Argon methane 5%
Ramp: 7.5°C/min until 160°C, hold 2.00 min 2°C/min until 240°C, hold 0.00 min 5°C/min until 280°C, hold 0.00 min 20°C/min until 325°C, hold 5.50 min	Pulse time: 0.60 min		
Post temp: 120°C	Purge flow: 55.5 ml/ml		
Run time: 64.08 min	Purge time 0.50 min		
	Total flow 60.2 ml/min		

8.5. Reagents

8.5.1. All chemicals are required to be pesticide grade or equivalent.

8.6. Standard Preparation – Primary standards are purchased as certified standards from several different vendors, typically at concentrations of 50µg/mL or 100µg/mL. Typically standards purchased from vendors come either with methanol or hexane as the solvent. Intermediate stock standards containing methanol will require a small amount (10-20% by volume) of acetone added as a carrier solvent to keep methanol and hexane miscible. The five calibration standards must be in hexane.

8.7. Intermediate standards

8.7.1. Pesticide Intermediate Stock – Commercially purchased pesticide standards are normally 100µg/mL. Make a 1µg/mL intermediate stock by diluting 50µL of the vendor standards and bring to volume at 5mL in hexane:acetone (80:20 v/v).

8.7.2. PCB/PBDE Intermediate Stock – Commercially purchased PCB standards are normally 100µg/mL while PBDE standards are normally 50µg/mL. Make a 1µg/mL intermediate stock by diluting 50µL of the PCB standards and 100µL of the PBDE standards to volume at 5mL in hexane.

8.7.3. Surrogate Intermediate Stock – Commercially purchased surrogate standards vary in concentration. Make a 1µg/mL intermediate stock by diluting an appropriate amount of the vendor standards and bring to volume at 5mL in hexane:acetone (80:20 v/v). Possible surrogate standards include 4,4'-dibromobiphenyl, 4-chloro-3-nitrobenzotrifluoride, Tetrachloro-m-xylene, 4,4'-dibromooctafluorobiphenyl, PCB#15, PCB#34, PCB#104, and PCB#134. The chosen surrogates will take into consideration target compounds from Table 1 on a project basis to avoid interferences between surrogates and target compounds. A minimum of three surrogates will be used per analysis.

8.7.4. Internal Standard (IS) Intermediate Stock -- Commercially purchased internal standard solutions vary in concentration. Make a 1µg/mL intermediate stock by diluting an appropriate amount of the vendor standards and bring to volume at 5mL in hexane:acetone (80:20 v/v). Possible internal standards include pentachloronitrobenzene, 1-bromo-2-nitrobenzene, 2, 2'-dinitrobiphenyl, PCB#96, PCB#103, and PCB#166. The chosen internal standards will take into consideration target compounds from Table 1 on a project basis to avoid interferences between internal standards and target compounds. A minimum of three internal standards will be used per analysis.

8.7.5. Calibration Standards -- Must be prepared at a minimum of five levels. The

lowest standard, generally 1ppb, should represent analyte concentrations near but not below their respective MDLs. The remaining standards should bracket the analyte concentrations expected in the sample extracts. Table 2 describes the preparation of the calibration standards.

8.7.6. Continuing Calibration Verification Standards (CCV) – The CCV standards are prepared like the calibration standards. CCVs are prepared near the midpoint level of the calibration curve, in the 10-25ng/mL range.

8.7.7. LFB/LFM Spike Sample – The spike solution is to be prepared in 10mL of hexane. It will be prepared at a concentration of 125ng/mL. One 1mL of this solution is spiked into the LFB, LFM, and LFMD. The final extract concentration will be 25ng/mL. Other concentrations may be used provided the final extract concentration falls within the calibration curve.

Table 2-Calibration Standard Preparation

Pesticide Intermediate	PCB/PBDE Intermediate	Surrogate Intermediate	IS Intermediate	Final Volume Hexane	Final Conc. Targets	Final Conc. IS
10µL	10µL	10µL	250µL	10mL	1ng/mL	25ng/mL
50µL	50µL	50µL	250µL	10mL	5ng/mL	25ng/mL
100µL	100µL	100µL	250µL	10mL	10ng/mL	25ng/mL
150µL	150µL	150µL	250µL	10mL	15ng/mL	25ng/mL
250µL	250µL	250µL	250µL	10mL	25ng/mL	25ng/mL
500µL	500µL	500µL	250µL	10mL	50ng/mL	25ng/mL
750µL	750µL	750µL	250µL	10mL	75ng/mL	25ng/mL

9. Sample Collection, Preservation, and Handling

9.1. Sediment

9.1.1. Sediment samples should be collected in 4-8oz containers, preferably glass containers. Samples should be labeled, at a minimum, with project name or number, sample ID, date and time of sampling. The required mass is usually project specific but typically a minimum of 50g is required.

9.1.2. Stored at -20^{o}C indefinitely unless project QAPPs storage requirements supersede this SOP.

9.2. Tissue

9.2.1. Tissue samples should be collected by wrapping the fish in aluminum foil and then double bagging with appropriately sized plastic storage bags.

9.2.2. Stored at -20°C indefinitely unless project QAPPs storage requirements supersede this SOP.

9.3. Extract storage

9.3.1. Extracts may be stored for up to 40 days at -20°C.

10. Procedure

10.1. Samples must be spiked with 25µL of the IS intermediate solution (1µg/mL) into the 1mL final volume sample extract just prior to bringing to final volume. This ensures an IS concentration of 25ng/mL in the final extract used for GC analysis.

10.2. Due to the low concentration of calibration standards used with a GC/µ-ECD, column adsorption may be a problem if the GC has not been used in a few days. It is recommended to deactivate the GC system by doing a priming injection with a high concentration standard followed by a solvent blank just prior to injecting the calibration standards.

10.3. Standards and samples are analyzed by gas chromatography using the same GC conditions. The "GC Operating Conditions" section summarizes the recommended operating conditions for the gas chromatograph for both the primary and confirmatory columns. For all runs, inject 5µL in the split/splitless inlet using the pulsed splitless mode.

10.4. On each GC, order the run so that the degradation standard is analyzed first, then the calibration standards bracketed by hexane blanks, followed by the samples with a hexane blank every ten samples. Insert check standards after every ten samples and at the end of the run, both bracketed by hexane blanks. Record data for each run in a logsheet (Appendix A)

10.5. Starting with the lowest concentration standard, analyze each calibration standard and tabulate peak area versus concentration in the standard. The results are used to generate a linear regression using internal standard.

10.6. The working calibration curve must be verified by the measurement of the continuing calibration verification (CCV) standard. CCVs are run after every ten samples and at the end of the batch to ensure the calibration is valid during the course of analyzing a batch of samples. Injections of method blank extracts, matrix spike extracts, LFB extracts, sample extracts, and other non-standards are counted in the total while standards and solvent blanks are not counted.

10.7. ChemStation Software

10.7.1. Using settings given in the GC methods section, a method is programmed so that it is appropriate for the desired analytes, injection port, column type, and detector. The methods can then be saved or copied as needed. The method dictates the time, pressures, and temperatures that will be used during analysis of a sample batch. Saving the method with a unique name that references the batch being analyzed is important because all calibration data are stored in the method. For each project, hard copies of relevant methods are printed out, both for quick reference and to ensure method consistency in the event of a hardware failure. Method calibration data (tables and curves) from each batch is printed out and included in reports. Refer to the ChemStation manual for details.

10.7.2. A sequence is created that details the sample identities, sample location, file storage information, sample amounts, and relevant dilution factors. It is important to save the sequence with a unique name appropriate to the batch being analyzed for reference purposes. The sequence data is printed out for each batch analyzed to be included in reports.

10.7.3. To be consistent, directories are named according to batch number. Data files are named according to batch number and the order in which the sample is analyzed. The sample identity is also stored in the data file for reference. Directory names and data file sets are limited to eight characters. All data are routinely backed up on network drives, which are backed up daily to tape as part of a disaster recovery program.

11. Analyte Identification

11.1. An analyte is identified by comparing a peak's retention time (RT) to that of the retention time of a reference chromatograph. If the retention of an unknown compound is within the retention time window of a standard compound, the identification is considered positive.

11.1.1. Calculating RT Windows

11.1.1.1. Inject three aliquots of a mid-point calibration solution over the course of 72 hours. Performing the injection in a shorter time window may cause RT windows that are too narrow.

11.1.1.2. Measure the RT in minutes out to three decimal points.

11.1.1.3. Calculate the mean (M) and standard deviation (SD) of the triplicate injections.

$$M = \frac{\sum_{i=1}^{n} X_i}{n}$$

where: Xi = sample measurement
n = number of measurements

$$SD = \sqrt{\frac{\sum_{i=1}^{n} (X_i - \overline{X})^2}{n-1}}$$

where: Xi = sample measurement
$\overline{X}$ = mean value of sample data set
n = number of measurements

11.1.1.4. RT windows are established by multiplying the measured SD by three. This constitutes the width of the entire RT window. If SD calculates to zero for an analyte (all measurements are the same), use a default SD value of 0.01min multiplied by three to give a default window of 0.03min.

11.1.1.5. RTs are adjusted per batch by setting the RT of the mid-point calibration standard as the center of each RT window. Alternatively the average of all the calibration standards can be used to set the mid-point of the calibration window per analyte.

11.2. The GC systems employ ChemStation software to acquire and analyze chromatographic data. All computerized data analyses must be reviewed carefully by the analyst to determine the accuracy and appropriateness of the quantitation and qualification performed by the data software system. The failure of the software to appropriately integrate is obvious from visual inspection of the chromatograph. Any error must be corrected by the analyst using the software tools available.

11.3. The review of the computerized analyses is done through a tool included in the ChemStation software called a batch review. During a batch review, each sample data file is reviewed and any erroneous integrations are corrected through manual integrations that are saved with each data file. Manual integration relies upon the experience of the analyst and will not be performed solely for the purpose of meeting QC criteria. After each data file has been reviewed, the batch is saved and the software requantitates the data based on any manual integrations that were saved. This data is then printed out and included with batch documentation. Refer to the ChemStation manual for details**.**

12. Data Quantification

12.1. Data analysis and calculations are performed by the ChemStation software using peak area. The software calculates the concentration of calibrated analytes by calculating response ratio and amount ratios between the compound and the ISTD. ChemStation software quantitates compounds using the following equations. Normally linear calibration using internal standard correction is chosen for calibration.

12.1.1. Linear using ISTD

12.1.1.1. Calculate the area ratio using the following equation. The area ratio is then renamed the response ratio in ChemStation terminology.

$$\text{Area Ratio} = \frac{area_X}{area_{ISTD}} = \text{Response Ratio}$$

$Area_X$ = Area of sample peak for quantitation
$Area_{ISTD}$ = Area of ISTD peak

Plug the area ratio into the equation for a line to calculate the sample specific amount ratio. The linear equation is the "calibration curve" in ChemStation terminology.

y = mx + b, where:

y = Area Ratio (or response ratio)
m = slope (given from calibration curve)
x = Amount Ratio
b = y-intercept (given from calibration curve)

12.1.1.2. The amount ratio and response ratio can then be entered into the following equation to calculate the sample specific concentration adjusted for internal standard recovery.

Concentration of X = (response ratio*RFx)*(amount of ISTD)*M*D

$$RF_x = \frac{amount\ ratio}{response\ ratio}$$

M = multiplier
D = dilution factor

12.1.2. Multiplication factors are included in the sequence table to allow ChemStation to calculate final concentrations that do no require manual adjustment for weight and extract final volumes.

12.1.3. Samples exceeding the upper calibration limit are diluted and reanalyzed until they fall inside the established calibration range.

12.1.4. Linear calibration requires a minimum of five calibration points.

13. Corrective Actions (CA)

CA are required when performance does not meet QC requirements and may include any of the following:

13.1. Inlet Maintenance

13.1.1. The liner and septa should be changed after each batch of samples or approximately after 50-60 injections.

13.1.2. The use of liners with glass wool reduces non-volatiles contaminating the analytical column.

13.1.3. Inlet Scrubbing

13.1.3.1. Cool injector and oven and remove liner.

13.1.3.2. Add a small amount of microgrit to methanol to create a slurry. Apply the slurry to the metal inlet body with a cotton swab. Scrub the inlet until clean.

13.1.3.3. Place beaker under inlet and remove all traces of microgrit by rinsing with copious amounts of methanol, followed by an acetone rinse and a hexane rinse. Agitating the inlet with a cotton swab to help loosen and rinse away the microgrit may be necessary.

13.1.3.4. Replace the gold seal and re-assemble the inlet.

13.1.4. Column Maintenance

13.1.4.1. Column contamination is reduced by using a 5m guard column, 0.25mm ID. If necessary, the front end of the guard column can be cut to remove possible contamination or the guard column can be replaced.

13.1.4.2. The front end, up to 1m, of the analytical column can be cut to remove

contamination.

13.1.4.3. Periodically check column connector for leaks and replace when necessary.

13.1.4.4. Baking the analytical column for 30 min up to 3 hours at 290° or 300°C can help to remove some contamination. Do not over bake at high temperature and monitor the baseline to ensure it is dropping, not rising which would indicate a leak.

13.1.4.5. If an injected sample contains residual acid both the guard column and analytical column will need to be replaced.

14. Quality Control

14.1. Initial Demonstration of Capability (IDOC)

14.1.1. Analyze a minimum of four replicate LFBs spiked at the intended calibration range midpoint or typical LFB concentration level.

14.1.2. Calculate the average recovery (%R), arithmetic mean (M), relative standard deviation (RSD), and standard deviation (SD) per analyte. The average recovery must pass the LFB limits and the RSD must pass the duplicate limits defined in the QC acceptance table below. The SD is useful for estimating method detection limit spiking.

14.1.2.1. $M = \frac{\sum_{i=1}^{n} X_i}{n}$

where: Xi = sample measurement
n = number of measurements

14.1.2.2. $SD = \sqrt{\frac{\sum_{i=1}^{n} (X_i - \overline{X})^2}{n-1}}$

where: Xi = sample measurement
$\overline{X}$ = mean value of sample data set
n = number of measurements

14.1.2.3. $RSD = \frac{SD}{\overline{X}} \times 100\%$

where: $\overline{X}$ = mean value of sample data set
n = number of measurements

14.2. Method Detection Limits (MDL)

14.2.1. Analyze a minimum of seven replicates spiked at 3-5 times the instrument noise level. The concentration equivalent to three times the SD of the IDOC replicates can be useful in determining MDL spike levels and instrument noise. Analyzing the MDLs over several days gives a better approximation of instrument performance.

14.2.2. Calculate the average recovery and standard deviation for each analyte. The MDL is calculated by multiplying the standard deviation by the students-t value for n-1 and a 99% confidence level. The students-t value for seven replicates (n-1=6) is 3.143. Average MDL recovery should meet acceptance limits for LFB in the QC table.

14.2.2.1. MDL = t(n-1, 1-α=0.99) X SD

14.2.3. Calculated MDLs should be no higher than the spike level. Spike levels should not be more than ten times the calculated MDL such that:

Calculated MDL < Spike Level < 10 X Calculated MDL

14.2.4. Reporting limits (RL) are generated from MDL data and can be set equal to or greater than the calculated MDL. RLs for this laboratory are set at the weight adjusted equivalent concentration (0.625 ng/g) based on the lowest GC calibration standard (1ng/mL).

14.3. Degradation Check -- DDT and endrin are easily degraded in the injection port. Breakdown occurs when the injection port liner is contaminated with high boiling point residue from sample injection or when the injector contains metal fittings. Check for degradation problems by injecting a standard containing only 4,4'-DDT and endrin. Presence of 4,4-DDE, 4,4'-DDD, endrin ketone, or endrin aldehyde indicates breakdown. If degradation of either DDT or DDE exceeds 20% using the following equations, take corrective action before proceeding with calibration.

14.3.1.1. % breakdown of DDT = $\frac{\sum DDE + DDD}{\sum DDE + DDD + DDT}$ X100%

14.3.1.2. % breakdown of endrin = $\frac{\sum aldehyde + ketone}{\sum aldehyde + ketone + endrin}$ X 100%

14.4. Calibration Standards – The calibration curve is injected following the passing degradation check. The curve fit is linear and sample concentrations are calculated using internal standard correction. The correlation coefficients for the curves must be $\geq$0.98.

14.5. Continuing Calibration Verification (CCV) Standards – The CCV checks verify instrument stability throughout the course of the GC analysis. Percent recovery for the check standards must be within ± 15% of the excepted concentration for at least 75% of all analytes.

14.6. Internal Standard (ISTD) – The ISTD serves as a normalizing factor and compensates for instrument drift. The percent recovery for each internal standard is calculated for each sample using the average area from the calibration curve as the theoretical value. The percent recovery must be within ± 30%. Low ISTD values bias data results high and high ISTD values bias data results low.

14.7. Laboratory Reagent Blanks (LRB) – LRB is a non-spiked sample that contains no tissue/sediment material, just reagent material. One LRB is extracted with each batch of samples. Concentrations for each analyte in the LRB must be less than 3x the MDL.

14.8. Laboratory Fortified Blanks (LFB) – LFB is a spiked sample that contains no tissue/sediment material, just reagent material. The LFB is spiked with a known amount of each analyte. The spike concentration can be adjusted to meet the goals of a particular project but ideally should be 3 to 5 times greater than the sample background. The percent recovery of the LFB must be within ± 30% recovery for at least 75% of all analytes. Precision is determined by calculating the relative percent difference, %RPD, between duplicate LFB samples. %RPD should be less than 50% for 75% of all analytes.

14.9. Surrogate: Calculate the percent recovery for each surrogate in each sample. The percent recovery for the surrogate in each sample must be within ± 30%.

14.10. Laboratory Fortified Matrix (LFM) and Duplicate (LFMD) – Spike two aliquots of the same sample with a known amount of each analyte. The LFM and LFMD are analyzed exactly like a sample, and the purpose is to determine whether the sample matrix contributes bias to the analytical results. Calculate the percent recovery for each LFM. Recoveries must be within ± 50% recovery for at least 75% of all analytes.

14.10.1. Precision is determined by calculating the relative percent difference, %RPD, between duplicate LFB samples. %RPD should be less than 50% for 75% of all analytes.

$$RPD\% = \frac{|x_1 - x_2|}{\left(\frac{x_1 + x_2}{2}\right)} \text{ X } 100\%$$

14.11. Standard Reference Material (SRM) – The purpose of the SRM is to provide a NIST certified second source standard to monitor extraction efficiency and instrument quantitation. Average recoveries must be ± 30% of the published SRM true value; not to exceed ±35% of true value for more than 30% of individual analytes.

14.12. Table 3 lists the QC parameters required for the procedure per batch. A batch consists of 19 samples, 5 QC samples, and a calibration curve.

Table 3-Quality Control Elements

Quality Element	Frequency	Acceptance Criteria	Corrective Action
Degradation Check	Once prior to calibration	20% each for DDT and endrin	1) Investigate and document. 2) If endrin, 4,4'-DDT, or their breakdown products were detected above RL, reanalyze the sample. 3) Corrective action prior to next calibration.
Initial Calibration	Once per batch	> 0.98[1]	1) Check standards and preparation. 2) Check system for leaks, poor injection repeatability. 3) Recalibrate.
LRB	Once per batch	<3X MDL[1]	1) Document high blank results. 2) Samples are reported if high blank analyte below RL in sample. 3) Samples with high blank and analyte above RL reanalyzed.
Standard Reference Material (SRM)	Once per batch	70-130% average recovery of true value; not to	1) Extract a second SRM and analyze. 2) If second SRM

Quality Element	Frequency	Acceptance Criteria	Corrective Action
		exceed ±35% of true value for more than 30% of individual analytes.	passes, report data with qualifier regarding SRM rerun. 3) If fails, entire batch must be re-extracted.
Check Standards (CCV)	Every 10 samples and end of sequence	85-115% average recovery; not to exceed ±30% for any one analyte	1) Rerun CCV once. 2) Recalibrate. 3) Rerun all samples back to the last passing CCV.
LFB	Once per batch	70-130% Recovery[1]	1) Rerun LFB. 3) Re-extract entire batch.
Surrogate	Every sample	70-130% Recovery	1) Reanalyze current sample extract or 2) Re-extract and rerun sample. 3) If now passing report data. 4) If still failing report data with S and I qualifier.
Internal Standard	Every sample	70-130% Recovery	1) Reanalyze current sample extract or 2) Re-extract and rerun sample. 3) If now passing report data. 4) If still failing report data with H' and I qualifier.
Duplicate (LFMD)	One set per batch	<30% RPD[1]	1) Document duplicate failure per analyte 2) Report data with D qualifier.
LFM	Once per batch	50-150% Recovery[1]	1) Document LFM failure per analyte. 2) Report data with M or M' qualifier.

[1]75% of all analytes

14.13. Data Qualifiers – Describe possible deviation from normal standard operating procedures or quality control measures. Typically used organic data qualifiers are listed in Table 4.

Table 4-Data Qualifiers

Qualifier Code	Definition
A	Value reported is the average of two or more determinations.
B	Analyte was detected in both the sample and the associated method blank. Result exhibits the potential for high bias.
B'	Analyte was detected in the associated method blank.
C	LFBD was analyzed instead of LFMD due to limited sample mass or volume.
D	Duplicate or replicate failed to meet acceptance limits.
D'	The % difference between the results for both columns exceeded acceptance limits.
E	Result is reported as less than the total result.
F	High concentration resulted in dilution and elevated detection limit
F'	Limited sample volume, turbidity, or other matrix effect resulted in elevated quant limit/reporting limit.
H	Result exhibits the potential for high bias.
H'	Low response for IS; possible high bias for detected compounds.
I	Matrix interference.
J	Result is estimated.
L	LFB failed to meet acceptance limits.
L'	LFBD failed to meet acceptance limits.
M	LFM recovery outside of acceptance limits.
M'	LFMD recovery outside of acceptance limits.
Q	Sample was received or analyzed outside of method established holding time.
R	Sample was received warm, was submitted in inappropriate container, or was improperly preserved.
S	One or more surrogate recoveries failed to meet acceptance limits.
T	Laboratory contamination is suspected.
U	Analyte not detected.
V	Compound in CCV had a high %R but results are <RL.
RR	Sample was or will be reanalyzed.
*****	Project specific comment.

15. Data Management

15.1. In a dual column analysis, the final reported values are composites of the data generated from both columns. A set of rules were developed to confirm the presence of a target analyte and determine how the analyte will be quantified. The rules are as follows:

15.1.1. The analyte had to be present on both columns in order to be reported as present. When an analyte is present on only one column, the peak is assumed to be due to a co-eluting interference and the value reported is <MDL.

15.1.2. When the analyte is present on both columns the reported value was determined using the following protocol:

15.1.2.1. When the values from both columns have a relative percent difference, RPD, <50%, i.e., the values from both columns are similar, then the final reported value is the average of both columns.

15.1.2.2. When the values from both columns have an RPD >50% then the data is evaluated as follows:

15.1.2.2.1. If one column had QC problems, then the column with the best QC is used for the final value.

15.1.2.2.2. If the QC data from both columns are similar, then the column with the lowest value is used for the final value.

15.1.2.2.3. The analyst's observations are also used to validate the quality of all data and to determine from which column the recorded value is taken.

15.2 Raw data, reports, and project communications (emails, etc..) will be placed in a 3-ring binder and labeled with project title and dates.

16. Waste Management

16.1. All waste material should be collected in a laboratory satellite waste container and sent to the Hazardous Waste Room (B-71) when filled.

17. References

17.1. USEPA Method 508.1, Determination of Chlorinated Pesticides, Herbicides, and Organohalides by Liquid-Solid Extraction and Electron Capture Detector, National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH 45268.

17.2. USA EPA. 1996. Method 8082 Polychlorinated biphenyls (PCBs) by Gas Chromatography. EPA SW846. EPA Office of Solid Waste and Emergency Response. Washington, D.C.

17.3. USA EPA. 1996. Method 8081 Organochlorine Pesticides by Gas Chromatography.

EPA SW846. EPA Office of Solid Waste and Emergency Response. Washington, D.C.

17.4. Agilent Technologies. 2001. Understanding Your Chemstation. Waldbronn, Germany

17.5. “40 CFR 136.6, Appendix B: Guidelines Establishing Test Procedures for the Analysis of Pollutants, Appendix B- Definition and Procedure for the Determination of the Method Detection Limit." (Current through 8/22/08). Text from: *Code of Federal Regulations*. Available from: e-CFR; Accessed 08/26/08.

STANDARD OPERATING PROCEDURES

For

ASE EXTRACTION AND CLEANUP OF WET TISSUE, SEDIMENT, AND BENTHIC MATERIALS FOR TRACE ORGANICS

Approved by:

_____

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Work Assignment 0-07 Chemical Analysis of Environmental Samples

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U.S. Environmental Protection Agency
NERL/EERD/MIRB
Cincinnati, OH

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ASE Extraction and Cleanup of Wet Tissue, Sediment, and Benthic Materials for Trace Organics

1. Scope and Application

1.1. This method is applicable to wet fish tissue, sediment, macroinvertebrates, periphyton, seston, and benthic materials such as leaves or similar material sometimes abbreviated FBOM or CPOM. Non-NRSA fish and similar organisms must be homogenized following the most recent version of SOP MIRB005, "Tissue Homogenization of Fish and Macroinvertebrates" prior to extraction. Fish requiring homogenization under NRSA guidelines must use the most recent version of MIRB-350, "Fish Tissue Preparation, Homogenization, and Distribution Procedures for the National Rivers and Streams Assessment Fish Tissue Indicator" prior to extraction. Sediment and similar materials must be sieved or otherwise prepared following the most recent version of SOP MIRB007, "Homogenization of Sediment, Seston, and Basal Resources" prior to extraction.

1.2. The procedure details specific cleanup steps to prepare samples for GC injection.

1.3. Extracted and cleaned samples are typically analyzed by GC/μECD for polychlorinated biphenyls (PCBs), organochlorine pesticides, and polybrominated diphenyl ethers (PBDEs) following SOP # 046, "GC Analysis of Organics".

2. Method Summary

2.1. Samples are prepared for extraction by mixing with granular sodium sulfate, termed the drying material. Sodium sulfate is used for tissue samples and sediment samples, although this SOP leaves an option to use hydromatrix as well. Samples using hydromatrix require GPC cleaning to remove background interference created by the hydromatrix. The sample is then loaded into an extraction cell.

2.2. The cell is heated, pressurized with the appropriate solvent, and extracted for seven minutes, using three static cycles.

2.3. The solvent is collected, concentrated, and dried with sodium sulfate.

2.4. Initial gross cleanup is performed by gel permeation chromatography (GPC).

2.5. Alumina cleanup is then performed to remove any trace amounts of contaminants left over from GPC, to yield a clean extract prior to GC analysis.

3. Definitions

3.1. CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.2. LABORATORY REAGENT BLANK (LRB) -- An aliquot of the blank matrix, typically sodium sulfate or hydromatrix, that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates, and sample preservatives that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.3. LABORATORY FORTIFIED BLANK (LFB) -- An aliquot of the blank matrix, typically sodium sulfate or hydromatrix, to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, including the use of sample preservatives, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.4. LABORATORY FORTIFIED SAMPLE MATRIX (LFM) and DUPLICATE (LFMD)-- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results.

3.5. INTERNAL STANDARD (ISTD) -- A pure analyte(s) added to a sample, extract, or standard solution in known amount(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same solution. The internal standard must be an analyte that is not a sample component.

3.6. SURROGATE ANALYTE (SUR) -- A pure analyte, which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in a known amount before extraction or other processing, and is measured with the same procedures used to measure other sample components. The purpose of the SUR is to monitor method performance with each sample throughout the entire extraction and analysis technique.

3.7. CONTINUING CALIBRATION VERIFICATION (CCV) -- A calibration standard containing one or more method analytes. The CCV is analyzed every ten samples to verify the accuracy of the existing calibration for those analytes.

3.8. METHOD DETECTION LIMIT (MDL) – The statistically calculated minimum amount of an analyte that can be measured with 99% confidence that the reported value is greater than zero.

3.9. GEL PERMEATION CHROMATOGRAPHY (GPC)-A size exclusion cleanup technique used to separate larger molecules (lipids, proteins) from smaller target analytes.

4. Health and Safety Warnings

4.1. The toxicity for all the chemicals used in this SOP have not been fully defined therefore the chemicals used should be treated as a potential health hazard. To prevent adverse health effects, minimize exposure to all chemicals and use the correct personal protection equipment.

4.2. Protective eyewear, lab coat, and nitrile gloves are required safety precautions for this procedure.

4.3. Caution should be taken when working around organic solvents, due to flammability and health issues from overexposure. See MSDS sheets for further information.

4.4. ASE cells are heated to >100^{o}C and care should be taken to avoid burns from recently heated cells. The ASE instrument should be powered down and the nitrogen turned off before doing maintenance to prevent burns and possible pinching from moving parts.

5. Target Compounds

5.1. All target compounds are listed in Table 1. Additional analytes may be added provided an initial demonstration of capability and MDL analysis are performed per analyte following quality control requirements.

Table 1. List of Target Organic Compounds, Internal Standards (ISTD), and Surrogates (Sur).

Compound	CAS Registry No.	IUPAC #
Aldrin	309-00-2	N/A
Alachlor	015972-60-8	N/A
α-Chlordane (cis)	5103-71-9	N/A
α-BHC	319-84-6	N/A
γ-Chlordane (trans)	5103-74-2	N/A
γ-BHC (Lindane)	58-89-9	N/A
Chlorpyrifos	2921-88-2	N/A
Cyanizine	21725-46-2	N/A
2,4'-DDD	53-19-0	N/A
4,4'-DDD	72-54-8	N/A
2,4'-DDE	3424-82-6	N/A
4,4'-DDE	72-55-9	N/A
2,4'-DDT	789-02-6	N/A
4,4'-DDT	55-29-3	N/A
Dacthal	1861-32-1	N/A
Dieldrin	60-57-1	N/A

Compound	CAS Registry No.	IUPAC #
Endosulfan I	959-98-8	N/A
Endosulfan II	33213-65-9	N/A
Endosulfan Sulfate	1031-07-8	N/A
Endrin	72-20-8	N/A
Endrin aldehyde	7421-93-4	N/A
Endrin ketone	53494-70-5	N/A
Heptachlor	76-44-8	N/A
Heptachlor epoxide	1024-57-3	N/A
Hexachlorobenzene	118-74-1	N/A
Metolachlor	51218-45-2	N/A
Mirex	2385-85-5	N/A
cis-Nonachlor	5103-73-1	N/A
trans-Nonachlor	39765-80-5	N/A
Oxychlordane	27304-13-8	N/A
Propachlor	1918-16-7	N/A
Terbacil	5902-51-2	N/A
Trifluralin	1582-09-8	N/A
4,4-Dibromobiphenyl	92-86-4	N/A (Sur)
Tetrachloro-m-xylene	877-9-8	N/A (Sur)
Pentachloronitrobenzene	82-68-8	N/A (ISTD)
2,4'-Dichlorobiphenyl	34883-43-7	8
2,2',5-Trichlorbiphenyl	37680-65-2	18
2,4,4'-Trichlorobiphenyl	7012-37-5	28
2,2',3,5'-Tetrachlorobiphenyl	41464-39-5	44
2,2',5,5'-Tetrachlorobiphenyl	35693-99-3	52
2,3',4,4'-Tetrachlorobiphenyl	32598-10-0	66
3,3',4,4'-Tetrachlorobiphenyl	32598-13-3	77
3,4,4',5-Tetrachlorobiphenyl	70362-50-4	81
2,2',3,6,6'-Pentachlorobiphenyl	73575-54-9	96 (ISTD)
2,2',4,5,5'-Pentachlorobiphenyl	37680-73-2	101
2,2',4,6,6'-Pentachlorobiphenyl	56558-16-8	104 (Sur)
2,3,3',4,4'-Pentachlorobiphenyl	32598-14-4	105
2,3,3',4',6-Pentachlorobiphenyl	38380-03-9	110
2,3,3',5,6-Pentachlorobiphenyl	74472-36-9	112 (Sur)
2,3',4,4',5-Pentachlorobiphenyl	31508-00-6	118
3,3',4,4',5-Pentachlorobiphenyl	57465-28-8	126
2,2',3,3',4,4'-Hexachlorobiphenyl	38380-07-03	128
2,2',3,4,4',5-Hexachlorobiphenyl	35065-28-2	138
2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1	153
2,3,4,4',5,6-Hexachlorobiphenyl	41411-63-6	166 (ISTD)
3,3',4,4',5,5'-Hexachlorobiphenyl	32774-16-6	169
2,2',3,3',4,4',5-Heptachlorobiphenyl	35065-30-6	170
2,2',3,4,4',5,5'-Heptachlorobiphenyl	35065-29-3	180
2,2',3,4',5,5',6-Heptachlorobiphenyl	52663-68-0	187
2,2',3,3',4,4',5,6-Octachlorobiphenyl	52663-78-2	195
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	40186-72-9	206
Decachlorobiphenyl	2051-24-3	209
2,2',4,4'-Tetrabromodiphenyl ether	5436-43-1	47
2,3',4,4'-Tetrabromodiphenyl ether	189084-61-5	66
2,2',4,4',5-Pentabromodiphenyl ether	60348-60-9	99
2,2',4,4',6-Pentabromodiphenyl ether	189084-64-8	100
2,2',4,4',5,5'-Hexabromodiphenyl ether	68631-49-2	153
2,2',4,4',5,6'-Hexabromodiphenyl ether	207122-15-4	154

Compound	CAS Registry No.	IUPAC #
2,2',3,4,4',5'-Hexabromodiphenyl ether	182677-30-1	138
2,2',3,4,4',5',6-Heptabromodiphenyl ether	207122-16-5	183

6. Interferences

6.1. Interferences during extraction are usually related to either contamination from reagents, solvents, and glassware/hardware or from compounds co-extracted from sample matrix which will vary depending on the individual matrix.

6.1.1. Interferences from reagents, solvents, and glassware.

6.1.1.1. Contamination from reagents and solvents can be minimized by using ultra-pure solvents and muffling reagents at 400°C for four hours.

6.1.1.2. Contamination from phthalate esters can be reduced by avoiding use of plastic materials. All containers used during extraction and cleanup should be glass.

6.1.1.3. Scrupulous cleaning of all glassware and hardware can significantly reduce sample contamination. Glassware should be treated to soap washing followed by DI and acetone rinsing.

6.1.2. Interferences co-extracted from sample matrix.

6.1.2.1. The best way to reduce interferences co-extracted from the sample matrix is to utilize adequate cleanup method that separates interferences from the target analytes.

6.1.2.2. The occurrence of elevated baselines and "hump-o-grams" is an indication of excessive contamination. These occurrences will effect quantification of data. Generally elevated baselines and hump-o-grams also indicate that the GC hardware will need to undergo thorough maintenance to remove the contamination from the GC system.

6.1.3. Glassware cleaning to eliminate contamination and carryover.

6.1.3.1. Due to the low concentration levels achievable by GC/u-ECD, contamination of the reagents, solvents, glassware and apparatus can cause severe interferences with method analytes. Such interferences can cause extra peaks or elevated baselines in the chromatographs. LRB analyzed to validate this cleaning procedure and ensure no method contamination.

6.1.3.2. Glassware must be carefully cleaned, ideally as soon as possible after use. Rinse with acetone after use. Wash with hot water and detergent, and

thoroughly rinse with tap and DI water. Rinse with acetone and bake dry in the muffle furnace at 300°C. Seal and store in a clean environment to prevent any accumulation of dust or other contaminants.

6.1.3.3. Use the 1L ASE solvent bottles because they seem to contribute less contamination to the extracts. Use the minimum amount of solvent necessary in the ASE solvent bottles.

6.1.3.4. Interfering contamination may occur when a sample containing low concentrations of compounds is analyzed immediately after a sample containing relatively high concentrations of compounds. Between-sample rinsing of the apparatus and shared glassware with hexane can minimize sample cross contamination.

7. Personnel Qualifications

7.1. Individuals performing this procedure are to be trained to use the Dionex Accelerated Solvent Extraction system for organic extraction.

7.2. Individuals performing this procedure are to be trained to use the GPC system for cleanup.

7.3. Individuals performing this procedure are to be trained in the use of alumina cleanup and the proper concentration of samples.

8. Equipment, Supplies, and Reagents

8.1. Equipment and Supplies:

8.1.1. Dionex ASE-200 Accelerated Solvent Extractor

8.1.2. Dionex Extraction Cells

8.1.3. Dionex Collection Vials

8.1.4. Waters 1525 Binary Pump

8.1.5. Waters 717 Autosampler

8.1.6. Waters 2487 UV Detector

8.1.7. Waters Fraction Collector III

8.1.8. GPC Autosampler vials (4mL)

8.1.9. GPC Collection Vials (40mL)

8.1.10. Analytical Balance and Printer

8.1.11. Mortar and Pestle Sets (24)

8.1.12. Supelco SPE Manifold with Glass Cartridges

8.1.13. Drying Tubes

8.1.14. KD Concentrator Tubes

8.1.15. N-Evap Nitrogen Evaporation System

8.2. Reagents

8.2.1. Sodium sulfate -- Anhydrous, ACS certified, 10-60 mesh, Ultra. Muffle at 400 °C for four hours.

8.2.2. Hydromatrix – granular diatomaceous earth, Varian.

8.2.3. Alumina-N -- Super I (EcoChrom), muffled at 400°C for four hours and deactivated to level III by adding 7% water (7g water added to 93g Alumina). Wait two hours after deactivation before use.

8.2.4. Hexane -- Pesticide quality or equivalent.

8.2.5. Isopropanol – Pesticide quality or equivalent.

8.2.6. Methylene chloride -- Pesticide quality or equivalent.

8.2.7. Acetone -- Pesticide quality or equivalent.

8.2.8. Nitrogen -- UHP

8.2.9. Elution Solvent -- Using a graduated cylinder, add 200mL of methylene chloride to a 1000mL volumetric flask. Bring up to volume with hexane.

8.3. Standard Preparation – Primary standards are purchased as certified standards from several different vendors, typically at concentrations of 50µg/mL or 100µg/mL. Typically standards purchased from vendors come either with methanol or hexane as the

solvent. Intermediate stock standards containing methanol will require a small amount (10-20% by volume) of acetone added as a carrier solvent to keep methanol and hexane miscible.

8.3.1. Pesticide Intermediate Stock – Commercially purchased pesticide standards are normally 100µg/mL. Make a 1µg/mL intermediate stock by diluting 50µL of the vendor standards and bring to volume at 5mL in hexane:acetone (80:20 v/v).

8.3.2. PCB/PBDE Intermediate Stock – Commercially purchased PCB standards are normally 100µg/mL while PBDE standards are normally 50µg/mL. Make a 1µg/mL intermediate stock by diluting 50µL of the PCB standards and 100µL of the PBDE standards to volume at 5mL in hexane.

8.3.3. Surrogate Intermediate Stock – Commercially purchased surrogate standards vary in concentration. Make a 1µg/mL intermediate stock by diluting an appropriate amount of the vendor standards and bring to volume at 5mL in hexane:acetone (80:20 v/v). Surrogate standards include 4,4'-dibromobiphenyl, Tetrachloro-m-xylene, PCB#104, and PCB#134. A minimum of three surrogates will be used per analysis. Spike in 100µL to each sample prior to sample extraction. Final concentration will be 20ng/mL

8.3.4. Internal Standard (IS) Intermediate Stock -- Commercially purchased internal standard solutions vary in concentration. Make a 1µg/mL intermediate stock by diluting an appropriate amount of the vendor standards and bring to volume at 5mL in hexane:acetone (80:20 v/v). Internal standards include pentachloronitrobenzene, PCB#96, and PCB#166. A minimum of three internal standards will be used per analysis. Spike in 25µL prior to bringing to 1mL final volume for a final concentration of 25ng/mL.

8.3.5. LFB/LFM Spike Sample – The spike solution is to be prepared in 10mL of hexane. It will be prepared at a concentration of 75ng/mL. One 1mL of this solution is spiked into the LFB, LFM, and LFMD. The final extract concentration will be 15ng/mL. Other concentrations may be used provided the final extract concentration falls within the calibration curve.

8.3.6. GPC Calibration Check Solution – Commercially purchased GPC check solutions are diluted according to vendor instructions. The GPC check solution contains corn oil, perylene, sulfur, bis(2-ethylhexyl) phthalate, and methoxychlor at concentrations specified in EPA method 3640A. Alternatively a higher level standard of the target analytes could be used as a GPC Calibration Check.

9. Sample Collection, Preservation, and Handling

9.1. Sediment

9.1.1. Sediment samples should be collected in 4-8oz containers, preferably glass containers. Samples should be labeled, at a minimum, with project name or number, sample ID, date and time of sampling. The required mass is usually project specific but typically a minimum of 50g is required.

9.1.2. Stored at -20^{o}C indefinitely unless project QAPPs storage requirements supersede this SOP.

9.2. Tissue

9.2.1. Tissue samples should be collected by wrapping the fish in aluminum foil and then double bagging with appropriately sized plastic storage bags.

9.2.2. Stored at -20^{o}C indefinitely unless project QAPPs storage requirements supersede this SOP.

9.3. Extract storage

9.3.1. Extracts may be stored for up to 40 days at -20^{o}C.

9.3.2. Samples currently in the extraction or cleanup process may be stored in a refrigerator at 4^{o}C $\pm$2^{o}C, up to twenty-four hours, otherwise they must be stored at -20^{o}C.

10. Procedure

10.1. Weigh 8.00g minimum for wet tissue samples or weigh 15.00g minimum for wet sediment samples. If the sample is very wet (more than 50% wet) or contains 7g or more of water, reduce the sample mass. Other types of wet tissue samples require 8g of mass or if the sample weight is less than 8.00g, weigh the entire sample of homogenized tissue and place in a clean glass mortar.

10.1.1. If only lipids are being determined an alternative extraction will be performed. The alternative method for “lipids only” is as follows:

10.1.1.1. Extract a smaller amount of material, approximately 3g of fish.

10.1.1.2. The solvent mixture is hexane/isopropanol at a ratio of 60:40.

10.1.1.3. Use Hydromatrix instead of sodium sulfate for the extraction.

10.1.1.4. All other extraction conditions remain unchanged.

10.2. Add 20g of baked sodium sulfate for wet tissue samples and 20g of Hydromatrix for wet sediment samples. This will be known as the drying material.

10.3. Grind sample with pestle until sample is well mixed with the drying material. Let samples dry for approximately one hour. Avoid leaving samples out for more than one hour or loss of more volatile pesticides (BHCs) may occur.

10.4. Spike the samples with the LFB/LFM spike and surrogate spike mixes prior to packing. The spike should be done with a 1mL syringe for the LFB and a 100µL syringe for the surrogate. Spikes should be added very slowly (dripped) onto the sample matrix.

10.5. Prepare the 33mL ASE extraction cells by screwing on one cap and inserting a cellulose filter. Use a dowel as a ramrod to ensure that the filter reaches the bottom. Check the filter to ensure it covers the entire bottom frit of the cell.

10.6. Carefully transfer the sample to a prepared ASE extraction cell. Dry-wash the mortar with one tablespoon of the drying material, and add wash and any additional required drying material to the cell before hand tightening top cap. Tamp down the sample with the ramrod to ensure good packing. Fill the extraction cell with drying material to within 1/4"of the top.

10.7. Process sufficient samples not to exceed 24 extractions per batch set. Follow all manufacturer operating and maintenance instructions.

10.8. Insert cells and 60 mL solvent collection tubes into the ASE 200. Load the tray slots in numerical order with all of the full sample cells, reserving the first slot for an ASE instrument blank. Hang the cells vertically in the tray slots from their top caps. Load the rinse tubes into the four open slots labeled R1 through R4, located between positions 1 and 24. Load the collection vial onto the corresponding vial tray position. Label the vials with laboratory assigned sample numbers and the types of sample. Log the serial numbers of the ASE cells, the sample identification numbers they contain, and their positions on the ASE.

Note: Check the end of each rinse tube to verify that the o-rings are in place and in proper condition. Install or replace if necessary. Do not use a wrench or other tool to tighten the cap.

10.9. For all tissue samples, extract with methylene chloride and hexane using the following method controls:

Preheat	0 min	Pressure		1000 psi
Heat	5 min	Temperature		100°C
Static	7 min	Sol A	Methylene chloride	50%
Flush%	130% vol	Sol B	Other	0%
Purges	90 sec	Sol C	Hexane	50%
Cycles	3	Sol D	Other	0%

10.10. For sediment samples, extract with acetone and hexane using the following method controls:

Preheat	0 min	Pressure		1500 psi
Heat	5 min	Temperature		125°C
Static	7 min	Sol A	Other	0%
Flush%	130% vol	Sol B	Acetone	50%
Purges	90 sec	Sol C	Hexane	50%
Cycles	3	Sol D	Other	0%

Note: Use automatic purge cycle between samples. Make sure that the nitrogen supply pressure is 150 psig. The ASE unit may not extract sample reliably with the nitrogen supply pressure below 150 psig. Use a schedule to have the ASE perform a rinse after each sample. A schedule controls the location of cells which will be collected, in which collection vial, the method used for extraction, whether a rinse is performed, and the solvent used for rinsing.

10.11. A rinse must be performed after each sample. An acetone rinse is effective in removing water from the ASE lines.

10.12. Allow the extracts to cool after the extractions are complete. Sample extract volumes collected from the ASE are typically 45-55mL. Discard the tissue or sediment samples into the laboratory solid waste container.

10.13. Clean the frits by sonicating them in acetone for 1 hour, followed by 5%HNO_3 for 1 hour, followed by a thorough DI water rinse and an acetone rinse. Do not use soap on the frits. Scrub cells in soap and tap water, followed by DI rinse and an acetone rinse.

10.14. Do not use squirt bottles since they contribute contamination to the extract. Solvents should be used directly from the solvent bottle by pouring the solvent into clean beakers just prior to use.

10.15. In the solvent collection vial, concentrate the ASE extracts to approximately 3 mL using an N-EVAP 111 nitrogen evaporator with a 50°C water bath until only hexane

remains. This is about 1cm from the bottom of the collection vial or from the top of the aqueous layer.

10.16. Inspect the samples prior to reaching 1cm. If there are multiple layers, there is likely a solvent layer, a water layer, and possibly a lipid layer (fish only). The layers formed are highly dependant on the solvents used during extraction. Determine which layer is the solvent layer. Do NOT let this layer evaporate completely because it will reduce recoveries, even if this means not bringing down to 1cm. There should always be at least 1cm of the solvent layer present in the vial to maintain the samples in solution.

10.17. Dry (water removal) the concentrated extract using a glass chromatography column, 1" diameter, packed with 20g of sodium sulfate and with a small amount of glass wool at the bottom. Rinse the sodium sulfate column with 5-10 mL of hexane. Use a vacuum flask and vacuum out excess hexane from column. Set rinsed sodium sulfate column over a 25mL graduated concentrator test tube. Pour the extract down the tube. Keep the collection vial above the tube and rinse the threads with a squirt of hexane from a pipette. Make sure the sample has soaked into the bed. If not, use a pipette bulb to force it into the bed.

10.18. Extremely wet samples may block the bed and the top of a pipet may be used to break the crust and let the solution soak into the bed. Rinse the inside of the collection vial with approximately 4 fills of the small pipet bulb (~5mL total). Pour this into the tube along the side, making sure it pools up briefly on top of the sodium sulfate. This ensures good rinsing of the sides of the tube and the sodium sulfate bed.

10.19. Repeat twice, making the total rinse about 15mL.

10.20. Make sure that the color moves through the drying tube and that the solvent exiting the tube is colorless. Extra rinsing may be necessary for very concentrated samples, but do not exceed the volume of the concentrator tube. Place a pipette bulb on top of the column and squeeze to remove all extract from the column. (Caution: remove pipette bulb before releasing pressure to avoid drawing the extract back up the column.) If by accident the tube becomes clogged at some point, force the solvent through and rinse with as much solvent as possible. DO NOT OVERFILL THE CONCENTRATOR TUBE.

10.21. In the concentrator tube, concentrate each solvent extract to approximately 5mL using an N-EVAP 111 nitrogen evaporator with a 50°C water bath. Use hexane to bring up to a final volume of 10mL and store in a vial with polypropylene cap.

10.22. Filter 4mL of the extract through a 25mm, 0.45µm PTFE syringe filter into a 4mL GPC vial. To perform this filtering step, attach the syringe filter to a 5mL Luer lock syringe and hold over the GPC vial. Remove the syringe barrel, then pipet

approximately 4mL into the syringe, replace the syringe barrel and filter into the GPC vial. It may be necessary to use a second filter for samples that contain large amounts of solids. Fill the 4mL GPC vial to the neck to ensure 4mL is collected for sample cleanup.

10.23. Save the unfiltered volume to be used for lipid testing.

10.24. Load "GPC cleanup" on the Breeze software. Prepare the GPC for analysis by priming the pumps and the injectors according to the instrument manufacturer's guidelines. Equilibrate the GPC for approximately 10 minutes. Typical back pressure readings can vary from 700-1100psi and may vary within this range over time. Sudden changes in pressure of more than 100psi either way may indicate a clog (high pressure) or a leak (low pressure). Large increases in pressure during injection are common and will subside back to pre-injection pressure after the injection is complete.

10.25. Document the GPC cleanup using the form in Appendix A.

10.26. Periodically verify the 5mL flow rate by collecting the eluent for 10 minutes in a graduated cylinder. The volume should be 90-110% of nominal or 45-55mL.

10.27. Inject 1000µL of the calibration check solution daily. Verify peak shapes and retention times of calibration mixture. Retention times should not vary by more than 5% from the last retention time measurement saved in the data files of the Breeze software. A concentrated standard ($\geq$1µg/mL) containing the pesticides/PBDEs/PCBs of interest will be injected weekly to verify collection times of target analytes. The specified collect times should be set to collect peaks from this standard. Daily use of the calibration check solution should indicate if any retention time shifting has occurred in between this weekly check.

10.28. Load the sample vials into the autosampler tray and set up a sequence to run the samples. The method is "GPC Cleanup" and the injection volume is 2000µL.

10.29. Set the fraction collector to collect one fraction from 14.5-20 minutes. This time may need to be adjusted slightly based upon results of the calibration mixture.

10.30. Transfer approximately half the cleaned GPC extract to a concentrator tube and concentrate under nitrogen with 50^{o}C heat. After the volume reduces to 2-5mL add the remaining GPC extract to the concentrator tube. Rinse the GPC collection vial with two 1-2mL portions of hexane and add to the concentrator tube.

10.31. Concentrate the extract down to approximately 2mL. Bring up to 5mL with hexane, mix using a vortex mixer, and concentrate to approximately 2mL. Extracts can be stored in the freezer in the concentrator tube (with caps).

10.32. Remove the samples from the freezer and let them come to room temperature.

10.33. Prepare an appropriate number of SPE tubes containing 3g of Alumina-N that has been muffled for 4 hours at 400°C and deactivated to level III two hours prior to use, and a small amount of glass wool in the bottom and approximately 0.5cm of sodium sulfate on the top. Pre-elute the column with 5mL of hexane and then with 5mL of the 20%/80% methylene chloride/hexane mix.

10.34. Pipet the entire concentrated extract onto the alumina column. Rinse the concentrator tube with two 1-2mL portions of hexane and add the rinses to the alumina column. Elute with 14mL of the methylene chloride/hexane mix. Once the extract has been eluted, place a pipette bulb on top of the SPE tube, and gently squeeze to remove excess solvent. (Caution: remove pipette bulb before releasing pressure to avoid drawing the extract back up the column.)

10.35. Move all samples to the N-EVAP 111 nitrogen evaporator with a 50°C water bath. During evaporation rinse sides of tube with a small amount of hexane. Concentrate the samples to 0.5mL and add 25µL of the IS solution. Adjust the volume to 1mL with hexane by rinsing the sides while filling. This step is important to ensure good recovery.

10.36. Sulfur cleanup should be performed for any sediment sample in the following steps prior to transferring to GC vials. Otherwise, transfer all other 1mL extracts into two separate GC vials containing glass inserts for GC analysis. Store in the freezer.

10.37. Samples that contain sulfur will need an additional cleanup using copper following EPA method 3660. Samples that contain sulfur can be identified by their gas chromatograms because sulfur can completely mask the region from the solvent peak through Aldrin. Recovery of PCB #77 and PCB #126 are reduced to approximately 30% and recovery of Endosulfan 2 is approximately 60%. For samples that contain sulfur, the following extra cleanup steps are required.

10.37.1. Clean the oxides from the copper powder by rinsing the powder with dilute (5%) nitric acid.

10.37.2. Rinse the copper with organic free water to remove the acid, then rinse with acetone and dry under nitrogen.

10.37.3. Take the 1mL alumina cleaned sample and transfer to a centrifuge tube. Add 2g of cleaned copper to the sample and mix for at least one minute.

10.37.4. Separate the sample from the copper using a disposable pipette. This sulfur-cleaned sample can then be transferred to two GC vials with glass inserts for analysis.

11. Analyte Identification

11.1. Analyte identification is not performed in this SOP.

12. Data Quantification

12.1. Data quantification is not performed in this SOP.

13. Corrective Actions

13.1. ASE Maintenance

13.1.1. ASE lines can become clogged with extraction material. Generally removing the lines and putting the "out" end on the solvent pump and reverse pumping solvent through the lines will sometimes remove the buildup. Otherwise replace the clogged line.

13.1.2. Check valves in the pump wear out and need to be replaced as needed.

13.1.3. Consult the instrument maintenance book for help in troubleshooting error codes and fixing these issues.

13.2. GPC Maintenance

13.2.1. The column/lines can become clogged, indicated by high back pressure. Changing the guard column or rinsing the lines (reverse rinse) may be necessary.

13.2.2. Methylene chloride is detrimental to the pump seals. Loss of pressure indicates seals are leaking and need replacing.

14. Quality Control

14.1. Initial Demonstration of Capability (IDOC)

14.1.1. Analyze a minimum of four replicate LFBs spiked at the intended calibration range midpoint or typical LFB concentration level.

14.1.2. Calculate the average recovery (%R), arithmetic mean (M), relative standard deviation (RSD), and standard deviation (SD) per analyte. The average recovery must pass the LFB limits and the RSD must pass the duplicate limits defined in the QC acceptance table from the most recent version of SOP MIRB-046, "GC Analysis

of Organics". The SD is useful for estimating method detection limit spiking.

14.1.2.1. $M = \frac{\sum_{i=1}^{n} X_i}{n}$

where: Xi = sample measurement
n = number of measurements

14.1.2.2. $SD = \sqrt{\frac{\sum_{i=1}^{n} (X_i - \overline{X})^2}{n-1}}$

where: Xi = sample measurement
$\overline{X}$ = mean value of sample data set
n = number of measurements

14.1.2.3. $RSD = \frac{SD}{\overline{X}} \times 100\%$

where: $\overline{X}$ = mean value of sample data set
n = number of measurements

14.2. Method Detection Limits (MDL)

14.2.1. Analyze a minimum of seven replicates consisting of reagent material (LRB) spiked at 3-5 times the instrument noise level. The concentration equivalent to three times the SD of the IDOC replicates can be useful in determining MDL spike levels and instrument noise. Analyzing the MDLs over several days gives a better approximation of instrument performance.

14.2.2. Calculate the average recovery and standard deviation for each analyte. Calculate the MDL by multiplying the standard deviation by the students-t value for n-1 and a 99% confidence level. The students-t value for seven replicates (n-1=6) is 3.143. Average MDL recovery should meet acceptance limits for LFB in the QC table.

14.2.2.1. $MDL = t(n-1, 1-\alpha=0.99) \times SD$

14.2.3. Calculated MDLs should be no higher than the spike level. Spike levels should not be more than ten times the calculated MDL such that:

Calculated MDL < Spike Level < 10 X Calculated MDL

14.2.4. Reporting limits (RL) are generated from MDL data and can be set equal to or greater than the calculated MDL. RLs for this laboratory are set at the weight adjusted equivalent concentration (0.625 ng/g) based on the lowest GC calibration standard (1ng/mL).

15. Data Management

15.1. All sample extraction, cleanup information, solvent tracking, and sample data are logged into a log sheet (Appendix A), which is attached to the final batch report, including completed GC, moisture, and lipid data.

15.2. Final data batch files are bundled together in a three-ring binder, with a project description on the cover.

16. Waste Management

16.1. All remaining solvents are to be placed in organic waste satellite containers and sent to the Hazardous Waste Disposal Room (B-71) once filled. Used sample matter, along with used alumina, sodium sulfate or Hydromatrix, may be disposed of in the solid waste container.

17. References

17.1. USA EPA. 1996. Method 3545A Pressurized Fluid Extraction. EPA SW846. EPA Office of Solid Waste and Emergency Response. Washington, D.C.

17.2. USA EPA. 1996. Method 3640A Gel-Permeation Cleanup. EPA SW846. EPA Office of Solid Waste and Emergency Response. Washington, D.C.

17.3. “40 CFR 136.6, Appendix B: Guidelines Establishing Test Procedures for the Analysis of Pollutants, Appendix B- Definition and Procedure for the Determination of the Method Detection Limit." (Current through 8/22/08). Text from: *Code of Federal Regulations*. Available from: e-CFR; Accessed 08/26/08.

17.4. USEPA Method 508.1, Determination of Chlorinated Pesticides, Herbicides, and Organohalides by Liquid-Solid Extraction and Electron Capture Detector, National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH 45268.