Organic Contaminant Analytical Methods of the National Status and Trends Program: 2000-2006

NOAA Technical Memorandum NOS NCCOS 30

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Cover photograph of shrimping fleet, Palacios, TX

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Organic Contaminant Analytical Methods of the National Status and Trends Program: Update 2000-2006

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1. INTRODUCTION

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This document describes the analytical methods used to quantify core organic chemicals in tissue and sediment collected as part of NOAA's National Status and Trends Program (NS&T) for the years 2000-2006. Organic contaminat analytical methods used during the early years of the program are described in NOAA Technical Memoranda NOS ORCA 71 and 130 (Lauenstein and Cantillo, 1993; Lauenstein and Cantillo, 1998) for the years 1984-1992 and 1993-1996, respectively. These reports are available from our website (http://www.ccma.nos.gov)

The methods detailed in this document were utilized by the Mussel Watch Project and Bioeffects Project, which are both part of the NS&T program. The Mussel Watch Project has been monitoring contaminants in bivalves and sediments since 1986 and is the longest active national contaminant monitoring program operating in U.S. costal waters. Approximately 280 Mussel Watch sites are sampled on a biennial and decadal timescale for bivalve tissue and sediment respectively. Similarly, the Bioeffects Assessment Project began in 1986 to characterize estuaries and near coastal environs. Using the sediment quality triad approach that measures; (1) levels of contaminants in sediments, (2) incidence and severity of toxicity, and (3) benthic macrofaunal conmmunities, the Bioeffects Project describes the spatial extent of sediment toxicity. Contaminant assessment is a core function of both projects. These methods, while discussed here in the context of sediment and bivalve tissue, were also used with other matricies including: fish fillet, fish liver, nepheloid layer, and suspended particulate matter.

The methods described herein are for the core organic contaminants monitored in the NS&T Program and include polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), butyltins, and organochlorines that have been analyzed consistently over the past 15-20 years. Organic contaminants such as dioxins, perfluoro compounds and polybrominated biphenyl ethers (PBDEs) were analyzed periodically in special studies of the NS&T Program and will be described in another document.

All of the analytical techniques described in this document were used by B&B Laboratories, Inc, an affiliate of TDI-Brook International, Inc. in College Station, Texas under contract to NOAA. The NS&T Program uses a performance-based system approach to obtain the best possible data quality and comparability, and requires laboratories to demonstrate precision, accuracy, and sensitivity to ensure results-based performance goals and measures.

REFERENCES

Lauenstein, G. G. and A. Y. Cantillo (eds.) (1998) Sampling and analytical methods of the National Status and Trends Program Mussel Watch Project 1993-1996 Update: TERL Trace Element Quantification Techniques, Volume III. NOAA Technical Memorandum NOS ORCA 71, Silver Spring, MD. 219 pp.

Lauenstein, G. G. and A. Y. Cantillo (eds.) (1993) Sampling and analytical methods of the National Status and Trends Program National Benthic Surveillance and Mussel Watch Projects 1984-1992: Comprehensive descriptions of elemental analytical methods, Volume III. NOAA Technical Memorandum NOS ORCA 71, Silver Spring, MD. 219 pp.

CHAPTER 2. EXTRACTION OF SEDIMENTS FOR AROMATIC AND CHLORINATED HYDROCARBONS AND POLYBROMINATED FLAME RETARDANTS 2000 – 2006

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ABSTRACT

Determining organic contaminant levels in sediments requires the extraction, isolation, and concentration of analytes from the matrix. Sediment samples are dried, pulverized, and homogenized prior to extraction. Sediments are extracted in dichloromethane using a Dionex® Accelerated Solvent Extractor. The extracts are purified using alumina/silica gel chromatography columns. The volume of the resultant eluent is reduced and analyzed for aromatic and chlorinated hydrocarbons and polybrominated flame retardants by gas chromatography/mass spectrometry and gas chromatography/electron capture detection.

1.0 INTRODUCTION

The procedure described is used to extract, isolate, purify, and concentrate aromatic and chlorinated hydrocarbons and polybrominated flame retardants from sediments. Contaminant concentrations in parts per billion or parts per trillion can be resolved in sediments. Sediment samples are homogenized, dried, and ground using a mortar and pestle. Approximately 15 g of dry sediment are extracted with 100% dichloromethane using a Dionex Accelerated Solvent Extractor (ASE200). The extracts are reduced in volume and then purified using alumina/silica gel column chromatography. Extracts are reduced to 1 mL and analyzed for PAHs, PCBs, pesticides, PBBs, and PBDEs by gas chromatography.

2.0 APPARATUS AND MATERIALS

2.1 EQUIPMENT

Dionex, ASE200 Accelerated Solvent Extractor (ASE) with 33 mL extraction cells

Water bath, capable of maintaining a temperature of 55-60 °C

Balance, top loading, tare capacity to 300 g, capable of weighing to 1 mg

Microbalance, capable of weighing to 1 μg

Calibrated weights, certified

Combustion furnace, electric, capable of combusting glassware at 400 °C for at least 4 hours

Oven, capable of maintaining 40 °C temperature maintenance

Conditioning oven, electric, gravity convection, capable of maintaining a stable temperature of up to 200 °C

Tumbler, Lortone rock tumbler or equivalent

Collection vials, 60 mL certified pre-cleaned with open screw caps and Teflon lined septa

Micropipettors, calibrated, 1% accuracy, disposable tips

250 mL flat bottom, boiling flasks

Borosilicate glass chromatography columns, 300 mm x 19 mm, with Teflon stopcock

Kurderna-Danish (K-D tubes), 25 mL, slow dry concentrator tubes

Synder columns, 3-ball

Boiling chips, Teflon

Glass wool

2.2 REAGENTS

Water (CAS 7732-18-5), gas chromatography/HPLC grade or equivalent purity

Acetone (CAS 67-64-1), pesticide grade or equivalent purity

Dichloromethane (CAS 75-09-2), pesticide grade or equivalent purity

Hexane (110-54-3), pesticide grade or equivalent purity

Pentane (109-66-0), pesticide grade or equivalent purity

Copper (CAS 7440-50-8), granular, 20-30 mesh, ACS reagent grade, purified with hydrochloric acid

Hydrochloric acid (7647-01-0), ACS reagent grade

Sodium sulfate (CAS 7757-82-6), anhydrous granular powder, ACS reagent grade, purified by combusting at 400 °C for at least 4 hours and stored at 120°C.

Alumina (CAS 1344-28-1), 80-325 mesh, basic or neutral, purified by combusting at 400°C for at least 4 hours and stored at 120 °C

Silica gel (CAS 1343-98-2), grade 923, 100-200 mesh, purified in an oven at 170 °C for at least 16 hours and stored at 170 °C

Nitrogen (CAS 7727-37-9), 99.8% purity

3.0 PROCEDURE

Sediment samples are frozen upon receipt from the field at –20°C in the contract laboratory. Prior to extraction, sediment samples are thawed and homogenized using a stainless steel spatula. A subsample is removed for percent moisture determination (McDonald *et al.*, 2006). At least 15 grams of sample are dried in an oven at 40 °C and then ground and homogenized using a mortar and pestle. Approximately 15 g of the dried sediment are extracted.

Sediments are extracted with dichloromethane using an ASE200. Dried samples are loaded into 33 mL stainless steel ASE extraction cells. Appropriate surrogate and spikes are added to the top of samples. The ASE extractor tubes are sealed and placed in the ASE cell carousel. The ASE conditions are: 100% dichloromethane as the extraction solvent, 2,000-psi solvent pressure, 100°C cell temperature, and 2 static cycles for 2 minutes each. Extracts are collected in 60 mL collection vials. The extracts are reduced to approximately 10 mL in the 60 mL collection vials in a 55-60 °C water bath. Extracts are then quantitatively transferred to Kurderna-Danish (K-D) tubes and the reduced to 1.0 mL in a 55-60 °C water bath. Quality control samples (e.g., blanks, duplicates, matrix spikes and standard reference materials) are prepared and extracted in the same manner as samples.

Extracts are purified using alumina/silica gel chromatography columns. Combusted and cooled alumina is deactivated by adding 1% (w/w) HPLC water and tumbled for at least 1 hour using a Lortone rock tumbler. Combusted, cooled silica gel is deactivated by adding 5% (w/w) HPLC water and tumbled for at least 1 hour using a Lortone rock tumbler. Borosilicate glass columns (300 mm x 19 mm) are filled with dichloromethane and packed from the bottom with: glass wool, 1-2 g of sodium sulfate, 10 g of deactivated alumina, 20 g of deactivated silica gel, and another 1-2 g of sodium sulfate. The dichloromethane is drained to the top of the column followed by the addition of 50 mL of pentane. The pentane is drained from the top of the upper sodium sulfate layer and discarded. The sample extract (approximately 1 mL) is added to the top of the column and eluted with 200 mL of a 50:50 mixture of pentane and dichloromethane at a flow rate of 1 mL/min. The eluent is collected in a 250 mL flat-bottom flask and reduced to approximately 10 mL in a 55-60 °C water bath. The extract is transferred to a 25 mL K-D container and reduced to 1 mL. The dichloromethane is exchanged with hexane and reduced to a final volume of 1 mL. The concentrate is transferred to a 2 mL amber vial containing pre-cleaned copper granules (copper granules are carefully mixed with concentrated hydrochloric acid followed by thorough rinsing with HPLC-grade water) and stored at 20 °C until analysis. High sulfur-containing samples may require additional copper granules. Additional pre-cleaned copper granules are added if the initial batch of copper granules turns black, indicating the presence of excess sulfur in the extract. Figure 2-1 shows a flow chart of the extraction and purification procedure.

4.0 QUALITY CONTROL (QC)

Solvents are verified to be contaminant-free by lot tests prior to use. All equipment and glassware used to extract samples are thoroughly cleaned by solvent rinsing or combustion at 400 °C. The calibration and accuracy of balances, weights, pipettors and thermometers are checked daily using certified weights and thermometers with calibrations traced to the National Institute of Standards and Technology (NIST). The calibration and accuracy of balances, weight, pipettors, and thermometers are verified yearly by an independent source. A series of quality control samples are processed with each batch of 20 samples or less. The following quality controls are used to ensure the accuracy and precision of sediment data.

- Surrogates. Solutions containing analytes that do not interfere with the analytes of interest are prepared at concentrations approximately 5 to 10 times the method detection limit (MDL). Specified surrogates are added to each sample extracted, including QC samples, at a specified volume (typically 100 μL) immediately prior to extraction.

- Method Blank. Method blanks are extractions of all support material used for extraction of samples, with the exception of sediment. A method blank is analyzed with each extraction batch of 20 or fewer samples. The method blank is extracted and analyzed in a manner identical to samples.

- Matrix Spike. Matrix spikes are extractions of sample matrix fortified with spikes of selected target analytes. Spikes are prepared at concentrations approximately 10 times the MDL. A matrix spike and matrix spike duplicate are analyzed with each extraction batch of 20 or fewer samples. Matrix spikes are extracted and analyzed in a manner identical to samples.

- Laboratory Duplicates. A sample is analyzed in duplicate with each extraction batch of 20 or fewer samples.

- Standard Reference Material (SRM). A sediment standard reference material from NIST (SRM 1941b) is analyzed with each extraction batch of 20 or fewer samples for aromatic and chlorinated hydrocarbons only.

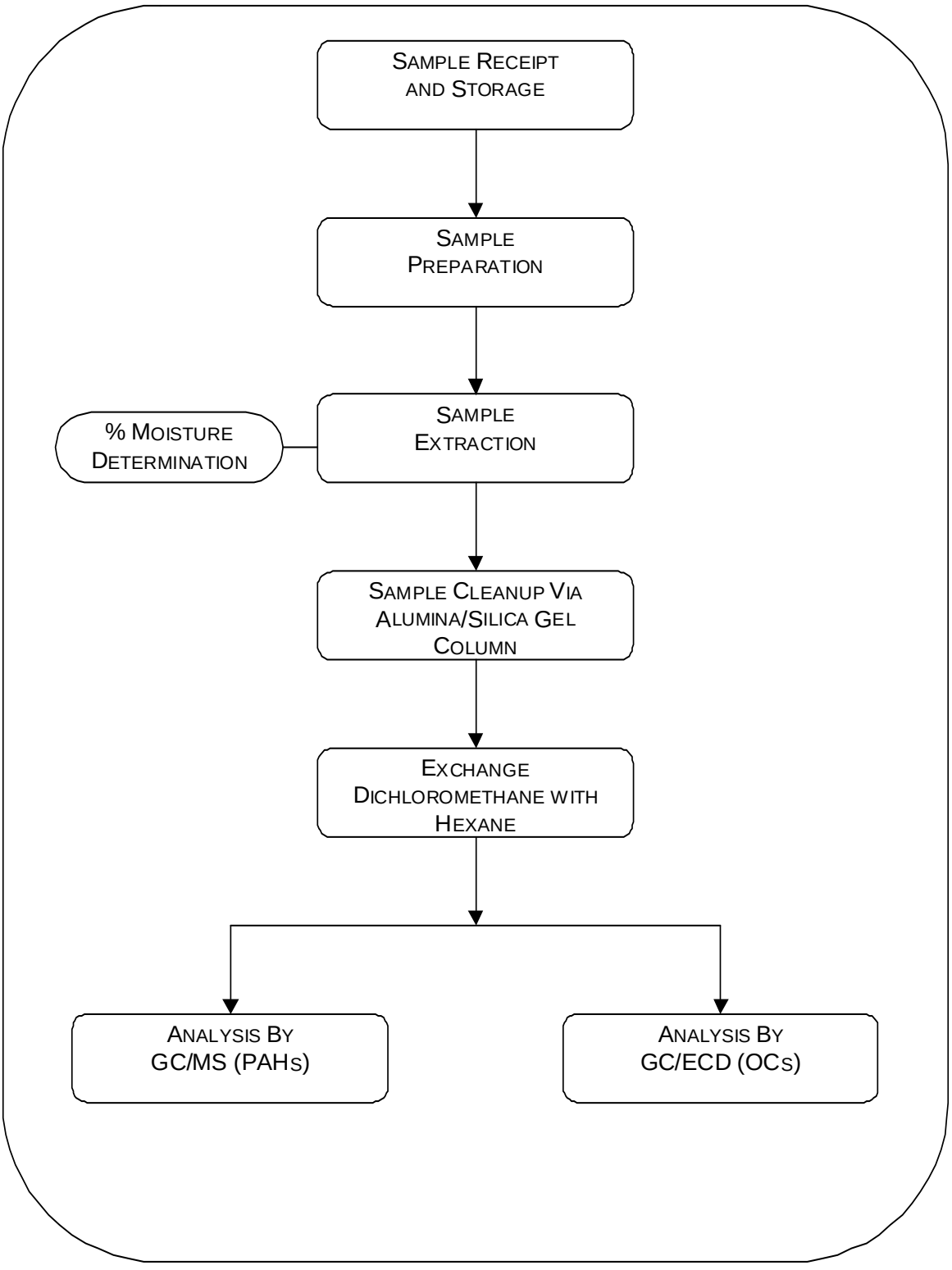

Figure 2-1. Methodology for extraction, isolation and quantification of sediment samples for polycyclic aromatic hydrocarbons (PAHs) and organic contaminants (OC).

5.0 REFERENCES

McDonald, S. J., D. S. Frank, J. A. Ramirez, B. Wang, and J. M. Brooks. 2006. Ancillary Methods of the National Status and Trends Program: 2000-2006 Update. Silver Spring, MD. NOAA Technical Memorandum NOS NCCOS 28. 17 pp.

CHAPTER 3. EXTRACTION OF BIOLOGICAL TISSUES FOR AROMATIC AND CHLORINATED HYDROCARBONS AND POLYBROMINATED FLAME RETARDANTS 2000 – 2006

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ABSTRACT

Determining organic contaminant levels in tissues require extraction, isolation, and concentration of analytes from the matrix. Tissue extracts require extensive purification procedures to remove lipids, which cause analytical interferences. Bivalves are shucked and homogenized. Aliquots of homogenized sample are chemically dried using Hydromatrix® and extracted in dichloromethane using a Dionex Accelerated Solvent Extractor. The extracts are purified using alumina/silica gel chromatography columns. The volume of the resultant eluent is further purified using a gel permeation column coupled to a high performance liquid chromatograph. The volume of the resultant eluant is reduced and analyzed for aromatic and chlorinated hydrocarbons and polybrominated flame retardants by gas chromatography.

1.0 INTRODUCTION

The procedure described is used to extract, isolate, purify, and concentrate aromatic, chlorinated hydrocarbons, and polybrominated flame retardant contaminants from tissues. Shell length and shell volume are determined for specimens collected at each location. Bivalves are shucked and multiple organisms are processed as one sample to ensure the sample is representative of a population at a given site and to have sufficient sample to complete the analyses. Tissue samples are homogenized using a stainless steel blender with titanium blades. Aliquots of approximately 15 g of wet tissue are chemically dried with Hydromatix®. The tissue/Hydromatix® mixtures are extracted with 100% dichloromethane using a Dionex Accelerated Solvent Extractor (ASE200) operated at 100 °C and 2,000 psi. The extracts are reduced to 3 mL by evaporative solvent reduction. A 100 μL aliquot is removed and weighed to determine lipid weight (McDonald *et al.*, 2006). The remaining sample portion is purified using alumina/silica gel column chromatography and gel permeation column (GPC)/high performance liquid chromatography (HPLC). After HPLC purification, the eluents are reduced to 0.5 mL and analyzed for PAHs, PCBs, pesticides, PBBs, and PBDEs by gas chromatography.

2.0 APPARATUS AND MATERIALS

2.1 EQUIPMENT

Dionex®, ASE200 Accelerated Solvent Extractor (ASE) with 33 mL extraction cells

Water bath, capable of maintaining a temperature of 55-60 °C

Balance, top loading, tare capacity to 300 g, capable of weighing to 1 mg

Microbalance, capable of weight to 1 μg

Calibrated weights, certified

Combustion furnace, electric, capable of combusting glassware at 400 °C for at least 4 hours

Oven capable of maintaining 40°C temperature maintenance

Conditioning oven, electric, gravity convection, capable of maintaining a stable temperature of up to 200 °C

Tumbler, Lortone rock tumbler or equivalent

HPLC system, Water Model 590 programmable solvent delivery module HPLC pump, Waters 717 plus autosampler, Waters UV absorbance detector, Waters 746 data module, Waters Fraction Collector, Phenogel 10μ GPC 100Å size exclusion columns and Phenogel 100Å guard column.

Glass fiber filter circles, 2.4 cm diameter

Collection vials, 60 mL certified pre-cleaned with open screw caps and Teflon lined septa

Micropipettors, calibrated, 1% accuracy, disposable tips

Zymark®, 50 mL concentration tubes

250 mL flat bottom, boiling flasks

Borosilicate glass chromatography columns, 300 mm x 19 mm, with Teflon stopcock

Kurderna-Danish (K-D) tubes, 25 mL, slow dry concentrator tubes

Synder columns, 3-ball

Boiling chips, Teflon

Glass wool

2.2 REAGENTS

Water (CAS 7732-18-5), gas chromatography/HPLC grade or equivalent purity

Acetone (CAS 67-64-1), pesticide grade or equivalent purity

Dichloromethane (CAS 75-09-2), pesticide grade or equivalent purity

Hexane (CAS 110-54-3), pesticide grade or equivalent purity

Pentane (CAS 109-66-0), pesticide grade or equivalent purity

Hydromatrix® (CAS 68855-54-9/14464-46-1), conditioned by combustion at 400 °C for at least 4 hours and stored at 120°C

Sodium sulfate (CAS 7757-82-6), anhydrous granular powder, ACS reagent grade, purified by combusting at 400°C for at least 4 hours and stored at 120°C.

Alumina (CAS 1344-98-2), 80-325 mesh, basic, purified by combusting at 400 °C for at least 4 hours and stored at 120 °C

Silica gel (CAS 1343-98-2), grade 923, 100-200 mesh, purified in an oven at 170 °C for at least 16 hours and store at 170°C

Nitrogen (CAS 7727-37-9), 99.8% purity

3.0 PROCEDURE

Shell length and volume are determined for all bivalves collected at each sampling site. The bivalves are then shucked and the soft tissue homogenized using a stainless steel Waring® blender. Homogenized tissue samples are frozen at –20 ºC until extraction. Prior to extraction, tissue samples are thawed and re-homogenized using a stainless steel spatula. A subsample is removed for percent moisture determination (McDonald *et al*., 2006).

Approximately 15 g of tissue are thoroughly mixed and ground with a sufficient quantity (approximately 40 g) of prepared (combusted) Hydromatrix® to "dry" the sample. The tissue samples must be thoroughly dry to optimize the extraction efficiency. Hydromatrix® chemically dries samples by binding moisture. The amount of Hydromatrix® necessary to dry a sample depends upon the amount of sample and the percent moisture in that sample.

Tissues are extracted with dichloromethane using an ASE200. The tissue/Hydromatix® mixture is loaded into 33 mL ASE extraction cells. Appropriate surrogates and spikes are added to the top of the samples. The ASE extractor tubes are sealed and placed in the ASE cell carousel. The ASE conditions are: 100% dichloromethane as the extraction solvent, 2,000-psi solvent pressure, 100 °C cell temperature, and 2 static cycles for 2 minutes each. Extracts are collected in 60 mL collection vials. The extracts are reduced to approximately 10 mL in the 60 mL collection vials in a 55-60 °C water bath. Extracts are then quantitatively transferred to Kurderna-Danish (K-D) tubes and the volume reduced to 3 mL in a 55-60 °C water bath. A 100 µL aliquot is removed and weighed to determine lipid content (McDonald *et al.*, 2006). Quality control samples (e.g., blanks,

duplicates, matrix spikes and standard reference materials) are prepared and extracted in the same manner as samples.

Extracts are initially purified using alumina/silica gel chromatography columns. Combusted and cooled alumina is deactivated by adding 1% (w/w) HPLC water and tumbled for at least 1 hour using a Lortone rock tumbler. Combusted and cooled silica gel is deactivated by adding 5% (w/w) HPLC water and tumbling for at least 1 hour using a Lortone rock tumbler. Borosilicate glass columns (300 mm x 19 mm) are filled with dichloromethane and packed from the bottom with: glass wool, 1-2 g of sodium sulfate, 10 g of deactivated alumina, 20 g of deactivated silica gel, and another 1-2 g of sodium sulfate. The dichloromethane is drained to the top of the column followed by the addition of 50 mL of pentane. The pentane is drained from the top of the upper sodium sulfate layer and discarded. The sample extract (approximately 3 mL) is added to the top of the column and eluted with 200 mL of a 50:50 mixture of pentane and dichloromethane at a flow rate of 1 mL/min. The eluent is collected in a 250 mL flat-bottom flask. The eluent is reduced to approximately 10 mL in a 55-60°C water bath. The extract is transferred to 25 mL K-D tubes and reduced to1-2 mL. The concentrate is transferred to 4 mL amber HPLC vials and brought up to 4 mL with dichloromethane.

The extract is further purified using HPLC. The extract is injected using a Waters, Model 717 Plus autosampler and eluted through one Phenogel 100Å guard column and two Phenogel 10µ GPC 100Å size exclusion columns with 100% dichloromethane at a flow rate of 7 mL per minute. Elution times for compounds of interest are monitored using standards and a UV absorbance detector (254 nm). The appropriate fraction is collected using a Waters Fraction Collector. The sample is collected in 50 mL Zymark tubes and reduced to 10 mL in a 50-60 ºC water bath. The extract is transferred to K-D tubes and reduced to 1.0 mL. The dichloromethane is exchanged with hexane and reduced to a final volume of 0.5 mL. The concentrate is transferred to 2 mL amber vials and stored at 20 °C until analysis. Figure 3-1 shows a flow chart of the extraction and purification procedure.

4.0 QUALITY CONTROL (QC)

Solvents are verified to be contaminant-free by lot tests prior to use. All equipment and glassware used to extract samples are thoroughly cleaned by solvent rinsing or combustion at 400 °C. The calibration and accuracy of balances, weights, pipettors and thermometers are checked daily using certified weights and thermometers with calibrations traced to the National Institute of Standards and Technology (NIST). The calibration and accuracy of balances, weight, pipettors and thermometers are verified yearly by an independent source. A series of quality control samples are processed with each batch of 20 samples or less. The following quality controls are used to ensure the accuracy and precision of tissue data.

- Surrogates. Solutions containing analytes that do not interfere with the analytes of interest are prepared at concentrations approximately 5 to 10 times the method detection limit (MDL). Specified surrogates are added to each sample extracted,

- Method Blank. Method blanks are extractions of all support material used for extraction of samples, with the exception of tissue. A method blank is analyzed with each extraction batch of 20 or fewer samples. The method blank is extracted and analyzed in a manner identical to samples.

- Matrix Spike. Matrix spikes are extractions of sample matrix fortified with spikes of selected target analytes. Spikes are prepared at concentrations approximately 10 times the MDL. A matrix spike and matrix spike duplicate are analyzed with each extraction batch of 20 or fewer samples. Matrix spikes are extracted and analyzed in a manner identical to samples.

- Laboratory Duplicates. A sample is analyzed in duplicate with each extraction batch of 20 or fewer samples.

- Standard Reference Material (SRM). A standard reference material from the NIST (SRM 1974a) is analyzed with each extraction batch of 20 or fewer samples for aromatic and chlorinated hydrocarbons.

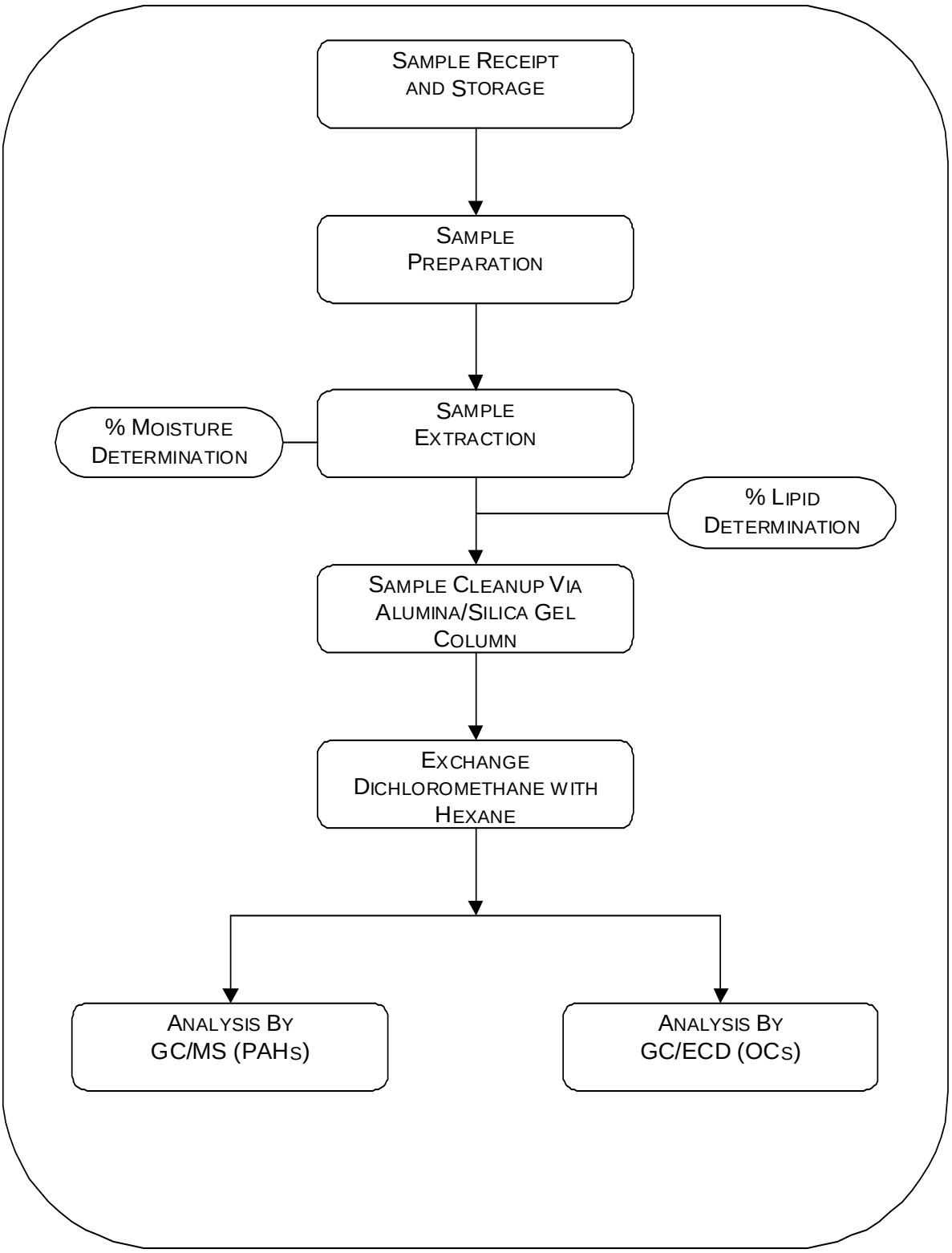

Figure 3-1. Methodology for extraction, isolation, and quantification of tissue samples for polycyclic aromatic hydrocarbons (PAHs) and organic contaminants (OC).

5.0 REFERENCES

McDonald, S. J., D. S. Frank, J. A. Ramirez, B. Wang, and J. M. Brooks. 2006. Ancillary Methods of the National Status and Trends Program: 2000-2006 Update. Silver Springs, MD. NOAA Technical Memorandums NOS NCCOS 28. 17 pp.

CHAPTER 4. QUANTITATIVE DETERMINATION OF CHLORINATED HYDROCARBONS USING GAS CHROMATOGRAPHY/ELECTRON CAPTURE DETECTION 2000 – 2005

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ABSTRACT

Selected chlorinated hydrocarbons, including polychlorinated biphenyls and pesticides, are detected using gas chromatograph/electron capture detector. This method is capable of detecting low concentration of chlorinated hydrocarbons in complex matrices such as tissues and sediments.

1.0 INTRODUCTION

A gas chromatograph/electron capture detector (GC/ECD), coupled to two capillary columns, is used to resolve and detect chlorinated hydrocarbons (polychlorinated biphenyls and pesticides) in tissues and sediments. Samples are injected into a temperature-programmed GC/ECD, operated in splitless mode. The capillary columns are DB-5 (30 m x 0.25 mm ID and 25 μm film thickness) and DB-17HT (30 m x 0.25 mm ID and 0.15 μm film thickness). The DB-17HT column is used for analyte confirmation. A data acquisition system continuously acquires and stores all data for quantitation. This method is capable of producing data at parts-per billion and parts-per trillion concentrations.

2.0 APPARATUS AND MATERIALS

2.1 EQUIPMENT

Gas chromatograph, split/splitless injection port and electronic pressure control, dual electron capture detectors, Agilent Technologies 5890-II

Data acquisition system, Agilent Technologies ChemStation, capable of continuous acquisition and storage of all data during analysis

Autosampler, capable of making 1 to 5 μL injections

Capillary columns, J&W DB-5®(30 m x 0.25 mm ID and 0.25 μm film thickness) or equivalent, and J&W DB-17HT®(30 m X 0.25 mm ID and 0.15 μm film thickness)

Micropipetters, calibrated, 1% accuracy, disposable tips

2.2 REAGENTS

Hexane (CAS 110-54-3), pesticide grade or equivalent purity

Helium (CAS 7440-59-7), 99.8% purity

95% Argon/5% Methane, 99.8% purity

2.3 STANDARDS

2.3.1 SURROGATE SPIKING SOLUTION

A surrogate spiking solution is prepared from a commercially available solution (Ultra Scientific) that is diluted with hexane to a concentration of 1,000 pg/μL. The surrogate spiking solution includes 4,4'-dibromooctaflurobiphenyl (DBOFB), 2,2',4,5',6 pentachlorobiphenyl (PCB 103), and 2,2',3,3',4,5,5'6 octachlorobiphenyl (PCB 198). Surrogate solution (100 μL) is added to all samples and quality control samples prior to extraction. Surrogate compounds are resolved from, but elute in close proximity to, the analytes of interest. The recovery of PCB 103 is used to correct analyte concentrations.

2.3.2 INTERNAL STANDARD SOLUTION

The internal standard solution is prepared from a commercially available solution (Ultra Scientific) of tetrachloro-m-xylene (TCMX) diluted with hexane to a final concentration of 1,000 pg/μL. The internal standard compound is resolved from, but elutes in close proximity to, the analytes of interest. The internal standard solution (100 μL) is added to all samples and quality control samples just prior to analysis. Internal standards are used to calculate relative response factors and specific analyte concentrations based on retention time.

2.3.3 MATRIX SPIKING SOLUTION

To prepare the matrix spiking solution, a certified solution (Accustandard) containing analytes of interest is purchased from commercial vendors and diluted with hexane (Table 4-1). The matrix spike solution is diluted to a concentration approximately 10 times the MDL and is added to all matrix spike samples.

2.3.4 CALIBRATION SOLUTION

Calibration solutions are prepared at 5 concentrations ranging from approximately 5 to 200 pg/μL (Table 2) by diluting a commercially prepared solutions (Ultra Scientific and Accustandard) containing the analytes of interest.

Table 4-1. Chlorinated hydrocarbons contained in matrix spike solution.

Compound Name	CAS	Spiking Solution Concentration (pg/μL)
1,2,4,5-Tetrachlorobenzene	95-94-3	40
1,2,3,4-Tetrachlorobenzene	634-66-22	40
Pentachlorobenzene	608-93-5	40
Pentachloroanisole	1825-21-4	40
Chlorpyrifos	2921-88-2	40
Hexachlorobenzene	118-74-1	40
α-HCH	319-84-6	40
β-HCH	319-85-7	40
γ–HCH (Lindane)	55-89-9	40
δ-HCH	319-86-8	40
Heptachlor	76-44-8	40
Heptachlor epoxide	1024-57-3	40
α-Chlordane (cis-)	5103-71-9	40
γ -Chlordane (trans-)	5103-74-2	40
Trans-nonachlor	39765-80-5	40
Cis-nonachlor	5103-73-1	40
Aldrin	309-00-2	40
Dieldrin	60-57-1	40
Endrin	72-20-8	40
Mirex	2385-85-5	40
2,4’ DDE	3424-82-6	40
4,4’ DDE	72-55-9	40
2,4’ DDD	53-19-0	40
4,4’ DDD	72-54-8	40
2,4’ DDT	789-02-6	40
4,4’ DDT	50-29-3	40
Endosulfan II	33213-65-9	40
Oxychlordane	27304-13-8	40
Endosulfan sulfate	1031-07-8	40
PCB 8	34883-43-7	40
PCB 18	37680-65-2	40
PCB 28	7012-37-5	40
PCB 44	41464-39-5	40
PCB 52	35693-99-3	40
PCB 66	32598-10-0	40
PCB 101	37680-73-2	40
PCB 105	32598-14-4	40
PCB 118	31508-00-6	40
PCB 128	38380-07-3	40
PCB 138	35065-28-2	40
PCB 153	35065-27-1	40
PCB 170	35065-30-6	40
PCB 180	35065-29-3	40
PCB 187	52663-68-0	40
PCB 195	52663-78-2	40
PCB 206	40186-72-9	40
PCB 209	2051-24-3	40

Table 4-2. Chlorinated hydrocarbons contained in calibration solutions and their approximate concentrations.

Compounds Contained in Calibration Solutions	CAS	Level 1 (pg/μL)	Level 2 (pg/μL)	Level 3 (pg/μL)	Level 4 (pg/μL)	Level 5 (pg/μL)
Internal Standard						
TCMX	877-9-8	100	100	100	100	100
Surrogates						
DBOFB	10386-84-2	5	20	40	80	200
PCB 103	60145-21-3	5	20	40	80	200
PCB 198	68194-17-2	5	20	40	80	200
Analytes						
1,2,4,5-Tetrachlorobenzene	95-94-3	5	20	40	80	200
1,2,3,4-Tetrachlorobenzene	634-66-22	5	20	40	80	200
Pentachlorobenzene	608-93-5	5	20	40	80	200
Pentachloroanisole	1825-21-4	5	20	40	80	200
Chlorpyrifos	2921-88-2	5	20	40	80	200
Hexachlorobenzene	118-74-1	5	20	40	80	200
α-HCH	319-84-6	5	20	40	80	200
β-HCH	319-85-7	5	20	40	80	200
γ–HCH (Lindane)	319-86-6	5	20	40	80	200
δ-HCH	58-89-9	5	20	40	80	200
Heptachlor	76-44-8	5	20	40	80	200
Heptachlor epoxide	1024-57-3	5	20	40	80	200
Oxychlordane	27304-13-8	5	20	40	80	200
α-Chlordane (cis-)	5103-71-9	5	20	40	80	200
γ-Chlordane (trans-)	5103-74-2	5	20	40	80	200
Trans-nonachlor	39765-80-5	5	20	40	80	200
Cis-nonachlor	5103-73-1	5	20	40	80	200
Aldrin	309-00-2	5	20	40	80	200
Dieldrin	60-57-1	5	20	40	80	200
Endrin	72-20-8	5	20	40	80	200
Mirex	2385-85-5	5	20	40	80	200
2,4'-DDE	3424-82-6	5	20	40	80	200
4,4'-DDE	75-55-9	5	20	40	80	200
2,4'-DDD	53-19-0	5	20	40	80	200
4,4'-DDD	72-54-8	5	20	40	80	200
2,4'-DDT	789-02-6	5	20	40	80	200
4,4'-DDT	50-29-3	5	20	40	80	200
Endosulfan II	33213-65-9	5	20	40	80	200
Endosulfan sulfate	1031-07-8	5	20	40	80	200

Table 4-2 cont'd. Chlorinated hydrocarbons contained in calibration solutions and their approximate concentrations.

Compounds Contained in Calibration Solutions	CAS	Level 1 (pg/µL)	Level 2 (pg/µL)	Level 3 (pg/µL)	Level 4 (pg/µL)	Level 5 (pg/µL)
PCB 8	34883-43-7	5	20	40	80	200
PCB 18	37680-65-2	5	20	40	80	200
PCB 28	7012-37-5	5	20	40	80	200
PCB 44	41464-39-5	5	20	40	80	200
PCB 52	35693-99-3	5	20	40	80	200
PCB 66	32598-10-0	5	20	40	80	200
PCB 101	37680-73-2	5	20	40	80	200
PCB 105	32598-14-4	5	20	40	80	200
PCB 118	31508-00-6	5	20	40	80	200
PCB 128	38380-07-3	5	20	40	80	200
PCB 138	35065-28-2	5	20	40	80	200
PCB 153	35065-27-1	5	20	40	80	200
PCB 170	35065-30-6	5	20	40	80	200
PCB 180	35065-29-3	5	20	40	80	200
PCB 187	52663-68-0	5	20	40	80	200
PCB 195	52663-78-2	5	20	40	80	200
PCB 206	40186-72-9	5	20	40	80	200
PCB 209	2051-24-3	5	20	40	80	200

3.0 QUANTITATIVE DETERMINATION OF CHLORINATED HYDROCARBONS BY GC/ECD

3.1 CALIBRATION

An ECD exhibits limited linearity, particularly for low concentrations. Consequently, a calibration must be established for each analytical run. An analytical run consists of samples and 5 calibration standards (approximately 5 to 200 pg/µL or 5 to 200 ng/mL) that are interspersed throughout the run. A calibration curve is established by analyzing the 5 interspersed calibration standards and fitting the data to the following quadratic equation.

$$x = \frac{-b_1 + \sqrt{b_1^2 - 4b_2(b_0 - Y)}}{2b_2}$$

Where:

x = the concentration of the analyte (ng/mL)

Y = the ratio of the area of the analyte to the area of the internal standard multiplied by the amount of the internal standard (ng)

b_2, b_1, b_0 = the coefficients for the quadratic equation

The data generated for each analyte in the calibration standards are subjected to the method of least squares to determine the coefficients for the corresponding quadratic

equation. Each analyte has different coefficients based on the relative response of the analyte compared to the internal standard, and as a function of the amount of the analyte. The injected concentration of the internal standard analyte is held constant for each set of calibration standards. In order for the calibration to be valid, each analyte must have a correlation coefficient greater than 0.997.

3.2 GC/ECD ANALYSIS

Sample analyses are completed only if the calibration meets previously described criteria. Samples are analyzed in analytical sets that consist of standards, samples, and quality control samples. Quality control (QC) samples are method blanks, laboratory duplicates, matrix spikes, and standard reference material (SRM). An autosampler is used to inject 1 or 5 μL of all samples, standards, and QC samples into the capillary column of the GC using the following instrument conditions. Slight modifications may be necessary depending upon the analysis.

Inlet: Splitless
Carrier gas: Helium, 1 mL/min

Temperatures:
- Injection port: 275 °C
- Detector: 325 °C

Oven program:
- Initial oven temp: 100 °C
- Initial hold time: 1 minute
- Ramp rate: 5 °C/min to 140 °C
- Hold time: 1 minute
- Ramp rate: 1.5 °C/min to 250 °C
- Hold time: 1 minute
- Final oven rate: 10 °C/min to 300 °C
- Final hold time: 5 minutes

3.3 ANALYTE IDENTIFICATION

The retention time of a sample analyte must fall within 15 seconds of the retention time for that analyte in a calibration standard or a retention index solution.

Chromatographic interferences may limit the ability to quantify peaks correctly and these data are reported but qualified to indicate interference.

4.0 QUANTITATION CALCULATIONS

Sample analyte concentrations are calculated based on the concentration and response of the internal standard (Table 4-2). The concentration (C) of each target analyte in the sample (ng/g) is calculated using the following equation:

$$C = \left(\frac{X}{W}\right)(V_e DF)$$

Where:

Ve = the final volume of the extract (mL)

X = the concentration of the analyte (ng/mL) as found from solving the quadratic equation

W = the sample weight (g)

DF = the dilution factor

$$DF = \frac{\text{Volume of Extract } (\mu L)}{\text{Volume of extract used to make dilution } (\mu L)}$$

Analyte concentrations are reported as corrected for surrogate recoveries. Percent surrogate recoveries ($SU_{Recovery}$) for each surrogate are calculated using the following equation:

$$SU_{Recovery} = \frac{C_{ESU}}{C_{SU}} x100$$

Where:

C_{ESU} = calculated surrogate concentration in the extract

C_{SU} = known concentration of surrogate added to extract

Analyte concentration corrections ($C_{Corrected}$) for surrogate recovery are calculated using the following equation:

$$C_{Corrected} \frac{C}{SU_{Recovery}} x\,100$$

5.0 QUALITY CONTROL (QC)

Samples are analyzed in analytical batches consisting of 19 samples or fewer and QC samples. The QC samples are a method blank, laboratory duplicate, matrix spike, matrix spike duplicate, and SRM. A method blank is a reagent blank prepared in the laboratory. A duplicate is a sample for which a second aliquot is analyzed. Matrix spikes are samples that are spiked with known concentrations of known analytes. The SRM used depends upon availability, matrix, and analytes. All SRMs are certified and traceable to the National Institute of Standards and Testing (NIST).

The validity of the data is monitored using defined QC criteria. The following QC criteria are used to evaluate analytical batches:

1) Calibration

- The calibration criteria (Section 3.1) must be met prior to data analyses. If the calibration criteria are not met, then the run is aborted and the instrument re-calibrated before further sample analysis.

2) Method Blank

- No more than two target analytes may exceed 3 times the concentration of the MDL.

- Exceptions are that if an analyte detected in the method blank exceeds 3 times the concentration of the MDL, but is not present in the associated samples, or if a sample analyte concentration is greater than 10 times that analyte concentration in the method blank, the result is qualified and reported.

- If a method blank exceeds these criteria then the source of contamination is determined and corrective action is taken before further sample analysis.

3) Matrix Spikes

- Analytes spiked into a matrix are considered valid only if they are spiked at concentrations equivalent to levels found in the sample.

- The average recovery for all valid spiked analytes in a matrix spike is between 60% and 120%. No more than two individual spiked analyte (valid) recoveries may exceed 40% - 120%, with the exception of chlorpyrifos and endosulfan sulfate.

- If the QC criteria are not met then the matrix spike sample failing the criteria will be re-analyzed and if the re-analyzed spike meets the criteria then the data are reported.

- If an analyte exceeds the criteria and is not present in the associated samples analyzed with the analytical batch, the result is qualified and reported.

- If upon re-analysis, QC criteria are still not met, the entire batch of samples is reanalyzed. If sufficient sample is unavailable to re-extract the matrix-spike, another sample may be selected or a blank-spike may be substituted.

- The average relative percent difference (RPD) for a valid matrix spike/matrix spike duplicate or blank spike/blank spike duplicate pair is 30%. No more than two individual analyte RPDs may exceed 35%.

4) Duplicate

- The average RPD between the duplicate and original sample, for analytes greater than 10 times the concentration of the MDL is 30%. The RPD for no more than two individual analytes may exceed 35%.

- If the QC criteria are not met then the sample pair failing the criteria will be re-analyzed and if the re-analyzed samples meet the criteria then the data are reported.

- If an analyte exceeds the criteria and is not present in the associated samples analyzed with the analytical batch, the result is qualified and reported.

- If upon re-analysis, QC criteria are still not met, the entire batch of samples is reanalyzed. If sufficient sample is unavailable to re-extract the duplicate pair, another sample may be selected.

5). Standard Reference Material

- The average recovery for target analytes in a SRM should not exceed 30% of the upper and lower bounds of the mean certified values. No more than two target analytes should deviate more than 35% from the upper or lower bounds of the mean certified values.

- If the QC criteria are not met then the SRM failing the criteria will be re-analyzed and if the re-analyzed SRM meets the criteria then the data are reported.

- If an analyte exceeds the criteria and is not present in the associated samples analyzed with the analytical batch, the result is qualified and reported.

- If upon re-analysis, QC criteria are still not met, the entire batch of samples is reanalyzed.

6). Surrogates

- The average recovery of surrogate compounds is between 50% and 150%.

- Exceptions are analytical interferences with the surrogates and diluted samples.

- If the average recovery of surrogates exceeds the criteria, and calculation and analytical errors are eliminated, the sample is re-analyzed. If sufficient sample is unavailable for re-extraction, the data are qualified and reported.

7). Method Detection Limit

- The method detection limit (MDL) is determined following the procedures outlined in the Federal Register (1984).

6.0 REFERENCES

Federal Registry (1984) Vol. 49, No. 209:198-199.

CHAPTER 5. QUANTITATIVE DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS USING SELECTED ION MONITORING GAS CHROMATOGRAPHY/MASS SPECTROMETRY 2000 – 2006

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ABSTRACT

Selected polycyclic aromatic hydrocarbons (PAHs), including alkylated homologues, are detected using a gas chromatograph/mass spectrometer in selected ion monitoring mode. This method is capable of detecting low concentrations of PAHs in complex matrices such as tissues and sediments.

1.0 INTRODUCTION

A gas chromatograph/mass spectrometer (GC/MS) in selected ion mode (SIM), coupled to a capillary column, is used to resolve and detect polycyclic aromatic hydrocarbons (PAHs) in tissues and sediments. Samples are injected into a temperature-programmed GC/MS, operated in splitless mode. The capillary column is an HP-5MS (60 m x 0.25 mm ID and 0.25 μm film thickness). The mass spectrometer is capable of scanning from 35 to 500 AMU every second or less and uses 70 electron volts energy in electron impact ionization mode. A data acquisition system continuously acquires and stores all data for quantitation. This method is capable of producing data at parts-per-billion concentrations.

2.0 APPARATUS AND MATERIALS

2.1 EQUIPMENT

Gas chromatograph, split/splitless injection port and electronic pressure control, Agilent Technologies 5890-II

Mass spectrometer, capable of scanning from 35 to 500 AMU, utilizing 70 electron volts of energy in impact ionization mode, Agilent Technologies 5972-MSD

Data acquisition system, Agilent Technologies ChemStation, capable of continuous acquisition and storage of all data during analysis

Autosampler, capable of making 1 to 5 μL injections

Capillary column, Agilent Technologies HP-5MS (60 m x 0.25 mm ID and 0.25 μm film thickness)

Micropipetters, calibrated, 1% accuracy, disposable tips

2.2 REAGENTS

Dichloromethane (CAS 75-09-02), pesticide grade or equivalent purity

Helium (CAS 7440-59-7), 99.8% purity

2.3 STANDARDS

2.3.1 SURROGATE SPIKING SOLUTION

The surrogate spiking solution is prepared from aliquots of pure compounds (Absolute Standards, Inc.) that are diluted with dichloromethane to a final concentration of 0.5 μg/mL. The surrogate spiking solution includes naphthalene-d_8, acenaphthalene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12} and perylene-d_{12}. Surrogate solution (100 μL) is added to all samples and quality control samples prior to extraction. Surrogate compounds are resolved from, but elute in close proximity to, the analytes of interest. Individual surrogate recoveries are used to correct specific analyte concentrations based on retention time.

2.3.2 INTERNAL STANDARD SOLUTION

The internal standard solution is made from aliquots of pure compounds (Cambridge Isotope Laboratories, Inc.) and diluted with dichloromethane to a final concentration of 0.5 μg/mL. The internal standard solution includes fluorine-d_{10}, pyrene-d_{10}, and benzo(a)pyrene-d_{12}. The internal standard compounds are resolved from, but elute in close proximity to, the analytes of interest. The internal standard solution (100 μL) is added to all samples and quality control samples just prior to instrument analysis. Internal standards are used to calculate relative response factors and specific analyte concentrations based on retention time.

2.3.3 MATRIX SPIKING SOLUTION

Certified solutions containing 2- to 5-ring PAH compounds are purchased from commercial vendors (Chiron, Aldrich and Absolute Standards) and diluted with dichloromethane to prepare the matrix spiking solution (Table 5-1). The matrix spiking solution is diluted to a concentration approximately 10 times the MDL and is added to all matrix spike samples.

2.3.4 CALIBRATION SOLUTION

Calibration solutions are prepared at 5 concentrations ranging from approximately 0.02 to 1 μg/mL (Table 5-2) by diluting commercially available certified solutions containing analytes of interest (Absolute Standards, Inc., Cambridge Isotope Laboratories, Inc., Chiron, Aldrich, and Absolute Standards).

2.3.5 RETENTION INDEX SOLUTIONS

The mid-level calibration standard, containing analytes at approximately 0.25 μg/mL, is used as a retention index solution to determine the retention times of unsubstituted compounds and certain substituted compounds. A crude oil/coal oil standard material is used as a retention index solution for the determination of retention times for the remaining alkyl homologues. The retention index solutions are also used to evaluate instrument retention time drift over time.

Table 5-1. Polycyclic aromatic hydrocarbons contained in the matrix-spiking solution.

Analyte	CAS	Spiking Solution Concentration (ng/mL)
Decalin	97-17-8	1.00
Naphthalene	91-20-3	1.06
2-Methylnaphthalene	91-57-6	1.05
1-Methylnaphthalene	90-12-0	1.06
Benzo[*b*]thiophene	95-15-8	1.86
Biphenyl	92-52-4	1.06
2,6-Dimethylnaphthalene	581-42-0	1.06
Acenaphthylene	208-96-8	1.02
Acenaphthene	83-32-9	1.10
1,6,7-Trimethylnaphthalene	2245-38-7	0.940
Dibenzofuran	132-64-9	1.00
Fluorene	86-73-7	1.05
Pentachlorophenol	87-86-5	4.00
Carbazole	86-74-8	1.00
Anthracene	120-12-7	0.801
1-Methylphenanthrene	832-69-9	1.05
Phenanthrene	85-07-8	1.06
Dibenzothiophene	132-65-01	1.00
Fluoranthene	206-44-0	1.06
Pyrene	129-00-0	1.06
Naphthobenzothiophene	239-35-0	1.00
Benz[*a*]anthracene	56-55-3	0.919
Chrysene	218-01-9	1.07
C30-Hopane	13849-96-2	1.00
Benzo[*b*]fluoranthene	200-99-2	1.06
Benzo[k]fluoranthene	207-08-9	1.05
Benzo[*e*]pyrene	192-97-2	1.06
Benzo[*a*]pyrene	50-32-8	0.955
Perylene	198-55-0	0.800
Indeno[*1,2,3-c,d*]pyrene	193-39-5	0.938
Dibenzo[*a,h*]anthracene	53-70-3	0.794
Benzo[*g,h,i*]perylene	191-24-2	0.945

Table 5-2. Polycyclic aromatic hydrocarbons contained in calibration solutions and their approximate concentrations.

Compounds Contained in Calibration Solutions	**CAS**	**Level 1 (µg/mL)**	**Level 2 (µg/mL)**	**Level 3 (µg/mL)**	**Level 4 (µg/mL)**	**Level 5 (µg/mL)**
Internal Standards						
Fluorene-d_{10}	NA	0.05	0.05	0.05	0.05	0.05
Pyrene-d_{10}	NA	0.05	0.05	0.05	0.05	0.05
Benzo[*a*]pyrene-d_{12}	NA	0.05	0.05	0.05	0.05	0.05
Surrogates						
Naphthalene-d_8	NA	0.02	0.10	0.25	0.50	1.00
Acenaphthene-d_{10}	NA	0.02	0.10	0.25	0.50	1.00
Phenanthrene-d_{10}	NA	0.02	0.10	0.25	0.50	1.00
Chrysene-d_{12}	NA	0.02	0.10	0.25	0.50	1.00
Perylene-d_{12}	NA	0.02	0.10	0.25	0.50	1.00
5β(H)-Cholane	80373-86-0	0.02	0.10	0.25	0.50	1.00
Analytes						
Naphthalene	91-20-3	0.021	0.106	0.267	0.531	1.06
2-Methylnaphthalene	91-57-6	0.021	0.104	0.263	0.524	1.05
1-Methylnaphthalene	90-12-0	0.021	0.105	0.265	0.527	1.05
Benzo[*b*]thiophene	95-15-8	0.037	0.184	0.464	0.928	1.86
Biphenyl	92-52-4	0.021	0.105	0.266	0.529	1.06
2,6-Dimethylnaphthalene	581-42-0	0.021	0.105	0.265	0.527	1.06
Acenaphthylene	208-96-8	0.020	0.101	0.255	0.508	1.02
Acenaphthene	83-32-9	0.022	0.109	0.276	0.548	1.10
1,6,7-Trimethylnaphthalene	2245-38-7	0.019	0.093	0.236	0.470	0.939
Dibenzofuran	132-64-9	0.020	0.100	0.250	0.500	1.00
Fluorene	86-73-7	0.021	0.105	0.264	0.526	1.05
Pentachlorophenol	87-86-5	0.250	0.500	1.000	1.500	2.00
Carbazole	86-74-8	0.020	0.100	0.250	0.500	1.00
Dibenzothiophene	132-65-01	0.020	0.100	0.250	0.500	1.00
Phenanthrene	85-01-8	0.021	0.105	0.266	0.528	1.06
Anthracene	120-12-7	0.016	0.080	0.201	0.400	0.800
1-Methylphenanthrene	832-69-9	0.021	0.104	0.263	0.523	1.05
Fluoranthene	206-44-0	0.021	0.106	0.267	0.530	1.06
Pyrene	129-00-0	0.021	0.105	0.266	0.530	1.06
Naphthobenzothiophene	239-35-0	0.020	0.099	0.250	0.500	1.00
Benz[*a*]anthracene	56-55-3	0.018	0.091	0.231	0.459	0.918
Chrysene	218-01-9	0.021	0.106	0.267	0.532	1.06
C_{30}-Hopane	13849-96-2	0.020	0.100	0.250	0.500	1.00
Benzo[*b*]fluoranthene	200-99-2	0.021	0.105	0.265	0.528	1.06
Benzo[*k*]fluoranthene	207-08-9	0.021	0.105	0.264	0.526	1.05
Benzo[*e*]pyrene	192-97-2	0.021	0.105	0.265	0.528	1.06
Benzo[*a*]pyrene	50-32-8	0.019	0.095	0.238	0.477	0.954
Perylene	198-55-0	0.016	0.080	0.201	0.400	0.799
Indeno[*1,2,3-cd*]pyrene	193-39-5	0.019	0.093	0.236	0.469	0.937
Dibenzo[*a,h*]anthracene	53-70-3	0.016	0.079	0.199	0.396	0.793
Benzo[*g,h,i*]perylene	191-24-2	0.019	0.094	0.237	0.472	0.944

3.0 QUANTITATIVE DETERMINATION OF PAHS BY GC/MS-SIM

3.1 MASS SPECTROMETER TUNING

Prior to calibration, the MS is autotuned using perfluorotributylamine (PFTBA) to criteria established by the instrument manufacturer.

3.2 INITIAL CALIBRATION

A 5-point relative response factor (RRF) calibration curve is established for analytes of interest prior to the analysis of samples and quality control (QC) samples (Table 5-2). A RRF is determined for each analyte for each calibration level using the following equation:

$$RRF = \frac{(A_A)(C_{IS})}{(A_{IS})(C_A)}$$

Where:
A_A = the area of the characteristic ion for the analyte to be measured
A_{IS} = the area of the characteristic ion for the specific internal standard
C_A = the known concentration of the analyte in the calibration solution (μg/mL)
C_{IS} = the known concentration of the internal standard in the calibration solution (μg/mg)

The response factors determined for each calibration level are averaged to produce a mean relative response factor ($\overline{RRF_i}$) for each analyte. The percent relative standard deviation (%RSD) for the 5 response factors must be less than or equal to 15%, for each analyte.

$$\%RSD = \frac{\text{Standard Deviation of the RRFs}}{\text{Average of the RRFs}} \times 100$$

Where:

$$\text{Standard Deviation} = \sqrt{\frac{\sum_{i=1}^{n}\left(x_i - \bar{x}\right)^2}{(n-1)}}$$

Where:
x_i = each RRF value used to calculate the mean RRF
$\bar{x}$ = the mean of n values
n = total number of values (5)

3.3 CONTINUING CALIBRATION

A mid-level calibration standard is analyzed at the beginning and end of each analytical set or every 10 samples (whichever is more frequent). The daily relative response factor

for each compound is compared to the mean relative response factor from the initial calibration curve and the average relative percent difference (RPD) of all analytes must be less than 25%. If the calibration check does not meet this criterion then the initial five-point calibration is repeated.

$$RPD = \frac{RRF_c - \overline{RRF_i}}{\overline{RRF_i}} x\,100$$

Where:

$\overline{RRF_i}$ = mean relative response factor from the most recent initial calibration (meeting technical acceptance criteria)

RRF_c = relative response factor from the continuing calibration standard

3.4 GC/MS-SIM ANALYSIS

The initial calibration of the GC/MS must meet the previously described criteria prior to sample analysis. Samples are analyzed in analytical sets that consist of standards, samples, and QC samples. Quality control samples are method blanks, laboratory duplicates, matrix spikes, and standard reference materials. An autosampler is used to inject 1 or 2 μL of all samples, standards, and QC samples into the capillary column of the GC using the following instrument conditions. Slight modifications may be necessary depending upon the analysis.

Inlet: Splitless
Carrier gas: Helium, 1 mL/min

Temperatures:
- Injection port: 300 °C
- Transfer line: 290 °C

Oven program:
- Initial oven temp: 60 °C
- Initial hold time: 0 minutes
- Ramp rate: 7 °C/min
- Final oven temp: 315 °C
- Final hold time: 22 minutes
- Total run time: 56 minutes

The effluent from the GC capillary column is routed directly into the ion source of the MS. The MS is operated in the selected ion monitoring mode (SIM) and includes the quantitation and confirmation masses for the PAHs listed in Table 5-3. For all compounds detected at a concentration above the MDL, the confirmation ion is checked to confirm the analyte's presence.

3.5 ANALYTE IDENTIFICATION

The extracted ion current profiles of the primary m/z and the confirmatory ion for each analyte must meet the following criteria:

The characteristic masses of each analyte of interest must be in the same scan or within one scan of each other. The retention time must fall within ±5 seconds of the retention time of the authentic compound or alkyl homologue grouping determined by the analysis of the daily calibration check or reference oil standard.

The alkylated PAH homologue groupings (e.g. C4-naphthalene) appear as a group of isomers. The pattern of each group and the retention time window for the group is established by the analysis of a reference oil standard. Each group of alkylated homologues is integrated in its entirety and the total area response is used to determine the concentration of the entire group.

The relative peak heights of the primary mass ion, compared to the confirmation or secondary mass ion, must fall within ±30 percent of the relative intensities of these masses in a reference mass spectrum (Table 5-3). The reference mass spectrum is obtained from the continuing calibration solution or the reference oil standard for the parent compounds and alkylated homologues, respectively. In some instances, a compound that does not meet secondary ion confirmation criteria may still be determined to be present in a sample after close inspection of the data by a qualified mass spectrometrist. Supportive data includes the presence of the confirmation ion, but at a ratio different then that indicated in Table 5-3.

Data not meeting the criteria established in this section are appropriately qualified or re-analyzed.

Table 5-3. Target analyte parameters.

Analyte	CAS	Reference to Internal Standard and Surrogate	Quantitation Ion	Confirmation Ion	% Relative Abundance of Confirmation Ion
Fluorene-d_{10} (I -1)	NA		176	174	85
Naphthalene-d_8 (S-1)	NA	I-1	136	134	11
Decalin	91-17-8	I-1, S-1	138	96	90
C1-Decalins	NA	I-1, S-1	152	ND	ND
C2-Decalins	NA	I-1, S-1	166	ND	ND
C3-Decalins	NA	I-1, S-1	180	ND	ND
C4-Decalins	NA	I-1, S-1	180	ND	ND
Naphthalene	91-20-30	I-1, S-1	128	127	13
2-Methylnaphthalene	91-57-6	I-1, S-1	142	141	80
1-Methylnaphthalene	90-12-0	I-1, S-1	142	141	80
C1-Naphthalenes	NA	I-1, S-1	142	141	ND
Acenaphthene-d_{10} (S-2)	NA	I-1	164	162	89
2,6-Dimethylnaphthalene	581-42-0	I-1, S-2	156	141	90
1,6,7-Trimethylnaphthalene	2245-38-7	I-1, S-2	170	155	102
C2-Naphthalenes	NA	I-1, S-2	156	141	ND
C3-Naphthalenes	NA	I-1, S-2	170	155	ND
C4-Naphthalenes	NA	I-1, S-2	184	169	ND
Benzothiophene	11095-43-5	I-1, S-2	134	89	10
C1-Benzothiophenes	NA	I-1, S-2	148	ND	ND
C2-Benzothiophenes	NA	I-1, S-2	162	ND	ND
C3-Benzothiophenes	NA	I-1, S-2	176	ND	ND
Biphenyl	92-52-4	I-1, S-2	154	152	30
Acenaphthylene	208-96-8	I-1, S-2	152	153	15
Acenaphthene	83-32-9	I-1, S-2	154	153	98
Dibenzofuran	132-64-9	I-1, S-2	168	139	25
Fluorene	86-73-7	I-1, S-2	166	165	95
C1-Fluorenes	NA	I-1, S-2	180	165	ND
C2-Fluorenes	NA	I-1, S-2	194	179	ND
C3-Fluorenes	NA	I-1, S-2	208	193	ND
Pyrene-d_{10} (I -2)	NA		212	210	15
Phenanthrene-d_{10} (S-3)	NA	I-2	188	184	15
Pentachlorophenol	87-86-5	I-2, S-3	266	268	70
Carbazole	86-74-8	I-2, S-3	167	139	10
Dibenzothiophene	132-65-01	I-2, S-3	184	152	18
C1-Dibenzothiophenes	NA	I-2, S-3	198	184	ND
C2-Dibenzothiophenes	NA	I-2, S-3	212	197	ND
C3-Dibenzothiophenes	NA	I-2, S-3	226	211	ND
C4-Dibenzothiophenes	NA	I-2, S-3	240	ND	ND
Phenanthrene	85-01-8	I-2, S-3	178	176	20
Anthracene	120-12-7	I-2, S-3	178	176	20
C1-Phenanthrene/anthracenes	NA	I-2, S-3	192	191	ND
C2-Phenanthrene/anthracenes	NA	I-2, S-3	206	191	ND
C3-Phenanthrene/anthracenes	NA	I-2, S-3	220	205	ND
C4-Phenanthrene/anthracenes	NA	I-2, S-3	234	219	ND
Naphthobenzothiophene	NA	I-2, S-3	234	ND	ND
C1-Naphthobenzothiophenes	NA	I-2, S-3	248	ND	ND
C2-Naphthobenzothiophenes	NA	I-2, S-3	262	ND	ND
C3-Naphthobenzothiophenes	NA	I-2, S-3	276	ND	ND
Fluoranthene	206-44-0	I-2, S-3	202	101	15
Pyrene	129-00-0	I-2, S-3	202	101	15
C1-Fluoranthene/pyrenes	NA	I-2, S-3	216	215	ND
C2-Fluoranthene/pyrenes	NA	I-2, S-3	230	ND	ND

Table 5-3. Target analyte parameters (cont'd).

Analyte	CAS	Reference to Internal Standard and Surrogate	Quantitation Ion	Confirmation Ion	% Relative Abundance of Confirmation Ion
C3-Fluoranthene/pyrenes	NA	I-2, S-3	244	ND	ND
Chrysene-d_{12} (S-4)	NA	I-2	240	236	20
Benz[*a*]anthracene	56-55-3	I-2, S-4	228	226	30
Chrysene	218-01-9	I-2, S-4	228	226	30
C1-Chrysenes/Benzo[*a*]anthracenes	NA	I-2, S-4	242	241	ND
C2-Chrysenes/Benzo[*a*]anthracenes	NA	I-2, S-4	256	241	ND
C3-Chrysenes/Benzo[*a*]anthracenes	NA	I-2, S-4	270	255	ND
C4-Chrysenes/Benzo[*a*]anthracenes	NA	I-2, S-4	284	269	ND
Benzo(a)pyrene-d_{12} (I-3)	NA		264	260	20
5β(H)-Cholane(S-6)	80373-86-0	I-3	217	ND	ND
C29-Hopane	53584-60-4	I-3, S-3	191	398	5
18α–Oleanane	30759-92-3	I-3, S-3	191	412	5
C30-Hopane	13849-96-2	I-3, S-3	191	412	5
Benzo[*b*]fluoranthene	200-99-2	I-3, S-4	252	253	30,
Benzo[*k*]fluoranthene	207-08-9	I-3, S-4	252	253	30,
Benzo[*e*]pyrene	192-97-2	I-3, S-4	252	253	30
Benzo[*a*]pyrene	50-32-8	I-3, S-4	252	253	30
Indeno[*1,2,3-cd*]pyrene	193-39-5	I-3, S-4	276	277	25,
Dibenzo[*a,h*]anthracene	53-70-3	I-3, S-4	278	279	25,
C1-Dibenzo[*a,h*]anthracenes	NA	I-3, S-4	292	ND	ND
C2-Dibenzo[*a,h*]anthracenes	NA	I-3, S-4	306	ND	ND
C3-Dibenzo[*a,h*]anthracenes	NA	I-3, S-4	320	ND	ND
Benzo[*g,h,i*]perylene	191-24-2	I-3, S-4	276	277	25,
Perylene-d_{12} (S-5)	NA	I-3	264	260	20
Perylene	198-55-0	I-3, S-5	252	253	20

ND = Not determined
(I-#) = Internal standard reference number
(S-#) = Surrogate reference number

4.0 QUANTITATION CALCULATIONS

Sample analyte concentrations are calculated based on the concentration and response of the internal standard compounds (Table 5-2). The equations in Section 3.2 are used to calculate the RRF of each analyte relative to the concentration and area of the internal standard in the initial calibration. Response factors for alkyl homologues are presumed equal to the response factor of the respective unsubstituted (parent) compound.

The mass (M_A) of each target analyte (ng), including alkyl homologues, is calculated using the following equation:

$$M_A = \frac{(A_A M_{IS})}{(A_{IS} \overline{RRF_i})}$$

Where:

A_A = the area of the characteristic ion for the analyte measured

A_{IS} = the area of the characteristic ion for the specific internal standard

M_{IS} = mass of internal standard added to the extract (ng)

$\overline{RRF_i}$ = the average relative response factor for the analyte from the current calibration

The concentration (C) of each target analyte in a sample (ng/g) is calculated using the following equation:

$$C = \frac{(M_A DF)}{(W)}$$

Where:

DF = the dilution factor applied to the extract

$$DF = \frac{\text{Volume of Extract } (\mu L)}{\text{Volume of extract used to make dilution } (\mu L)}$$

W = the sample weight (g)

Analyte concentrations are reported as corrected for individual surrogate recoveries. The corrections for each compound are based on the surrogates referenced in Table 5-3. Percent surrogate recoveries ($SU_{Recovery}$) for each surrogate are calculated using the following equation:

$$SU_{Recovery} = \frac{C_{ESU}}{C_{SU}} x100$$

Where:

C_{ESU} = calculated surrogate concentration in the extract

C_{SU} = known concentration of surrogate added to extract

Analyte concentration corrections ($C_{corrected}$) for surrogate recovery are calculated using the following equation:

$$C_{Corrected} \frac{C}{SU_{Recovery}} x\,100$$

5.0 QUALITY CONTROL (QC)

Samples are analyzed in analytical batches consisting of 19 samples or fewer and QC samples. The QC samples are a method blank, laboratory duplicate, matrix spike, matrix spike duplicate, and standard reference material (SRM). A method blank is a reagent blank prepared in the laboratory. A duplicate is a sample for which a second aliquot is analyzed. Matrix spikes are samples that are spiked with known analyte concentrations. The SRM is a reference material with known analyte concentrations. All SRMs are certified and traceable to National Institute of Standards and Technology (NIST). The SRM used depends upon availability, matrix, and analytes.

The validity of the data is monitored using defined QC criteria. The following QC criteria are used to evaluate analytical batches:

1) Calibration

- The calibration criteria (Section 3.2) must be met prior to data analyses. If the calibration criteria are not met, then the run is aborted and the instrument re-calibrated before further sample analysis.

2) Method Blank

- No more than two target analytes may exceed 3 times the concentration of the MDL.

- Exceptions are that if an analyte detected in the method blank exceeds 3 times the concentration of the MDL but is not present in the associated samples, or if a sample analyte concentration is greater than 10 times that analyte concentration in the method blank, the result is qualified and reported.

- If a method blank exceeds these criteria then the source of contamination is determined and corrective action is taken before further sample analysis.

3) Matrix Spikes

- Analytes spiked into a matrix are considered valid only if they are spiked at concentrations equivalent to levels found in the sample.

- The average recovery for all valid spiked analytes in a matrix spike is between 60% and 120%. No more than two individual spiked analyte (valid) recoveries may exceed 40%-120%, with the exception of decalin and biphenyl.

- If the QC criteria are not met then the matrix spike sample failing the criteria will be re-analyzed and if the re-analyzed spike meets the criteria then the data are reported.

- If an analyte exceeds the criteria and is not present in the associated samples analyzed with the analytical batch, the result is qualified and reported.

- If upon re-analysis, QC criteria are still not met, the entire batch of samples is reanalyzed. If sufficient sample is unavailable to re-extract the matrix-spike, another sample may be selected or a blank-spike may be substituted.

- The average RPD for a valid matrix spike/matrix spike duplicate or blank spike/blank spike duplicate pair is 30%. No more than two individual analyte RPDs may exceed 35%.

4) Duplicate

- The average RPD between the duplicate and original sample, for analytes greater than 10 times the concentration of the MDL, is 30%. The RPD for no more than two individual analytes may exceed 35%.

- If the QC criteria are not met then the sample pair failing the criteria will be re-analyzed and if the re-analyzed samples meet the criteria then the data are reported.

- If an analyte exceeds the criteria and is not present in the associated samples analyzed with the analytical batch, the result is qualified and reported.

- If upon re-analysis, QC criteria are still not met, the entire batch of samples is reanalyzed. If sufficient sample is unavailable to re-extract the duplicate pair, another sample may be selected.

5) Standard Reference Material

- The average recovery for target analytes in a SRM should not exceed 30% of the upper and lower bounds of the mean certified values. No more than two target analytes should deviate more than 35% from the upper or lower bounds of the mean certified values.

- If the QC criteria are not met then the SRM failing the criteria will be re-analyzed and if the re-analyzed SRM meets the criteria then the data are reported.

- If an analyte exceeds the criteria and is not present in the associated samples analyzed with the analytical batch, the result is qualified and reported.

- If upon re-analysis, QC criteria are still not met, the entire batch of samples is reanalyzed.

6) Surrogates

- The average recovery of surrogate compounds is between 50% and 150%.

- Exceptions are analytical interferences with the surrogates and diluted samples.

- If the average recovery of surrogates exceeds the criteria, and calculation and analytical errors are eliminated, the sample is re-analyzed. If sufficient sample is unavailable for re-extraction, the data are qualified and reported.

7) Method Detection Limit

- The method detection limit (MDL) is determined following the procedures outlined in Federal Register (1984).

6.0 REFERENCES

Federal Registry (1984) Vol. 49, No. 209:198-199.

CHAPTER 6. EXTRACTION OF SEDIMENTS FOR BUTYLTINS 2000 - 2006

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ABSTRACT

Determining organic contaminant levels in sediments require extraction, isolation, and concentration of analytes from the matrix. Sediments are dried, pulverized, and homogenized prior to extraction. Sediments are extracted in a tropolone/hexane or tropolone/dichloromethane mixture using sonication. The extracts are reduced in volume and hexylmagnesium bromide (Grignard reagent) is added, followed by neutralization with hydrochloric acid. The organic fraction is reduced in volume and purified using silica gel/florisil chromatography columns. The volume of the resultant eluent is reduced and analyzed for butyltins by gas chromatography/flame photometric detection.

1.0 INTRODUCTION

Butyltins (BTs), including monobutyltin, dibutyltin, tributyltin, and tetrabutyltin are contaminants of concern in the estuarine environment. The procedure described is used to extract, isolate, purify, and concentrate BT contaminants from sediments. Contaminant concentrations in parts per billion can be resolved in sediments. Sediment samples are homogenized, dried, and ground using a mortar and pestle. Approximately 10 g of dry sediment are extracted in 60 mL of 0.1% tropolone/hexane or 0.1% tropolone/dichloromethane using a sonic probe. The liquid is decanted and the extraction procedure is repeated twice more. The combined extracts are reduced in volume. The samples are treated with hexylmagnesium bromide (Grignard reagent) and then neutralized with hydrochloric acid (HCl). The organic layer is drawn off, reduced in volume, and then purified using silica gel/florisil columns. The eluent is reduced to 10 mL from which 2 mL is prepared for the analysis of BTs by gas chromatography/flame photometric detection (GC/FPD).

2.0 APPARATUS AND MATERIALS

2.1 EQUIPMENT

Solvent reduction apparatus, Zymark TurboVap LV concentration workstation

Sonicator, Tekmar TMX500 sonicator

Balance, top loading, tare capacity to 300 g, capable of weighing to 1 mg

Microbalance, capable of weight to 1 μg

Calibrated weights, certified

Combustion furnace, electric capable of combusting glassware at 400 °C for at least 4 hr

Oven capable of maintaining 40 °C temperature

Conditioning oven, electric, gravity convection, capable of maintaining a stable temperature of up to 200 °C

Concentration tubes, Zymark® 60 mL borosilicate glass

Concentration tubes, 60 mL certified pre-cleaned with open screw caps and Teflon lined septa

Micropipettors, calibrated, 1% accuracy, disposable tips

Beakers, 150 mL

Erlenmeyer flasks, 250 mL

Amber extract vials, 2.0 mL, with teflon-lined screw caps

Glass wool

2.2 REAGENTS

Silica gel (CAS 1343-98-2)/Florisil (CAS 1343-88-0) columns, Resteck, 16 g florisil and 5 g silica gel

Dichloromethane (CAS 75-09-2), pesticide grade or equivalent purity

Hexane (CAS 110-54-3), pesticide grade or equivalent purity

Hydrochloric acid (CAS 7647-01-0), Tracepur® Plus or equivalent purity

Copper (CAS 7440-50-8), granular, 20-30 mesh, ACS reagent grade, purified

Tropolone (CAS 533-75-5), 98% purity

Grignard reagent, hexylmagnesium bromide (CAS 3761-92-0)

Nitrogen (CAS 7727-37-9), 99.8% purity

3.0 PROCEDURE

Sediment samples are frozen upon receipt at –20 °C until extraction. Prior to extraction, sediment samples are thawed and homogenized using a stainless steel spatula. A subsample is removed for percent moisture determination (McDonald *et al.*, 2006). At least 10 g of sample are dried in an oven at 40 °C and then ground and homogenized using a mortar and pestle. Approximately 10 g of the dried sediment are extracted.

Dried samples are weighed into 150 mL beakers to which 60 mL of 0.1% tropolone in either hexane or dichloromethane is added. Appropriate surrogates and spikes are added

to beakers prior to extraction. The sediment/tropolone mixture is sonicated at 50% power, 1 second on and 0.5 second off, for a total of 3 minutes. The extract is decanted into a 250 mL Erlenmeyer flask. The extraction is repeated two more times. Quality control (QC) samples (e.g., blanks, duplicates, matrix spikes, and certified reference materials) are prepared in the same manner as samples.

The combined extracts are reduced to 10 mL using a Zymark TurboVap LV concentration station set at 40 °C and 15 psi. An aliquot of extract (between 20 and 45 mL) is added to a 60 mL concentration tube and reduced in volume. Additional aliquots of extract are added to the tube as the volume is decreased until the sample extract has been reduced to approximately 10 mL. Purified copper granules are carefully mixed with concentrated hydrochloric acid, followed by thorough rinsing with HPCL grade water. Copper is added to the concentration tube to remove sulfur. Copper is added until it no longer turns black, indicating that all sulfur has been bound. Samples extracted in the tropolone/dichloromethane mixture are back-extracted into hexane. The samples are quantitatively transferred to a clean 60 mL concentration tube and reduced to 10 mL. The butyltin in the sample extracts are then hexylated by adding 1 mL of hexylmagnesium bromide (Grignard reagent). The sample headspace is purged using nitrogen, the tubes are capped and shaken for 1 hour on a shaker table. After shaking, the Grignard reagent is neutralized by adding 40 mL of 10% HCl to each tube in an ice bath. The tubes are shaken and the upper organic layer is transferred to a 50 mL Zymark concentration tube. The acid fraction is rinsed twice more using 10 mL of hexane, each time transferring the organic layer to the concentration tube. The extract is then reduced to 2 – 4 mL using a Zymark TurboVap LV set at 40 °C and 15 psi.

The concentrated extract is purified using silica gel/florisil chromatography columns. The chromatography columns contain 16 g of florisil and 5 g silica gel and are conditioned by flushing with 30 mL of hexane. The sample and solvent rinses are added to the top of column and 125 mL of hexane is added and eluted until the column is dry. The eluent is collected in 250 mL Erlenmeyer flasks. The eluent is quantitatively transferred to a Zymark concentration tube and reduced to a final volume of 10 mL. Approximately 2 mL of each extract is transferred to a clean 2 mL amber vial and stored at –20 °C until analysis. Internal standard is added immediately prior to instrument analysis. Figure 9-1 shows a flow chart of the extraction and purification procedure.

4.0 QUALITY CONTROL (QC)

Solvents are verified to be contaminant-free by lot tests prior to use. All equipment and glassware used to extract samples are thoroughly cleaned by solvent rinsing or combustion at 400 °C. The calibration and accuracy of balances, weights, pipettors, and thermometers are checked daily using certified weights and thermometers with calibrations traced to the National Institute of Standards and Technology (NIST). The calibration and accuracy of balances, weight, pipettors, and thermometers are verified yearly by independent sources. A series of quality control samples are processed with each batch of 20 samples or less. The following quality controls are used to ensure the accuracy and precision of tissue data.

- Surrogates. Solutions containing analytes that do not interfere with the analytes of interest are prepared at concentrations approximately 5 to 10 times the method detection limit (MDL). Specified surrogates are added to each sample extracted, including QC samples, at a specified volume (typically 100 μL), immediately prior to extraction.

- Method Blank. Method blanks are extractions of all support material used for extraction of samples, with the exception of sediment. A method blank is analyzed with each extraction batch of 20 or fewer samples. The method blank is extracted and analyzed in a manner identical to samples.

- Matrix Spike. Matrix spikes are extractions of sample matrix fortified with spikes of selected target analytes. Spikes are prepared at concentrations approximately 10 times the MDL. A matrix spike and matrix spike duplicate are analyzed with each extraction batch of 20 or fewer samples. Matrix spikes are extracted and analyzed in a manner identical to samples.

- Laboratory Duplicates. A sample is analyzed in duplicate with each extraction batch of 20 or fewer samples.

- Certified Reference Material (CRM). A standard reference material (CRM PACS-2) is analyzed with each extraction batch of 20 or fewer samples.

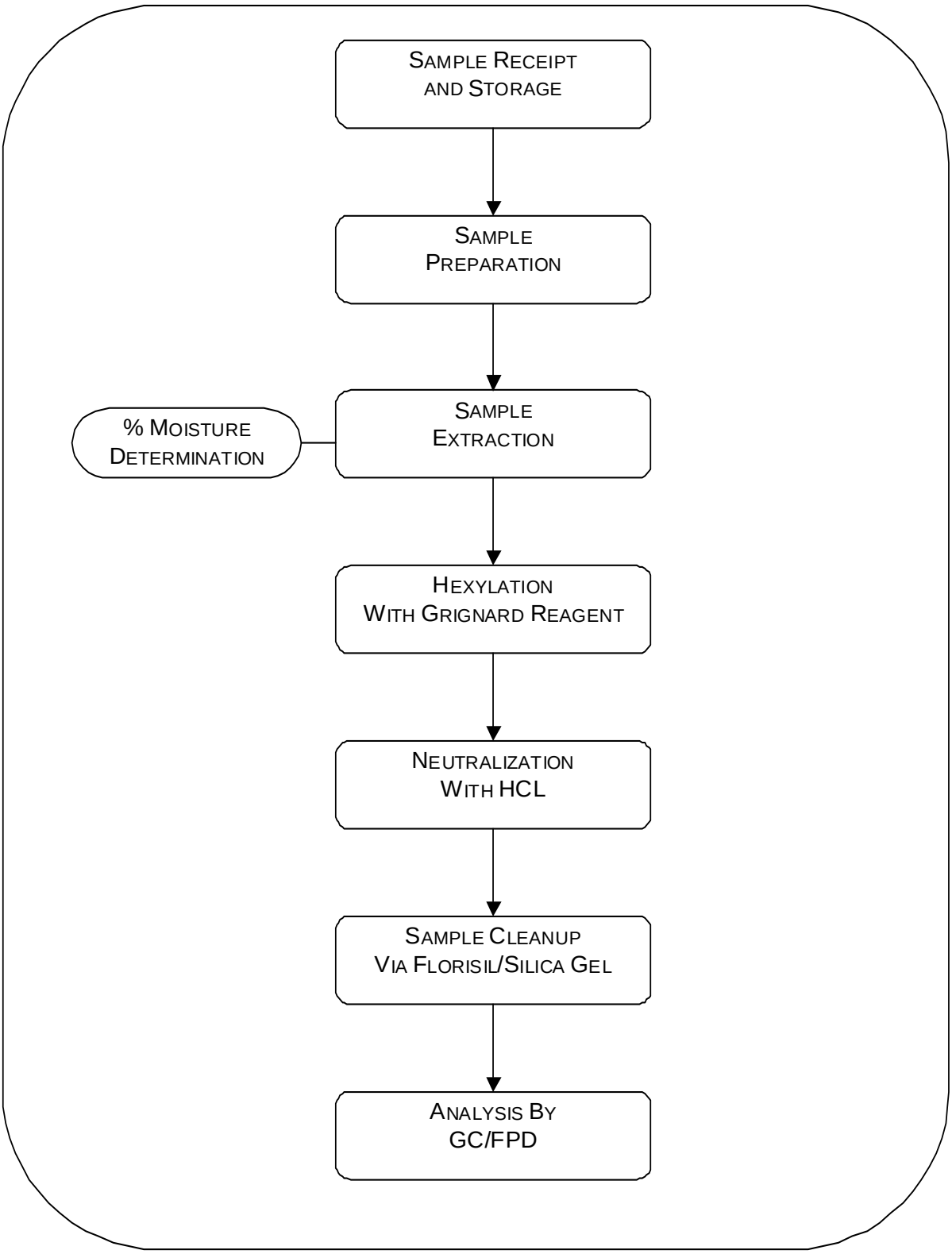

Figure 6-1. Methodology for extraction, isolation, and quantification of sediment samples for butyltins.

CHAPTER 7. EXTRACTION OF BIOLOGICAL TISSUES FOR BUTYLTINS 2000 - 2006

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ABSTRACT

Determining organic contaminant levels in tissues require extraction, isolation, and concentration of analytes from the matrix. Bivalves are shucked and homogenized. Aliquots of homogenized sample are chemically dried using Hydromatrix® and extracted in a tropolone/ hexane or tropolone/dichloromethane mixture using a tissuemizer. The extracts are reduced in volume and hexylmagnesium bromide (Grignard reagent) is added, followed by neutralization with hydrochloric acid. The organic fraction is reduced in volume and purified using silica gel/florisil chromatography columns. The volume of the resultant eluent is reduced and analyzed for butyltins by gas chromatography/flame photometric detection.

1.0 INTRODUCTION

Butyltins (BTs), including monobutyltin, dibutyltin, tributyltin, and tetrabutyltin are contaminants of concern in the estuarine environment. The determination of these compounds at low concentrations in tissue is necessary to accurately monitor spatial and temporal changes in U.S. coastal waters. The procedure described is used to extract, isolate, purify, and concentrate BT contaminants from tissues. Contaminant concentrations in parts-per billion can be resolved in lipid-rich tissues. Shell length and shell volume are determined for specimens collected at each location. Bivalves are then shucked and multiple organisms are processed as one sample to ensure the sample is representative of a population at a given site and to have sufficient sample to complete the analyses. Tissue samples are homogenized using a stainless steel blender outfitted with titanium blades. Aliquots of approximately 10 g of wet tissue are chemically dried with Hydromatrix® or sodium sulfate. The tissue/drying agent mixtures are extracted in 60 mL of 0.1% tropolone in either hexane or dichloromethane using a Tekmar® tissumizer. The liquid is decanted and the extraction procedure is repeated twice more. The combined extracts are reduced in volume. The samples are treated with hexyl magnesium bromide (Grignard reagent) and then neutralized with hydrochloric acid (HCl). The organic layer is drawn off, reduced in volume, and then purified using silica/florisil columns. The eluent is reduced to 10 mL, from which 2 mL is prepared for the analysis of BTs by gas chromatography/flame photometric detection (GC/FPD).

2.0 APPARATUS AND MATERIALS

2.1 EQUIPMENT

Solvent reduction apparatus, Zymark TurboVap LV concentration workstation

Tissumizer, Tekmar®, with stainless steel probes

Balance, top loading, tare capacity to 300 g, capable of weighing to 1 mg

Microbalance, capable of weight to 1 μg

Calibrated weights, certified

Combustion furnace, electric, capable of combusting glassware at 400 °C for at least 4 hours

Oven capable of 40 °C temperature maintenance

Conditioning oven, electric, gravity convection, capable of maintaining a stable temperature of up to 200 °C

Concentration tubes, Zymark® 60 mL borosilicate glass

Concentration tubes, 60 mL certified pre-cleaned tubes with open screw caps and Teflon lined septa.

Micropipettors, calibrated to 100 μL, 1% accuracy, disposable tips

Centrifuge bottles, 200 mL

Erlenmeyer flasks, 250 mL

2 mL amber extract vials with teflon-lines screw caps

Glass wool

2.2 REAGENTS

Silica gel (CAS 7343-88-0)/Florisil (CAS 1343-88-0) columns, Resteck, 16 g florisil and 5 g silica gel

Dichloromethane (CAS 75-09-2), pesticide grade or equivalent purity

Hexane (CAS 110-54-3), pesticide grade or equivalent purity

Hydrochloric acid (CAS 7647-01-0), Tracepur® Plus or equivalent purity

Hydromatrix® (CAS 68855-54-9/14464-46-1), conditioned by combustion at 400 °C for at least 4 hours and stored at 120 °C

Sodium sulfate (CAS 7757-82-6), anhydrous granular powder, ACS reagent grade, purified by combusting at 400°C for at least 4 hours and stored at 120 °C.

Hydrochloric acid (CAS 7647-07-0)

Topolone (CAS 5533-75-5), 98% purity

Grignard reagent, hexylmagnesium bromide (CAS 3761-92-0)

Nitrogen, (CAS 7727-37-9) 99.8% purity

3.0 PROCEDURE

Shell length and volume are determined for all bivalves collected at each sampling site. The bivalves are then shucked and the soft tissue is homogenized using a stainless steel Waring® blender with titanium blades. Homogenized tissue samples are frozen at –20 °C until extraction. Prior to extraction, tissue samples are thawed and re-homogenized using a stainless steel spatula. A subsample is removed for percent moisture determination (McDonald *et al*., 2006).

Approximately 10 g wet tissue and 20 g of prepared Hydromatrix® or prepared sodium sulfate are weighed into a 200 mL centrifuge bottle. The tissues samples must be thoroughly dry to optimize the extraction efficiency. Hydromatrix® and sodium sulfate chemically dry samples by binding moisture. Appropriate surrogates and spikes are added to the centrifuge bottle prior to extraction. The samples are extracted in 60 mL of 0.1% tropolone, in hexane or dichloromethane, using a Tekmar® tissumizer. The samples are macerated for 2 min. The liquid is decanted into a 250 mL Erlenmeyer flask. The extraction is repeated two more times. Quality control (QC) samples (e.g., blanks, duplicate, matrix spikes) are prepared in the same manner as samples.

The combined extracts are reduced to 10 mL using a Zymark TurboVap LV concentration station set at 40 °C and 15 psi. An aliquot of extract (between 20 and 45 mL) is added to a 60 mL concentration tube and reduced in volume. Additional aliquots of extract are added to the tube as the extract volume is decreased until the sample extract has been reduced to approximately 10 mL. Samples extracted in the tropolone/dichloromethane mixture are back extracted into hexane. Samples are quantitatively transferred to a clean 60 mL concentration tube and reduced to 10 mL. BTs in the sample extracts are then hexylated by adding 1 mL of hexylmagnesium bromide (Grignard reagent). The sample headspace is purged using nitrogen and then the tubes are capped and shaken for 1 hour on a shaker table. After shaking the Grignard reagent is neutralized by adding 40 mL of 10% HCl to each tube, in an ice bath. The tubes are shaken and the upper organic layer is transferred to a 50 mL Zymark concentration tube. The acid fraction is rinsed twice more using 10 mL of hexane, each time transferring the organic layer to the concentration tube. The extract is then reduced to 2 – 4 mL using a Zymark TurboVap LV set at 40 °C and 15 psi.

The concentrated extract is purified using silica gel/florisil chromatography columns. The chromatography columns contain 16 g of florisil and 5 g silica gel and are conditioned by flushing with 30 mL of hexane. The sample and solvent rinses are added to the top of the column and 125 mL of hexane is added and eluted until the column is

dry. The eluent is collected in 250 mL Erlenmeyer flasks. The eluent is quantitatively transferred to a Zymark concentration tube and reduced to a final volume of 10 mL. Approximately 2 mL of each extract is transferred to a clean 2 mL amber vial and stored at –20 °C until analysis. Internal standard is added immediately prior to instrument analysis. Figure 10-1 shows a flow chart of the extraction and purification procedure.

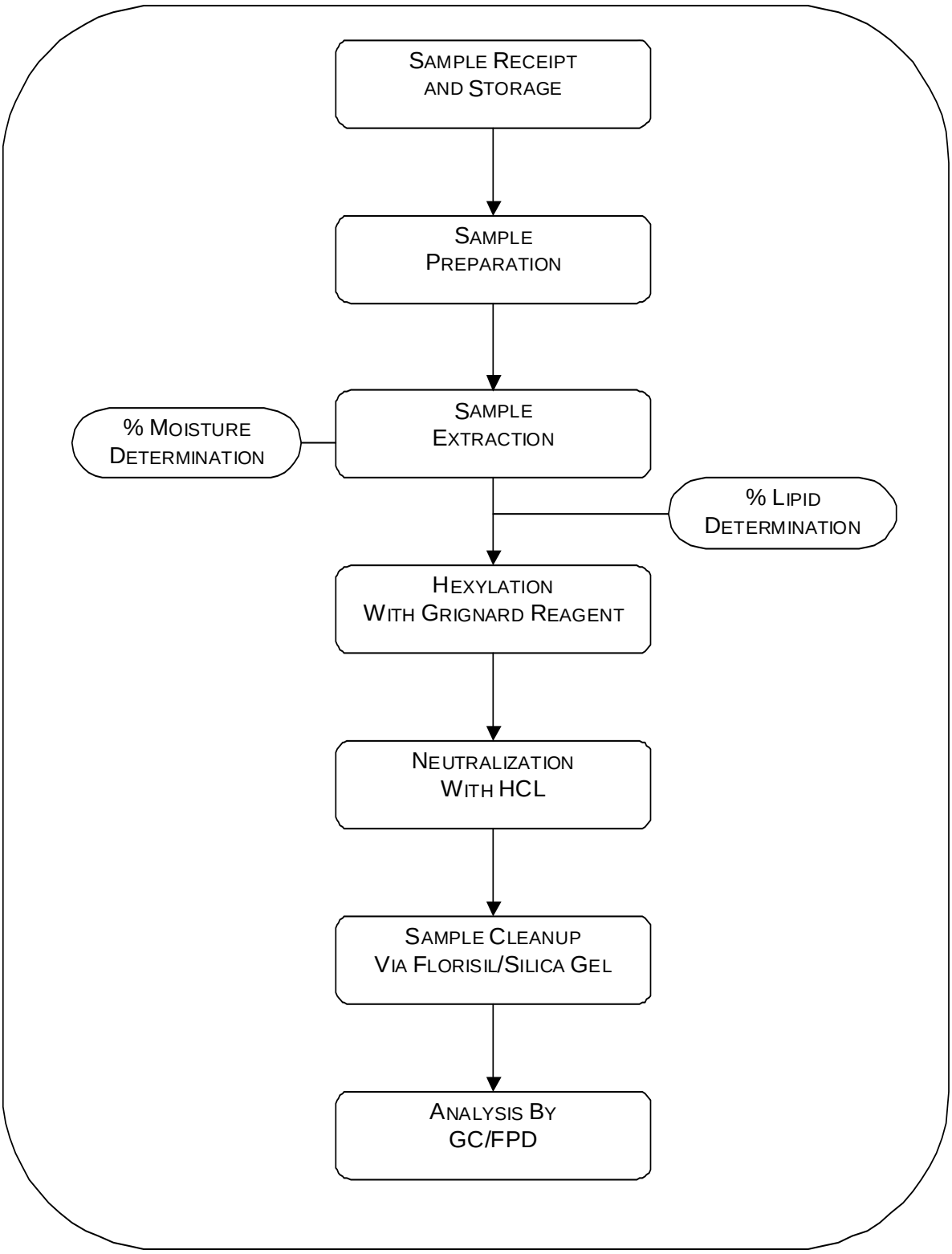

Figure 7-1. Methodology for extraction, isolation, and quantification of tissue samples for butyltins.

4.0 QUALITY CONTROL (QC)

Solvents are verified to be contaminant-free by lot tests prior to use. All equipment and glassware used to extract samples are thoroughly cleaned by solvent rinsing or combustion at 400 °C. The calibration and accuracy of balances, weights, pipettors, and thermometers are checked daily using certified weights and thermometers with calibrations traced to the National Institute of Standards and Technology (NIST). The calibration and accuracy of balances, weight, pipettors, and thermometers are verified yearly by an independent source. All samples are shipped and received under chain-of-custody. A series of quality control samples are processed with each batch of 20 samples or less. The following quality controls are used to ensure the accuracy and precision of tissue data.

- Surrogates. Solutions containing analytes that do not interfere with the analytes of interest are prepared at concentrations approximately 5 to 10 times the method detection limit (MDL). Specified surrogates are added to each sample extracted, including QC samples, at a specified volume (typically 100 μL) immediately prior to extraction.

- Method Blank. Method blanks are extractions of all support material used for extraction of samples, with the exception of tissue. A method blank is analyzed with each extraction batch of 20 or fewer samples. The method blank is extracted and analyzed in a manner identical to samples.

- Matrix Spike. Matrix spikes are extractions of sample matrix fortified with spikes of selected target analytes. Spikes are prepared at concentrations approximately 10 times the MDL. A matrix spike and matrix spike duplicate are analyzed with each extraction batch of 20 or fewer samples. Matrix spikes are extracted and analyzed in a manner identical to samples.

- Laboratory Duplicates. A sample is analyzed in duplicate with each extraction batch of 20 or fewer samples.

CHAPTER 8. QUANTITATIVE DETERMINATION OF BUTYLTINS USING GAS CHROMATOGRAPHY/FLAME PHOTOMETRIC DETECTION 1999 -2006

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ABSTRACT

Selected butyltins (BTs) are detected using a gas chromatograph/flame photometric detector. This method is capable of detecting low concentrations of monobutyltin, dibutyltin, tributyltin, and tetrabutyltin in complex matrices such as tissues and sediments.

1.0 INTRODUCTION

A gas chromatograph/flame photometric detector (GC/FPD), coupled to a capillary column, is used to resolve and detect butyltins (BTs) in tissues and sediments. Samples are injected into a temperature-programmed GC/FPD. The capillary column is a DB-5 (30 m x 0.25 mm ID and 0.25 μm film thickness). The data acquisition system continuously acquires and stores all data for quantitation. This method is capable of producing data at parts per-billion-concentrations.

2.0 APPARATUS AND MATERIALS

2.1 EQUIPMENT

Gas chromatograph equipped with large volume injector, electronic pressure control, and a flame photometric detector with a tin selective 610 nm filter, Thermoquest 2000 Series Trace GC

Data acquisition system, Thermoquest Chromquest software, capable of continuous acquisition and storage of all data during analysis

Autosampler capable of making 1 to 250 μL injections

Capillary column, J&W Scientific DB-5 (30 m x 0.25 mm ID and 0.25 μm film thickness)

Desolvation column, Supelco fused silica intermediate polarity (15 m long by 0.53 mm ID)

Micropipetters, calibrated, 1% accuracy, disposable tips

2.2 REAGENTS

Hexane (CAS 110-54-3), pesticide grade or equivalent purity

Helium (CAS 7440-59-7), 99.8% purity

Hydrogen (CAS1333-74-0), 99.8% purity

Nitrogen (CAS 7727-37-9), 99.8% purity

Air (CAS 132259-10-0), 99.8% purity

2.3 STANDARDS

2.3.1 SURROGATE SPIKING SOLUTION

The surrogate spiking solution is prepared with aliquots of pure compounds (Restek) diluted with hexane to a final concentration of 646 ng Sn/mL. The surrogate spiking solution includes tripentyltin chloride. The surrogate solution (100 μL) is added to all samples and quality control (QC) samples prior to extraction. The surrogate is resolved from, but elutes in close proximity to, the analytes of interest. The recovery of the surrogate is used to correct analyte concentrations based on retention time.

2.3.2 INTERNAL STANDARD SOLUTION

The internal standard solution is made from an aliquot of pure compound (Restek) and diluted with hexane to a final concentration of 1,015 ng Sn/mL. The internal standard compound is tetra-n-propyltin. The internal standard compound is resolved from, but elutes in close proximity to, the analytes of interest. The internal standard solution (100 μL) is added to all samples and QC samples just prior to analysis. The internal standard is used to calculate relative response factors and specific analyte concentrations based on retention time.

2.3.3 MATRIX SPIKING SOLUTION

A certified solution containing monobutyltin (MBT), dibutyltin (DBT), tributyltin (TBT) and tetrabutyltin (TeBT) is purchased from a commercial vendor (Restek**)** and diluted with hexane to prepare the matrix spiking solution (Table 8-1). The matrix spike solution is diluted to approximately 10 times the MDL and is added to all matrix spike samples.

2.3.4 CALIBRATION SOLUTION

Calibrations solutions are prepared at 5 concentrations ranging from approximately 0.5 to 50 ng Sn/mL (Table 8-2) by diluting a commercially prepared solution (Restek) containing the analytes of interest.

2.3.5 RETENTION INDEX SOLUTIONS

The mid-level calibration standard is used as a retention index solution to determine the retention times of analytes of interest.

Table 8-1. Butyltins Contained in the Matrix-Spiking Solution.

Analyte	CAS	Spiking Solution Concentration (ng Sn/mL)
Tetrabutyltin	1461-25-2	683.9
Tributyltin chloride	1461-22-9	729.4
Dibutyltin dichloride	683-18-1	781.4
Butyltin trichloride	1118-46-3	841.4

Table 8-2. Butyltins Contained in Calibration Solutions and their Approximate Concentrations.

Compounds Contained in Calibration Solutions	CAS	Level 1 (ng Sn/mL)	Level 2 (ng Sn/mL)	Level 3 (ng Sn/mL)	Level 4 (ng Sn/mL)	Level 5 (ngSnmL)
Internal Standard						
Tetra-n-propyltin	2176-98-9	10.15	10.15	10.15	10.15	10.15
Surrogates						
Tripentyltin chloride	3342-67-4	0.5	2.5	5.0	25	50
Tri-n-propyltin	995-25-5	0.5	2.5	5.0	25	50
Analytes						
Tetrabutyltin	1461-25-2	0.5	2.5	5.0	25	50
Tributyltin chloride	1461-22-9	0.5	2.5	5.0	25	50
Dibutyltin dichloride	683-18-1	0.5	2.5	5.0	25	50
Butyltin trichloride	1118-46-3	0.5	2.5	5.0	25	50

3.0 QUANTITATIVE DETERMINATION OF BUTYLTINS BY GC/FPD

3.1 INITIAL CALIBRATION

A 5-point relative response factor (RRF) calibration curve is established for analytes of interest prior to the analysis of samples and quality control (QC) samples. An RRF is determined for each analyte for each calibration level using the following equation:

$$RRF = \frac{(A_A)(C_{IS})}{(A_{IS})(C_A)}$$

Where:

A_A = the area of the analyte to be measured

A_{IS} = the area of the specific internal standard

C_A = the known concentration of the analyte in the calibration solution (ng Sn/mL)

C_{IS} = the known concentration of the internal standard in the calibration solution (ng Sn/mL)

The response factors determined for each calibration level are averaged to produce a mean relative response factor ($\overline{RRF_i}$) for each analyte. The percent relative standard deviation (%RSD) for the 5 response factors must be less than or equal to 15% for each analyte.

$$\%RSD = \frac{\text{Standard Deviation of the RRFs}}{\text{Average of the RRFs}} \times 100$$

Where:

$$\text{Standard Deviation} = \sqrt{\frac{\sum_{i=1}^{n}\left(x_i - \bar{x}\right)^2}{(n-1)}}$$

Where:

x_i = each RRF value used to calculate the mean RRF

$\bar{x}$ = the mean of n values

n = total number of values (5)

3.2 CONTINUING CALIBRATION

A mid-level calibration standard is analyzed at the beginning and end of each analytical set, or every 10 samples (whichever is more frequent). The daily relative response factor for each compound is compared to the mean relative response factor from the initial calibration curve and the average percent difference (RPD) of all analytes must be less than 15%. If the calibration check does not meet this criterion then the initial five-point calibration is repeated.

$$RPD = \frac{RRF_c - \overline{RRF_i}}{\overline{RRF_i}} \times 100$$

Where:

$\overline{RRF_i}$ = mean relative response factor from the most recent initial calibration (meeting technical acceptance criteria)

RRF_c = relative response factor from the continuing calibration standard

3.3 GC/FPD ANALYSIS

The initial calibration of the GC/FPD must meet the previously described criteria prior to sample analysis. Samples are analyzed in analytical sets that consist of standards, samples, and QC samples. Quality control samples are method blanks, laboratory duplicates, blank spikes, matrix spikes, and standard reference materials. An autosampler is used to inject 50 μL of all samples, standards, and QC samples into the capillary column of the GC using the following instrument conditions. Slight modifications may be necessary depending upon the analysis.

Inlet:	Large volume with solvent vent
Carrier gas:	Helium, 1.9 mL/min
Detector gas:	Hydrogen 90 mL/min:Air 105 mL/min
Make up gas:	Nitrogen 20 mL/min

Temperatures:

Base Temp:	250°C
FPD:	160°C

Oven program:

Initial oven temp:	65°C
Initial hold time:	0.18 minutes
Ramp rate:	10°C/min
Final oven temp:	240°C
Final hold time:	4 minutes

3.4 ANALYTE IDENTIFICATION

The retention time of a sample analyte must fall within 15 seconds of the retention time for the analyte in a calibration standard or retention index solution.

Data not meeting established criteria are appropriately qualified or are re-analyzed.

4.0 QUANTITATION CALCULATIONS

Sample analyte concentrations are calculated based on the concentration and response of the internal standard. The equations in section 3.1 are used to calculate the RRF of each analyte relative to the concentration and area of the internal standard in the initial calibration.

The mass (M_A) of each target analyte (ng), is calculated using the following equation:

$$M_A = \frac{\left(A_A M_{IS}\right)}{\left(A_{IS}\overline{RRF_i}\right)}$$

Where:

A_A = the area of the analyte measured

A_{IS} = the area of the specific internal standard

M_{IS} = mass of internal standard added to the extract (ng)

$\overline{RRF_i}$ = the average relative response factor for the analyte from the current calibration

The concentration (C) of each target analyte in a sample (in ng Sn/g) is calculated using the following equation:

$$C = \frac{(M_A V_E DF)}{(V_{inj} W)}$$

Where:

Ve = final volume of the extract (μL)

Vinj = volume of the sample injected onto the column (μL)

W = the weight of the sample (g)

DF = the dilution factor applied to the extract

$$DF = \frac{\text{Volume of Extract } (\mu L)}{\text{Volume of extract used to make dilution } (\mu L)}$$

Analyte concentrations are reported as corrected for surrogate recovery. Percent surrogate recovery ($SU_{Recovery}$) is calculated using the following:

$$SU_{Recovery} = \frac{C_{ESU}}{C_{SU}} x100$$

Where:

C_{ESU} = calculated surrogate concentration in the extract (ng Sn/mL)

C_{SU} = known concentration of surrogate added to extract (ng Sn/mL)

Analyte concentration corrections ($C_{corrected}$) for surrogate recovery are calculated using the following equation:

$$C_{Corrected} \frac{C}{SU_{Recovery}} x\,100$$

5.0 QUALITY CONTROL (QC)

Samples are analyzed in analytical batches consisting of 19 samples or fewer and QC samples. The QC samples are a method blank, laboratory duplicate, matrix spike, matrix spike duplicate, and standard reference material (SRM), if available. A method blank is a reagent blank prepared in the laboratory. A duplicate is a sample for which a second aliquot is analyzed. Matrix spikes are samples that are spiked with known concentrations of known analytes. The SRM is a reference material with known analyte concentrations. All SRMs are certified and traceable to the Canadian Research Council (NRC). A SRM

is not available for tissues. The validity of the data is monitored using defined QC criteria. The following QC criteria are used to evaluate analytical batches:

1). Calibration

- The calibration criteria (Section 3.2) must be met prior to data analyses. If the calibration criteria are not met, then the run is aborted and the instrument re-calibrated before further sample analysis.

2). Method Blank

- No more than one target analyte may exceed 3 times the concentration of the MDL.

- Exceptions are that if an analyte detected in the method blank exceeds 3 times the concentration of the MDL but is not present in the associated samples, or if a sample analyte concentration is greater than 10 times that analyte concentration in the method blank, the result is qualified and reported.

- If a method blank exceeds these criteria then the source of contamination is determined and corrective action is taken before further sample analysis.

3). Matrix Spikes

- Analytes spiked into a matrix are considered valid only if they are spiked at concentrations equivalent to levels found in the sample.

- The average recovery for all valid spiked analytes in a matrix spike is between 60% and 120%. No more than one individual spiked analyte (valid) recoveries may exceed 40%-120%, with the exception of monobutyltin.

- If the QC criteria are not met then the matrix spike sample failing the criteria will be re-analyzed and if the re-analyzed spike meets the criteria then the data are reported.

- If an analyte exceeds the criteria and is not present in the associated samples analyzed with the analytical batch, the result is qualified and reported.

- If upon re-analysis, QC criteria are still not met, the entire batch of samples is reanalyzed. If sufficient sample is unavailable to re-extract the matrix-spike, another sample may be selected or a blank-spike may be substituted.

- The average RPD for a valid matrix spike/matrix spike duplicate or blank spike/blank spike duplicate pair is 30%. No more than two individual analyte RPDs may exceed 35%.

4). Duplicate

- The average RPD between the duplicate and original sample, for analytes greater than 10 times the concentration of the MDL, is 30%. The RPD for no more than two individual analytes may exceed 35%.

- If the QC criteria are not met then the sample pair failing the criteria will be re-analyzed and if the re-analyzed samples meet the criteria then the data are reported.

- If an analyte exceeds the criteria and is not present in the associated samples analyzed with the analytical batch, the result is qualified and reported.

- If upon re-analysis, QC criteria are still not met, the entire batch of samples is reanalyzed. If sufficient sample is unavailable to re-extract the duplicate pair, another sample may be selected.

5). Standard Reference Material

- The average recovery for target analytes in a SRM should not exceed 30% of the upper and lower bounds of the mean certified values. No more than two target analytes should deviate more than 35% from the upper or lower bounds of the mean certified values.

- If the QC criteria are not met then the SRM failing the criteria will be re-analyzed and if the re-analyzed SRM meets the criteria then the data are reported.

- If an analyte exceeds the criteria and is not present in the associated samples analyzed with the analytical batch, the result is qualified and reported.

- If upon re-analysis, QC criteria are still not met, the entire batch of samples is reanalyzed.

6). Surrogates

- The average recovery of surrogate compounds is between 50% and 150% with the exception of monobutyltim.

- Exceptions are analytical interferences with the surrogates and diluted samples.

- If the average recovery of surrogates exceeds the criteria, and calculation and analytical errors are eliminated, the sample is re-analyzed. If sufficient sample is unavailable for re-extraction, the data are qualified and reported.

7). Method Detection Limit

- The method detection limit (MDL) is determined following the procedures outlined in Federal Register (1984).

6.0 REFERENCES

Federal Registry (1984) Vol. 49, No. 209:198-199.

APPENDIX A. METHOD DETECTION LIMITS

Table A-1. Reporting units for organic contamiants measured by the Mussel Watch Project.

Analysis	Reporting Unit
BT Analysis by GC/FPD	ng Sn/dry g
PAH Analysis by GC/MS	ng/dry g
PCB and PEST Analysis by GC/ECD	ng/dry g

Table A-2. Mussel Watch Project tissue butyltin method limits of detection (ng Sn/g dry weight).

	2000	2001	2002	2003	2004
Sample size	**1.15 g**	**1.15 g**	**1.15 g**	**1.15 g**	**1.15 g**
Monobutyltin	1.95	1.95	1.95	0.56	0.56
Dibutyltin	3.6	3.6	3.6	4.88	4.88
Tributyltin	4.83	4.83	4.83	6.82	6.82
Tetrabutyltin	2.73	2.73	2.73	6.53	6.53

Table A-3. Mussel Watch Project sediment butyltin method limits of detection (ng Sn/g dry weight).

	2003	2004
Sample size	**1.15 g**	**15.0 g**
Monobutyltin	2.54	0.71
Dibutyltin	0.40	0.41
Tributyltin	0.27	0.56
Tetrabutyltin	0.21	0.49

Table A-4. Mussel Watch Project tissue polycyclic aromatic hydrocarbon method limits of detection (ng/g dry weight).

	2000	2001	2002	2003	2004
Sample size	**3 g**	**2.5 g**	**2.5 g**	**2.1 g**	**2.1 g**
Decalin				5.9	5.9
C1-Decalin				5.9	5.9
C2-Decalin				5.9	5.9
C3-Decalin				5.9	5.9
C4-Decalin				5.9	5.9
Naphthalene	2.2	2.3	2.3	9.03	9.03
C1-Naphthalenes	1.6	4.6	4.6	9.03	9.03
C2-Naphthalenes	1.4	4.6	4.6	9.03	9.03
C3-Naphthalenes	1.4	4.6	4.6	9.03	9.03
C4-Naphthalenes	1.4	4.6	4.6	9.03	9.03
Benzothiophene				3.94	3.94
C1-Benzothiophene				3.94	3.94
C2-Benzothiophene				3.94	3.94
C3-Benzothiophene				3.94	3.94
Biphenyl	1.5	2	2	2.45	2.45
Acenaphthylene	0.7	0.5	0.5	2.22	2.22
Acenaphthene	0.6	0.4	0.4	2.12	2.12
Dibenzofuran				2.22	2.22
Fluorene	0.7	0.6	0.6	2.48	2.48
C1-Fluorenes	1.5	1.1	1.1	2.48	2.48
C2-Fluorenes	1.5	1.1	1.1	2.48	2.48
C3-Fluorenes	1.5	1.1	1.1	2.48	2.48
Anthracene	2	1.7	1.7	1.18	1.18
Phenanthrene	2.2	1.5	1.5	3.63	3.63
C1-Phenanthrenes/Anthracenes	4.4	2.9	2.9	3.63	3.63
C2-Phenanthrenes/Anthracenes	4.4	2.9	2.9	3.63	3.63
C3-Phenanthrenes/Anthracenes	4.4	2.9	2.9	3.63	3.63
C4-Phenanthrenes/Anthracenes	4.4	2.9	2.9	3.63	3.63
Dibenzothiophene	3.5	0.5	0.5	1.78	1.78
C1-Dibenzothiophenes	7.1	1.1	1.1	1.78	1.78
C2-Dibenzothiophenes	7.1	1.1	1.1	1.78	1.78
C3-Dibenzothiophenes	7.1	1.1	1.1	1.78	1.78
Fluoranthene	2.1	0.8	0.8	9.02	9.02
Pyrene	1.9	1.4	1.4	5.71	5.71
C1-Fluoranthenes/Pyrenes	4.1	1.6	1.6	9.02	9.02
C2-Fluoranthenes/Pyrenes				9.02	9.02
C3-Fluoranthenes/Pyrenes				9.02	9.02

Table A-4 (cont'd). Mussel Watch Project tissue polycyclic aromatic hydrocarbon method limits of detection (ng/g dry weight).

	2000	2001	2002	2003	2004
Sample size	**3 g**	**2.5 g**	**2.5 g**	**2.1 g**	**2.1 g**
Naphthobenzothiophene				2.94	2.94
C1-Naphthobenzothiophene			2.94	2.94	5.9
C2-Naphthobenzothiophene			2.94	2.94	5.9
C3-Naphthobenzothiophene			2.94	2.94	5.9
Benz[*a*]anthracene	3.3	1	1	3.16	3.16
Chrysene	3.8	0.9	0.9	5.08	5.08
C1-Chrysenes	6.6	1.8	1.8	5.08	5.08
C2-Chrysenes	6.6	1.8	1.8	5.08	5.08
C3-Chrysenes	6.6	1.8	1.8	5.08	5.08
C4-Chrysenes	6.6	1.8	1.8	5.08	5.08
Benzo[*b*]fluoranthene	1.6	0.8	0.8	3.83	3.83
Benzo[*k*]fluoranthene	1.2	0.7	0.7	2.82	2.82
Benzo[*e*]pyrene	1.6	1.9	1.9	2.68	2.68
Benzo[*a*]pyrene	1.2	1.3	1.3	1.63	1.63
Perylene	1.5	2	2	5.35	5.35
Indeno[*1,2,3-c,d*]pyrene	1.2	1	1	3.34	3.34
Dibenzo[*a,h*]anthracene	1	0.4	0.4	2.36	2.36
C1-Dibenzo[*a,h*]anthracene				2.36	2.36
C2-Dibenzo[*a,h*]anthracene				2.36	2.36
C3-Dibenzo[*a,h*]anthracene				2.36	2.36
Benzo[*g,h,i*]perylene	1.4	0.7	0.7	2.39	2.39
2-Methylnaphthalene	1.1	0.6	0.6		
1-Methylnaphthalene	0.7	0.3	0.3		
2,6-Dimethylnaphthalene	0.7	0.5	0.5		
1,6,7-Trimethylnaphthalene	1.2	1	1		
1-Methylphenanthrene	1.2	1.3	1.3		

Table A-5. Mussel Watch Project sediment polycyclic aromatic hydrocarbon method limits of detection (ng/g dry weight).

	2003	2004
Compound	**15.0 g**	**15.0 g**
Decalin	0.17	0.17
C1-Decalin	0.35	0.35
C2-Decalin	0.35	0.35
C3-Decalin	0.35	0.35
C4-Decalin	0.35	0.35
Naphthalene	0.17	0.17
C1-Naphthalenes	0.33	0.33
C2-Naphthalenes	0.35	0.35
C3-Naphthalenes	0.35	0.35
C4-Naphthalenes	0.35	0.35
Benzothiophene	0.17	0.17
C1-Benzothiophene	0.35	0.35
C2-Benzothiophene	0.35	0.35
C3-Benzothiophene	0.35	0.35
Biphenyl	0.14	0.14
Acenaphthylene	0.19	0.19
Acenaphthene	0.13	0.13
Dibenzofuran	0.2	0.2
Fluorene	0.19	0.19
C1-Fluorenes	0.39	0.39
C2-Fluorenes	0.39	0.39
C3-Fluorenes	0.39	0.39
Carbazole	0.33	0.33
Anthracene	0.19	0.19
Phenanthrene	0.14	0.14
C1-Phenanthrenes/Anthracenes	0.29	0.29
C2-Phenanthrenes/Anthracenes	0.29	0.29
C3-Phenanthrenes/Anthracenes	0.29	0.29
C4-Phenanthrenes/Anthracenes	0.29	0.29
Dibenzothiophene	0.15	0.15
C1-Dibenzothiophenes	0.31	0.31
C2-Dibenzothiophenes	0.31	0.31
C3-Dibenzothiophenes	0.31	0.31
Fluoranthene	0.21	0.21
Pyrene	0.19	0.19
C1-Fluoranthenes/Pyrenes	0.39	0.39
C2-Fluoranthenes/Pyrenes	0.39	0.39
C3-Fluoranthenes/Pyrenes	0.39	0.39

	2003	2004
Compound	**15.0 g**	**15.0 g**
Naphthobenzothiophene	0.2	0.2
C1-Naphthobenzothiophene	0.41	0.41
C2-Naphthobenzothiophene	0.41	0.41
C3-Naphthobenzothiophene	0.41	0.41
Benz[a]anthracene	0.13	0.13
Chrysene	0.17	0.17
C1-Chrysenes	0.35	0.35
C2-Chrysenes	0.35	0.35
C3-Chrysenes	0.35	0.35
C4-Chrysenes	0.35	0.35
Benzo(b)fluoranthene	0.29	0.29
Benzo(k)fluoranthene	0.23	0.23
Benzo(e)pyrene	0.31	0.31
Benzo(a)pyrene	0.22	0.22
Perylene	1.38	1.38
Indeno[*1,2,3-c,d*]pyrene	0.28	0.28
Dibenzo(a,h)anthracene	0.15	0.15
C1-Dibenzo[*a,h*]anthracene	0.31	0.31
C2-Dibenzo[*a,h*]anthracene	0.31	0.31
C3-Dibenzo[*a,h*]anthracene	0.31	0.31
Benzo[*g,h,i*]perylene	0.14	0.14
2-Methylnaphthalene		
1-Methylnaphthalene		
2,6-Dimethylnaphthalene		
1,6,7-Trimethylnaphthalene		
1-Methylphenanthrene		

Table A-6. Mussel Watch Project pesticide tissue method limits of detection (ng/g dry weight).

	2000	2001	2002	2003	2004
Sample size	**3 g**	**2.5 g**	**2.5 g**	**2.1 g**	**2.1 g**
Aldrin	0.23	0.25	0.25	0.24	0.24
Dieldrin	0.27	0.37	0.37	0.22	0.22
Endrin	0.38	0.24	0.24	0.21	0.21
Heptachlor	0.33	0.28	0.28	0.25	0.25
Heptachlor-Epoxide	0.28	0.3	0.3	0.23	0.23
Oxychlordane	0.52	0.27	0.27	0.28	0.28
Alpha-Chlordane	0.59	0.29	0.29	0.23	0.23
Gamma-Chlordane	0.71	0.29	0.29	0.27	0.27
Trans-Nonachlor	0.44	0.22	0.22	0.22	0.22
Cis-Nonachlor	0.4	0.26	0.26	0.24	0.24
Alpha-HCH	0.43	0.25	0.25	0.23	0.23
Beta-HCH	0.27	0.26	0.26	0.23	0.23
Delta-HCH	0.43	0.23	0.23	0.23	0.23
Gamma-HCH	0.37	0.25	0.25	0.22	0.22
2,4'-DDD	0.34	0.29	0.29	0.22	0.22
4,4'-DDD	0.57	0.34	0.34	0.2	0.2
2,4'-DDE	0.28	0.25	0.25	0.21	0.21
4,4'-DDE	0.51	0.24	0.24	0.22	0.22
2,4'-DDT	0.3	0.25	0.25	0.25	0.25
4,4'-DDT	0.47	0.25	0.25	0.21	0.21
1,2,3,4-Tetrachlorobenzene	0.26	0.29	0.29	0.33	0.33
1,2,4,5-Tetrachlorobenzene	0.38	0.27	0.27	0.3	0.3
Hexachlorobenzene	0.51	0.24	0.24	0.25	0.25
Pentachloroanisole	0.32	0.28	0.28	0.18	0.18
Pentachlorobenzene	0.28	0.26	0.26	0.22	0.22
Endosulfan II	0.72	0.26	0.26	0.25	0.25
Endosulfan I	0.72	0.26	0.26	0.25	0.25
Endosulfan Sulfate	0.72	0.29	0.29	0.27	0.27
Mirex	0.42	0.28	0.28	0.23	0.23
Chlorpyrifos	0.47	0.28	0.28	0.25	0.25

Table A-7. Mussel Watch Project pesticide sediment method limits of detection (ng/g dry weight).

	2003	2004
Sample size	**15.0 g**	**15.0 g**
Aldrin	0.1	0.1
Dieldrin	0.06	0.06
Endrin	0.12	0.12
Heptachlor	0.09	0.09
Heptachlor-Epoxide	0.18	0.18
Oxychlordane	0.05	0.05
Alpha-Chlordane	0.04	0.04
Gamma-Chlordane	0.05	0.05
Trans-Nonachlor	0.04	0.04
Cis-Nonachlor	0.07	0.07
Alpha-HCH	0.09	0.09
Beta-HCH	0.07	0.07
Delta-HCH	0.08	0.08
Gamma-HCH	0.05	0.05
2,4'-DDD	0.07	0.07
4,4'-DDD	0.11	0.11
2,4'-DDE	0.05	0.05
4,4'-DDE	0.04	0.04
2,4'-DDT	0.1	0.1
4,4'-DDT	0.08	0.08
1,2,3,4-Tetrachlorobenzene	0.12	0.12
1,2,4,5-Tetrachlorobenzene	0.08	0.08
Hexachlorobenzene	0.05	0.05
Pentachloroanisole	0.06	0.06
Pentachlorobenzene	0.06	0.06
Endosulfan II	0.1	0.1
Endosulfan I	0.1	0.1
Endosulfan Sulfate	0.11	0.11
Mirex	0.04	0.04
Chlorpyrifos	0.1	0.1

Table A-8. Mussel Watch Project polychlorinated biphenyl tissue method limits of detection (ng/g dry weight).

	2000	2001	2002	2003	2004
Sample size	**3 g**	**2.5 g**	**2.5 g**	**2.1 g**	**2.1 g**
PCB8	0.77	0.39	0.39	0.36	0.36
PCB18	0.8	0.52	0.52	0.44	0.44
PCB28	0.54	0.45	0.45	0.22	0.22
PCB44	0.63	0.35	0.35	0.4	0.4
PCB52	0.26	0.48	0.48	0.24	0.24
PCB66	0.67	0.43	0.43	0.34	0.34
PCB101	0.43	0.66	0.66	0.32	0.32
PCB105	0.85	0.41	0.41	0.33	0.33
PCB118	0.35	0.67	0.67	0.25	0.25
PCB128	0.83	0.43	0.43	0.54	0.54
PCB138	0.58	0.87	0.87	0.43	0.43
PCB153	0.97	0.67	0.67	0.49	0.49
PCB170	0.51	0.81	0.81	0.32	0.32
PCB180	0.84	0.58	0.58	0.24	0.24
PCB187	0.45	0.62	0.62	0.31	0.31
PCB195	0.34	0.33	0.33	0.27	0.27
PCB206	0.33	0.31	0.31	0.29	0.29
PCB209	0.32	0.36	0.36	0.25	0.25

Table A-9. Mussel Watch Project polychlorinated biphenyl sediment method limits of detection (ng/g dry weight).

	2003	2004
Sample size	**15.0 g**	**15.0 g**
PCB8	0.1	0.1
PCB18	0.06	0.06
PCB28	0.05	0.05
PCB44	0.1	0.1
PCB52	0.05	0.05
PCB66	0.04	0.04
PCB101	0.04	0.04
PCB105	0.1	0.1
PCB118	0.06	0.06
PCB128	0.11	0.11
PCB138	0.06	0.06
PCB153	0.06	0.06
PCB170	0.06	0.06
PCB180	0.06	0.06
PCB187	0.04	0.04
PCB195	0.04	0.04
PCB206	0.04	0.04
PCB209	0.07	0.07

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