NOAA Technical Memorandum NOS ORCA 71

National Status and Trends Program
for Marine Environmental Quality

Sampling and Analytical Methods of the National Status and Trends Program National Benthic Surveillance and Mussel Watch Projects 1984-1992

Volume IV

Comprehensive Descriptions of Trace Organic Analytical Methods

Silver Spring, Maryland
July, 1993

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Coastal Monitoring and Bioeffects Assessment Division
Office of Ocean Resources Conservation and Assessment
National Ocean Service

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G. G. Lauenstein and A. Y. Cantillo
(Editors)

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United States Department of Commerce — Ronald H. Brown, Secretary
National Oceanic and Atmospheric Administration — D. James Baker, Under Secretary
National Ocean Service — W. Stanley Wilson, Assistant Administrator

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PREFACE

The quantification of environmental contaminants and their effects by the National Oceanic and Atmospheric Administration's National Status and Trends Program began in 1984. Polycyclic aromatic hydrocarbons, butyltins, polychlorinated biphenyls, DDTs and other chlorinated pesticides, trace and major elements, and a number of measures of contaminant effects are quantified in estuarine and coastal samples. Two of the major monitoring components in this program are: the National Benthic Surveillance Project, which is responsible for quantification of contamination in fish tissue and sediments, and measurement of the biological significance of environmental contamination; and the Mussel Watch Project, which monitors pollutant concentrations by quantifying contaminants in mollusk bivalves and sediments. A series of four documents describing the methods for sample collection and preparation, and chemical biological and ancillary measurements made by these two projects have been developed. The evolution of methods, method detection limits, and the Quality Assurance Project are also discussed in these documents.

This document is Volume IV of this series. It contains detailed descriptions of the analytical methods used for the determination of trace organic compounds in sediments and tissues by laboratories participating in the NS&T Program (Tables P.1 and P.2).

Table P.1. Laboratories analyzing National Status and Trends Program National Benthic Surveillance Project samples for trace organic compounds and pertinent chapters in this document. Names listed with laboratories are authors of pertinent chapters.

National Benthic Surveillance Project

Year	1984-1987	1988-present
Northeast Coast	NEFSC Gadbois *et al.*	NWFSC Sloan *et al.*
Southeast and Gulf Coasts	SEFSC Fortner and Sivertseen	NWFSC Sloan *et al.*
West Coast	NWFSC MacLeod *et al.*	NWFSC Sloan *et al.*

NEFSC - NOAA/NMFS/Northeast Fisheries Science Center, Gloucester, MA.
SEFSC - NOAA/NMFS/Southeast Fisheries Science Center, Charleston, SC.
NWFSC - NOAA/NMFS/Northwest Fisheries Science Center, Seattle, WA.

Table P.2. Laboratories analyzing National Status and Trends Program Mussel Watch Project samples for trace organic compounds and pertinent chapters in this document. Names listed with laboratories are authors of pertinent chapters.

Mussel Watch Project

Year	1986-1987	1988	1989	1990-present
East Coast	Battelle (Peven and Uhler)	Battelle (same)	Battelle (same)	Battelle (same)
Gulf Coast	TAMU (Wade *et al.*)	TAMU (same)	TAMU (same)	TAMU (same)
West Coast				
California	SAIC (Peven and Uhler)	SAIC (same)	SAIC (same)	Battelle (same)
Oregon	Battelle (Peven and Uhler)	Battelle (same)	Battelle (same)	Battelle (same)
Washington	Battelle (Peven and Uhler)	Battelle (same)	Battelle (same)	Battelle (same)
Alaska	Battelle (Peven and Uhler)	NS -	Battelle (same)	Battelle (same)
Hawaii	SAIC (Peven and Uhler)	NS -	SAIC (same)	Battelle (same)

NS - Not sampled.
Battelle - Battelle Ocean Sciences, Duxbury, MA, and Sequim, WA.
TAMU - Geochemical and Environmental Research Group of Texas A&M University, College Station, TX.
SAIC - Science Applications International Corporation, Inc.

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Editors

Coastal Monitoring and Bioeffects Assessment Division
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INTRODUCTION

The National Status and Trends (NS&T) Program began in 1984 with the National Benthic Surveillance Project (NBSP). Analytical methods for the quantification of organic contaminants by the NS&T NBSP were developed by MacLeod *et al*. (1984) at the Northwest Fisheries Science Center of NOAA's National Marine Fisheries Service (NMFS) in Seattle, WA. These methods were prescribed for use by the cooperating NMFS laboratories of the NS&T NBSP. These laboratories were: NMFS/Northeast Fisheries Science Center, Gloucester, MA; NMFS/Southeast Fisheries Science Center, Charleston, SC; and NMFS/NWFSC, Seattle, WA. The MacLeod *et al*. (1984) methods were updated in 1985 (MacLeod *et al*., 1985).

The NS&T Mussel Watch Project (MWP) began in 1986. The contract laboratories were: Battelle Ocean Sciences, Duxbury, MA, and Sequim, WA; the Geochemical and Environmental Research Group of Texas A&M University, College Station, TX; and Science Applications International Corporation, Inc. By 1986, the NS&T cooperating laboratories involved in the NBSP and the MWP were allowed to use any method if it could be proven that the proposed alternate procedure was equal to or better than that originally prescribed. To ensure that data remained comparable among laboratories, the scope of the NS&T Quality Assurance effort pertaining to the quantification of organic contaminants was increased.

The method of MacLeod *et al*. (1985) is placed first in this volume because all the NMFS and Battelle Ocean Sciences laboratories participating in the NS&T Program relied heavily upon it. Readers should note that the methods developed by MacLeod *et al*., (1985) are no longer used by NMFS laboratories participating in the NS&T Program and <u>are not</u> to be considered NOAA protocols for the quantification of organic contaminants.

References

MacLeod, W. D., Jr., D. W. Brown, A. S. Friedman, O. Maynes, and R. Pearce (1984) Standard analytical procedures of the NOAA National Analytical Facility, 1984-85: Extractable Toxic Organic Compounds. NOAA Tech. Memo. NMFS F/NWC-64. NOAA/NMFS/NWAFC, Seattle, WA. 110 pp.

MacLeod, W. D., Jr., D. W. Brown, A. J. Friedman, D. G. Burrows, O. Maynes, R. W. Pearce, C. A. Wigren, and R. G. Bogar (1985) Standard analytical procedures of the NOAA National Analytical Facility, 1985-86: extractable toxic organic compounds. Second Edition. NOAA Tech. Memo. NMFS F/NWC-92. NOAA/NMFS/NWAFC, Seattle, WA. 121 pp.

Standard Analytical Procedures of the NOAA National Analytical Facility, 1985-1986 (Revised) Extractable Toxic Organic Compounds

W. D. MacLeod Jr., D. W. Brown, A. J. Friedman, D. G. Burrows, 0. Maynes,
R. W. Pearce, C. A. Wigren, and R. G. Bogar
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Revised/Edited by A. Cantillo, C. Sloan, and G. Lauenstein

ABSTRACT

This document describes the analytical methods for trace organic compounds in sediments and tissues developed by the NOAA/National Marine Fisheries Service/National Analytical Facility. These methods were used in many NOAA projects and formed the basis of the NOAA National Status and Trends Program trace organic analytical methodology. This document is a revision of NOAA Technical Memorandum NMFS F/NWC-92.

1. PREFACE

The analytical procedures for marine environmental samples described in this document were the result of nine years of methods development and application by the National Analytical Facility (NAF) of the National Oceanic and Atmospheric Administration (NOAA). These procedures were the basis of the trace organic analytical methodology used in the National Status and Trends (NS&T) Program. This is a revised version of the Second Edition, NOAA Tech. Memo. NMFS F/NWC-92 (MacLeod *et al.,* 1985), which in turn incorporates additions to and revisions of NOAA Tech. Mem. NMFS F/NWC-64, which is hereby superseded. NOAA Tech. Memo. NMFS F/NWC-92 served as the initial laboratory manual for use by analytical chemists working on the NS&T National Benthic Surveillance Project and National Mussel Watch Project.

[This method is no longer used in the NS&T program. Extensive modifications to these procedures have been made by NOAA laboratories and contractors since publication of NOAA Technical Memorandum NMFS F/NWC-92, which was superseded by NOAA Technical Memorandum NMFS F/NWC-153. This method description has been edited for clarification purposes only. Ed. Note.]

2. INTRODUCTION

Numerous studies demonstrate associations between organic chemical contamination of the aquatic environment and impacts on environmental health and, potentially, on human health. If the results of one study are to be compared with those of another, uniform analytical methods for the chemicals will be required. To meet this need, NOAA's NAF prepared this Technical Memorandum as a methods manual for extractable organic chemicals in marine sediments and tissues. It applies specifically to the organic analytes selected for monitoring by the NS&T Program at its inception in 1984 (Table IV.1).

Table IV.1. Organic chemicals determined in 1985 as part of the NOAA National Status and Trends Program [Polychlorinated biphenyl (PCB) congener nomenclature uses method of Ballschmitter and Zell (1980)].

Polycyclic aromatic hydrocarbons

Biphenyl
Naphthalene
1-Methylnaphthalene
2-Methylnaphthalene
2,6-Dimethylnaphthalene
Acenaphthene
Fluorene
Phenanthrene
1-Methylphenanthrene
Anthracene
Fluoranthene
Pyrene
Benz[*a*]anthracene
Chrysene
Benzo[*a*]pyrene
Benzo[*e*]pyrene
Perylene
Dibenz[*a,h*]anthracene

DDT and metabolites

2,4'-DDD	4,4'-DDD
2,4'-DDE	4,4'-DDE
2,4'-DDT	4,4'-DDT

Chlorinated pesticides other than DDT

Aldrin	Heptachlor epoxide
cis-Chlordane	Hexachlorobenzene
Dieldrin	Mirex
gamma-HCH*	*trans*-Nonachlor
Heptachlor	

Polychlorinated biphenyls congeners

PCB 7◊	2,4-Dichlorobiphenyl
PCB 31	2,4',5-Trichlorobiphenyl
PCB 47	2,2',4,4'-Tetrachlorobiphenyl
PCB 101	2,2',4,5,5'-Pentachlorobiphenyl
PCB 153	2,2',4,4',5,5'-Hexachlorobi= phenyl
PCB 185	2,3,4,5,6,2',5'-Heptachloro= biphenyl
PCB 194	2,3,4,5,2',3',4',5'-0ctachloro= biphenyl
PCB 206	2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl

Coprostanol

* Also known as Lindane.

◊ *[PCB 7 was the reference PCB congener supplied by NAF, during the first years of the NS&T Program, this congener was used to determine the response factor for the quantification of dichlorobiphenyls. The development of PCB quantitation in the NS&T Program is discussed in detail in Volume I. Eds. Note]*.

Over the years, NAF methods have found wide application in environmental studies concerning the Strait of Juan de Fuca (MacLeod *et al.* 1977; Brown *et al.* 1979), the New York Bight (MacLeod *et al.* 1981), and Puget Sound (Malins *et al.* 1980, 1982), among others.

[Subsequent to publication of NOAA Technical Memorandum NMFS F/NWC-92, these procedures were also used in environmental studies concerning Puget Sound (e.g., Varanasi et al., 1985) and the vicinity of Los Angeles (Malins et al., 1987), and in cooperative projects with the US Army Corps of Engineers Seattle District (e.g. Chan et al., 1986). Ed. Note.]

As these methods are neither simple nor inexpensive, it is most important that the best analytical techniques available be employed and that improvements be continually sought. Thus, evolution of the methodology is assured. This manual will be updated periodically as methods improve.

[As stated above, new methodology has been developed by the NS&T laboratories and the methods described in this document are no longer in use. Ed. Note.]

2.1. Quality of Analytical Data

Horwitz and co-workers (1980) observed that the uncertainty in the analytical results in interlaboratory comparisons increases in a regular progression as the concentrations of the particular analyte descend from fractions of a percent to parts-per-million to parts-per-billion (ppb). According to their studies, standard deviations for interlaboratory comparisons of means around 10 ppb should not be expected to be better than 35% of the grand mean. Our experience (MacLeod *et al.* 1982) has shown this thesis to be realistic, but it often dismays or confounds statisticians, modelers, and administrators. Nevertheless, the issue must be faced, and the best possible precision must be secured for the analytical results.

In implementing these procedures during the first year of the NS&T Program, the following quality assurance protocols have been observed. First, the procedures were validated statistically in NAF's laboratories, consistent with the Horwitz model. Then, NAF distributed calibrating solutions and previously analyzed sample extracts to the participating laboratories for testing their gas chromatographs. Once consistent and satisfactory results were obtained, interim reference materials were supplied to the laboratories to assess the proficiencies of the overall analytical procedures. This was repeated on a continuing basis throughout the performance of analyses. Our constant goal is to have interlaboratory standard deviations that conform as closely as possible to the Horwitz model.

2.2. Summary of Analytical Procedures

In general, analyses of sediment and organisms follow the scheme shown in Figure IV.1, as summarized below:

Thaw the sample (if frozen), remove excess water, and weigh the wet sample (approximately 10 g for sediment or 3 g for tissue) to the nearest 0.01 g. Add the extraction solvent (dichloromethane) and internal standards (I-Stds), then mix/grind/extract sample with sodium sulfate three times under dichloromethane. Combine the solvent extracts and concentrate them by boiling. Chromatograph the extract concentrate on silica gel and alumina, and collect fractions eluted with pentane (fraction SA1) and 50% dichloromethane in pentane (fraction SA2). For sediment samples only, continue to elute with dichloromethane followed by 10% methanol in dichloromethane, then collect fraction SA3 eluted with 20% methanol in dichloromethane. Concentrate fraction SA2 by boiling and then chromatograph it on precalibrated Sephadex LH-20. Collect the second fraction from Sephadex chromatography (fraction SA2-L2), and concentrate it to 1 mL if from sediment, or to 0.1 mL if from tissue. Analyze fraction SA2-L2 from sediment and tissue (except liver) for aromatic hydrocarbons by capillary gas chromatography (GC) with a flame ionization detector (FID). Analyze fraction SA2-L2 from all samples for chlorinated hydrocarbons using an electron-capture detector (ECD). If hexachlorobenzene is found, also analyze fraction SA1 for this compound as with fraction SA2-L2. Concentrate fraction SA3 from sediments and analyze it by GC/FID for coprostanol.

The procedures summarized above are presented in detail in the sections that follow, each of which deals with a major analytical operation.

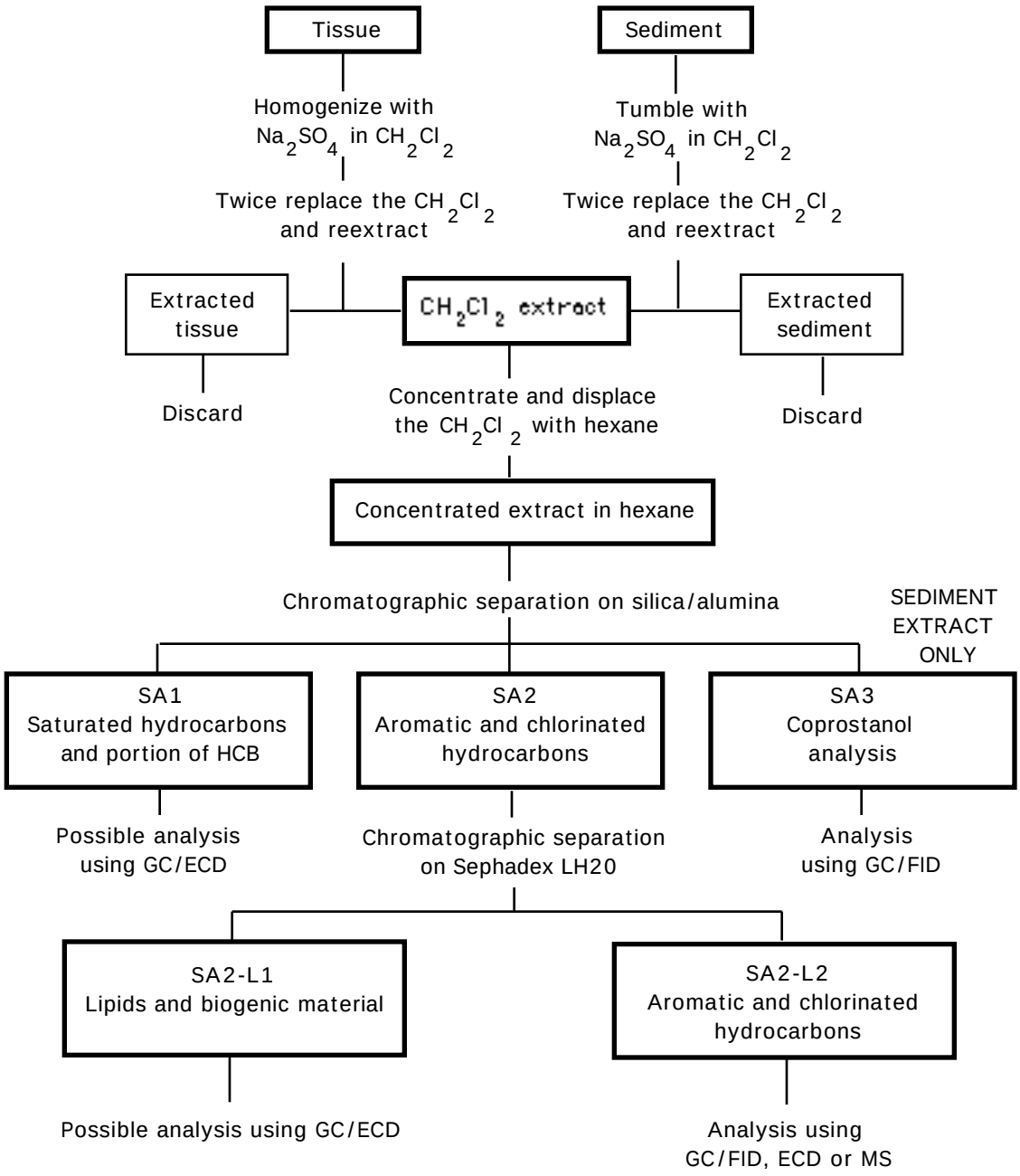

Figure IV.1. Schematic of analytical procedures.

3. MATERIALS

3.1. Instruments and accessories

3.1.1. Gas chromatograph

Gas Chromatograph (GC), Hewlett-Packard 5880A, including capillary column inlet system. Hewlett-Packard, Avondale, PA.
- Autosampler, Hewlett-Packard 7672A
- 5880A Series GC terminal, Level Four with BASIC
- Cartridge tape unit
- Flame-ionization detector (FID)
- Electron-capture detector (ECD)

The following modifications were made to the GC system. A graphite O-ring was placed around the injector insert instead of the usual Viton O-ring. A Viton O-ring was installed beneath the septum. A slot was cut in two 1 x 1-inch, 1/32-inch thick aluminum plates. These plates were inserted from opposite sides around the injection port, just above the gas lines, and between the septum retainer assembly and the insert retainer assembly. A 1/16-inch tube was installed to blow compressed air gently onto the cooling fins.

3.1.2. Gas cylinders and accessories

Molecular sieve traps, Hydro-Purge ASC-I. Coast Engineering Laboratory, Gardena, CA.
Regulators, 2-stage oxygen traps. J & W Scientific, Inc., Folsom, CA.

3.1.3. GC column and accessories

Brush for cleaning fused-silica liner
Column, DB-5 fused silica column, 30-m, 0.25 mm i.d., 0.25 µm film thickness, 122-5032. J & W Scientific, Inc., Folsom, CA.
Diamond-tip etcher
Ferrules; 0.4-mm graphite, 2004. J & W Scientific, Inc., Folsom, CA.
Flow meters, 4068 (suitable for all gases used). Alltech, Deerfield, IL.
Glass wool
Jeweler's loupe, 10x
Leak detector, Snoop. Nupro Co., Willoughby, OH.
O-ring, 0.208-inch i.d., Viton. Parker Seal Co.
Septum, 9.5 mm, blue, 6514. Alltech Associates, Deerfield, IL.
Adapters and connectors. Swagelok Co., Solon, OH.
Syringe, 10-µL, 701N, 80300. Hamilton, Reno, NV.
Tubing, 1/8-in o.d., copper
Vials, 2-mL, 96-000099-00. Varian, San Fernando, CA.
Vials, 100-µL, conical, Teflon-faced silicon septum and screw cap, 986281. Wheaton, Millville, NJ.

3.2. Chemicals

3.2.1. Solvents

All solvents must be checked for purity using GC-ECD and GC-FID analysis before use.

Cyclohexane (C_6H_6) [110-82-7]
Dichloromethane (CH_2Cl_2) [75-09-2]
Hexamethylbenzene ($C_{12}H_{18}$) [87-85-4]
Hexane (C_6H_{14}) [110-54-3]
Methanol (CH_30H) [67-56-1]
Pentane (C_5H_{12}) [109-66-0]

3.2.2. Reagents and other chemicals

Azulene (cyclopentacycloheptene, $C_{10}H_{18}$) [275-51-4]
Copper (Cu), reagent grade, fine granular
Sodium sulfate (Na_2S0_4) [7757-82-6], reagent grade, anhydrous granular

3.2.3. Standards

These compounds are 95% pure or better.

1-Methylnaphthalene
1-Methylphenanthrene
2,4'-DDD
2,4'-DDE
2 4'-DDT
4,4'-DDD
4,4'-DDE
4,4'-DDT
4,4'-Dibromooctafluorobiphenyl
5α-Androstan-3β-ol
cis-Chlordane
Acenaphthene
Acenaphthene-d_{10}
Aldrin
Anthracene
Benzo[*a*]pyrene
Benzo[*e*]pyrene
Benz[*a*]anthracene
Biphenyl
Chrysene
Coprostanol
Dibenz[*a,h*]anthracene
Dieldrin
Fluoranthene
Fluorene
Heptachlor
Heptachlor epoxide
Hexachlorobenzene
gamma-HCH
Mirex
Naphthalene
Naphthalene-d_8
PCB 7
PCB 31
PCB 47
PCB 101
PCB 153
PCB 185
PCB 194
PCB 206
Perylene
Perylene-d_{12}
Phenanthrene
Pyrene
Tetrachloro-m-xylene
trans-Nonachlor

3.2.4. Air and gases

Air, breathing air, USP Grade E (or equivalent)
Argon:methane, 95:5 (v:v) Grade P-5
Helium, grade 4.5 (purified, 99.995%)
Hydrogen, grade 5 (ultra pure, 99.999%)
Nitrogen, grade 4.5 (purified, 99.995%)

3.3. Column packings

Alumina, 80-200 mesh, F-20. Sigma Chemical Co., St. Louis, MO.
Copper, fine granular, 4649. Mallinckrodt, Darmstadt, Germany.
Sand, Ottawa, kiln dried, 30-40 mesh, SX0075-3. EM Science, Gibbstown, NJ.
Silica gel, Amicon No. 84040
Size-exclusion gel, Sephadex LH-20 25-100 µ particle size. Sigma Chemical Co., St. Louis, MO.

3.4. Labware

Adapter, 45/50-STJ to 24/40-STJ
Balance, analytical, 2200-g capacity, 0.0001-g readability, Mettler AT100
Balance, top-loading, ≥1000-2000 g capacity, 0.01 g readability, Mettler PL1200
Boiling chips, Teflon, Chemplast, Chemware. Norton, Worcester, MA.
Bottle, 4-L standard solvent equipped with volumetric dispenser, 50-mL Brinkman Dispensette, 50030407
Bottle, 250-mL tumbler/centrifuge, amber, Boston round, with Teflon cap
Carboy, 20-L, glass with Teflon-lined stopper
Centrifuge, cups to accommodate 250-mL bottles and 100-mL tubes
Clamp, Hoffman
Column, 3-ball Snyder, 24/40-STJ
Column, chromatography, 30 cm, 19-mm i.d.
Condenser, Corning 2400 or Kimble 18140
Desiccator with desiccant
Fittings, miscellaneous
Flask, 1-L Erlenmeyer, with stopper, 24/40-STJ
Flask, 5-L round bottom receiver, with a 24/40-STJ port
Flask, 22-L round bottom boiling, with 24/40-STJ and 71/60-STJ ports, and a thermometer well
Flask, 22-L round bottom receiver, with a 45/50-STJ port
Flask, 500-mL Erlenmeyer, with stopper, 24/40-STJ
Foil, aluminum, 12-inch width
Forceps
Fractionation column, 5 cm x 50 cm, 24/40-STJ, packed with 7-mm lengths of 6-mm glass tubing
Funnel adapter, 200-mm o.d., long-stem, 45/50-STJ to 24/40-STJ
Funnel, 200-mm o.d., long-stem
Funnel, powder, 15 mm o.d. stem
Graduated cylinder, 2-L TC, Kimax Single Metric Scale TC, 200392000
Heating mantle for 22-L flask
Lab Jax, 59298-1. Baxter, McGaw Park, IL.
Oven, drying (120°C)
Pipet filler, 3-valve, rubber, for volumetric pipet
Pipet, 50-mL
Pipet, transfer, Pasteur style, with rubber bulbs
Rock tumbler, modified NF-l, (Belt guard was removed). Lortone Inc., Seattle, WA.
Spatula, stainless steel
Stillhead with 10/30-STJ thermometer port
Syringe, 100-µL, 710N 80600. Hamilton, Reno, NV.
Syringe, 1000-µL, 1001 LTN. Hamilton, Reno, NV.
Temperature controllers, automatic, models 63RC and 74. YSI, Yellow Srings, OH.
Three-way receiver valve, 8-mm bore Teflon stopcock
Timing clock
Tissue macerator, SDT-1819. Tekmar, Cincinnati, OH.
Transformer, Variac
Tube heater, modified, aluminum inserts bored to fit 25-mL concentrator tube and glass shroud, Kontes 720000-0000
Tube, concentrator, 25-mL 19/22-STJ Kontes, with stopper, 570050-2525
Vials, 2-mL GC vials
Vortex Genie, S8233. American Scientific Products, McGaw Park, IL.
Wash bottle, 500-mL, Teflon (for use with dichloromethane)
Water bath
Weighing pan, aluminum. D2165. Baxter, McGaw Park, IL.
White correction fluid
Yellow fluorescent and/or incandescent lights
Yellow transparent acetate sheeting for laboratory windows

3.5. Internal-standard solutions and solvents

3.5.1. Internal standard solutions

3.5.1.1. HMB GC internal standard solution

The HMB (hexamethylbenzene) GC/I-Std (gas chromatography internal standard) is prepared to contain 100 ng/µL of hexamethylbenzene in hexane.

3.5.1.2. TCMX GC internal standard solution

The TCMX (tetrachloro-m-xylene) GC/I-Std is prepared to contain 2 ng/µL of tetrachloro-m-xylene in hexane.

3.5.1.3. AH internal standard solution

The AH (aromatic hydrocarbon) GC/I-Std is prepared to contain 50 ng/µL of each of the following compounds in hexane:

Naphthalene-d_8
Acenaphthene-d_{10}
Perylene-d_{12}

3.5.1.4. PES internal standard solution

The PES (pesticides) I-Std is prepared to contain 1 ng/µL of 4,4'-dibromooctafluorobiphenyl in hexane.

3.5.1.5. COP internal standard solution

The COP (coprostanol) I-Std is prepared to contain 50 ng/µL of 5α-Androstan-3ß-ol in hexane.

3.5.2. Calibration solutions

3.5.2.1. AH GC calibration check solution

The AH calibration check solution is prepared to contain 5 ng/µL in hexane of each of the following compounds:

1-Methylnaphthalene
1-Methylphenanthrene
Acenaphthene
Acenaphthene-d_{10} (I-Std)
Anthracene
Benzo[*a*]pyrene
Benzo[*e*]pyrene
Benz[*a*]anthracene
Biphenyl
Chrysene
Dibenz[*a,h*]anthracene
Fluoranthene
Fluorene
Hexamethylbenzene (GC/I-Std)
Naphthalene
Naphthalene-d_8 (I-Std)
Perylene
Perylene-d_{12} (I-Std)
Phenanthrene
Pyrene

3.5.2.2. PES calibration check solution

The PES calibration check solution is prepared to contain 0.1 ng/µL in hexane of each of the following compounds:

2,4'-DDD
2,4'-DDE
2,4'-DDT
4,4'-DDD
4,4'-DDE
4,4'-DDT
4,4'-Dibromooctafluorobiphenyl (I-Std)
cis-Chlordane
Dieldrin
Heptachlor
Heptachlor epoxide
Hexachlorobenzene
gamma-HCH
Mirex
PCB 7
PCB 31
PCB 47
PCB 101
PCB 153
PCB 185
PCB 194
PCB 206
Tetrachloro-m-xylene (GC/I-Std)
trans-Nonachlor

3.5.2.3. COP GC calibration check solution

The COP calibration check solution is prepared to contain 5 ng/µL of each of the following compounds in hexane:

Hexamethylbenzene (GC/I-Std)
5α-Androstan-3ß-ol
Coprostanol

3.5.3. Spike solutions

3.5.3.1. AH spike solution

The AH spike solution is prepared to contain 50 ng/µL in hexane of each of the following compounds:

1-Methylnaphthalene
1-Methylphenanthrene
2-Methylnaphthalene
2,6-Dimethylnaphthalene
Acenaphthene
Anthracene
Benzo[*a*]pyrene
Benzo[*e*]pyrene
Benz[*a*]anthracene
Biphenyl
Chrysene
Dibenz[*a,h*]anthracene
Fluoranthene
Fluorene
Naphthalene
Perylene
Phenanthrene
Pyrene

3.5.3.2. PES spike solution

The PES spike solution is prepared to contain approximately 1 ng/µL in hexane of each of the following compounds:

2,4'-DDD	gamma-HCH
2,4'-DDE	Mirex
2,4'-DDT	PCB 7
4,4'-DDD	PCB 31
4,4'-DDE	PCB 47
4,4'-DDT	PCB 101
Aldrin	PCB 153
cis-Chlordane	PCB 185
Heptachlor	PCB 194
Heptachlor epoxide	PCB 206
Hexachlorobenzene	*trans*-Nonachlor

3.5.3.3. COP spike solution

The COP spike solution is prepared to contain 50 ng/µL of coprostanol in hexane.

3.5.4. Solvents

3.5.4.1. 6:4 Cyclohexane-methanol azeotrope preparation

Wash all glassware, including the distillation apparatus, twice with dichloromethane before each run. Distillation apparatus is shown in Figure IV.2.

Attach a 5-L round-bottom flask to the rear port of a 3-way receiver valve, and set the receiver valve to collect the distillate into the flask. Attach the 22-L round-bottom receiver flask to the front port of the 3-way receiver valve.

Place a heating mantle around the 22-L boiling flask. Place the stillhead on the top of the fractionation column, and fit the column into the 24/40-STJ port of the boiling flask. Align the stillhead outlet and the condenser inlet fittings, then secure the ball and socket joint with the Hoffman clamp.

Wash the glass sensor probe of the Model 74 temperature controller twice with dichloromethane, and set it firmly into the 10/30-STJ port at the top of the stillhead. Fill the thermometer well of the boiling flask with corn oil, then insert the probe of the Model 63RC temperature controller into the thermometer well.

Place a large funnel in the 71/60-STJ port, then fill the boiling flask with 10 L of cyclohexane and 8 L of methanol. Add 40-50 Teflon boiling chips and attach the flask to the fractionation column. Turn on the condenser cooling water. Set the Variac at 60, the Model 74 temperature controller at 55.5°C, and the Model 63RC temperature controller at 68°C. Start the distillation by switching on the timer.

Collect 3 L of 6:4 azeotrope forerun during the first 6 hr in the 5-L receiver flask. Then switch the receiver valve to collect most of the distillate in the 22-L receiver during the next 24 hr.

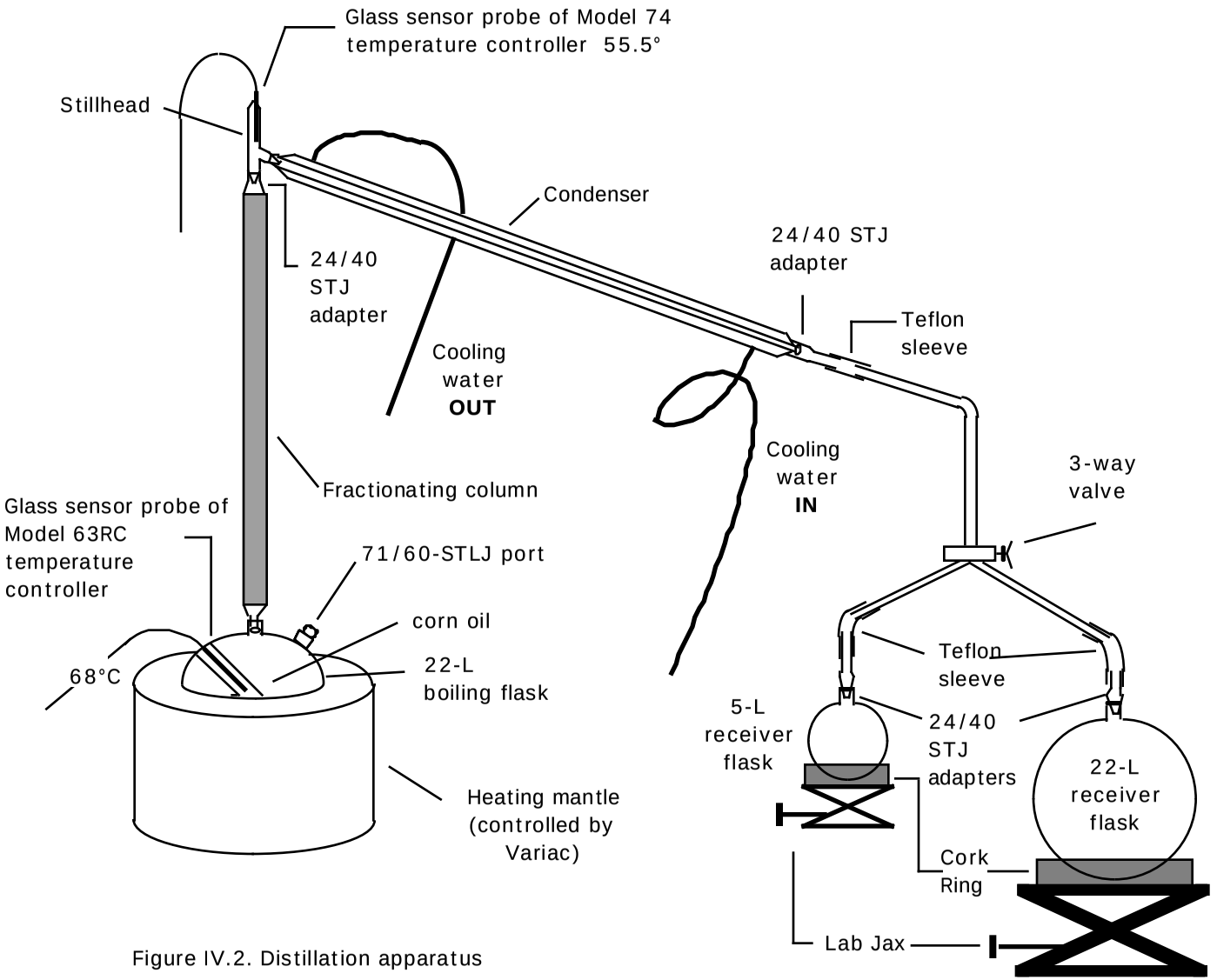

Figure IV.2. Distillation apparatus

As the solvent temperature in the boiling flask rises toward 65°C, distillation slows. Only a small amount (approximately 1 L) of mostly methanol, will remain undistilled. At this time, switch the timer off to stop the distillation.

Allow the distillation apparatus to cool and then disassemble it. Transfer the undistilled solvent and the forerun to 4-L glass bottles and set aside for recycling.* Discard the boiling chips. Wash the apparatus and the 22-L boiling flask twice with dichloromethane. Reassemble the apparatus as before and pour only the 6:4 azeotropic distillate (a 2-phase mixture) from the 22-L receiver back into the boiling flask. Add 40-50 boiling chips.

Make sure that the cooling water is flowing. All settings remain the same. Switch timer on, and distill 1 L of forerun into the 5-L flask. Switch the receiver valve so as to collect most of the distillate in the 22-L receiver. Distill until the solvent level (approximately 1 L) reaches the bottom of the thermometer well. Switch off the timer.

Allow the apparatus to cool. Remove the large receiver flask. Discard the boiling chips and set the undistilled solvent aside for recycling.

3.5.4.2. Redistilled methanol

[The methanol used in the following sections must be of high purity. If acceptable methanol is not available commercially, it can be prepared by distilling commercial methanol or by redistilling methanol from 6:4 cyclohexane:methanol azeotrope. Ed. Note.]

To prepare redistilled methanol, add one tenth volume of carbon-filtered distilled water to a volume of 6:4 cyclohexane:methanol azeotrope. Allow the phases to separate in a separatory funnel. Drain the lower phase (water and methanol) into a boiling flask. The upper phase remaining in the separatory funnel, mostly cyclohexane, can be recycled. Adjust the Variac to setting 70, the Model 74 temperature controller to 66°C, and the Model 63RC temperature controller to 75°C. Distill pure methanol through the fractionation column. Check the methanol purity using the procedure described in Section 3.5.5.1.

3.5.4.3. Preparation of 6:4:3 cyclohexane:methanol:dichloromethane solvent

Prepare a sample of 6:4:3 solvent for purity testing by pipetting 200 mL each of the upper and lower layers of the 6:4 cyclohexane:methanol azeotrope into a 1-L Erlenmeyer flask. Add 120 mL of dichloromethane to the flask and mix well. Check the purity of the solvent using the procedure described in Section 3.5.5.2.

If the purity of the sample from the step above is acceptable, proceed to the next step. Otherwise, return the remaining 6:4 azeotrope to the boiling flask for redistillation.

Transfer 200 mL of the upper layer and 200 mL of the lower layer of the 6:4 azeotrope into a carboy in 2-L increments, noting the total volume. Multiply the total volume of the 6:4 azeotrope by 0.30. This is the volume of dichloromethane to be added to the 6:4 azeotrope to make the 6:4:3 solvent. Add the calculated amount of dichloromethane to the carboy.

Stopper the carboy and mix the 6:4:3 solvent until it is completely homogeneous. Transfer the 6:4:3 solvent into 4-L solvent bottles for storage until use.

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* The boiling flask residues and foreruns may be saved and recycled in a subsequent distillation. However, they should not be recycled more than twice.

3.5.5. Solvent purity testing

When a solvent sample (except methanol) is analyzed using gas chromatography, no GC peaks should occur within 0.1 min of an analyte peak. Moreover, no peaks after the retention time of naphthalene for GC analysis using a flame ionization detector (FID) or tetrachloro-m-xylene for GC analysis using an electron capture detector (ECD) should give a deflection of greater than 5% on the GC chart. Methanol, used solely for washing, should show no GC peaks greater than 100% of the GC chart after the retention time of naphthalene (GC/FID) or tetrachloro-m-xylene (GC/ECD). If any peaks are observed, then the solvents must be purified prior to use.

3.5.5.1. Methanol and redistilled methanol purity testing

Duplicate samples* and a blank must be analyzed.

3.5.5.1.1. Extraction

Add 100 mL of methanol or redistilled methanol and 25 mL of dichloromethane to a 500-mL separatory funnel. Swirl the funnel for a few seconds to mix well. Add 250 mL of carbon-filtered, distilled water to the separatory funnel, and shake it vigorously for 2 min. Allow the phases to separate well.

Drain the lower (dichloromethane) phase into a 500-mL 24/40-STJ Erlenmeyer flask with stopper, leaving behind any emulsion layer. Save the contents of the flask. This is flask A.

Add 10 mL of dichloromethane to the separatory funnel and shake it vigorously for 2 min. Allow the phases to separate well. Drain the lower phase (dichloromethane) into flask A, including any emulsion layer. Discard the contents (methanol and water) of the separatory funnel.

Pour the extract from flask A back into the separatory funnel. Wash the flask with 3-4 mL of dichloromethane and add the washings to the separatory funnel. Repeat the washing step of the flask with dichloromethane. This flask is no longer needed.

Repeat the above steps starting with the addition of 250 mL of carbon-filtered distilled water to the separatory funnel. Use a clean Erlenmeyer flask, labeled B, but do not include the emulsion layer in the last step.

3.5.5.1.2. Concentration

Add 3-4 boiling chips to the flask B and attach a Snyder column. Concentrate the extract in a 60°C water bath to between 10 and 15 mL. Transfer the extract to a labeled 25-mL concentrator tube with stopper.

Add a boiling chip to the tube and, using the tube heater, concentrate the sample to between 0.9 and 1.0 mL. Add 50 µL of HMB GC/I-Std solution and 50 µL of TCMX GC/I-Std solution to the extract and mix on the Vortex Genie for 2 sec at setting 8 - 10.

* Each sample is considered to be one 100-mL portion of methanol.

Transfer equal amounts of the extract to two 2-mL GC vials, cap the vials, and label them. Store one of the vials as a reserve. Save for GC analysis. Purity must be assured before proceeding to sample extraction.

3.5.5.1.3. Procedure for blank

Proceed as in Section 3.5.5.1.1 and 3.5.5.1.2 above, except omit the addition of 100 mL of methanol and perform only a single analysis.

3.5.5.2. 6:4:3 Cyclohexane:methanol:dichloromethane solvent purity testing

Analyze duplicate samples and a blank.

3.5.5.2.1. Extraction

Transfer 100 mL of the 6:4:3 solvent to a 500-mL Erlenmeyer flask with stopper. Add 3-4 boiling chips and attach a Snyder column to the flask.

Concentrate the sample in a 75°C water bath to between 10 and 15 mL. Transfer the sample to a 25-mL concentrator tube with stopper. Do not wash the flask with dichloromethane.

Add a boiling chip and 1 mL of redistilled methanol to the tube and, using the tube heater, concentrate the sample to between 0.9 and 1.0 mL. Add 7 mL of high purity hexane to the tube and concentrate the sample to between 0.9 and 1.0 mL.

Add 50 µL of HMB GC/I-Std solution and 50 µL of TCMX GC/I-Std solution to the sample, and mix on the Vortex Genie at setting 8-10 for 2 sec.

Transfer equal amounts of the sample to two 2-mL GC vials, cap the vials, and label them. Store one of the vials as a reserve. Save for GC analysis.

3.5.5.2.2. Procedure for blank

Prepare a blank by adding 1 mL of redistilled methanol and 7 mL of high purity hexane to a Kontes concentrator tube. Concentrate the solvents to between 0.9 and 1.0 mL.

Add 50 µL of HMB GC/I-Std solution and 50 µL of TCMX GC/I-Std solution to the sample, and mix on the Vortex Genie at setting 8-10 for 2 sec.

Transfer equal amounts of the sample to two 2-mL GC vials, cap the vials, and label them. Store one of the vials as a reserve. Save for GC analysis.

3.5.5.3. Dichloromethane

Analyze duplicate samples plus a sample of the lot currently in use.

Transfer 350 mL of dichloromethane to a 500-mL Erlenmeyer flask with stopper. Add 3-4 Teflon boiling chips and attach a Snyder column to the flask.

Reduce the sample volume in a 60°C water bath to between 10 and 15 mL to concentrate any solvent impurities present. Transfer the sample to a 25-mL concentrator tube with stopper. Do not wash the flask with dichloromethane.

Add a boiling chip to the tube, and using the tube heater, reduce the sample volume to between 0.9 and 1.0 mL.

Add 50 µL of HMB GC/I-Std solution and 50 µL of TCMX GC/I-Std solution to the sample, and mix on the Vortex Genie at setting 8-10 for 2 sec.

Transfer equal amounts of the sample to two 2-mL GC vials, cap the vials, and label them. Store one of the vials as a reserve. Save for GC analysis.

3.5.5.4. Pentane

Analyze duplicate samples of each lot to be tested as well as the lot currently in use.

Transfer 100 mL of pentane to a 500-mL Erlenmeyer flask with stopper. Add 3-4 boiling chips and attach a Snyder column to the flask.

Reduce the sample volume in a 55°C water bath to between 10 and 15 mL. Transfer the sample to a 25-mL concentrator tube with stopper. Do not wash the flask with dichloromethane.

Add a boiling chip and 1 mL of redistilled methanol to the tube and, using the tube heater, reduce the volume to between 0.9 and 1.0 mL.

Add 50 µL of HMB GC/I-Std solution and 50 µL of TCMX GC/I-Std solution to the sample and mix on the Vortex Genie at setting 8-10 for 2 sec.

Transfer equal amounts of the sample to two 2-mL GC vials, cap the vials, and label them. Store one of the vials as a reserve. Save for GC analysis.

3.5.5.5. Hexane

Analyze duplicate samples for each lot to be tested, plus a sample of the hexane lot currently in use.

Add 25 mL of hexane to a concentrator tube. Add a boiling chip to the tube and, using the tube heater, reduce the sample volume to between 0.9 and 1.0 mL.

Add 50 µL of HMB GC/I-Std solution and 50 µL of TCMX GC/I-Std solution to the sample and mix on the Vortex Genie at setting 8-10 for 2 sec.

Transfer equal amounts of the sample to 2 labeled GC vials and cap the vials. Save for GC analysis.

3.6. Lot testing and calibration of silica gel/alumina column packing

3.6.1. Column packing activation

3.6.1.1. Silica gel

Heat the silica gel at 700°C for 18 hr. Store it at 170°C. Allow it to cool to room temperature in a desiccator before weighing and use.

[The silica gel is heated to remove contaminants. Ed. Note.]

3.6.1.2. Alumina

Activate the alumina by heating at 120°C for 2 hr. Allow it to cool to room temperature in a desiccator just before weighing and use.

[The alumina is activated by heating for 2 hr to obtain consistent activity whenever it is used. Ed. Note.]

3.6.1.3. Copper

Less than one hr before use, activate the copper by covering it with concentrated HCl, stirring with a glass rod, and allowing it to stand for 5 min. Wash the copper twice with methanol and then three times with dichloromethane. Leave the copper covered with dichloromethane to avoid contact with air.

[When copper is activated, the oxidized sites are removed so it can efficiently remove sulfur from the sample extracts by forming copper sulfide. If sulfur is not removed from the extracts, it interferes with quantitation of chlorinated hydrocarbons by GC/ECD. Ed. Note.]

3.6.1.4. Sand

Soak the sand in aqua regia overnight. The aqua regia is a 1:3 v:v mix of ACS reagent grade nitric and hydrochloric acids. The sand is washed three times each with water, methanol and dichloromethane, dried, and stored at 120°C.

[The sand is soaked in aqua regia to remove active sites Ed. Note.]

3.6.1.5. Silica gel/alumina calibration extract

Extract 10 samples each of control (clean) sediment and of control mussel using procedures in Section 5 and 6. Combine 2 mL each of the 20 extracts and add 10 mL of AH spike solution, 1 mL of PES spike solution, and coprostanol spike solution to give a final concentration of approximately 2 µg/mL. The final volume should be approximately 55 mL.

[The individual extracts are combined and spike solutions added to provide an extract pool for calibrating multiple silica/alumina columns and to allow the same elution volumes to be used for both tissues and sediments. Ed. Note.]

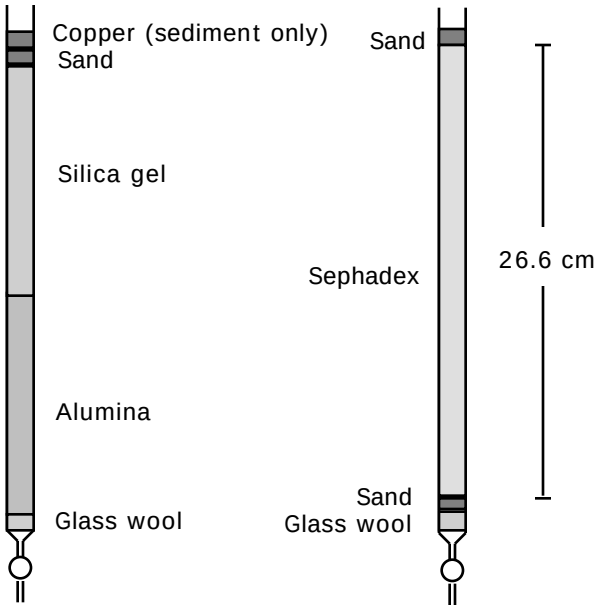

Figure IV.3. Schematic diagram of silica gel/alumina and Sephadex column packings.

3.6.2. Column preparation

The laboratory ambient temperature must be lower than 80°F (27°C). Prepare the columns just prior to use. On warm days proceed more slowly to avoid vapor bubbles. Column schematics are shown in Figure IV.3.

Fit a 19-mm i.d. column with a stopcock and add 100 mL of dichloromethane. Insert a glass-wool plug, 5 to 15 mm high. Tap the plug down with a glass rod to remove any bubbles.

Place 10 g of activated alumina in a 250-mL beaker and slowly add 20 mL of dichloromethane. Gently swirl the beaker for 30 sec and let it stand for 5 min to remove all air bubbles until used in a subsequent step.

Add 20 g of activated silica gel to a second beaker. Slowly add 40 mL of dichloromethane to the beaker. Gently swirl the beaker for 30 sec and let it stand for 5 min to remove all air bubbles until used in a subsequent step.

Place a curved-stem funnel into the column reservoir so that the funnel tip hangs well off-center. The funnel will be used to add the slurries and solvents.

Swirl the alumina to resuspend the solids and pour the slurry through the funnel into the column. Wash the beaker with approximately 5 mL of dichloromethane and add the washings to the column. Repeat the wash twice. Place the beaker under the column. After the particles settle, open the stopcock for 30 sec to allow the alumina to pack more tightly, then close the stopcock.

Add the silica gel following the steps used for the alumina. After the silica gel has settled, open the stopcock.

While the solvent is still draining, add 1 cc of acid-washed sand and 7.5 cc of activated copper through the powder funnel. Lower the solvent level to the packing top, then close the stopcock.

Add 50 mL of pentane to the column. Lower to the packing top, then close the stopcock. Discard the eluates collected thus far.

3.6.3. Column calibration

Using a 2500-µL syringe, place 2 mL of the silica-gel/alumina calibration extract on top of the packing. Place a 25-mL concentrator tube, labeled SA1.1, beneath the column. Open the stopcock, lower to the packing top, then close the stopcock.

Add 0.5 mL of the remaining pentane to the packing. Open the stopcock. Lower to the packing top, and close the stopcock. Repeat this step again.

Add the remainder of the pentane (approximately 49 mL) to the column and elute at approximately 3 mL/min until 20 mL has been collected in the concentrator tube. Close the stopcock and discard the eluate. This is the column dead volume.

Place the concentrator tube under the column again and collect 15 mL of eluate. Close the stopcock and set aside the tube for use in the fraction concentration step in Section 3.6.4.

Replace the tube with one labeled SA1.2 and collect 2.0 mL. Close the stopcock and set aside the tube for use in the fraction concentration step in Section 3.6.4.

Using tubes labeled successively SA1.3 to SA1.11, repeat the step above nine times. Add 200 mL of 1:1 dichloromethane:pentane (v:v) to the column when the pentane in the column drains to the packing top. Set aside the tubes for use in the fraction concentration step in Section 3.6.4.

Place a concentrator tube labeled SA2.1 under the column and collect 20 mL of eluate. Close the stopcock and set aside the tube for use in the fraction concentration step in Section 3.6.4.

Using tubes labeled successively SA2.2 to SA2.10, and repeat the fraction collection process nine times. Add 50 mL of dichloromethane to the column when the level of the dichloromethane:pentane solution reaches the packing top.

Place a 500-mL Erlenmeyer flask labeled "waste" under the column and lower the remaining solvent to the packing top.

Add 25 mL of 10% methanol in dichloromethane to the column and lower to the packing top at approximately 2 mL/min. Close the stopcock. Discard the contents of the waste flask and replace it with a 500-mL Erlenmeyer flask with stopper labeled SA3.

Add 30 mL of 20% methanol in dichloromethane to the column and elute all of the solvent into the SA3-labeled flask.

3.6.4. Fraction concentration

Add 3-4 Teflon boiling chips and attach a Snyder column to the SA3 flask. Concentrate the fraction in a 70°-75°C water bath to between 10 and 15 mL. Transfer the fraction to a concentrator tube labeled SA3.

Wash down the SA3 flask with 3-4 mL of dichloromethane and add the washings to the concentrator tube. Repeat the washing step once.

Add a boiling chip to each concentrator tube (SA1.1 - SA1.11, SA2.1 - SA2.10, and SA3) and concentrate each fraction to between 0.9 and 1.0 mL using the tube heater.

Add 7 mL of hexane to the SA3 tube and 2 mL of hexane to each of the other tubes. Concentrate each fraction to between 0.9 and 1.0 mL using the tube heater.

Add 50 µL of HMB GC/I-Std solution and 50 µL of TCMX GC/I-Std solution to each fraction and mix each on the Vortex Genie at setting 8-10 for 2 sec.

Transfer each fraction to labeled 2-mL GC vials, cap the vials, and save for GC analysis.

Establish the elution volumes using GC analysis for the SA1, SA2 and SA3 fractions, such that all alkanes elute in the SA1 fraction; coprostanol and androstanol elute in the SA3 fraction; and all other analytes and internal standards elute in the SA2 fraction. The elution volumes for the SA1, SA2, and SA3 fractions are used in Section 8.

3.7. Sephadex LH-20 column preparation and calibration

3.7.1. Calibration solutions

3.7.1.1. Azulene/perylene calibration solution

Add approximately 500 mg of azulene and approximately 50 mg of perylene to approximately 50 mL of 6:4:3 solvent to produce a deeply colored solution. Make sure that the azulene and perylene are completely dissolved.

[The azulene/perylene calibration solution is used to evaluate the chromatographic performance of the Sephadex LH-20 column. See Section 3.7.3. Ed. Note.]

3.7.1.2. Sediment/tissue calibration extract

Extract 10 samples each of control sediment and of control mussel tissue using procedure described in Section 3.6.3. Chromatograph these samples on silica gel/alumina as described in Sections 5 and 6. Combine the 20 SA2 fractions (approximately 2 mL each) and add 1 mL of PES spike solution and 10 mL of AH spike solution to the combined fractions. Concentrate this to 10 mL and add sufficient methanol and dichloromethane to make a 6:4:3 hexane:methanol:dichloromethane solution.

[The individual SA2 fractions are combined and spike solutions added to provide an extract pool for calibrating multiple Sephadex columns and to allow the same elution volumes to be used for both tissues and sediments. Ed. Note.]

3.7.2. Column preparation

Column schematic is shown in Figure IV.3.

Swell the Sephadex overnight in 6:4:3 solvent.

Fit a 19-mm i.d. x 30-cm column with a stopcock, add 10 mL of the 6:4:3 solvent and a 5 to 10-mm glass wool plug. Tap the plug to remove any air bubbles.

Add approximately 1 mL of acid-washed sand to the column and tap the column gently so that the sand forms a smooth layer on top of the glass wool.

Pour approximately 80 mL of swelled Sephadex gel through the funnel into the column until the gel fills the column and about one fourth of the reservoir. Allow 10 min for the Sephadex to settle. Open the stopcock, and elute 80 mL of solvent to ensure firm packing. Add more solvent as needed. Leave 30 mL of solvent in the column reservoir. Cover the top with aluminum foil and allow the packing to settle overnight.

Open the stopcock and elute 10 mL of solvent, then close the stopcock. Remove the excess Sephadex packing from the top of the column with a transfer pipet until the height of the Sephadex packing is 26.5 cm.

Gently add approximately 1 mL of sand to the top of the packing so that it forms an even layer. The column may be tapped or tilted slightly to get an even layer of sand.

Examine the packing for air bubbles. If bubbles are evident, elute approximately 250 mL of warm (approximately 35°C) solvent through the column. If the bubbles persist, recycle the packing. (See Section 3.7.6.)

3.7.3. Column calibration with azulene/perylene

Place a 100-mL graduated cylinder beneath the column. Using a transfer pipet, carefully remove any excess 6:4:3 solvent from the top of the packing as the composition of the solvent may have changed due to evaporation.

Using a transfer pipet, carefully apply 2 mL of the azulene/perylene calibration solution on top of the column. Use a circular motion to disperse the solution just above the packing. Drip the solution slowly down the column wall so as not to disturb the packing.

Open the stopcock, drain to the packing top, and close the stopcock. Add approximately 0.5 mL of 6:4:3 solvent to the top of the column. Lower to the packing top and close the stopcock. Repeat this step.

Add 100 mL of solvent, and open the stopcock. Elute the solvent until all of the perylene has emerged. Use the Mineralight UV light to monitor the perylene.

If the azulene emerges in the 50-65 mL range and the perylene emerges in the 60-80 mL range without distinct tailing, proceed to the next step. Otherwise, recycle the packing.

Discard the eluate. Add 50 mL of solvent to the column and flush the packing by eluting 50 mL into the cylinder. Again, discard the eluate. The column is now ready for the next calibration step (Section 3.7.4).

If the column is to be stored, maintain 30-50 mL of solvent in the column reservoir and cover the top with aluminum foil. If the solvent separates into 2 phases, remove it with a transfer pipet, add 80 mL of fresh solvent, and elute 50 mL.

3.7.4. Column calibration with sediment/tissue calibration extract

Select one representative column for every 10 columns made. Use the representative column for this procedure.

Remove any excess 6:4:3 solvent from the column reservoir with a transfer pipet.

Wash the column tip with dichloromethane and place a 50-mL graduated cylinder under the column. Using a transfer pipet, carefully add 2 mL of the sediment/tissue calibration extract to the column. Use a circular motion to disperse the extract just above the packing, dripping it slowly down the column wall so as not to disturb the packing. Lower to the packing top and close the stopcock.

Add approximately 0.5 mL of the 6:4:3 solvent to the column. Lower to the packing top and close the stopcock. Repeat this step once.

Add 200 mL of the 6:4:3 solvent to the column and collect 25 mL of eluate in the cylinder. Close the stopcock and discard the eluate.

Place a concentrator tube labeled L1.0 under the column and collect 5.0 mL of eluate. Close the stopcock and set the tube aside for Section 3.7.5.

Place a concentrator tube labeled L1.1 under the column and collect 1.0 mL of eluate. Close the stopcock and set the tube aside for Section 3.7.5.

Repeat the step above fourteen times, labeling the successive fractions L1.2 through L1.15.

Replace the last tube with a 50-mL graduated cylinder labeled L2.0 and collect 50 mL of eluate. Close the stopcock and transfer the eluate to an Erlenmeyer flask labeled L2.0.

Wash down the graduated cylinder with 3-4 mL of dichloromethane and add the washings to the Erlenmeyer flask. Repeat this step once. Set the flask aside for Section 3.7.5.

Place a concentrator tube labeled L2.1 under the column and collect 10 mL of eluate. Close the stopcock and set aside the tube for Section 3.7.5.

Repeat step above four times, labeling the successive fractions L2.2 through L2.5.

Place a graduated cylinder under the column and flush the packing by eluting 50 mL of the 6:4:3 solvent. Discard this eluate.

3.7.5. Fraction concentration

Add 3-4 boiling chips to the Erlenmeyer flask labeled L2.0, attach a Snyder column, and concentrate the fraction in a 75°C water bath to between 10 and 15 mL.

Transfer fraction L2.0 to a labeled concentrator tube. Wash down the flask with 3-4 mL of dichloromethane, and add the washings to the flask. Repeat the wash step once.

Add 1 mL of methanol and a boiling chip to each tube from Section 3.7.4 and the above step. Using the tube heater, concentrate each fraction to between 0.9 and 1.0 mL. Add 7 mL of hexane to each tube, and concentrate to between 0.9 and 1.0 mL.

Add 50 µL of HMB GC/I-Std solution and 50 µL of TCMX GC/I-Std solution to each fraction. Mix each fraction on the Vortex Genie for 2 sec at setting 8-10.

Transfer each fraction to a labeled 2-mL GC vial and save for GC analysis.

Verify by GC analysis that the analytes are separated from lipids. Establish the elution volumes so as to leave all analytes and internal standards in the L2 fraction.

[Lipids are identified by their broad GC peaks which can shift the retention times of other compounds.

The elution volumes established above are used for the batch of ten Sephadex columns prepared. The volume after the dead volume and before the analytes elute is the volume used for the SA2-L1 fraction in Section 9.2. The volume in which the analytes elute is the volume used for the SA2-L2 fraction in Section 9.2. Ed. Note.]

3.7.6. Recycling Sephadex column packing

This is an optional procedure.

Remove any solvent in the column reservoir. Empty the column packing into a 500 mL beaker. Rinse the column with dichloromethane to remove all the Sephadex particles, the sand, and the glass wool.

Add enough dichloromethane to the beaker to float the Sephadex particles in the upper half of the beaker. Remove all glass wool with forceps. Cover the beaker and let it stand for 1-2 hr.

Decant the floating Sephadex particles into a fritted glass funnel attached to an aspirator, leaving the sand in the beaker. Aspirate the dichloromethane from the Sephadex particles and set the Sephadex aside. Allow the Sephadex particles to swell overnight in 6:4:3 solvent before reusing.

4. SUGGESTED SAMPLE COMPOSITE PREPARATION

The methods described below are additional procedures which NAF adopted. Though they were built upon years of experience, they do not have the advantage of years of testing behind them. Thus, they were offered provisionally with the view that they might be of use to those wishing tentative recommendations. No claims were made as to their statistical validity.

4.1. Sediment composites

Remove the samples from the freezer and allow them to thaw completely. Decant the supernatant water from the top of each sample. Discard all pebbles, shells, biota, and other detritus. Using a spatula, stir each sample to homogenize thoroughly.

Remove approximately 15 g of each sample and place it into a 4-oz jar. Stir the resulting mixture thoroughly to form a homogeneous composite. Return the unused portion of each sample to the freezer.

Line the jar cap with Teflon sheeting. Cap the jar, label it with the appropriate composite sample designation, and store it in the freezer until needed.

4.2. Tissue composites

Remove the samples from the freezer and allow them to thaw completely. Using forceps and scissors, remove approximately one half of each sample and place it into a rinsed 2-oz bottle. Return the remaining half of each sample to its original container and store in the freezer.

If the combined weight of the sample portions forming the composite sample is less than 10 g, macerate and mix the composite in the 2-oz bottle using a spatula until it is thoroughly homogenized.

If the weight is greater than 10 g, macerate and mix the composite in the 2-oz bottle using the Tekmar Tissumizer for 1 min at a setting of 50.

Line the bottle top with rinsed Teflon sheeting. Cap the composite sample bottle, label it with the appropriate composite sample designation, and store it in the freezer until needed.

5. SEDIMENT EXTRACTION

5.1. Blanks and calibration solutions

Blanks and calibrations solutions are prepared for each set of samples processed as a unit.

5.1.1. Spiked blank

For each set of samples prepare a spiked blank (reagent spike)* by adding to an empty 250-mL amber centrifuge bottle with Teflon cap 100 mL of dichloromethane, 100 µL of AH I-Std solution, 100 µL of PES I-Std solution, 100 µL of COP I-Std solution, 100 µL of PES spike solution, 100 µL of AH spike solution, and 100 µL of COP spike solution.

5.1.2. Field blank

If the sample set requires a field blank (sediment blank),Δ prepare this by washing an empty sediment sample container 3 times with 10 mL of dichloromethane. Combine the washings in a bottle and add 70 mL of dichloromethane.

* The reagent spike is defined as a sample that is treated the same as a sediment sample except the spike solutions are added instead of sediment at the beginning of the procedure.

Δ The sediment blank is defined as a sample that is treated the same as a sediment sample except that the washings of an empty sediment sample container are added instead of a sediment sample. The empty container is one which has been exposed to air at the site of sample collection.

5.1.3. Blank

For each set of samples prepare a blank (reagent blank)[◊] by adding to an empty 250-mL amber centrifuge bottle with Teflon cap 100 mL of dichloromethane, 100 µL of AH I-Std solution, 100 µL of PES I-Std solution, and 100 µL of COP I-Std solution.

5.1.4. Analyte calibration solutions

Prepare two AH/PES analyte calibration solutions by adding to each of two 2-mL GC vials 600 µL of hexane, 100 µL of AH spike solution, 100 µL of AH I-Std solution, 100 µL of PES spike solution, and 100 µL of PES I-Std solution.

Prepare two COP analyte calibration solutions by adding to each of two 2-mL GC vials 800 µL of hexane, 100 µL of COP I-Std solution, and 100 µL of COP spike solution.

5.2. Sample extraction

A schematic diagram of this procedure is shown in Figure IV.4.

Decant the excess water from the sediment and homogenize it by stirring. Discard all pebbles, shells, biota, and other detritus. Weigh 10 ± 0.5 g of sediment to the nearest 0.01 g into a tared 250-mL amber tumbler/centrifuge bottle with Teflon cap. Store the remaining sample in a freezer.

Centrifuge each sample bottle at more than 1500 rpm for 5 min. Decant and discard the supernatant.

Add 100 mL of dichloromethane, 100 µL of AH I-Std solution, 100 µL of PES I-Std solution, and 100 µL of COP I-Std solution to each sediment sample. Make certain that the standard solutions are placed directly into the dichloromethane to avoid loss by evaporation.

Add 50 g of dichloromethane-washed anhydrous Na_2S0_4 to each sediment and blank bottle. Screw each bottle cap on tight enough to prevent leakage. Tape the cap to the bottle crosswise over the top with 2 strips of masking tape. Manually shake each bottle until the contents are loose, then roll for 16 hr (overnight) on a tumbler at 100 to 250 rpm.

Remove the tape from each bottle. If the sample does not immediately settle, centrifuge at 1500 rpm for 5 min.

Decant each extract into a labeled 500-mL Erlenmeyer flask with stopper. Add 100 mL of dichloromethane to each sediment or blank sample and repeat above steps, except roll each bottle for 6 hr (during the day instead of overnight). Decant the second extract into the flask from the first extraction.

Repeat the extraction step except roll each bottle for 16 hr (overnight). Add the third extract to those of the first and second.

[◊] The reagent blank is defined as a sample that is treated the same as a sediment sample in the laboratory except that no sediment is added at the beginning of the procedure.

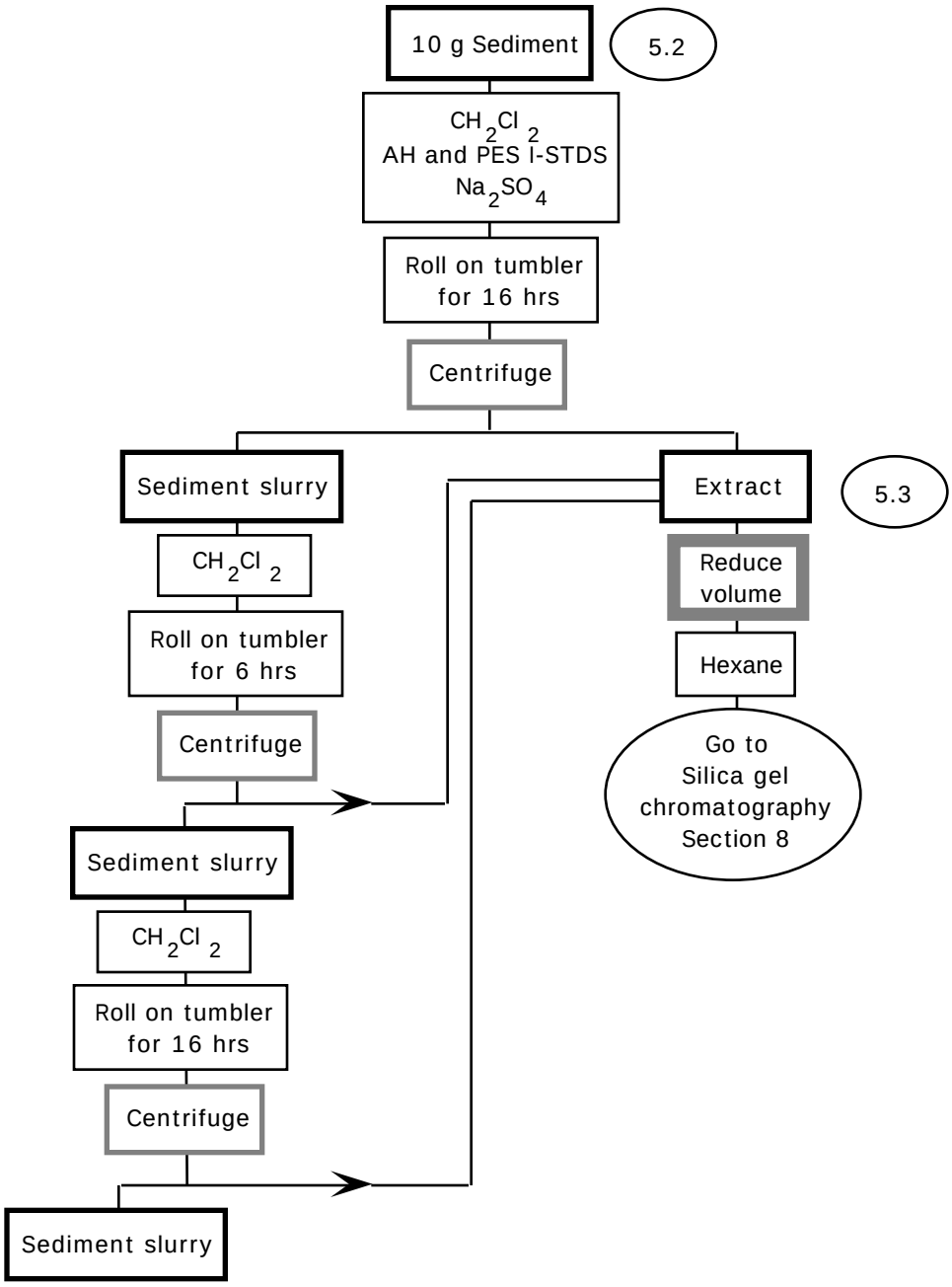

Figure IV.4. Sediment extraction scheme.

5.3. Extract concentration

Add 3-4 Teflon boiling chips to the flasks containing the combined dichloromethane extracts and attach a Snyder column. Concentrate the extract in a 60°C water bath to between 10 and 15 mL and transfer it to a labeled 25-mL concentrator tube with stopper. Wash down the flask with 3-4 mL of dichloromethane and add the washings to the tube. Repeat the washing step.

Add a boiling chip to each tube and, using a tube heater, concentrate the extract to between 0.9 and 1.0 mL. Add 3 mL of hexane to each tube and concentrate the extract to 2 mL using the tube heater.

Proceed to Silica Gel/Alumina chromatography (Section 8).

6. TISSUE EXTRACTION

[Liver samples are not analyzed for aromatic hydrocarbons, so the AH I-Std and AH Spike solutions do not need to be used for liver samples. The mixed function oxidase system in fish liver metabolizes AHs into more water soluble compounds which are excreted into the bile. Ed. Note.]

6.1. Blanks and calibration solutions

Blanks and calibrations solutions are prepared for each set of samples processed as a unit.

6.1.1. Spiked blank

For each set of samples prepare a spiked blank (reagent spike)* by adding to a 100 mL centrifuge tube with Teflon-lined cap containing 35 mL of dichloromethane 20 µL of AH I-Std solution, 20 µL of PES I-Std solution, 20 µL of AH spike solution, and 20 µL of PES spike solution.

6.1.2. Field blank

If the sample set requires a field blank (tissue blank),Δ prepare this by washing down the empty sample container 3 times with 10 mL of dichloromethane. Combine the washings in a centrifuge tube and add 5 mL more of dichloromethane.

6.1.3. Blank

For each set of samples prepare a blank (reagent blank)◊ by adding to an empty 100-mL centrifuge tube with Teflon-lined cap 35 mL of dichloromethane, 20 µL of AH I-Std solution, and 20 µL of PES I-Std solution.

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* The reagent spike is defined as a sample that is treated the same as a tissue sample except the spike solutions are added instead of tissue at the beginning of the procedure.

Δ The tissue blank is defined as a sample that is treated the same as a tissue sample except that the washings of an empty tissue sample container are added instead of a tissue sample. The empty container is one which has been exposed to air at the site of sample collection.

◊ The reagent blank is defined as a sample that is treated the same as a tissue sample in the laboratory except that no tissue is added at the beginning of the procedure.

6.1.4. Analyte calibration solutions

Prepare two AH/PES analyte calibration solutions by adding to each of two 2-mL GC vials 900 µL of hexane, 20 µL of AH I-Std solution, 20 µL of PES I-Std solution, 20 µL of AH spike solution, and 20 µL of PES spike solution.

6.2. Sample extraction

A schematic diagram of this procedure is shown in Figure IV.5.

Weigh 3 ± 0.5 g of tissue sample to the nearest 0.01 g into a 100-mL centrifuge tube with Teflon-lined cap. Be careful to place the sample on the bottom and not the sides of the tube. Store the remaining sample in a freezer.

To each tissue sample in a centrifuge tube add 35 mL of dichloromethane, 20 µL of AH I-Std solution, and 20 µL of PES I-Std solution. Make certain that the solutions are placed into the dichloromethane to avoid loss by evaporation.

Add 25 g of dichloromethane-washed anhydrous Na_2S0_4 to each tissue and blank sample tube. Macerate/extract the sample in the tube for 1 min with the Tekmar Tissumizer at setting 100. Then continue at setting 50 for 2 min. Avoid splattering the tissue.

Wash down the probe with dichloromethane, collecting the washings in the centrifuge tube. Centrifuge the sample for 5 min at 2000 rpm. Decant the extract into a labeled 500-mL Erlenmeyer flask with stopper.

Add 35 mL of dichloromethane to the centrifuge tube. Macerate and combine with the previous extract in the Erlenmeyer flask.

Wash the Na_2S0_4 sample mixture by adding 10 mL of dichloromethane to the tube and mixing on the Vortex Genie at setting 5-6 for 5-10 sec. Centrifuge and combine with the previous extracts in the Erlenmeyer flask.

6.3. Extract concentration

Add 3-4 Teflon boiling chips to the Erlenmeyer flask containing the combined dichloromethane extracts and attach a Snyder column. Concentrate the extract in a 60°C water bath to between 10 and 15 mL and transfer it to a labeled 25-mL graduated tube with stopper. Wash down the flask with 3-4 mL of dichloromethane and add the washings to the tube. Repeat the washing step.

Add a boiling chip to the tube and using a tube heater, reduce the volume of the extract to between 0.9 to 1.0 mL. Add 3 mL of hexane to the tube and reduce the volume to 2 mL using the tube heater.

Proceed to Silica Gel/Alumina Chromatography (Section 8).

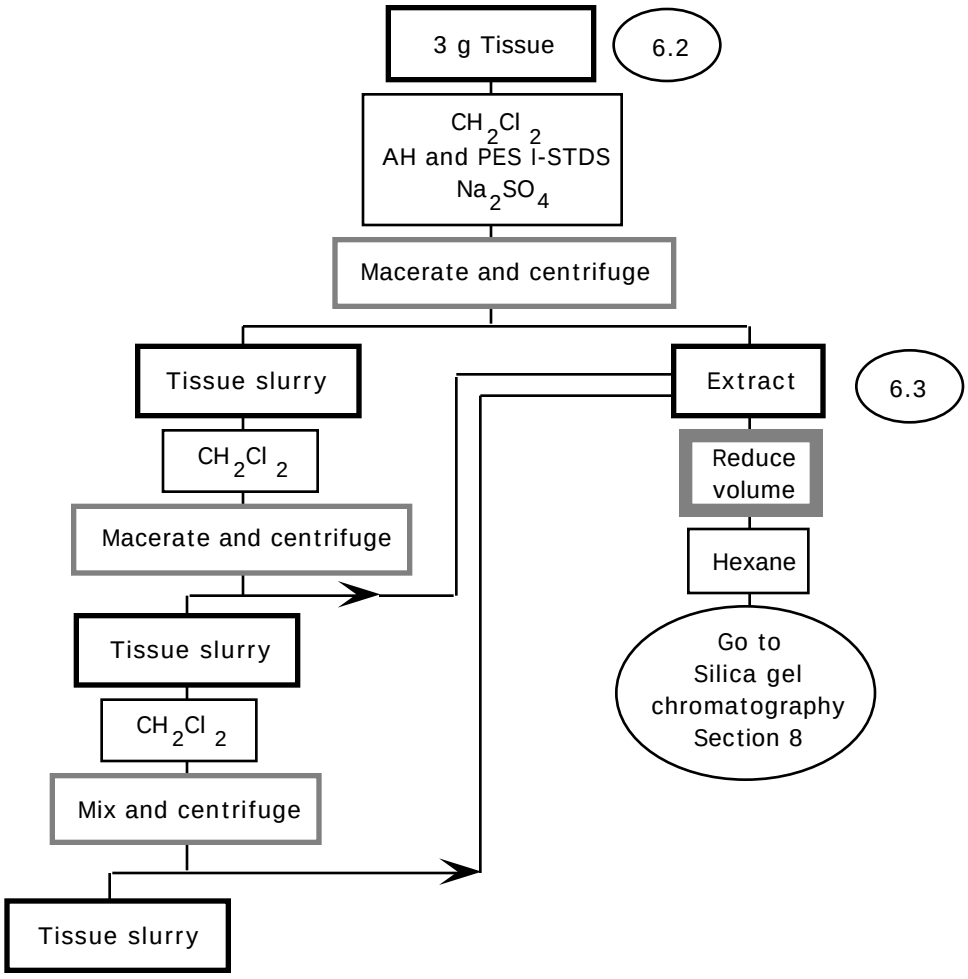

Figure IV.5. Tissue extract scheme.

7. DRY WEIGHT DETERMINATION

7.1. Sediment

Etch the sample number on the tab of the aluminum weighing pan. Place up to 3 aluminum pans on one half of a 9-inch strip of 12-inch wide aluminum foil. Fold the aluminum foil over the weighing pans to form an envelope. Close the envelope, but do not seal it, and then place it in the drying oven overnight at 120°C.

Cool the envelope containing the pans in a desiccator for 30 min. Remove each pan from the envelope and weigh the pan to the nearest 0.01 g. This is the pan weight. Handle the weighing pans with forceps.

Open the sediment sample container and discard all pebbles, shells, biota, and other detritus. Stir the sediment sample with a spatula to homogenize it. Add 10 ± 0.5 g of the sediment to the pan. Record the weight to the nearest 0.01 g. This is the wet weight.

Return the weighing pan to the foil envelope and close the envelope as before, but do not seal it. Dry the sample in the drying oven for 24 hr. Remove the sample from the oven and cool it in the desiccator for 30 min. Weigh the sample and pan. This is the dry weight.

7.2. Tissue

Proceed as above for sediments except weigh pan to nearest 0.1 mg. Use a spatula to spread approximately 0.5 g of tissue onto each pan. Record the dry weight and wet weight to the nearest 0.1 mg.

7.3. Dry weight calculation

Calculate percent dry weight (Dry Wt %) as follows:

$$\text{Dry Wt. \%} = \left(\frac{\text{Dry wt. - Pan wt.}}{\text{Wet wt. - Pan wt.}}\right) 100\%$$

8. SILICA GEL/ALUMINA CHROMATOGRAPHY

8.1. Column preparation

Column schematic is shown in Figure IV.3.

Prepare the columns just prior to use. The laboratory temperature must be below 80°F (27°C). On warm days proceed more slowly to avoid vapor bubbles.

Fit a 19-mm i. d. x 30-cm column with a stopcock, add 100 mL of dichloromethane and a 5 to 15-mm glass wool plug. Tamp the plug with a glass rod to remove any air bubbles.

Mix 10 g of activated alumina and 20 mL of dichloromethane in a beaker. Gently swirl the beaker for 30 sec, and let it stand for 5 min to remove all air bubbles until used.

Mix 20 g of activated silica gel and 40 mL of dichloromethane in a beaker. Gently swirl the beaker for 30 sec and let it stand for 5 min or until used to remove all air bubbles.

Place a curved-stem funnel into the column reservoir so that the funnel tip hangs well off-center. Swirl the alumina beaker to resuspend the particles and pour the slurry into the column. Wash the beaker with approximately 5 mL of dichloromethane and add the washings to the column. Repeat this step twice, then place the beaker under the column tip.

After the particles settle, open the stopcock for 30 sec to allow the alumina to pack more tightly, then close the stopcock.

Add the silica gel following the procedure for alumina.

After the particles settle, open the stopcock. While the solvent still drains, add the sand through the powder funnel. For sediment samples, then add the 7.5 cc of activated copper. Lower the solvent to the packing top, then close the stopcock.

Add 50 mL of pentane to the column. Lower to the packing top, then close the stopcock. Discard the eluates collected thus far.

8.2. Column chromatography of extracts

A schematic diagram of this procedure is shown in Figure IV.6.

Rinse the silica gel/alumina column tip with dichloromethane, remove the waste beaker from beneath the column, and replace it with a 50-mL graduated cylinder.

With a transfer pipet, cautiously transfer the sediment or tissue extract to the top of the packing. Lower to the packing top, then close the stopcock.

Rinse the tube that contained the sample extract with 0.5 mL of pentane and add the washings to the top of the packing. Lower to the packing top, then close the stopcock. Repeat this step once.

Rinse the sample tube with approximately 0.5 mL of 1:1 dichloromethane:pentane and hold the washings in the sample tube for use below.

[These washings ensure quantitative transfer of the more polar analytes. The washings are added to the column after pentane chromatography so that the dichloromethane does not change the polarity of the solvent (pentane). Ed. Note.]

Use approximately 2 mL of pentane to wash down the column wall. Lower to the packing top, then close the stopcock. Repeat this step once.

Add 40 mL of pentane and adjust the flow to approximately 3 mL/min. Collect 20 mL of eluate in a graduated cylinder. Close the stopcock. Discard the contents of the cylinder.

Replace the graduated cylinder with a concentrator tube labeled SA1. Partially open the stopcock and continue eluting until the volume of the SA1 fraction has been collected (see Section 3.6.4), then close the stopcock.

Set aside the SA1-labeled tube for Section 8.2.2.

[The SA1 fraction containing the alkanes is not quantified in the NS&T Program and is included here for information only. Ed. Note.]

Place a flask labeled SA2 under the column. Lower to the packing top, then close the stopcock. Add the washings from the tube saved above to the top of the packing. Lower to the packing top, then close the stopcock.

Wash down the tube with 0.5 mL of the 1:1 dichloromethane:pentane and add the washings to the top of the packing. Lower to the packing top, then close the stopcock.

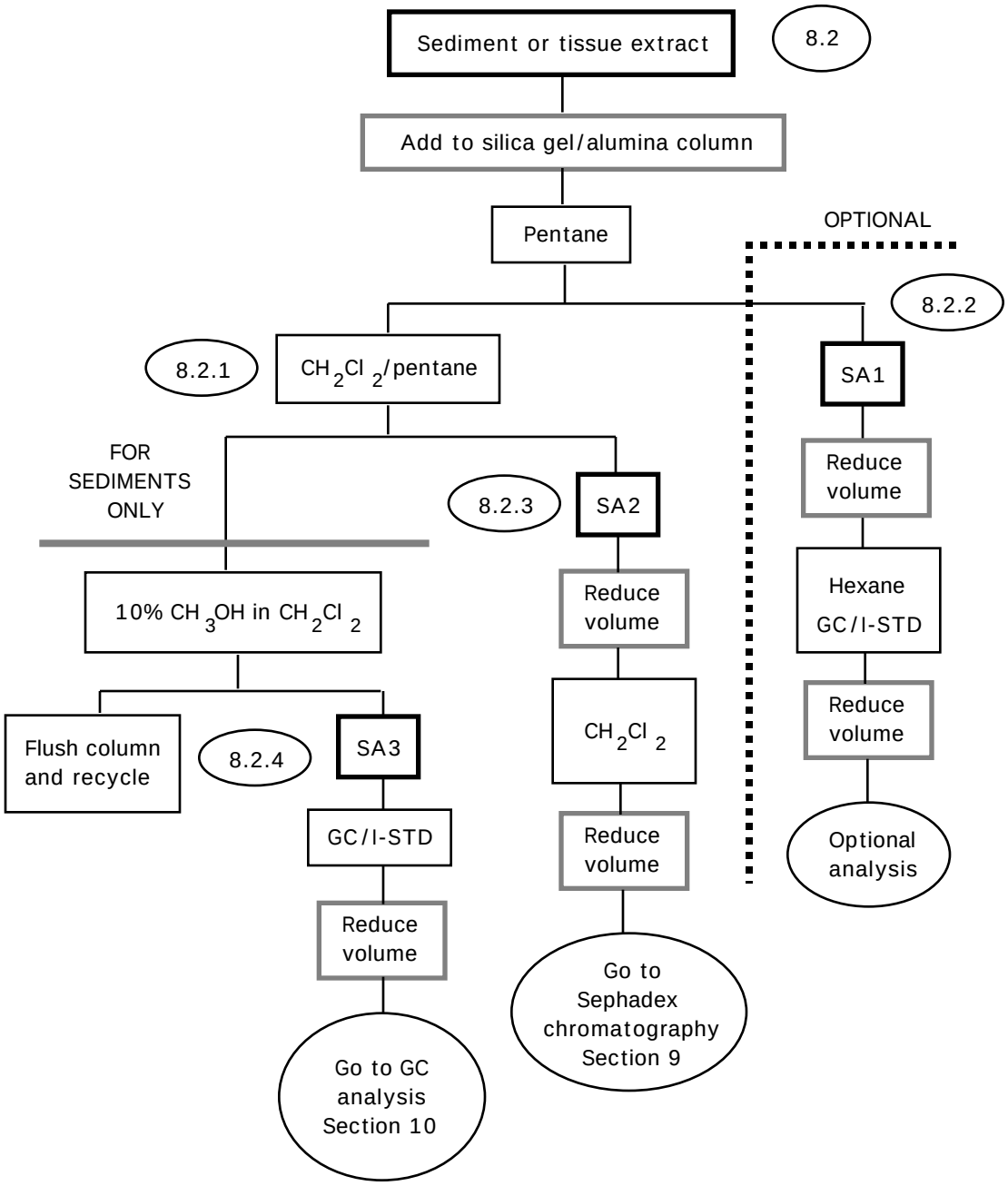

Figure IV.6. Silica gel/alumina chromatography scheme.

Add to the column the remaining 1:1 dichloromethane:pentane and partially open the stopcock. Lower to the packing top and close the stopcock.

Set aside the SA2-labeled flask for use in Section 8.2.3.

8.2.1. Special instructions for sediment samples

Place a waste flask under the column and add 50 mL of dichloromethane to the column. Lower to the packing top and close the stopcock.

Add 25 mL of 10% methanol in dichloromethane to the column. Lower to the packing top at approximately 2 mL/min, and close the stopcock.

Discard the contents of the waste flask and replace the flask with one labeled SA3, the coprostanol-containing fraction.

Add 30 mL of 20% methanol in dichloromethane to the column. Elute the remaining solvent in the reservoir into the SA3-labeled flask and set it aside for Section 8.2.4.

8.2.2. Concentration of fraction SA1 (optional)

Fraction SA1 contains the saturated hydrocarbons and possibly a portion of hexachlorobenzene.

Add a Teflon boiling chip to the concentrator tube labeled SA1, and using the tube heater, concentrate the SA1 fraction to between 0.9 mL and 1.0 mL. Add 2 mL of hexane to the tube and concentrate the fraction to between 0.9 mL and 1.0 mL.

Add 10 µL of TCMX GC/I-Std solution to the tube and mix for 2 sec on the Vortex Genie at setting 8-10.

Transfer the concentrate into a 2-mL GC vial, label it as SA1, cap the vial, and store it in the freezer for possible GC analysis.

8.2.3. Concentration of fraction SA2

Fraction SA2 contains the aromatic and chlorinated hydrocarbons.

Add 3-4 Teflon boiling chips to the SA2-labeled Erlenmeyer flask and attach a Snyder column. Concentrate the SA2 fraction in a 60°C water bath to between 10 and 15 mL and transfer it to a graduated tube.

Wash down the flask with 3-4 mL of dichloromethane and add the washings to the tube. Repeat this step once.

Continue concentrating the SA2 fraction in the same manner as the SA1.

Add appropriate amounts of methanol and dichloromethane to make approximately 2.3 mL of a solution of 6:4:3 hexane:methanol:dichloromethane (v:v:v).

[There is no performance difference between hexane and cyclohexane. Ed. Note.]

Proceed to Sephadex LH-20 chromatography (Section 9).

8.2.4. Concentration of fraction SA3 (sediment samples only)

Fraction SA3 contains coprostanol and is used only during sediment sample analysis.

Concentrate the fraction in the SA3-labeled flask in the same manner as for fraction SA2, except use a 75°C bath and add 7 mL of hexane instead of 2 mL.

Add 50 µL of HMB GC/I-Std solution to the tube and mix for 2 sec on the Vortex Genie at setting 8-10.

Transfer the concentrate into a 2-mL GC vial, label it as SA3, and cap the vial.

Add 50 µL of HMB GC/I-Std solution to the COP analyte-calibration solution vials from Section 5.1.4.

Proceed to GC analysis for coprostanol.

9. SEPHADEX LH-20 CHROMATOGRAPHY

9.1. Special Instructions

The sample extract must be completely dissolved in the solvent (no layers), with the total volume no larger than 2.3 mL.

[2.3 mL is the volume of sample for which the Sephadex column was calibrated in Section 3.7. Ed. Note.]

The fraction volumes are dependent on the column calibration. Occasionally check the column calibration.

[Check the column calibration after approximately 50 uses or if the column packing is damaged. Ed. Note.]

When removing or adding solvent or extract, extreme care must be used to avoid disturbing the column packing.

During column storage, maintain 30-50 mL of the solvent in the column reservoir and cover the top with aluminum foil to minimize evaporation. If the solvent in the reservoir separates into 2 phases, remove it and replace it with 80 mL of fresh 6:4:3 solvent, then elute 50 mL.

9.2. Column chromatography of extracts

A schematic diagram of this procedure is shown in Figure IV.7.

Remove the excess solvent from the top of the column using a transfer pipet and discard.* Add 10 mL of 6:4:3 solvent to the column. Lower to the packing top, and close the stopcock. Discard the eluate.

* This is done in case there has been a change in the 6:4:3 solvent concentration ratio during storage of the column.

Wash the column tip with dichloromethane and place a 50-mL graduated cylinder under the column.

Using a transfer pipet, carefully apply the 2-mL SA2 extract from Section 8.2.3 to the column. Use a circular motion to dispense the sample immediately above the packing. Drip the solution slowly down the column wall so as not to disturb the packing.

Lower to the packing top and close the stopcock.

Wash down the sample tube with 0.5 mL of the 6:4:3 solvent and apply the washings to the column. Lower to the packing top and close the stopcock. Repeat this step once.

Wash down the column wall with 3 mL of the 6:4:3 solvent. Lower to the packing top and close the stopcock. Repeat this step once.

Carefully add approximately 150 mL of the 6:4:3 solvent to the column (add more as needed) without disturbing the packing. Collect 25 mL of eluate in the 50-mL graduated cylinder. Close the stopcock and discard this eluate.

Replace the cylinder with a 25-mL graduated cylinder labeled SA2-L1. Open the stopcock, collect the amount calibrated in Section 3.7.4 for fraction SA2-L1, then close the stopcock. Fraction SA2-L1 contains lipid and biogenic materials. It is not used in the NS&T Program analysis protocol.

Place a 100-mL graduated cylinder labeled SA2-L2 under the column. Open the stopcock and collect the amount calibrated in Section 3.7.4 for fraction SA2-L2. Close the stopcock and transfer the eluate to a 500-mL Erlenmeyer flask with stopper labeled SA2-L2.

Wash down the graduated cylinder with 3-4 mL of dichloromethane and add the washings to the flask. Repeat the above step once and set the flask aside for Section 9.3.

Place a waste flask under the column and elute 50 mL of solvent to flush the column. Discard this eluate.

The column is now ready for the next sample.

9.3. Concentration of fraction SA2-L2

Add 3-4 Teflon boiling chips to the flask from Section 9.2 and attach a Snyder column. Concentrate the SA2-L2 fraction in a 75°C water bath to between 10 and 15 mL and transfer to a 25-mL concentrator tube.

Wash down the flask with 3-4 mL of dichloromethane and add the washings to the tube. Repeat this step once.

Add 1 mL of methanol and a Teflon boiling chip to the concentrator tube and, using the tube heater reduce the volume to between 0.9 and 1.0 mL.

Add 7 mL of hexane to the concentrator tube and reduce the volume again to between 0.9 and 1.0 mL.

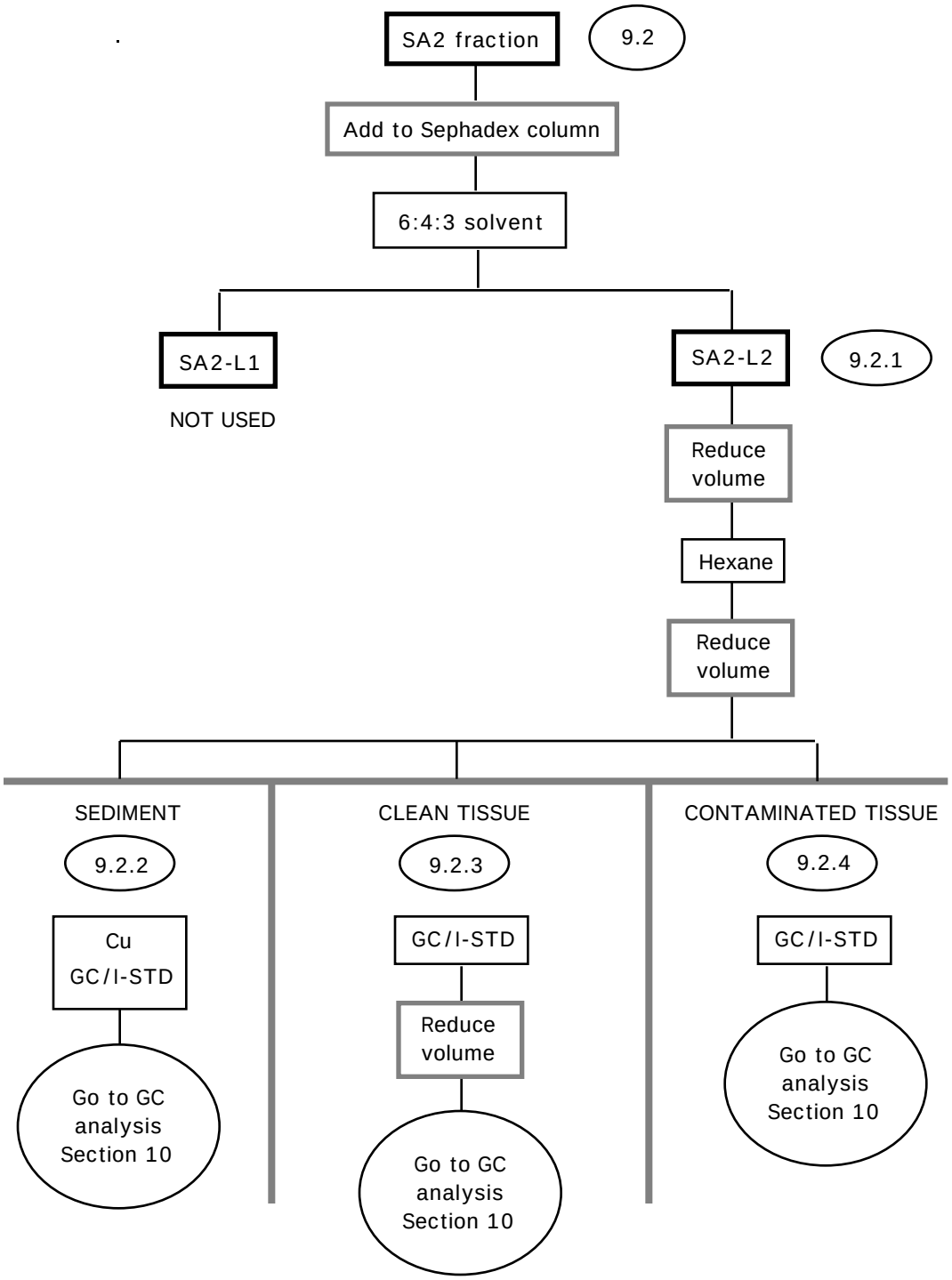

Figure IV.7. Sephadex chromatography scheme.

Proceed to Section 9.3.1 for a sediment sample to Section 9.3.2 for a clean tissue sample, and to Section 9.3.3 for a contaminated tissue sample.

9.3.1. Concentration of fraction SA2-L2 from sediment

Add grains of activated copper to the SA2-L2 tube from Section 9.3 until no further discoloring occurs, then stopper the tube and let it stand overnight in a refrigerator.

Add 50 µL of HMB GC/I-Std solution and 50 µL of TCMX GC/I-Std solution to the tube. Mix on the Vortex Genie at setting 8-10 for 2 sec.

Transfer equal amounts of the SA2-L2 fraction to two 2-mL GC vials, cap the vials, and label them. Store one of these vials in the refrigerator as a reserve.

Add 50 µL of HMB GC/I-Std solution and 50 µL of TCMX GC/I-Std solution to the AH/PES analyte-calibration solution vials from Section 5.1 4.

Proceed to GC analysis.

9.3.2. Concentration of fraction SA2-L2 from a clean tissue sample

[Liver samples are not analyzed for aromatic hydrocarbons, so HMB does not need to be used for liver samples. Ed. Note.]

This procedure cannot be used for highly contaminated tissue samples because they might saturate the ECD.

Add 10 µL of HMB GC/I-Std solution and 10 µL of TCMX GC/I-Std solution to the SA2-L2 tube from Section 9.3. Mix on the Vortex Genie at setting 8-10 for 2 sec.

Transfer half of the SA2-L2 fraction to a 2-mL GC vial, cap the vial, and label it. Store it in the refrigerator as a reserve.

Using a pipet, transfer a portion of the remaining tissue fraction SA2-L2 to a 2-mL GC vial. Place this vial under a gentle stream of nitrogen gas piped through dichloromethane-washed Teflon, stainless-steel, or glass tubing, and slowly evaporate to one half volume.

Repeat the step above until the entire contents of the tube have been transferred to the conical GC vial. The volume of the concentrated fraction should be approximately 0.1 mL. Cap the vial and label it.

Add 10 µL of HMB GC/I-Std solution and 10 µL of TCMX GC/I-Std solution to the analyte-calibration solution vials from Section 6.1 4.

Proceed to GC analysis.

9.3.3. Concentration of fraction SA2-L2 from a contaminated tissue sample

This procedure should be used to analyze tissue samples that have moderate to high concentrations of PCBs or DDTs.

Add 10 µL of HMB GC/I-Std solution and 10 µL of TCMX GC/I-Std solution to the SA2-L2 tube from Section 9.3. Mix on the Vortex Genie at setting 8-10 for 2 sec.

Transfer approximately 0.1 mL of fraction SA2-L2 into a 2-mL GC vial. Cap the vial and label it.

Add 10 µL of HMB GC/I-Std solution and 10 µL of TCMX GC/I-Std solution to the analyte-calibration solution vials from Section 6.1.

Proceed to GC analysis.

Place the remaining SA2-L2 fraction in a GC vial, and store the vial in the refrigerator as a reserve.

9.4. Recycling of column packing

When the column no longer maintains its calibration with azulene/perylene, recycle the packing using procedure in Section 3.7.6.

[Columns can be used up to 50 times. See Sections 9.1 and 3.7.3. Ed. Note.]

10. GAS CHROMATOGRAPHY ANALYTICAL PROCEDURES

10.1. Instrument settings

[GC analysis was done using a Hewlett-Packard HP 5880 with an FID detector for hydrocarbons or an ECD detector for compounds containing elements other than and in addition to H and C (i.e., halogenated pesticides and PCBs). It is routinely operated using a 4:1 split ratio and a fused silica capillary column with DB-5 bonded phase. All gases were purified through molecular-sieve traps; oxygen must be removed from the helium and the argon/methane using oxygen traps. All gas-line fittings must be free of leaks. The attenuation of the integrator was adjusted for the required sensitivity. Ed. Note.]

10.2. ECD operation

The instrument settings used for ECD detectors are listed in Table IV.2. The oven temperature profile is shown in Figure IV.8.

10.3. FID operation

The instrument settings used for FID detectors are listed in Table IV.3. The oven temperature profile is shown in Figure IV.9. Note the significant difference in temperature profile and in detector purge gas.

10.4. Injection of sample concentrates and blanks

The autosampler automatically injects a specified volume of sample into the GC, eliminating operator error associated with this process. The sample vial frequency used during the GC analysis is listed in Table IV.4. The first hexane-filled vial is used as a warm up for the system. The subsequent hexane-filled vials are used to insure no carryover of material from one analysis to another. The hexane-filled vials in the even-numbered slots are not analyzed; they are for rinsing the syringe only.

Table IV.2. Instrument description and conditions for tissue and sediment extract analysis using the ECD detector.

Gas Chromatograph: Hewlett-Packard HP 5880 with ^{63}Ni electron capture detector (ECD)

Instrument settings:

Injection volume:	2 µL
Injection technique:	splitless
Splitter closing time:	0.5 min
Detector temp.:	320°C
Injector temp.:	300°C
Recorder range:	(depends on ECD)
Chart speed:	0.7 cm/min
Carrier gas:	He
Linear gas velocity:	33 cm/sec at 300°C
Carrier gas flow:	~1.5 mL/min (varies with temperature)
Detector purge gas:	5% CH_4, 95% Ar
Detector purge flow:	30 mL/min
Septum purge flow:	10 mL/min
Split vent flow:	40 mL/min

Temperature Program:

Initial temp.:	3 min at 50°C
First rate:	4°C/min to 170°C
Isothermal pause:	0 min
Second rate:	1°C/min to 210°C
Isothermal pause:	0 min
Third rate:	4°C/min to 300°C
Isothermal pause:	10 min at 300°C

Column:

Material:	fused silica tubing
Length:	30 m
Int. Diameter:	0.25 mm
Stationary phase:	DB-5
Phase Composition:	5% phenyl, 95% methyl polysiloxane
Film Thickness:	0.25 µm

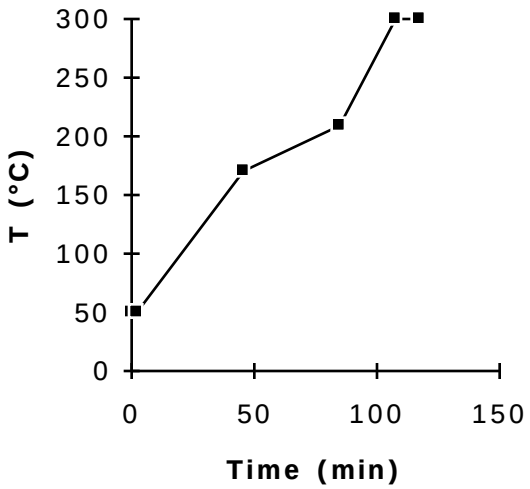

Figure IV.8. GC temperature profile for tissue and sediment extract analysis using an ECD detector.

Table IV.3. Instrument description and conditions for tissue and sediment extract analysis using an FID detector.

Gas Chromatograph: Hewlett-Packard HP 5880 with flame ionization detector (FID)

Instrument settings:		Temperature Program:	
Injection volume:	2 µL	Initial temp.:	3 min at 50°C
Injection technique:	splitless	First rate:	4°C/min to 300°C
Splitter closing time:	0.5 min	Isothermal pause:	0 min
Detector temp.:	320°C	Second rate:	none
Injector temp.:	300°C	Isothermal pause:	0 min
Recorder range:	(depends on FID)	Third rate:	none
Chart speed:	0.7 cm/min	Isothermal pause:	10 min at 300°C
Carrier gas:	He		
Linear gas velocity:	33 cm/sec at 300°C	Column:	
Carrier gas flow:	~1.5 mL/min (varies with temperature)	Material:	fused silica tubing
		Length:	30 m
Detector purge gas:	nitrogen	Int. Diameter:	0.25 mm
Detector purge flow:	30 mL/min	Stationary phase:	DB-5
Septum purge flow:	10 mL/min	Phase Composition:	5% phenyl, 95% methyl polysiloxane
Split vent flow:	40 mL/min		
Air flow:	240 mL/min	Film Thickness:	0.25 µm

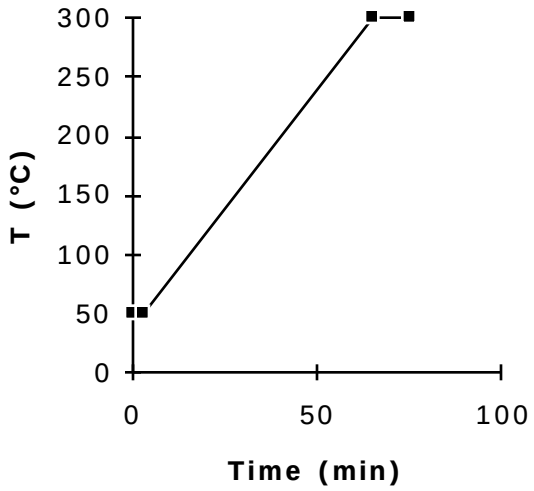

Figure IV.9. GC temperature profile for tissue and sediment extract analysis using the FID detector.

Table IV.4. Sample vial frequency used for GC analysis using the Hewlett-Packard Autosampler 7672A.

Place the following vials in odd-numbered slots in the autosampler tray and additional hexane-filled vials in the even-numbered slots.

Hexane-filled vial	Sample
2nd (of 2) analyte calibration soln.	Sample
1st (of 2) analyte calibration soln. (FIRST RUN)	Sample
Hexane-filled vial	1st (of 2) analyte calibration soln. (THIRD RUN)
Sample	Hexane-filled vial
Sample	Sample
Sample	Sample
Sample	Sample
1st (of 2) analyte calibration soln. (SECOND RUN)	Sample
Hexane-filled vial	Spiked blank sample
Sample	1st (of 2) analyte calibration soln. (FOURTH RUN)

10.5. Verification of stable GC performance

Use 6 vials containing GC calibration check solutions in the odd-numbered slots of the autosampler and hexane-filled vials in the even numbered slots. Use the results for the calculations in Section 10.6.

10.6. GC reproducibility and calibration mixture verification

To assess the reproducibility of analysis, use the second run of the first analyte calibration (AC) solution vial analysis results as the reference for calculating the relative responses of the other AC vial analyses. Do this by calculating for each analyte the ratio of the response factor in an AC analysis to that for the AC reference analysis, and express the result as a percent. The response factor for an individual analysis is defined as R_2/R_3, where,

$$R_2 = \frac{\text{analyte concentration in the AC vial (ng/µL)}}{\text{GC-I-Std concentration in the AC vial (ng/µL)}}$$

and

$$R_3 = \frac{\text{analvte peak area from the analvsis of the AC vial}}{\text{GC-I-Std peak area from the analysis of the AC vial}}$$

If the AC reference analysis is denoted with a "°", then the ratio of the response factors, expressed as a percent, reduces to:

$$\text{Ratio of response factors} = \frac{R_2^\circ\ R_3}{R_3^\circ\ R_2}\ 100\%$$

where the undenoted R_2 and R_3 stand for the corresponding parameters of the AC analysis being compared to the AC reference analysis. A deviation >5% from 100% indicates a problem with the GC system (e.g., a leaking septum, a loose ferrule, or a worn out or dirty column). Such problems should be rectified before proceeding with analyses of the extract fractions.

To check the integrity of the solutions in the AC vials, calculate for each analyte the ratio of the response factor for the GC calibration check solution to that of the last AC vial. Use the same formula given above, except the last AC vial results takes the place of the undenoted AC vial and the GC calibration check solution vial takes the place of the AC reference vial, with appropriate changes in the definitions of the $R_2°$ and $R_3°$ parameters (i.e., substitute "GC calibration check solution" for "AC reference vial"). A deviation >5% from 100% indicates a problem with the solution in the last AC vial, and perhaps with the other AC vials.

11. ANALYTE AND INTERNAL-STANDARD CONCENTRATION CALCULATIONS

11.1. Analyte concentration calculations

Identify the analyte peaks in the chromatograms of the extract fractions by comparing them with the analyte retention times in the chromatogram of the AC reference mixture. Fractions analyzed by GC/ECD that show the presence of PCBs will have PCB peaks from congeners present in the sample in addition to those corresponding to the PCB congeners in the standards. Representative extracts need to be analyzed by GC/MS to identify the chlorination level of these peaks and verify the other analytes indicated by retention time comparisons.

The GC/MS chromatograms are used to label the peaks in the GC/ECD chromatogram. Generally, the GC/MS is not as sensitive as the GC/ECD, so the sample extract fraction may have to be concentrated to as little as 20 µL for GC/MS analysis. Analyze a tenfold concentrated AC solution for the chlorinated compounds listed in Table IV.5. Determine the sum ($\sum$) of the selected ion areas (A) and the total ion current (TIC) for each analyte. Calculate the response ratio, RR, for each analyte standard by the equation,

$$RR = \frac{TIC}{\sum A}$$

For a multicomponent GC/MS peak, estimate the percent of each analyte using the areas of the selected ions indicated in Table IV.5. For example, for a 2-component GC peak containing analytes x and y (peak x+y), set the MS data system to determine the sum ($\sum$) of the ion areas (A) for analyte x, ($\sum A_x$), and analyte y, ($\sum A_y$). Calculate the percentage of x, % x, in peak x+y by the equation:

$$\%\ x = \frac{(\sum A_x)\ (RR_x)}{(\sum A_x)\ (RR_x) + (\sum A_y)\ (RR_y)}$$

Then calculate % y by substituting ($\sum A_y$) (RR_y) for ($\sum A_x$) (RR_x) in the numerator.

The peak areas of each PCB congener (Table IV.1) with the same number of chlorine atoms (isomer set) are to be summed to give the total area for that set (e.g., the dichlorobiphenyls). Because the calibration standard contains only one isomer for each set, use the response of that isomer as a surrogate standard to calculate the amounts of the other isomers in the set. In addition, report separately the concentration of each calibration isomer in each extract.

Table IV.5. Selected ions used for estimating proportions of analytes in multicomponent GC/MS peaks.

Analytes:	Selected Ions (m/z)
Dichlorobiphenyls (set)	222, 224
Trichlorobiphenyls (set)	256, 258, 260
Tetrachlorobiphenyls (set)	290, 292, 294, 296
Pentachlorobiphenyls (set)	324, 326, 328, 330
Hexachlorobiphenyls (set)	358, 360, 362, 364, 366
Heptachlorobiphenyls (set)	392, 394, 396, 398, 400
Octachlorobiphenyls (set)	426, 428, 430, 432, 434, 436
Nonachlorobiphenyls (set)	460, 462, 464, 466, 468, 470
DDE's (set)	246, 248
DDD's and DDT's (set)	235, 237
trans-Nonachlor	405, 407, 409, 411, 413
cis-Chlordane	371, 373, 375, 377, 379
Aldrin	261, 263, 265, 267
Dieldrin	79
Mirex	270, 272, 274, 276
Hexachlorobenzene	282, 284, 286, 288
gamma-HCH	181, 183, 185
Heptachlor	100
Heptachlor epoxide	351, 353, 355, 357
Internal Standards:	
Tetrachloro-m-xylene (TCMX)	242, 244, 246
Dibromooctafluorobiphenyl (DBOFBP)	454, 456, 458

All extracted sulfur (S_8) must be removed from the fractions before analysis for PCBs because the S_8 molecule interferes with the GC/ECD and GC/MS responses.

The internal standards (I-Stds) added to the sample at the beginning of the extractions are used to adjust for analyte losses during sample work-up. Use Equation A to calculate the analyte concentration in an aquatic sediment or tissue sample, dry weight basis:

Equation A

$$\frac{\text{ng of the analyte}}{\text{g sample (dry wt)}} = \frac{R_1 R_2}{R_3} \frac{\text{ng I-Std added to sample}}{\text{sample wt.}} \frac{100\%}{\text{dry wt.}}$$

where

$$R_1 = \frac{\text{analyte peak area from analysis of the extract fraction}}{\text{I-Std peak area from analysis of the extract fraction}},$$

$$R_2 = \frac{\text{analyte concentration in the AC reference vial (ng/µL)}}{\text{I-Std concentration in the AC reference vial (ng/µL)}},$$

and

$$R_3 = \frac{\text{analyte peak area from analysis of the AC reference vial}}{\text{I-Std peak area from analysis of the AC reference vial}}.$$

To calculate the results for the chlorinated analytes, use dibromooctafluorobiphenyl as the internal standard. In the calculations for aromatic hydrocarbons, use naphthalene-d_8 as the internal standard for naphthalene, 2-methylnaphthalene, and 1-methylnaphthalene. Use perylene-d_{12} as the internal standard for benz[*a*]anthracene and the aromatic hydrocarbons below it in Table IV.1. Calculate all other aromatic hydrocarbons analytes in Table IV.2 using acenaphthene-d_{10} as the internal standard.

[The aromatic hydrocarbon Internal standards were chosen for calculating the analytes that elute In the same region of the chromatogram. Ed. Note.]

To calculate the percent recovery of each internal standard use equation B that makes use of the GC/I-Std (HMB and/or TCMX) added to the extract fraction just before it is transferred to the GC vial. If less than 50% of the internal standard is recovered, re-extract and reanalyze the unused portion of the sample.

Equation B

$$\%\ \text{recovery of I-Std} = \frac{R_1\ R_2}{R_3}\ \frac{\text{ng GC/I-Std added to the fraction}}{\text{ng I-Std added to the sample}}\ 100\%$$

where

$$R_1 = \frac{\text{I-Std peak area from analysis of the extract fraction}}{\text{GC/I-Std peak area from analysis of the extract fraction}},$$

$$R_2 = \frac{\text{I-Std concentration in the AC reference vial (ng/µL)}}{\text{GC/I-Std concentration in the AC reference vial (ng/µL)}}$$

and

$$R_3 = \frac{\text{I-Std peak area from analysis of the AC reference vial}}{\text{GC/I-Std peak area from analysis of the AC reference vial}}.$$

11.2. Spiked blank calculations

Identify the analyte peaks in the chromatograms of the spiked blanks by comparing them with the analyte retention times obtained from the chromatogram of the AC reference vial. Calculate the percent (%) recovery of the analytes added to the spiked blanks using the equation below. Calculation of I-Std recovery is unchanged.

$$\%\ \text{recovery of analyte} = \frac{R_1\ R_2}{R_3}\ \frac{\text{ng I-Std added to the blank sample}}{\text{ng analyte added to the blank sample}}\ 100\%$$

where R_1 R_2, and R_3 correspond to the definitions given above.

11.3. Sample chromatograms and calculations

Chromatograms of an AH/PES spike vial using the FID and ECD detectors are shown in Figures IV.10 and IV.11. The analyte peaks are identified in both figures. The selected peak areas and retention times are listed in Tables IV.6 and IV.7.

11.3.1. Percent recovery calculation of an I-Std in a sample extract fraction

The percent recovery of naphthalene-d_8, one of the internal standards, in a sample extract fraction was calculated as shown below. The peak areas were obtained from the GC chromatogram data in Tables IV.6: hexamethylbenzene (HMB), 60.90; naphthalene-d_8 (DNPH), 70.99; and naphthalene (NPH), 83.51.

Using the formulas in Section 11.1,

$$R_1 = \frac{\text{I-Std peak area from analysis of the extract fraction}}{\text{GC/I-Std peak area from analysis of the extract fraction}}$$

Table IV.6. Selected peak areas and retention times of an AH/PES spike vial chromatogram using the FID detector.

Analyte	Acronym	Retention time (min)	Peak area
Naphthalene-d_8	DNPH	15.92	70.99
Naphthalene	NPH	16.02	83.51
2-Methylnaphthalene	2MN	20.05	63.27
1-Methylnaphthalene	1MN	20.64	77.33
Biphenyl	BPH	23.04	75.91
2,6-Dimethylnaphthalene	DMN	23.85	74.11
Hexamethylbenzene	HMB	25.50	60.90
Acenaphthene-d_{10}	DACE	26.26	71.65
Acenaphthene	ACE	26.44	68.75
Fluorene	FLU	29.49	75.01
Phenanthrene	PHN	35.13	75.24
Anthracene	ANT	35.40	71.88
1-Methylphenanthrene	1MPH	38.99	75.08
Fluoranthene	FLA	42.35	77.34
Pyrene	PYR	43.58	74.42
Benz[*a*]anthracene	BAA	51.04	59.93
Chrysene	CHR	51.27	57.34
Benzo[*a*]pyrene	BEP	58.60	54.81
Benzo[*e*]pyrene	BAP	58.84	51.26
Perylene-d_{12}	DPER	59.16	49.81
Perylene	PER	59.28	44.77
Dibenz[*a,h*]anthracene	DBA	64.45	33.94

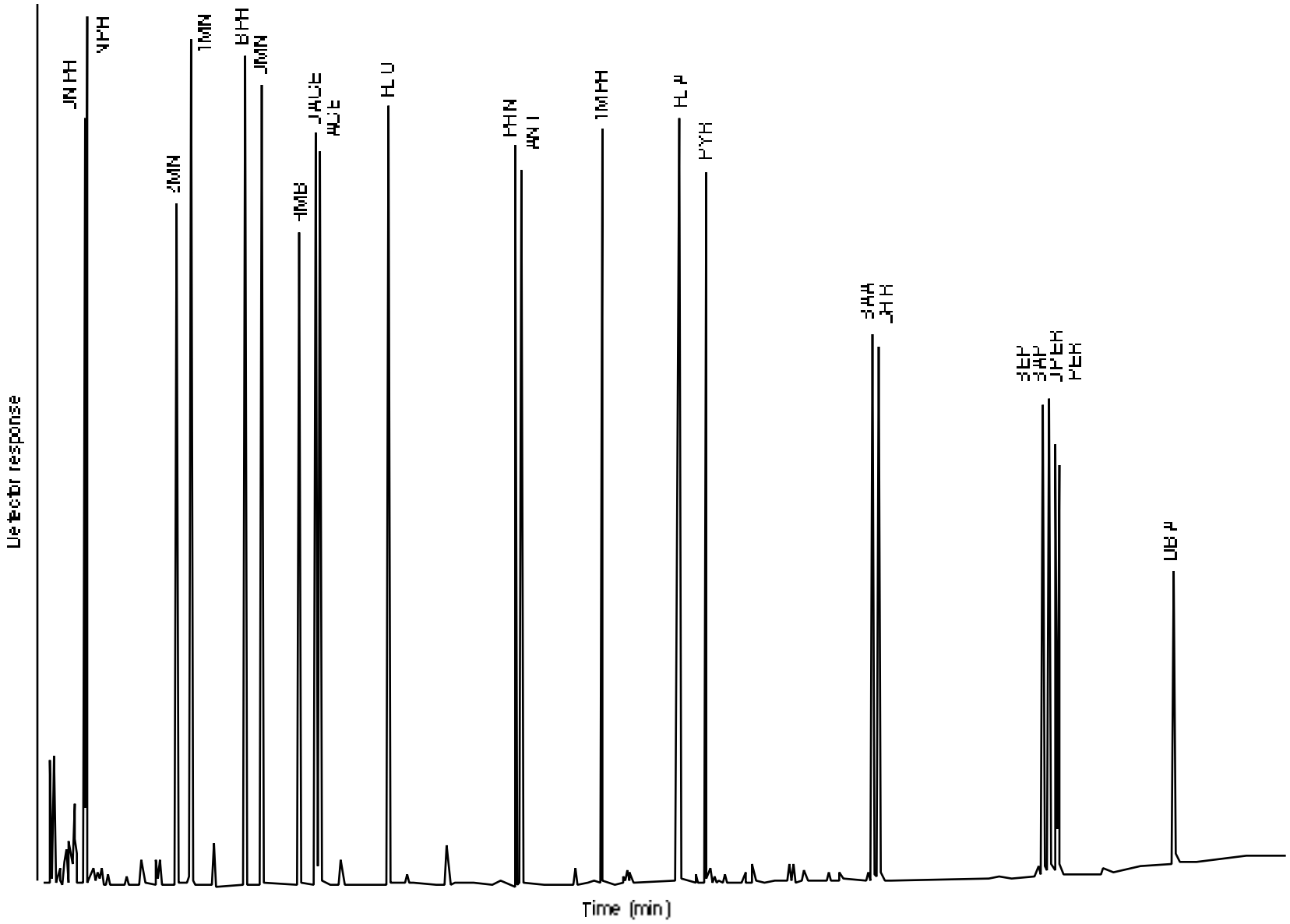

Figure IV.10. AHPES spike water chromatogram using the FD detector (chromatogram starts at 14 min after injection for the sake of clarity). Analyte acronyms and retention times can be found in Table IV.6.

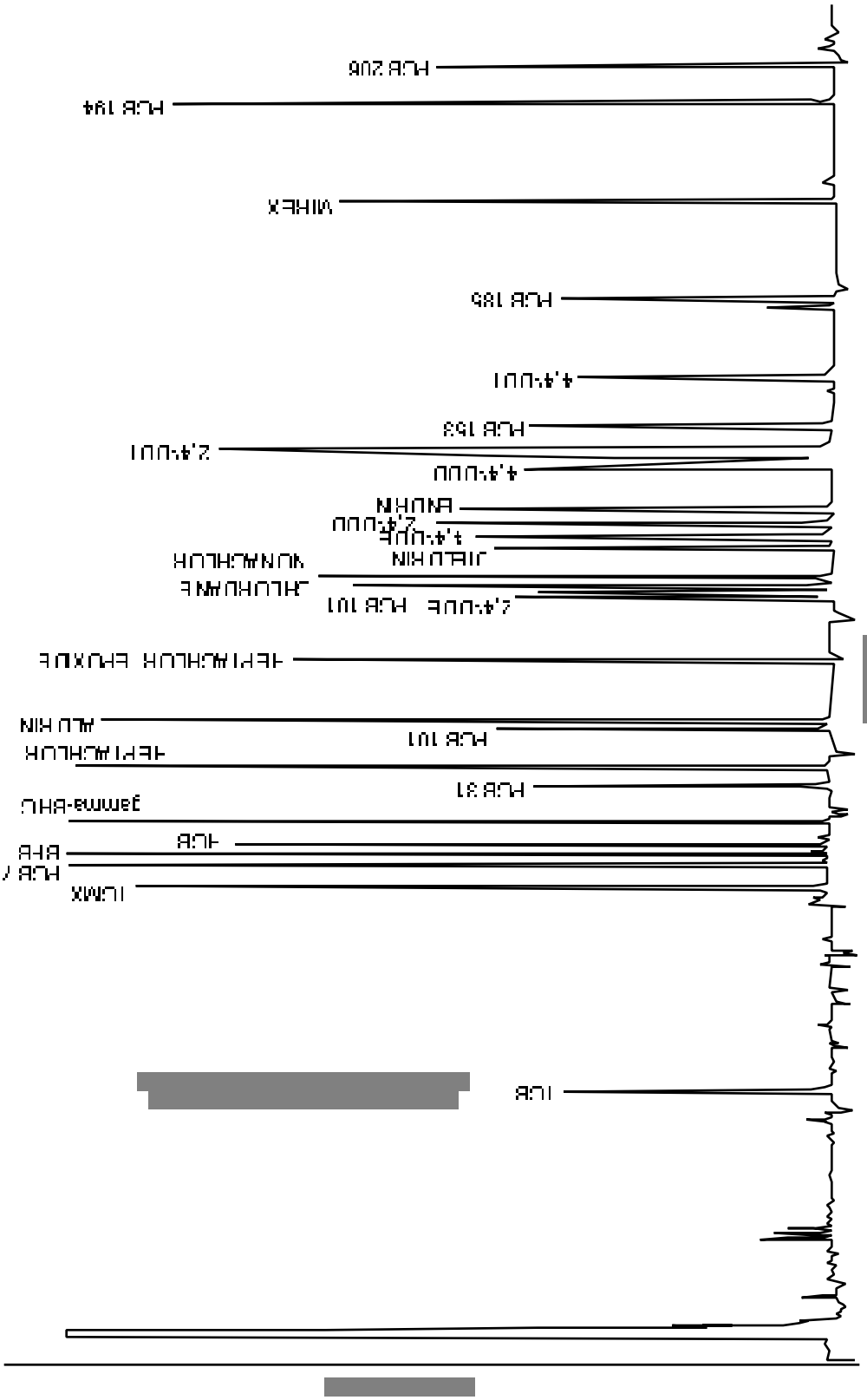

Figure IV.11. AH rPES spike vial chromatogram using the ECD detector. Analyte acronyms and retention times can be found in Table IV.7.

Table IV.7. Selected peak areas and retention times of an AH/PES spike vial chromatogram using the ECD detector.

Analyte	Acronym	Retention time (min)	Peak area
Tetrachlorobenzene *	TCB	17.69	1260.93
Tetrachloro-m-xylene	TCMX	31.16	3111.17
PCB 7		32.63	4140.23
4,4'-Dibromooctafluorobiphenyl	BFB	33.26	3084.66
Hexachlorobenzene		33.88	2829.00
gamma-HCH		35.45	3415.79
PCB 31		39.55	2019.46
Heptachlor		40.71	5179.14
PCB 47		43.26	2550.62
Aldrin		43.66	5343.76
Heptachlor epoxide		47.72	4280.34
2,4'-DDE		51.41	2713.27
PCB 101		51.73	2844.98
cis-Chlordane		52.11	4297.99
trans-Nonachlor		52.76	4723.09
Dieldrin		54.69	3241.71
4,4'-DDE		55.38	3309.12
2,4'-DDD		56.26	3632.70
Endrin		57.21	3612.66
2,4'-DDD		60.72	3291.76
2,4'-DDT		61.02	6718.20
PCB 153		62.65	3343.78
4,4'-DDT		65.89	2755.64
PCB 185		71.01	3161.67
Mirex		77.49	4620.29
PCB 194		84.01	4373.62
PCB 206		86.40	2525.73

* Internal standard used after initial method development.

$$R_1 = \frac{\text{DNPH peak area from analysis of the extract fraction}}{\text{HMB peak area from analysis of the extract fraction}}$$

$$R_1 = \frac{42.33}{45.68} = 0.92$$

Similarly,

$$R_2 = \frac{\text{I-Std concentration in the AC reference vial (ng/µL)}}{\text{GC/I-Std concentration in the AC reference vial (ng/µL)}}$$

$$R_2 = \frac{\text{[DPNH] in the AC reference vial (ng/µL)}}{\text{[HMB] in the AC reference vial (ng/µL)}}$$

$$R_2 = \frac{1.0}{1.0} = 1.0$$

and,

$$R_3 = \frac{\text{I-Std peak area from analysis of the AC reference vial}}{\text{GC/I-Std peak area from analysis of the AC reference vial}}.$$

$$R_3 = \frac{\text{DPNH peak area from analysis of the AC reference vial}}{\text{HMB peak area from analysis of the AC reference vial}}.$$

$$R_3 = \frac{70.99}{60.90} = 1.166.$$

The percent recovery of the DNPH I-Std is calculated using

$$\%\ \text{recovery of analyte} = \frac{R_1\ R_2}{R_3}\ \frac{\text{ng HMB added to the sample fraction}}{\text{ng I-Std added to the sample}}\ 100\%$$

$$\%\ \text{recovery of analyte} = \frac{(0.92)\ (1.0)}{1.166}\ \frac{1000}{1000}\ 100\% = 79\ \%$$

11.3.2. Calculation of an analyte concentration in a sample extract fraction

The peak areas obtained from GC chromatogram data of an SA2-L2 sample extract solution are listed in Tables IV.8 and IV.9. The chromatograms are not shown due to their complexity. Not all analyte peaks were found and/or identified. The peak areas obtained from the GC chromatogram data in Tables IV.8: hexamethylbenzene (HMB), 45.68; naphthalene-d_8 (DNPH), 42.23; and naphthalene (NPH), 87.02. The dry sample weight was 1.572 g and the percent dry weight was 29.93%. The concentration of naphthalene in the extract SA2-L2 solution was calculated as follows:

Table IV.8. Selected peak areas and retention times of an AH/PES SA2-L2 sample extract solution chromatogram using the FID detector.

Analyte	Retention time (min)	Peak area
Naphthalene-d_8	15.92	42.23
Naphthalene	16.05	87.02
1-Methylnaphthalene	20.62	65.27
Hexamethylbenzene	25.50	45.68
Acenaphthene-d_{10}	26.26	44.46
1-Methylphenanthrene	39.00	19.94
Benzo[*a*]pyrene	58.88	24.60
Perylene-d_{12}	59.15	27.17

Table IV.9. Selected peak areas and retention times of an AH/PES SA2-L2 sample extract solution chromatogram using the FID detector (not all analytes were identified in chromatogram).

Analyte	Retention time (min)	Peak area
TCB	17.69	1716.54
Tetrachloro-m-xylene	31.16	4691.35
PCB 7	32.64	16035.00
4,4'-Dibromooctafluorobiphenyl	33.27	5301.44
Hexachlorobenzene	33.89	987.21
PCB 47	43.19	1002.82
Aldrin	43.64	4527.09
2,4'-DDE	51.33	290.53
PCB 101	51.74	883.50
cis-Chlordane	52.16	544.63
4,4'-DDE	55.39	828.00
PCB 153	62.68	1644.13
4,4'-DDT	65.84	476.98

$$R_1 = \frac{\text{NPH peak area from analysis of the extract fraction}}{\text{DNPH I-Std peak area from analysis of the extract fraction}}$$

$$R_1 = \frac{87.02}{42.23} = 2.06$$

$$R_2 = \frac{\text{NPH concentration in the AC reference vial (ng/µL)}}{\text{DNPH concentration in the AC reference vial (ng/µL)}}.$$

$$R_2 = \frac{1.0 \text{ ng/µL}}{1.0 \text{ ng/µL}} = 1.0$$

and

$$R_3 = \frac{\text{NPH peak area from analysis of the AC reference vial}}{\text{DNPH peak area from analysis of the AC reference vial}}$$

$$R_3 = \frac{83.51}{70.99} = 1.176.$$

Therefore, using Equation A

$$\frac{\text{ng of analyte}}{\text{g sample (dry wt)}} = \frac{R_1 R_2}{R_3} \quad \frac{\text{ng I-Std added to sample}}{\text{sample wt.}} \quad \frac{100\%}{\text{dry wt.}}$$

$$\frac{\text{ng of analyte}}{\text{g sample (dry wt)}} = \frac{(2.06)(1.0)}{1.176} \quad \frac{1000 \text{ ng}}{1.572 \text{ g}} \quad \frac{100\%}{29.93\%} = 3700 \text{ ng NPH/g dry weight.}$$

12. CONCLUSIONS

The trace organic analytical procedures developed by NOAA/NMFS/NAF have been described in detail for use by analytical chemists. These procedures were used for analysis of samples collected as part of the NOAA National Status and Trends Program and are the basis of the currently used methodology.

13. ACKNOWLEDGMENTS

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Northwest Fisheries Science Center Organic Analytical Procedures

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ABSTRACT

This document describes the analytical methods for the analysis of trace organic compounds in sediments and tissues, developed and currently used by the Environmental Conservation Division of the Northwest Fisheries Science Center. Detailed descriptions are presented for the composition, extraction, and high performance liquid chromatography (HPLC) cleanup of marine sediment and tissue and for the quantification of aromatic hydrocarbons and/or chlorinated hydrocarbons by gas chromatography/mass spectrometry (GC/MS) and/or gas chromatography/electron capture detection (GC/ECD), plus quantification of coprostanol in sediment by gas chromatography/flame ionization detection (GC/FID). Detailed descriptions are also given for determining the percent dry weight of tissues and sediments, and for determining the percent lipid content of tissues. Formulas are given for calculating the concentrations of analytes and the percent recoveries of internal standards, as well as for percent dry weight and percent lipid determinations. Also included is information pertaining to the required laboratory supplies and instruments. These methods are used as part of the National Status and Trends Program and are used by several other government and private agencies for numerous projects. This document includes the latest revisions to the methods described in Krahn *et al.* (1988a), which updated the methods of MacLeod *et al.* (1985).

1. INTRODUCTION

The Environmental Chemistry Program (formerly the National Analytical Facility) of the Environmental Conservation Division, Northwest Fisheries Science Center (National Oceanic and Atmospheric Administration/National Marine Fisheries Service) continues to develop and employ state-of-the-art methods for analyzing marine environmental samples for trace organic contaminants. As improvements to procedures are validated, they are incorporated into our analytical protocols used for the National Status and Trends Program's National Benthic Surveillance Project, in which selected aromatic hydrocarbons (AHs), chlorinated hydrocarbons (CHs), and coprostanol are quantified in fish stomach contents, fish livers, and/or sediments (Table IV.10). The description given here reflects modifications made to previously published methods (MacLeod *et al.*, 1985; Krahn *et al.*, 1988b; and Krahn *et al.*, 1989).

The analyses of sediment and tissue samples follow the diagram shown in Figure IV.12, as summarized below. Sediment and tissue were extracted by homogenization with an organic solvent and a drying agent, and the solvent extract separated from the solid matrix by centrifugation. The sediment extracts were split into two portions: one for isolation and quantification of AHs and CHs, and the other for isolation and quantification of coprostanol. Fish livers were not analyzed for AHs. Fish liver extracts were split into two portions: one for isolation and quantification of CHs, and one for percent lipid analysis. Fish stomach contents

Table IV.10. Organic chemicals determined as part of the National Benthic Surveillance Project. [Nomenclature of polychlorinated biphenyl (PCB) congeners uses the method of Ballschmitter and Zell (1980)].

Aromatic Hydrocarbons (AHs)	**Sewage tracer**
Naphthalene	Coprostanol
1-Methylnaphthalene	
2-Methylnaphthalene	**DDT and metabolites**
Biphenyl	
2,6-Dimethylnaphthalene	2,4'-DDD 4,4'-DDD
Acenaphthylene	2,4'-DDE 4,4'-DDE
Acenaphthene	2,4'-DDT 4,4'-DDT
1,6,7-Trimethylnaphthalene	
Fluorene	**Chlorinated pesticides other than DDT**
Phenanthrene	
Anthracene	Aldrin Dieldrin
1-Methylphenanthrene	*cis*-Chlordane *trans*-Nonachlor
Fluoranthene	Heptachlor gamma-HCH
Pyrene	Heptachlor epoxide Mirex
Benz[*a*]anthracene	Hexachlorobenzene
Chrysene	
Benzo[*b*]fluoranthene	**Polychlorinated biphenyls congeners**
Benzo[*k*]fluoranthene	
Benzo[*e*]pyrene	PCB 8, PCB 18, PCB 28, PCB 29, PCB 44,
Benzo[*a*]pyrene	PCB 50, PCB 52, PCB 66, PCB 77, PCB 87,
Perylene	PCB 101, PCB 104, PCB 105, PCB 118, PCB
Indeno[1,2,3-*cd*]pyrene	126, PCB 128, PCB 138, PCB 153, PCB 154,
Dibenz[*a,h*]anthracene	PCB 170, PCB 180, PCB 187, PCB 188, PCB
Benzo[*ghi*]perylene	195, PCB 200, PCB 206, PCB 209

were analyzed for both AHs and CHs and did not require lipid analysis, therefore the extracts were not split. The portions of sediment or tissue extracts for analyses for AHs and/or CHs were filtered through columns of silica/alumina. The filtered sample extracts were then concentrated, and a portion of each extract chromatographed on size-exclusion HPLC columns, using dichloromethane as the mobile phase. A fraction containing AHs and CHs was collected, and the solvent volume exchanged to hexane while being reduced to a final volume of 150 µL or 250 µL. The concentrated AH/CH fraction was analyzed by GC/MS using sequenced selected ion monitoring to determine AHs and/or GC/ECD to determine CHs (Burrows *et al.*, 1990). Selected samples were analyzed by GC/MS for confirmation of CHs. The portions of sediment extracts for analysis of coprostanol were chromatographed on a polyamino-cyano HPLC column, using methyl-t-butyl ether as the mobile phase. A fraction containing coprostanol was collected, and the solvent exchanged to hexane while being reduced to a volume of 1 mL. The coprostanol

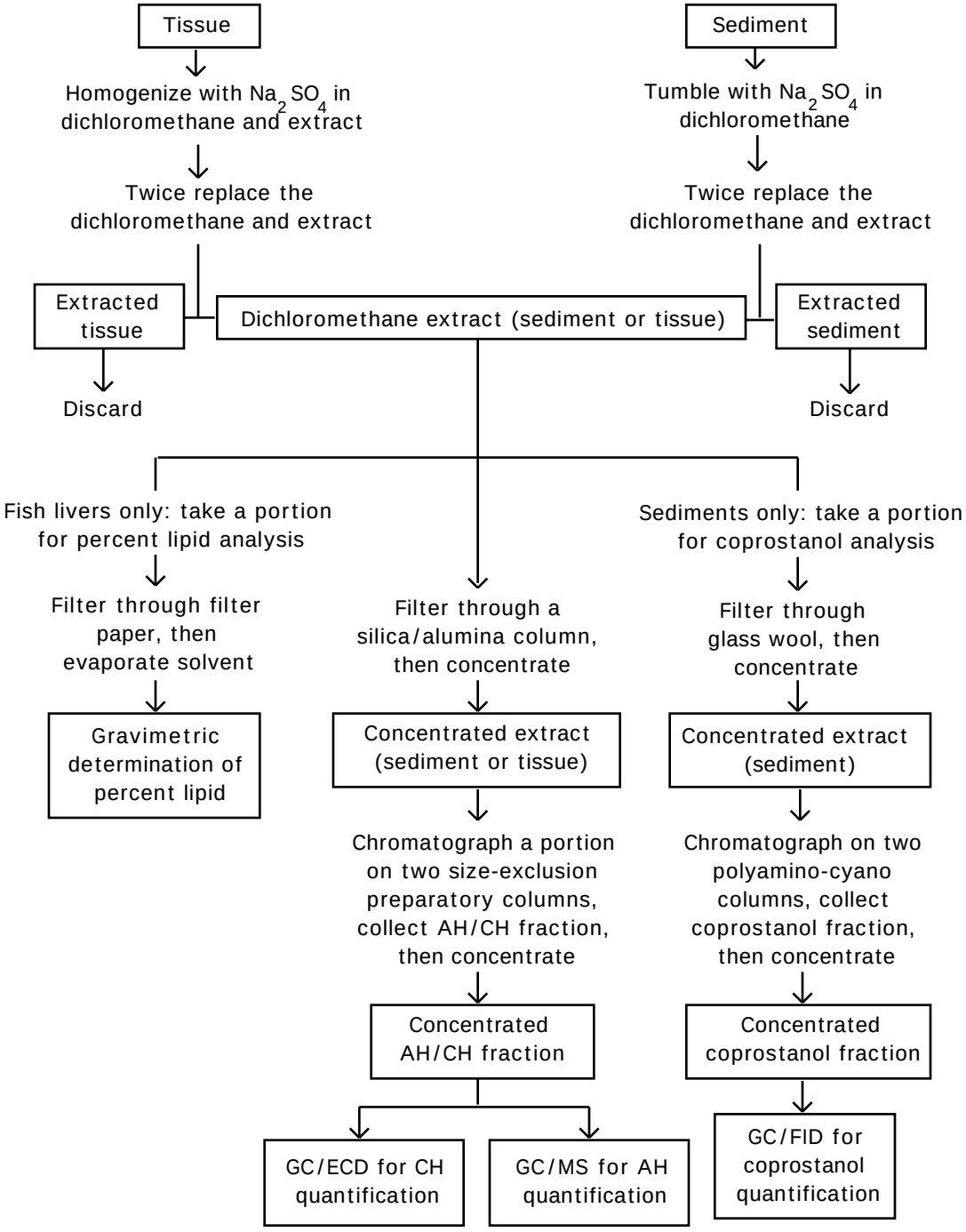

Figure IV.12. Flow diagram of extract cleanup.

fraction was then derivatized using BSTFA and further concentrated to a final volume of 100 µL. The concentrated fraction was analyzed by GC with a flame ionization detector (GC/FID) to determine coprostanol. For quality assurance, sets of 12 samples included: (1) a method blank, (2) a sample of reference material or a spiked blank, and, frequently, (3) a replicate of one of the samples.

2. MATERIALS

2.1. Instruments and accessories

2.1.1. Gas chromatographs, detectors, and data systems

Hewlett-Packard 5890 Series II GC/ECD (A graphite/Vespel O-ring is placed around the injector insert instead of a Viton O-ring.), PE Nelson Turbochrom data system, and Hewlett-Packard 7673 autosampler for analysis of chlorinated hydrocarbons. Hewlett-Packard Co., Avondale, PA. PE Nelson, Cupertino, CA.

Hewlett-Packard 5890 Series II GC/MSD/HP-UX ChemStation, and Hewlett-Packard 7673 autosampler - for analysis of aromatic hydrocarbons

Hewlett-Packard 5880A GC/FID (A graphite/Vespel O-ring is placed around the injector insert instead of a Viton O-ring), Level 4 integrator/terminal, PE Nelson Turbochrom data system, capillary-column inlet system, and Hewlett-Packard 7672 autosampler - for analysis of coprostanol

2.1.2. Gases and accessories

Air, Ohio-breathing, GCA Grade E (or equivalent)

Argon/methane, 95:5 (v:v)

Filter regulator, one for each primary air line, 2Z436A. Speedaire, Dayton Electric Mfg Co., Chicago, IL.

Helium, grade 4.5, purified, 99.9959%

Hydrogen, grade 5, ultra-pure, 99.9999%

Indicating moisture traps, one for each primary gas line, 700-5124. J & W Scientific Inc., Rancho Cordova, CA.

Indicating oxygen traps, one for each GC plus one additional for each ECD, 4004. Alltech Associates, Inc., Deerfield, IL.

Moisture trap, 2 for each primary air line, 700-404 with 1 pint absorbent refill, 018-1200. J & W Scientific Inc., Rancho Cordova, CA.

Moisture trap, two for each primary air line, F504-02AH. Watts Fluidair, Inc., Kittery, ME.

Nitrogen, grade 4.5, purified, 99.995%

Oxygen traps, disposable, one each for the primary He line and primary Ar/CH_4 line, S/P G5301-3. American Scientific Products, McGaw Park, IL.

Regulators, one for each gas cylinder, two-stage

Regulators, one for each primary gas line, one-stage

Split vent trap, one each for the split vent and septum purge outlets, 5879 with charcoal refill, 5872. Alltech Associates Inc., Deerfield, IL.

2.1.3. GC column and accessories

Adapters, Swagelok, 1/8-in brass

Brush, small-diameter, for cleaning glass insert

Capillary cleaving tool, 2-3740m. Supelco Inc., Bellefonte, PA.

Capillary column nuts, 05921-21170. Hewlett-Packard Co., Avondale, PA.

Connectors, Swagelok, as needed.

Copper tubing, 1/8-in o.d.

Ferrule, 40% graphite:60% Vespel, 1/4" to 6-mm reducing, shaped to fit the glass

insert and the weldment assembly, RF-400/6MM-VG2. Alltech Associates, Inc., Deerfield, IL.

Ferrule, 40% graphite:60% Vespel, 1/4", for sealing make-up gas adapter assembly to detector, SF-400-VG2. Alltech Associates, Inc., Deerfield, IL.

Ferrules, 15% graphite:85% Vespel, 1/16", no-hole, 100/0-VG1. (Use a #78 or #79 drill bit in a micro-drill press to drill a hole in the ferrule for the column.) Alltech Associates, Inc., Deerfield, IL.

Ferrules, Swagelok, 1/8-in brass

FID cleaning kit, 9301-0985. Hewlett-Packard Co., Avondale, PA.

Flowmeter, digital, 4068. Alltech Associates, Inc., Deerfield, IL.

Flowmeter FID adapter, 19301-60660. Hewlett-Packard Co., Avondale, PA.

Forceps, stainless-steel

Gas leak detector, liquid soap type, for air and N_2

Gas leak detector, thermal-conductivity type, 21-150. Gow-Mac Instrument Co., Bridgewater, NJ.

GC column, fused-silica, DB-5, 30-m x 0.25-mm, 0.25-µm film thickness, 122-5032, J & W Scientific Inc., Rancho Cordova, CA.

Glass wool, pesticide grade, 2-0409. Supelco, Inc., Bellefonte, PA.

Jeweler's loupe, 10X or 20X

Puller/Installer, 2-2406M. Supelco, Inc., Bellefonte, PA.

Ruler, 6 to 7 in. long with mm calibration

Septum, 11-mm, Thermogreen LB-2, 2-0654. Supelco, Inc., Bellefonte, PA.

Stainless-steel tubing, 1/8-in o.d.

Stopwatch, analog

Syringe, 10-µL, 701N. Hamilton Co., Reno, NV.

Syringe, 10-µL, . 80377, Hamilton Co., Reno, NV, available only from Hewlett-Packard as 9301-0725. Hewlett-Packard Co., Avondale, PA.

Tee, Swagelok, 1/8-in brass

2.1.4. HPLC system and accessories

Actuator, electronic, equipped with Rheodyne 7010 six-port injection valve, 732. Alcott Chromotography, Norcross, GA.

Autosampler, 231/401. Gilson Co., Middleton, WI.

Filter, in-line, 2-µm particle size, 7302. Rheodyne, Inc., Cotati, CA.

Fraction collector, 201. Gilson Co., Middleton, WI.

Helium degas system

Hydrocarbon trap filled with activated charcoal, 14634. Alltech Associates, Inc., Deerfield, IL.

Regulator, two-stage with stainless-steel diaphragm

Trap, heated oxygen/moisture, 2-3800M. Supelco Inc., Bellefonte, PA.

Trap, indicating oxygen, 4004. Alltech Associates, Inc., Deerfield, IL.

HPLC pump, 8800. Spectra-Physics, San Jose, CA.

Injection valve, six-port, 7030. Rheodyne, Inc., Cotati, CA.

Integrator, 4290. Spectra-Physics, San Jose, CA.

Polyaminocyano (PAC) columns, two each in series, Partisil, 250 x 4.6-mm, 10-µm particle size, 00G-0226-E0. Phenomenex, Inc., Torrance, CA.

Refractive index detector, LC-25. Perkin-Elmer, Norwalk, CT.

SEC guard column, Phenogel, 50 x 7.8-mm, 100-Å pore size, 10-µm particle size, 03B-2090-K0. Phenomenex, Inc., Torrance, CA.

Size-Exclusion (SEC) columns, two each in series, Phenogel, 250 x 22.5-mm, 100-Å pore size, 10-µm particle size, 00G-0642-Q0. Phenomenex, Inc., Torrance, CA.

UV/VIS detector, 8450. Spectra-Physics, San Jose, CA.

2.2. Chemicals

2.2.1. Solvents

The following solvents must be checked for purity by GC-ECD and GC/MS prior to use.

Dichloromethane (CH_2Cl_2) [75-09-2]
Hexane (C_6H_{14}) [110-54-3]
Methyl-t-butyl ether (MTBE) [$(CH_3)_2COCH_3$ [1634-04-4]

2.2.2. Reagents and other chemicals

N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) ($C_8H_{18}Si_2F_3NO$) [21149-38-2] with 1% trimethylchlorosilane (TMCS) [$(CH_3)_3SiCl$] [75-77-4] solution
Copper [744-50-8], reagent grade, fine granular, 4649. Mallinckrodt, Darmstadt, Germany.
Diatomaceous earth filtrate, Hyflo Super Cel, H333-3. Fisher Scientific, Pittsburgh, PA.
Hydrochloric acid (HCl) [7647-01-0], concentrated, reagent grade
Methanol (CH_3OH) [67-56-1], high-purity GC grade
Nitric acid (HNO_3) [52583-42-3], concentrated, reagent grade
Pyridine (C_5H_5N) [110-86-1], silylation grade
Sodium sulfate (Na_2S0_4) [7757-82-6], reagent grade, anhydrous granular

2.3. Column packings

Alumina, F-20, 80-200 mesh. Sigma Chemicals, St. Louis, MO.
Glass wool, Pyrex, 8-µm pore size, 3950. Corning Inc., Corning, NY.
Sand, Ottawa, kiln-dried, 30-40 mesh, SX0075-3. EM Science, Gibbstown, NJ.
Silica gel, Amicon 84040. Amicon, Beverly, MA.

2.4. Labware

Aluminum foil
Balances, analytical
Beakers, assorted sizes
Boiling chips, Teflon
Bottles, amber, 250-mL, Boston round with 24-mm mouth, narrow-mouth
Bottles, amber, 250-mL, Boston round with 28-mm mouth, wide-mouth
Caps, plastic, 28-mm, Teflon-lined to fit wide-mouth amber bottles
Caps, Teflon, 24-mm to fit narrow-mouth amber bottles
Centrifuge, with cups to accommodate 250-mL bottles and 100-mL tubes
Chromatography column, plain, 22-mm i.d. x 25-cm length, 5884-998. Ace Glass Inc., Vineland, NJ.
Desiccator with desiccant
Forceps, stainless-steel
Funnels, powder
Glass rods, 1.6-cm diameter, 20-cm and 45-cm lengths
Heating module for 2-mL GC vials
Jars, 4-oz and 2-oz with Teflon-lined plastic lids
Microdispensers, digital, 30-µL, 100-µL, and 250-µL, with glass bores
Muffle furnace
Ovens, drying, 50°C, 120°C, and 170°C
Pipets, transfer, Pasteur-style, with rubber bulbs
Rock tumbler, modified to accommodate 250-mL bottles
Rotary evaporator with 50-mL pear-shaped flasks, ROTOVAPOR-R. Brinkman Buchi, Westbury, NY.
Sample-concentrating apparatus. N_2 tank, two-stage regulator with needle-valve

outlet, 1/8-in Teflon tubing, and Pierce Reacti-Vap 18780 evaporating unit. Pierce Chemical Co., Rockford, IL.
Scissors, stainless-steel
Spatulae, stainless-steel
Spoons, measuring, 5-cc and 7.5-cc
Steam table
Syringes, 100-µL, 500-µL, and 1000-µL
Teflon sheeting, 10-mil thickness to line caps and lids
Tissue homogenizers, with stainless-steel probes, Tissumizer SDT 182EN. Tekmar, Cincinnati, OH.
Tube heater with glass-cylinder shroud, modified to accept 50-mL tubes, Kontes 720000-0000
Tubes, centrifuge, 50-mL, conical with Teflon-lined plastic caps
Tubes, centrifuge, 100-mL with Teflon-lined aluminum-foil caps
Vacuum filtration apparatus: Buchner funnel, 5-cm; rubber stopper, 12, 2-hole; Pyrex container to accommodate stopper and contain the 50-mL pear-shaped flask; filter paper, Whatman 1.
Vials, GC, 2-mL with 250-µL inserts, caps, and septa
Vials, GC, 2-mL with caps and septa
Vortex Genie, S8233. American Scientific Products, McGaw Park, IL.
Wash bottle, 500-mL, Teflon, for use with dichloromethane
Water bath
Weighing pans, aluminum

2.5. Standard solutions

2.5.1. Internal-standard solutions

Those from the National Institute of Standards and Technology (NIST) are so designated; all others are prepared in this laboratory. Concentrations given in parentheses are approximate to actual concentrations since those will vary slightly from batch to batch.

2.5.1.1. AH internal-standard solutions

Sediment AH GC I-std (gas chromatography internal standard) - hexamethylbenzene (HMB) (40 ng/µL) in hexane

Tissue AH GC I-std - HMB (16 ng/µL) in hexane

Sediment AH I-std (NIST) - naphthalene-d_8, acenaphthene-d_{10}, perylene-d_{12}, benzo[*a*]pyrene-d_{12} (50 ng/µL) in hexane

Tissue AH I-std - naphthalene-d_8, acenaphthene-d_{10}, perylene-d_{12}, benzo[*a*]pyrene-d_{12} (5 ng/µL) in hexane

Sediment AH HPLC I-std (high-performance liquid chromatography internal standard) - biphenyl-d_{10}, fluorene-d_{10}, phenanthrene-d_{10} (50 ng/µL) in hexane

Tissue AH HPLC I-std - biphenyl-d_{10}, fluorene-d_{10}, phenanthrene-d_{10} (5 ng/µL) in hexane

2.5.1.2. CH internal-standard solutions

CH GC I-std (NIST) - tetrachloro-m-xylene (TCMX) (2 ng/µL) in hexane

CH I-std - 4,4'-dibromooctafluorobiphenyl, 2,4-dibromobiphenyl (1 ng/µL) in hexane

CH HPLC I-std - tetrachloro-o-xylene (TCOX) (1 ng/µL) in hexane

2.5.1.3. Coprostanol internal-standard solutions

COP GC I-std (NIST) - HMB (80 ng/µL) in hexane

COP I-std - 5a-androstan-17b-ol (50 ng/µL) in hexane

COP HPLC I-std - benzo[*e*]pyrene-d_{12} (50 ng/µL) in hexane

2.5.2. Calibration solutions

AH/CH HPLC calibration solution - biphenyl (3 ng/µL), 4,4'-dibromooctafluorobiphenyl (1.5 ng/µL), perylene (1.5 ng/µL) in dichloromethane

COP HPLC calibration solution - coprostanol (180 ng/µL), benzo[*e*]pyrene-d_{12} (4 ng/µL) in MTBE

AH GC calibration-check solutions for sediments - AHs (see list in Table IV.10), plus HMB, biphenyl-d_{10}, fluorene-d_{10}, phenanthrene-d_{10}, naphthalene-d_8, acenaphthene-d_{10}, perylene-d_{12}, benzo[*a*]pyrene-d_{12} (Level 1 at 0.1 ng/µL except HMB at 5 ng/µL, Level 2 at 0.2 ng/µL except HMB at 5 ng/µL, Level 3 at 1 ng/µL except HMB at 5 ng/µL, Level 4 at 3 ng/µL except HMB at 5 ng/µL, Level 5 at 6 ng/µL except HMB at 5 ng/µL) in hexane

AH GC calibration-check solutions for tissues - AHs (see list in Table IV.10), plus HMB, biphenyl-d_{10}, fluorene-d_{10}, phenanthrene-d_{10}, naphthalene-d_8, acenaphthene-d_{10}, perylene-d_{12}, benzo[*a*]pyrene-d_{12} (Level 1 at 0.003 ng/µL except HMB at 3 ng/µL, Level 2 at 0.006 ng/µL except HMB at 3 ng/µL, Level 3 at 0.015 ng/µL except HMB at 3 ng/µL, Level 4 at 0.3 ng/µL except HMB at 3 ng/µL, Level 5 at 3 ng/µL) in hexane

CH GC calibration-check solutions - CHs (see list in Table IV.10), plus TCMX, TCOX, dibromooctafluorobiphenyl, dibromobiphenyl (Level 1 at 0.003 ng/µL except TCMX at 0.5 ng/µL, Level 2 at 0.01 ng/µL except TCMX at 0.5 ng/µL, Level 3 at 0.03 ng/µL except TCMX at 0.5 ng/µL in hexane, Level 4 at 0.1 ng/µL except TCMX at 0.5 ng/µL, Level 5 at 0.5 ng/µL) in hexane

Coprostanol GC calibration-check solution - coprostanol, HMB, benzo[*e*]pyrene-d_{12}, androstanol (5 ng/µL) in hexane

2.5.3. Spike solutions

AH spike solution - AHs (see list in Table IV.10) (6 ng/µL) in hexane

CH spike solution - CHs (see list in Table IV.10) (0.15 ng/µL) in hexane

COP spike solution (NIST) - coprostanol (50 ng/µL) in hexane

2.6. Purity testing

New glassware was used during analyses. All glassware and materials contacting the sample were washed with dichloromethane. Duplicate samples of the lot of dichloromethane being tested and of the lot currently in use were analyzed for impurities.

2.6.1. Sodium sulfate

Thirty grams of sodium sulfate were weighed into a labeled 100-mL centrifuge tube, 35 mL of dichloromethane was added to the tube and the sodium sulfate ground with a glass rod for 5 min.

The sample was mixed on a Vortex Genie for 1 min at setting 4-6. The sample was tightly capped and allowed to sit overnight in a refrigerator.

The sample was centrifuged for 5 min at 2000 rpm. The sample extract was decanted into a 50-mL centrifuge tube. No dichloromethane washes were used.

A boiling chip was added to the tube, and using the tube heater, the extract was concentrated to 1 mL.

The sample was transferred to a labeled GC vial, a small boiling chip was added, and using the steam table, the extract was concentrated to 170 µL.

Thirty microliters of Tissue AH GC I-std solution and 50 µL of CH GC I-std solution were added to the sample, and mixed on a Vortex Genie for 2 sec at setting 4-6.

Half of the extract was transferred to a GC vial with an insert labeled "AH" and the other half to a GC vial with an insert labeled "CH."

GC/MS analysis of the AH fraction and GC/ECD of the CH fraction is described in Section 10.

2.6.2. Hexane

Twenty-five milliliters of hexane was added to a 50-mL centrifuge tube. A boiling chip was added to the tube, and using the tube heater, the sample volume was reduced to 0.9 - 1.0 mL.

Fifty microliters each of Sediment AH GC I-std and CH GC I-std solutions were added to the sample, and mixed on a Vortex Genie for 2 sec at setting 4-6.

Equal amounts of the sample were transferred to two labeled GC vials. One of the vials was saved as a reserve. The other vial was used for GC/MS and GC/ECD analyses.

2.6.3. Dichloromethane

One hundred milliliters of dichloromethane was added to a wide-mouth 250-ml bottle. Three to four boiling chips were added to the bottle, and using the steam table, the sample volume was reduced to 15-20 mL.

The sample was transferred to a 50-mL tube. No dichloromethane washes were used. A boiling chip was added to the tube, and using the tube heater, the extract was concentrated to 1 mL.

The sample was transferred to a labeled GC vial, a small boiling chip was added, and using the steam table, the extract was concentrated to 170 µL.

Thirty microliters of Sediment AH GC I-std solution and 50 µL of CH GC I-std solution were added to the sample, and mixed on a Vortex Genie for 2 sec at setting 4-6.

Half of the extract was transferred to a GC vial with an insert labeled "AH", and the other half to a GC vial with an insert labeled "CH."

GC/MS analysis of the AH fraction and GC/ECD analysis of the CH fraction is described in Section 10.

2.7. Activation of column packings

2.7.1. Silica gel

The silica gel was activated by heating it at 700°C for 18 hr, then stored at 170°C. It was allowed to cool to room temperature in a desiccator just before weighing and use.

2.7.2. Alumina

The alumina was activated by heating it at 120°C for 2 hr. It was allowed to cool to room temperature in a desiccator just before weighing and use.

2.7.3. Copper

Less than one hour before use, the copper was activated by covering it with concentrated HCl, stirring with a glass rod, and allowing it to stand for 5 min. The copper was then washed three times each with water, methanol, and dichloromethane, in that order. The copper was stored covered with dichloromethane until use to avoid contact with air.

2.7.4. Sand

The sand was soaked in *aqua regia* overnight. *Aqua regia* is a 1:3 v:v mixture of concentrated HNO_3 and concentrated HCl. The sand was washed three times each with water, methanol and dichloromethane, in that order, then dried, and stored at 120°C.

2.7.5. Glass wool

The glass wool was prepared for use by heating it at 400°C for 18 hr. It was stored in a covered glass container at room temperature until use.

2.8. Lot testing and calibration of silica gel and alumina

2.8.1. Silica gel

2.8.1.1. Column calibration

Four silica gel spiked blanks were prepared by adding to each of four wide-mouth 250-mL bottles, 85 mL of dichloromethane, 100 µL of CH I-std solution, 100 µL of AH I-std solution (NIST), 1000 µL of CH spike solution, and 500 µL of PAH spike solution.

Two CH analyte-calibration solutions were prepared by adding to each of two GC vials labeled "CH Spike Vial" in addition to the sample number, 1000 µL of CH spike solution, 100 µL of CH I-std solution, and 100 µL of hexane.

Two AH analyte-calibration solutions were prepared by adding to each of two GC vials, labeled "AH Spike Vial" in addition to the sample number, 700 µL of hexane, 500 µL of PAH spike solution, and 100 µL of AH I-std solution (NIST).

Four silica gel/alumina columns were prepared for the spiked blanks using the current lot of alumina for all four columns. The current lot of silica gel was used for two of the columns and the lot being tested for the other two.

Each column was prepared by plugging a chromatography column with glass wool tamped down with a glass rod, then adding 10 cc of alumina, followed by 20 cc of silica gel, and 5 cc of sand.

Fifty milliliters of dichloromethane was slowly added to the column and allowed to drain into a waste container. This step was repeated once, then the tip of the column was rinsed with dichloromethane.

A second wide-mouth 250-mL bottle labeled "f_1" in addition to the sample number, was placed under the column.

A spiked blank was slowly decanted into the column and allowed to drain into the second bottle.

The first bottle was washed with approximately 5 mL of dichloromethane, and the washings slowly decanted into the column. This step was repeated twice.

The column wall was washed with 25 mL of dichloromethane, and the washings allowed to drain into the bottle.

After the solvent finished draining into the bottle, a 50-mL tube labeled "f_2" in addition to the sample number, was placed under the column. Ten milliliters of dichloromethane were added to the column and the fraction collected.

The step immediately above was repeated once, collecting another 10-mL fraction, except this fraction was labeled "f_3", in addition to the sample number.

The fractions were further processed as described in Section 2.8.1.4.

2.8.1.2. Silica purity check

Four silica gel blanks were analyzed by preparing four columns with only glass wool and 20 cc of silica gel each, using the current lot of silica gel for two of the columns and the lot being tested for the other two.

Fifty milliliters of dichloromethane was slowly added to the column and allowed to drain into a waste container. This step was repeated once, then the tip of the column was rinsed with dichloromethane.

A wide-mouth 250-mL bottle labeled with the sample number was placed under the column.

One hundred milliliters of dichloromethane was added to the column and allowed to drain into the bottle.

The column wall was washed with 35 mL of dichloromethane, and the washings allowed to drain into the bottle.

The blanks were further processed as described in Section 2.8.1.4.

2.8.1.3. Solvent blank preparation

Two silica gel solvent blanks were analyzed by preparing two columns with glass wool only.

Fifty milliliters of dichloromethane was slowly added to the column and allowed to drain into a waste container. This step was repeated once, then the tip of the column was rinsed with dichloromethane.

A wide-mouth 250-mL bottle labeled with the sample number was placed under the column.

One hundred milliliters of dichloromethane was added to the column and allowed to drain into the bottle.

The column wall was washed with 35 mL of dichloromethane, and the washings allowed to drain into the bottle.

The blanks were further processed as described in Section 2.8.1.4.

2.8.1.4. Concentration of samples

Six to eight boiling chips were added to the bottle containing the eluents for each of the samples above (spiked blanks, blanks, and solvent blanks).

Each sample was concentrated to 15-20 mL using a steam table, then transferred to an appropriately labeled 50-mL tube.

The bottle was washed with approximately 5 mL of dichloromethane, and the washings added to the tube. This step twice was repeated twice.

A boiling chip was added to the tube from the step immediately above and to the tubes containing the f_2 and f_3 fractions from the spiked blank columns, and, using a tube heater, each sample was concentrated to 1 mL.

Two milliliters of hexane and another boiling chip were added to each tube, and using a tube heater, the samples were concentrated to 1 mL.

To each spiked blank sample, 300 µL of CH GC I-std solution and 200 µL of Sediment AH GC I-std solution were added, and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

To each blank and solvent blank sample, 50 µL of CH GC I-std solution and 30 µL of Tissue AH GC I-std solution were added, and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

To each of the CH analyte-calibration solutions, 300 µL of CH GC I-std solution was added, and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

To each of the AH analyte-calibration solutions, 200 µL of Sediment AH GC I-std solution was added, and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

Each of the fractions from the spiked blank samples was transferred to a labeled GC vial and the volume brought to 1500 µL, either by adding hexane or by evaporating the excess under a gentle stream of nitrogen gas. Approximately half of each fraction was transferred to a GC vial labeled "CH" in addition to the sample number. The vial containing the remaining half was labeled "AH."

Each blank and solvent blank sample was transferred to a labeled GC vial and brought to 200 µL, either by adding hexane or by evaporating the excess under a gentle stream of nitrogen gas. Approximately half of each fraction was transferred to a GC vial labeled "CH" in addition to the sample number. The vial containing the remaining half was labeled "AH."

GC/ECD analysis of the CH analyte-calibration solutions and all of the CH fractions is described in Section 10.2. GC/FID analysis of the AH analyte-calibration solutions and the spiked blank AH fractions is described in Section 10.4, except the GC oven temperature program for AHs was used. GC/MS analysis of the AH analyte-calibration solutions, blank and solvent blank AH fractions is described in Section 10.3.

2.8.2. Alumina

2.8.2.1. Column calibration

Four alumina spiked blanks were prepared by adding to each of four 250-mL wide-mouth bottles, 85 mL of dichloromethane, 100 µL of CH I-std solution, 100 µL of AH I-std solution (NIST), 1000 µL of CH spike solution, and 500 µL of PAH spike solution.

Two CH analyte-calibration solutions were prepared by adding to each of two GC vials labeled "CH Spike Vial" in addition to the sample number, 1000 µL of CH spike solution, 100 µL of CH I-std solution, and 100 µL of hexane.

Two AH analyte-calibration solutions were prepared by adding to each of two GC vials labeled "AH Spike Vial" in addition to the sample number, 700 µL of hexane, 500 µL of PAH spike solution, and 100 µL of AH I-std solution (NIST).

Four silica gel/alumina columns were prepared for the spiked blanks using the current lot of silica gel for all four columns. The current lot of alumina was used for two of the columns and the lot being tested for the other two.

Each column was prepared by plugging a chromatography column with glass wool tamped down with a glass rod, then adding 10 cc of alumina, followed by 20 cc of silica gel and 5 cc of sand.

Fifty milliliters of dichloromethane was added slowly to the column, and allowed to drain into a waste container. This step was repeated once. The tip of the column was then rinsed with dichloromethane.

A second wide-mouth bottle, labeled "f_1" in addition to the sample number, was placed under the column.

A spiked blank was slowly decanted into the column and allowed to drain into the second bottle.

The first bottle was washed down with approximately 5 mL of dichloromethane and the washings slowly decanted into the column. This step was repeated twice.

The column wall was washed down with 25 mL of dichloromethane and the washings allowed to drain into the bottle.

After the solvent finished draining into the bottle, a 50-mL tube labeled "f_2" in addition to the sample number, was placed under the column. Ten milliliters of dichloromethane was added to the column and the fraction collected.

The step immediately above was repeated once, collecting another 10-mL fraction, except this fraction was labeled "f_3" in addition to the sample number.

The fractions were further processed as described in Section 2.8.2.4.

2.8.2.2. Alumina purity check

Four alumina blanks were generated by preparing four columns with only glass wool and 10 cc of alumina each, using the current lot of alumina for two of the columns and the lot being tested for the other two.

Fifty milliliters of dichloromethane was added to each column and allowed to drain into a waste container. This step was repeated once. The tip of the column was then rinsed with dichloromethane.

A wide-mouth bottle labeled with the sample number was placed under the column.

One hundred milliliters of dichloromethane was added to the column and allowed to drain into the bottle.

The column wall was washed with 35 mL of dichloromethane and the washings were allowed to drain into the bottle.

The blanks were further processed as described in Section 2.8.2.4.

2.8.2.3. Solvent blank preparation

Two alumina solvent blanks were generated by preparing two columns with glass wool only.

Fifty milliliters of dichloromethane was added to each column and allowed to drain into a waste container. This step was repeated once. The tip of the column was then rinsed with dichloromethane.

A wide-mouth bottle labeled with the sample number was placed under the column.

One hundred milliliters of dichloromethane were added to the column, and allowed to drain into the bottle.

The column wall was washed with 35 mL of dichloromethane and the washings were allowed to drain into the bottle.

The blanks were further processed as described in Section 2.8.2.4.

2.8.2.4. Concentration of samples

Six to eight boiling chips were added to the bottle containing the eluent of each of the samples above (spiked blanks, blanks, and solvent blanks).

Using a steam table, each sample was concentrated to 15-20 mL, then transferred to a labeled 50-mL tube.

The bottle was washed down with approximately 5 mL of dichloromethane and the washings added to the tube. This step was repeated twice.

A boiling chip was added to each tube from the step immediately above and to the tubes containing the f_2 and f_3 fractions from the spiked blank columns, and the sample volumes reduced to 1 mL using a tube heater.

Two milliliters of hexane and another boiling chip were added to each tube, and using a tube heater, the sample volume reduced to 1 mL.

Three hundred microliters of CH GC I-std solution and 200 µL of Sediment AH GC I-std solution were added to the spiked blank samples, and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

Fifty microliters of CH GC I-std solution and 30 µL of Tissue AH GC I-std solution were added to the blank and solvent blank samples, and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

Three hundred microliters of CH GC I-std solution was added to the CH analyte-calibration solutions, and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

Two hundred microliters of Sediment AH GC I-std solution was added to the AH analyte-calibration solutions, and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

Each of the fractions from the spiked blank samples were transferred to a labeled GC vial and the volume brought to 1500 µL, either by adding hexane or by evaporating the excess under a gentle stream of nitrogen gas. Approximately half of each fraction was transferred to a GC vial labeled "CH" in addition to the sample number. The other half was transferred to a vial labeled "AH."

Each of the blank and solvent blank samples were transferred to labeled GC vials and the volumes brought to 200 µL, either by adding hexane or by evaporating the excess under a gentle stream of nitrogen gas. Approximately half of each fraction was transferred to a GC vial labeled "CH" in addition to the sample number. The other half was transferred to a vial labeled "AH."

GC/ECD analysis of the CH analyte-calibration solutions and all of the CH fractions is described in Section 10.2. GC/FID analysis of the AH analyte-calibration solutions and the spiked blank AH fractions is described in Section 10.4, except the GC oven temperature

program for AHs was used. GC/MS analysis of the AH analyte-calibration solutions and the blank and solvent blank AH fractions is described in Section 10.3.

3. PREPARATION OF COMPOSITE SAMPLES

3.1. Preparation of composite samples for sediments

All samples were removed from the freezer and allowed to thaw completely. The standing water was decanted from the top of each sample. Using a spatula, each sample was stirred to homogenize thoroughly, and all pebbles, shells, biota, and other detritus discarded.

Using a spatula, approximately 15 g of each sample was placed in a 4-oz jar, and the contents mixed thoroughly to form a homogeneous composite. The unused portion of each sample was returned to the freezer.

The jar was capped, labeled with the appropriate composite sample designation, and stored in the freezer until needed.

The number of each sample used to prepare the composite sample was recorded.

3.2. Preparation of composite samples for tissues

The samples were removed from the freezer, and using dichloromethane-rinsed utensils, immediately transferred from their containers to a dichloromethane-rinsed 2-oz jar labeled with the proper composite sample designation. In the case of liver tissues, the samples were kept as cold as possible, allowing them to thaw only enough to be easily removed from their containers.

The composite sample was homogenized by cutting it into small pieces using a pair of dichloromethane-rinsed scissors and blending it, or if the composite was exceptionally large, using a Tekmar Tissumizer at a low setting. After the composite was thoroughly homogenized, the jar was capped, labeled with the appropriate composite sample designation, and stored in the freezer until needed.

4. EXTRACTION OF SEDIMENT SAMPLES

4.1. Extraction of samples for AHs, CHs, and coprostanol

Sediments were customarily analyzed in sets of twelve samples each. In addition to the 8-9 regular sediment samples in each set, there were one or more method blank samples, one spiked blank sample, one reference sediment sample, one field blank sample, and one duplicate sediment sample.

For each sample to be extracted, except the field blank, 100 mL of dichloromethane was added to a narrow-mouth 250-mL bottle with a Teflon cap. The bottle for each sample was labeled "SEDIMENT" in addition to the sample number.

The field blank was prepared at this point in the analytical procedure by washing down the empty sample container three times with 10-mL aliquots of dichloromethane, adding the combined washes to a narrow-mouth bottle, then adding an additional 70 mL of dichloromethane.

To each bottle from the step immediately above, 60 g of sodium sulfate, 7.5 cc of activated copper, 100 µL of AH I-std solution, 100 µL of CH I-std solution, and 100 µL of COP I-std solution were also added.

To the bottle for the spiked blank, 500 µL of PAH spike solution, 1000 µL of CH spike solution, and 100 µL of COP spike solution were added.

The blank, spiked blank, and/or field blank bottles received nothing more.

Two AH analyte-calibration solutions were prepared by adding to each of two GC vials, labeled "AH Spike Vial" in addition to the sample number, 500 µL of PAH spike solution, 180 µL of hexane, and 100 µL of AH I-std solution.

Two CH analyte-calibration solutions were prepared by adding to each of two GC vials, labeled "CH Spike Vial" in addition to the sample number, 1000 µL of CH spike solution and 100 µL of CH I-std solution.

Two COP analyte-calibration solutions were prepared by adding to each of two GC vials, labeled "COP Spike Vial" in addition to the sample number, 200 µL of hexane, 100 µL of COP spike solution, and 100 µL of COP I-std solution.

The excess water from the sediment was decanted and the sample stirred to homogenize it. All pebbles, seaweed, wood, crabs, and other detritus was discarded. Using a spatula and powder funnel, and being careful not to splash from the bottle, approximately 10 ± 0.5 g of sediment to the nearest 0.01 g was weighed into each of the "SEDIMENT" labeled bottles.

To avoid clumping and hardening of the sodium sulfate, the following two steps were performed immediately after adding the sediment to the bottles.

Each bottle was capped, screwing the Teflon cap on just tight enough to prevent leakage. Each bottle was shaken manually until the contents were loose. If the bottle cap leaked during manual shaking, a rinsed 3"x3" square of Teflon sheeting was placed over the mouth of the bottle before recapping. The Teflon sheeting was secured tightly around the neck of the bottle using a rubber band.

Each bottle was rolled for 16 hr (overnight) on the tumbler at 100-250 rpm.

Each bottle was centrifuged for 5 min at 1500 rpm. Each extract was decanted into a wide-mouth 250-mL bottle labeled "AH/CH EXTRACT", in addition to the sample number.

Fifty milliliters of dichloromethane was added to each sample, and the above three steps repeated, except each bottle was rolled for 6 hr (i.e., during the day). The second extract was decanted into the bottle labeled "AH/CH EXTRACT."

The extraction step above was repeated immediately, except each bottle was rolled for 16 hr (overnight). The third extract was decanted into the bottle labeled "AH/CH EXTRACT."

To each AH/CH EXTRACT from the step immediately above, 100 µL each of Sediment AH HPLC I-std, CH HPLC I-std, and COP HPLC I-std solutions were added, and the bottles swirled to thoroughly mix the contents.

To each of the AH analyte-calibration solutions, 100 µL of Sediment AH HPLC I-std solution was added and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

To each of the CH analyte-calibration solutions from the EXTRACTION section, 100 µL of CH HPLC I-std solution was added and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

To each of the COP analyte-calibration solutions from the EXTRACTION section, 100 µL of COP HPLC I-std solution was added and the contents mixed them on a Vortex Genie for 2 sec at setting 4-6.

Approximately 50 mL of the AH/CH EXTRACT were transferred into a second wide-mouth bottle, which was labeled "COP EXTRACT" in addition to the sample number.

The AH/CH and the COP EXTRACTs were further processed as described in the Pre-cleanup Sections 4.2 and 4.4.

4.2. Precleanup of AH/CH extracts

A cleanup column was prepared by adding a 10-15-mm plug of glass wool to a chromatography column and tamping it down firmly with a glass rod. Ten cubic centimeters of alumina, followed by 20 cc of silica gel and 5 cc of sand were added to the column.

Fifty milliliters of dichloromethane was slowly added to the column, and allowed to drain into a waste container. This step was repeated once. The tip of the column was then rinsed with dichloromethane.

A wide-mouth 250-mL bottle, labeled "AH/CH" in addition to the sample number, was placed under the column and the extract slowly decanted from the bottle labeled "AH/CH EXTRACT" into the column.

The bottle was washed with approximately 5 mL of dichloromethane and the washings decanted into the column. This step was repeated twice.

The column wall was washed with 35 mL of dichloromethane, and the washings allowed to drain into the bottle.

4.3. Concentration of AH/CH extracts

Six to eight boiling chips were added to the bottle labeled "AH/CH", and, using a steam table, the extract volume was reduced to 15-20 mL.

The extract was transferred to a 50-mL centrifuge tube labeled "AH/CH" in addition to the sample number.

The bottle was washed with approximately 5 mL of dichloromethane and the washings added to the tube. This step was repeated twice.

A boiling chip was added to the tube, and, using a tube heater, the extract volume was reduced to 1 mL

Activated copper was added to the tube, a few grains at a time, until no further discoloring of the copper occurred. The tube was capped and stored overnight in a refrigerator.

The AH/CH extract was transferred to a GC vial and the volume brought to 750 µL, either by adding dichloromethane or by evaporating the excess under a gentle stream of nitrogen gas.

SEC-HPLC chromatography is described in Section 7.

4.4. Precleanup of COP extracts

Six to eight boiling chips were added to the bottle labeled "COP EXTRACT", and, using a steam table, the extract volume was reduced to 15-20 mL.

A cleanup column was prepared by adding a 10-15-mm plug of glass wool to a chromatography column, tamping it down firmly with a glass rod, and then adding 5 cc of sand.

The column was washed with approximately 20 mL of dichloromethane, and the washings allowed to drain into a waste container. This step was repeated twice. The tip of the column was then rinsed with dichloromethane.

A 50-mL tube, labeled "COP" in addition to the sample number, was placed under the column and the concentrated extract slowly decanted into the column.

The bottle was washed with approximately 5 mL of dichloromethane and the washings decanted into the column. This step was repeated twice.

The column wall was washed with approximately 5 mL of dichloromethane, and the washings allowed to drain into the tube. This step was repeated once.

4.5. Concentration of COP extracts

A boiling chip was added to the tube containing the COP extract, and, using a tube heater, the extract volume was reduced to 1 mL.

Two milliliters of MTBE was added to the tube and the volume reduced to 1 mL.

Activated copper was added to the tube, a few grains at a time, until no further discoloring of the copper occurred. The tube was capped and stored overnight in a refrigerator.

The COP extract was transferred to a GC vial, labeled "COP EXTRACT" in addition to the sample number, and the volume brought to 250 µL by evaporating the solvent under a gentle stream of nitrogen gas.

PAC-HPLC chromatography is described in Section 8.

5. EXTRACTION OF TISSUE SAMPLES

Tissues were customarily analyzed in sets of twelve samples each. In addition to the 8-9 regular tissue samples in each set, there were one or more method blank samples, one spiked

blank sample, one matrix spike sample, one reference tissue sample, one field blank sample, and one duplicate tissue sample.

5.1. Extraction of samples for AHs and CHs

For composite samples larger than 1.5 g, 0.5 g was used for the Dry Weight Determination and the remainder was extracted for analysis of AHs and CHs.

For each sample to be extracted, the tissue was weighed to the nearest 0.01 g into a labeled 100-mL centrifuge tube with a Teflon-lined foil cap. Care was taken to place the sample on the bottom and not the sides of the tube. The remaining sample was stored in a freezer.

Thirty-five milliliters of dichloromethane was added to each sample tube, except the field blank.

The field blank was prepared at this point in the analytical procedure by washing down the empty sample container three times with 10-mL aliquots of dichloromethane, adding the combined washings to an empty centrifuge tube, then adding 5 mL more of dichloromethane to the tube.

One hundred microliters each of Tissue AH I-std and CH I-std solutions were added to each tube.

To the tube of the spiked blank or matrix spike, 50 µL of PAH spike solution, 100 µL of DBT spike solution, and 1000 µL of CH spike solution were also added.

Two CH analyte-calibration solutions were prepared by adding to each of two GC vials labeled "CH Spike Vial" in addition to the sample number, 1000 µL of CH spike solution and 100 µL of CH I-std solution.

Two low-level AH analyte-calibration solutions were prepared by adding to each of two GC vials labeled "AH Spike Vial" in addition to the sample number, 500 µL of hexane, 50 µL of PAH spike solution, 100 µL of DBT spike solution, and 100 µL of AH I-std solution.

Twenty five grams of sodium sulfate was added to each tube.

The sample in the tube was macerated/extracted for 0.5 min with a Tissumizer at setting 80. The speed was reduced to setting 60 and maceration continued for 1.5 min. Care was taken to avoid spattering the tissue.

The Tissumizer probe was rinsed with approximately 5 mL of dichloromethane and the washings collected in the tube.

The sample was centrifuged for 5 min at 2,000 rpm and the extract decanted into a wide-mouth 250-mL bottle, labeled "AH/CH EXTRACT" in addition to the sample number.

Thirty-five milliliters of dichloromethane was added to each sample tube, and the three steps immediately above were repeated, combining the second extract with the first.

The sodium sulfate/sample mass was washed by adding 10 mL of dichloromethane to the tube and then swirling the tube to thoroughly mix the contents. The washings were decanted into the bottle.

One hundred microliters of CH HPLC I-std solution and 50 µL of Tissue AH HPLC I-std solution were added to the bottle and the contents swirled thoroughly.

One hundred microliters of CH HPLC I-std solution was added to each of the CH analyte-calibration solutions from the Extraction section, and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

Fifty microliters of Tissue AH HPLC I-std solution was added to each of the low-level AH analyte-calibration solutions from the Extraction section, and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

The AH/CH EXTRACT was further processed as described in the Precleanup Section 5.4.

5.2. Extraction of samples for CHs and lipid

Tissues were customarily analyzed in sets of twelve samples each. In addition to the 8-9 regular tissue samples in each set, there may be one or more method blank samples, one spiked blank sample, one matrix spike sample, one reference tissue sample, one field blank sample, and one duplicate tissue sample. For composite samples larger than 3.0 g, 0.5 g were used for Dry Weight Determination, 0.2 g were taken for triglyceride analysis, and 3 g were extracted and the extract used for CHs and lipid. For samples of between 2.5 - 3.0 g, 0.5 g was used for Dry Weight Determination. The remainder of the sample was extracted and the extract used for CHs and lipid. No triglyceride analysis was performed. For samples of between 1.5 - 2.5 g, 0.5 g were used for Dry Weight Determination, the remainder was extracted, and the total extract used for CHs. No triglyceride or lipid analyses were performed. For samples smaller than 1.5 g, the total amount was extracted and all of the extract used for CH analysis. No other analysis was performed.

Using a spatula, and being careful to place the sample on the bottom and not the sides, the appropriate amount of tissue (see above) weighed to the nearest 0.01 g was placed in a labeled 100-mL centrifuge tube with a Teflon-lined foil cap.

For samples larger than 1.5 g, approximately 0.5 g were set aside for Dry Weight Determination, and the remaining portion was stored in a freezer.

Thirty-five milliliters of dichloromethane was added to each tube, except for the field blank.

The field blank was prepared at this step in the analytical procedure by washing down the empty sample container three times with 10-mL aliquots of dichloromethane, adding the combined washes to a labeled 100-mL centrifuge tube with Teflon lined foil cap, then adding 5 mL of dichloromethane.

One hundred microliters of CH I-std solution was added to each tube.

One thousand microliters of CH spike solution was also added to the tube for the spiked blank and/or matrix spike.

Two CH analyte-calibration solutions were prepared by adding to each of two GC vials labeled "CH Spike Vial" in addition to the sample number, 1000 µL of CH spike solution and 100 µL of CH I-std solution.

For the blanks and each sample of 2.5 g or more, two wide-mouth 250-mL bottles with Teflon-lined caps were prepared: one bottle was labeled "CH EXTRACT", in addition to the sample number; the other bottle was labeled "Lipid EXTRACT", in addition to the sample number. Each bottle was tared. For each sample smaller than 2.5 g, one wide-mouth 250-mL bottle with a Teflon-lined cap was assembled and labeled "CH EXTRACT" in addition to the sample number.

Twenty-five grams of sodium sulfate was added to each sample.

The sample in the tube was macerated/extracted for 0.5 min with a Tissumizer at setting 80. The speed was reduced to setting 60 and maceration continued for 1.5 min. Care was taken to avoid spattering the tissue.

The Tissumizer probe was rinsed with approximately 5 mL of dichloromethane and the washings collected in the tube.

The sample was centrifuged for 5 min at 2,000 rpm and the extract decanted into the bottle labeled "CH EXTRACT", in addition to the sample number. Care was taken not to transfer any solids.

Thirty-five milliliters of dichloromethane was added to each sample tube, and the three steps immediately above were repeated, combining the second extract with the first.

The sodium sulfate/sample mass was washed by adding 10 mL of dichloromethane to the tube and then swirling the tube to thoroughly mix the contents. The washings were decanted into the bottle labeled "CH EXTRACT", being careful to leave all solids in the tube. The bottle was recapped.

Fifty microliters of CH HPLC I-std solution was added to the bottle, and the contents swirled to thoroughly mix the contents.

One hundred microliters of CH HPLC I-std solution was added to each of the CH analyte-calibration solutions from the Extraction section, and the solution mixed on a Vortex Genie for 2 sec at setting 4-6.

For the blanks and samples of 2.5 g or more, the bottle containing the total CH EXTRACT was weighed and approximately 30 ml of the CH EXTRACT was transferred into the bottle labeled "Lipid EXTRACT" in addition to the sample number. The bottles were capped, and the bottle containing the Lipid EXTRACT was reweighed.

The lipid and CH extracts were further processed as described in Sections 5.3 and 5.4 respectively.

5.3. Concentration of lipid extracts

Six to eight boiling chips were added to the bottle containing the Lipid EXTRACT, and, using a steam table, the extract volume was reduced to 15-20 mL; 7.5 cc of diatomaceous earth filtrate was added to the bottle and swirled.

A tared pear-shaped flask was placed in the filtering container of the vacuum filtration apparatus and the Büchner funnel positioned so that the tip of the funnel was just below the mouth of the flask. A filter paper was placed in the funnel.

The vacuum source was activated and the filter paper wetted with dichloromethane. A seal formed between the funnel and the paper when the paper could no longer be moved. The Lipid EXTRACT was decanted from the bottle into the Büchner funnel.

The bottle was washed with approximately 5 mL of dichloromethane and the washings decanted into the funnel. This step was repeated twice.

Using the rotary evaporator, the dichloromethane was evaporated from the extract in the flask. The temperature of the water bath was set at 35-40°C. The solvent was evaporated at as fast a rotation as possible without splashing the sample. When water condensed on the outside of the flask, the flask was lowered into the bath, being careful not to boil the extract. If boiling occurred, the flask was quickly lifted out of the water bath and either the temperature of the bath lowered or air was bled into the system to stop the boiling.

When the dichloromethane evaporated and any remaining liquid was thick and oily, the vacuum was broken, the flask was removed, and placed in a drying oven at 50°C for 2 hr.

The flask was cooled in a desiccator, and weighed. The weight of the dried lipid was then calculated.

Using the weights of the CH EXTRACT bottle and the Lipid EXTRACT bottle, the fraction (F_X) of the total CH EXTRACT that was used for the Lipid EXTRACT was calculated as follows:

$$F_X = \frac{\text{Wt. of Lipid extract and bottle - Wt. of bottle}}{\text{Wt. of total CH extract and bottle - Wt. of bottle}} .$$

Finally, the percent lipid content of the original sample was calculated, as follows:

$$\text{Percent Lipid} = \frac{\text{Wt. of lipid}}{(F_X \text{ extract taken}) \text{ (Orig. sample wt.)}} 100\%$$

5.4. Pre-cleanup of AH/CH and CH extracts

A cleanup column was prepared by adding a 10-15-mm plug of glass wool to a chromatography column and tamping it down with a glass rod, then adding 10 cc of alumina, followed by 20 cc of silica gel and 5 cc of sand.

Fifty milliliters of dichloromethane was added slowly to the column, and allowed to drain into a waste container. This step was repeated once. The tip of the column was then rinsed with dichloromethane.

Another wide-mouth 250-mL bottle, labeled "AH/CH" or "CH" in addition to the sample number, was placed under the column. The extract of samples prepared in either Sections 5.1 or 5.2 was slowly decanted into the column and allowed to drain into the bottle.

The first bottle was washed with approximately 5 mL of dichloromethane, and the washings slowly decanted into the column. This step was repeated twice, and the washings allowed to drain into the bottle.

The column wall was washed down with 35 mL of dichloromethane, and the washings allowed to drain into the bottle.

The AH/CH extract was concentrated as described in Section 5.5, and the CH extract as described in Section 5.6.

5.5. Concentration of AH/CH extracts

Six to eight boiling chips were added to the bottle containing the AH/CH extract from the Pre-cleanup Section, and using a steam table, the extract volume was reduced to 15-20 mL

The extract was transferred to a 50-mL tube.

The bottle was washed with approximately 5 mL of dichloromethane, and the washings added to the tube. This step was repeated twice.

A boiling chip was added to the tube, and, using a tube heater, the extract volume was reduced to 1 mL.

Using a pipet, the extract was transferred to a GC vial labeled "EXTRACT" in addition to the sample number, and the volume brought to 1 mL under a gentle stream of nitrogen gas.

SEC-HPLC CHROMATOGRAPHY is described in Section 7.

5.6. Concentration of CH extracts

Six to eight boiling chips were added to the bottle containing the CH extract from the Precleanup Section, and, using a steam table, the extract volume was reduced to 15-20 mL.

The extract was transferred to a 50-mL tube.

The bottle was washed with approximately 5 mL of dichloromethane, and the washings were added to the tube. This step was repeated twice.

A boiling chip was added to the tube, and using a tube heater, the extract volume was reduced to 1 mL.

Using a pipet, the extract was transferred to a GC vial labeled "EXTRACT" in addition to the sample number, and the volume brought to 500 µL under a gentle stream of nitrogen gas.

SEC-HPLC CHROMATOGRAPHY is described in Section 7.

6. DRY WEIGHT DETERMINATION

6.1. Dry weight determination for sediment samples

The sample number was etched on the tab of an aluminum weighing pan.

An envelope was made from a 10 x 18-inch piece of aluminum foil, and up to six of the numbered weighing pans were placed in it. The envelope was closed but not sealed, and then placed in a drying oven at 120°C overnight.

The envelope containing the pans was removed from the oven and cooled in a desiccator for 30 min.

Each pan was weighed to the nearest 0.1 mg. The pans were handled with forceps.

Using a spatula, each sample was stirred to homogenize thoroughly, and all pebbles, shells, biota, and other detritus discarded.

Ten ± 0.5 grams of the sediment were placed in each the pan and the weight recorded to the nearest 0.1 mg. This is the wet weight.

The weighing pans were returned to the foil envelope. The envelope was closed as before, but not sealed, then placed in a drying oven at 120°C for 24 hr.

The envelope was removed from the oven and allowed to cool in a desiccator for 30 min. The sample was weighed again to determine the dry weight.

6.2. Dry weight determination for tissue samples

The procedure followed was the same as above for sediments, except approximately 0.5 g of tissue sample was used.

6.3. Dry weight calculation

The dry weight percent (Dry Wt %) was determined as follows:

$$\text{Dry wt. \%} = \frac{\text{Dry wt.- Pan wt.}}{\text{Wet wt.- Pan wt.}} \ 100\%.$$

7. SEC-HPLC CHROMATOGRAPHY

7.1. Calibration of the SEC-HPLC system

A schematic of the HPLC system is shown in Figure IV.13, and the analytical sequence is listed in Table IV.11.

A fresh bottle of dichloromethane was installed in the solvent reservoir holder, and the solvent inlet filter and He gas diffuser placed at the bottom of the reservoir. The dichloromethane was degassed for approximately 10 min prior to calibration.

The flow rate of the solvent (mobile phase) was set at 7 mL/min and the pump initialized until the system pressure stabilized.

Empty GC vials without caps were placed in the first (back) row of the autosampler rack. Three to four vials of AH/CH HPLC calibration solution were taken from the refrigerator, uncapped, and placed in the second row of the autosampler tray.

Three to four 50-mL tubes were placed in the rack of the fraction collector to collect the fractions from the runs of the calibration solution.

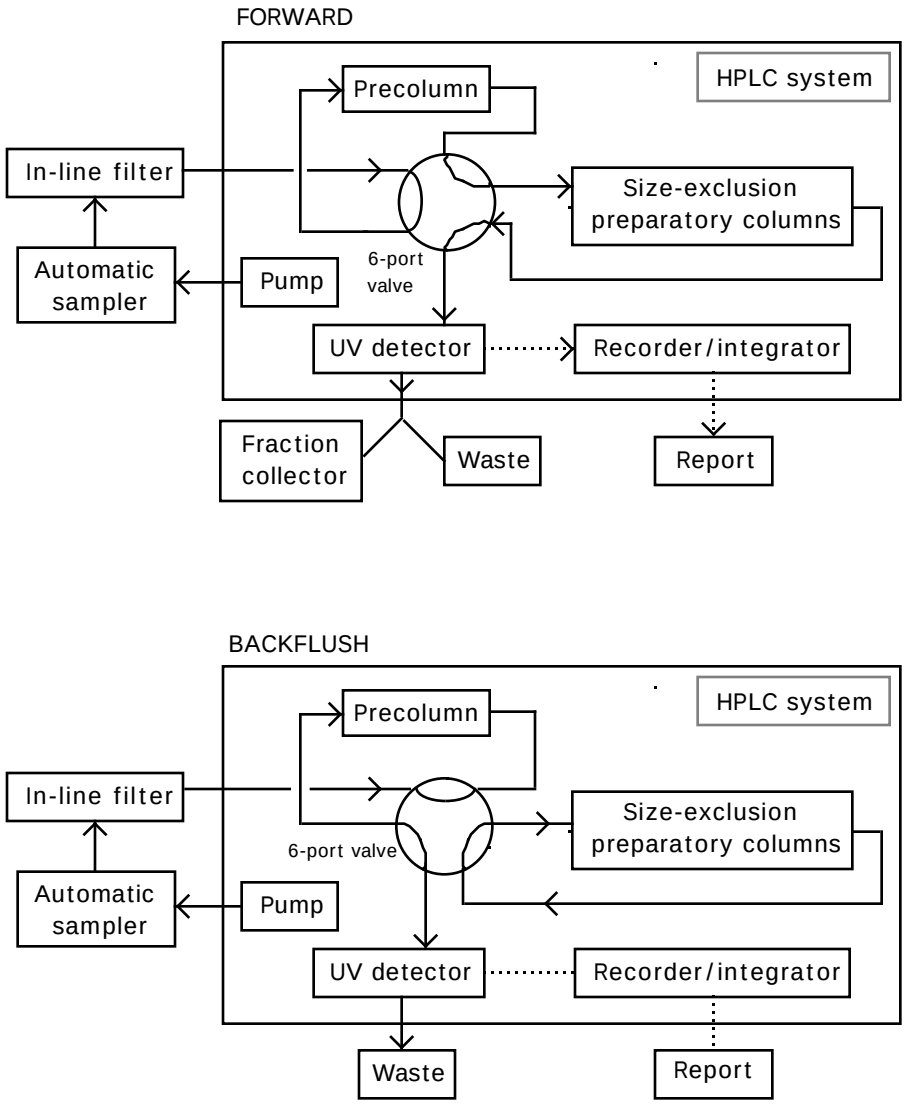

Figure IV.13. Normal and backflush operational diagram of HPLC system (Solid lines with arrows indicate direction of solvent flow. Dashed lines indicate electrical connections.).

The autosampler sequence began by rinsing the sample loop and the injection needle, then aspirating 30 µL of air, and 250 µL of the calibration solution. With each injection, the autosampler automatically placed the pump, integrator, and fraction collector into a processing sequence.

For each use of the calibration solution, the retention times of 4,4'-dibromooctafluorobiphenyl (DOB; first peak) and perylene (PER; third peak), and the system pressure were recorded.

Table IV.11. Instrument analysis sequence of HPLC.

The flow diagram and time table must be adjusted for the user's particular instrument.

Time (min)	Instrument	Activity/instruction
<0	Autosampler	Sample loop loaded; see 18 min (below)
0	HPLC	Flow = 7 mL/min; oven = 40°C; solvent = 100% methylene chloride
0	Autosampler	Sample injected; signal sent to HPLC to start data system
0-20	HPLC	Isocratic elution
1	Autosampler	Injection port rinsed; needle rinsed
~14-19	Fraction collector	Fraction collected
18	Autosampler	New sample picked up; loop loaded
20	HPLC	Data system ends integration

When the retention times of the DOB and PER peaks in the calibration solution have stabilized (3-4 applications), the system was ready to perform sample analyses. After the injection needle was rinsed, the autosampler program was stopped. Tubes from the fraction collector were emptied into the TOXIC WASTE receptacle. The rinse vials from the autosampler were placed in a fume hood, the solvent allowed to evaporate, and the vials discarded. The vials of calibration solution were removed from the autosampler tray, recapped, and set aside until needed for sample runs.

Collection time for the AH/CH or CH fraction were determined based on retention times of DOB and PER from the last use of the AH/CH HPLC calibration solution.

7.2. Isolation of AH/CH or CH fractions

A stable solvent (mobile phase) flow rate of 7 mL/min was established.

Fifty-milliliter tubes labeled with the appropriate sample numbers were placed in the rack of the fraction collector.

Empty GC vials without caps were placed in the first (back) row of the autosampler tray.

Sample vials for the autosampler tray were prepared by removing the cloth labels and relabeling the vials using a felt pen. The caps were replaced on the sample vials with caps having septa made from 10-mil Teflon sheeting.

Vials of AH/CH HPLC calibration solution were placed in positions 8 and 15 of the second row of the autosampler tray. A GC vial filled with dichloromethane was placed in position 1 as the HPLC blank, followed by the method blank in position 2, then in numerical order, the sample extracts from the Concentration of extracts sections.

The HPLC blank and the method blank were processed through the system with the UV detector range set at 0.05 Absorption Units Full Scale (AUFS). The detector range was changed to 2.0 AUFS for sediment extracts and to 0.50 AUFS for tissue extracts. The wavelength on the detector was set at 254 nm. Column time for samples and calibration solutions was 20 min.

For analysis of the calibration solution, the UV detector range was set at 0.05 AUFS, and the retention times of DOB and PER and the system pressure recorded.

The collection time for the AH/CH or CH fraction (the time is the same for either fraction) was determined based on the retention times for DOB and PER from the last use of the AH/CH HPLC calibration solution, and recorded.

If the retention time for DOB changed by ±0.05 min, the starting time for collection of the fraction was changed. If the retention time for PER changed ±0.05 min with respect to the new retention time for DOB, the collection time for the fraction was changed.

When samples containing sulfur or particulates were processed, the system was backflushed for 2-3 min after all the fractions were collected.

The vials containing the sample extracts were recapped with the original caps, and stored in a freezer.

The tubes containing the AH/CH or CH fractions were capped and stored for processing for GC analysis.

8. PAC-HPLC CHROMATOGRAPHY

8.1. Calibration of the PAC-HPLC system

A fresh bottle of MTBE was installed in the solvent reservoir holder, making sure the solvent inlet filter and the He gas diffuser were at the bottom of the reservoir. The MTBE was degassed for approximately 10 min prior to performing the calibration.

The flow rate of the solvent (mobile phase) was set at 2 mL/min and the pump started. The system pressure was allowed to stabilize.

Empty GC vials without caps were placed in the first (back) row of the autosampler tray.

Three to four vials of COP HPLC calibration solution were removed from the refrigerator, uncapped, and placed in the second row of the autosampler tray.

Three to four 50-mL tubes were placed in the rack of the fraction collector to collect the fractions from processing of the COP calibration solution.

The autosampler sequence began with rinsing of the sample loop and the injection needle, aspiration of 30 µL of air, followed by 100 µL of the calibration solution. After each injection, the autosampler placed the pump, integrator, and fraction collector in the processing sequence.

For each analysis of the calibration solution, the retention times of benzo[*e*]pyrene-d_{12} (dBeP; by UV detector) and coprostanol (COP; by RI detector), and the system pressure were recorded.

When the retention times of the dBeP and COP peaks in the calibration solution stabilized (3-4 runs), the system was ready for samples. The injection needle was rinsed, and the autosampler program stopped. The tubes from the fraction collector were emptied into the TOXIC WASTE receptacle. The rinse vials from the autosampler were placed in a fume hood, the solvent allowed to evaporate, then discarded. The vials of calibration solution were removed from the autosampler tray, recapped, and set aside until needed for sample analyses.

The collection time for the COP fraction was determined based on the retention times for dBeP and COP from the last analysis of the COP HPLC calibration solution.

8.2. Isolation of COP fractions

A stable solvent (mobile phase) flow rate of 2 mL/min was established.

Fifty-milliliter tubes labeled with the appropriate sample numbers were placed in the even numbered positions of the fraction collector rack. Fifty-milliliter tubes for waste collection were placed in the odd numbered positions and in position 14 for the first analysis of the calibration solution.

Empty GC vials without caps were placed in the first (back) row of the autosampler.

The COP extracts from the concentration section were transferred to GC vials with 250-µL inserts and with caps having septa made from 10-mil Teflon sheeting.

Vials of COP HPLC calibration solution were placed in positions 7 and 15 of the second row of the autosampler tray. A GC vial filled with MTBE was placed in position 1 as the HPLC Blank, followed by the method blank in position 2, then in numerical order, the sample extracts.

The HPLC blank, method blank, the sample extracts, and calibration solutions were analyzed with the UV detector set at 2.0 AUFS. The analysis time for samples and calibration solutions was 18 min.

The collection time for the COP fraction was based on the retention times for dBeP and COP from the last analysis of the COP HPLC calibration solution, and the fraction collection times were recorded. Also, during the first minute of each run, a waste fraction was collected to rinse the port of the fraction collector.

After the fraction collector collected the COP fractions from the first seven analyses, the sample fractions were capped, the fraction from the calibration solution was disposed in the TOXIC WASTE receptacle, and the remaining labeled sample tubes placed in the empty spaces of the rack in numerical order to collect the next seven fractions.

Note that when the fraction collector was collecting, flow through the cell of the detector was interrupted, so for the COP HPLC calibration solutions analyses, the retention times were monitored for dBeP only. If the dBeP retention time shifted by more than 0.05 min, the collection time was changed for that fraction.

Before the last COP HPLC calibration check solution was injected, the fraction collector was reset. This allowed the monitoring of the signal from the detector for the COP retention time.

After the COP fraction from each sample was collected, the HPLC column was backflushed in order to rapidly remove any remaining polar compounds.

After the last fraction was collected, the tubes containing the fractions from the calibration runs and the waste fractions were emptied into the TOXIC WASTE receptacle.

The tubes containing the COP fractions were capped and stored for processing for GC analysis.

9. PREPARATION OF SAMPLES FOR GAS CHROMATOGRAPHY

9.1. Concentration of AH/CH fractions from sediment

A boiling chip was added to the tube containing the AH/CH fraction from SEC-HPLC CHROMATOGRAPHY, and, using a tube heater, the fraction volume was reduced to 1 mL.

Two milliliters of hexane was added to the tube, and the volume was reduced to 1 mL.

The fraction was transferred to a labeled GC vial. The vial was placed under a gentle stream of nitrogen gas, and the solvent was slowly evaporated to a volume of 175 µL.

Thirty microliters of Sediment AH GC I-std solution and 45 µL of CH GC I-std solution were added to the GC vial, and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

One hundred twenty microliters of Sediment AH GC I-std solution was added to each of the AH analyte-calibration solutions from the Extraction section, and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

Two hundred sixty microliters of CH GC I-std solution was added to each of the CH analyte-calibration solutions from the Extraction section, and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

Approximately half of the fraction was transferred to a GC vial with an insert. The vial was labeled "AH" in addition to the sample number.

The remainder of the fraction was transferred to a GC vial with an insert. This vial was labeled "CH" in addition to the sample number.

The next procedure was GC/MS with the AH analyte-calibration solutions and AH fractions, while GC/ECD was used for the CH analyte-calibration solutions and the CH fractions.

9.2. Concentration of AH/CH fractions from tissue

A boiling chip was added to the tube containing the AH/CH fraction from SEC-HPLC CHROMATOGRAPHY, and, using a tube heater, the volume was reduced to 1 mL.

Two milliliters of hexane was added to the tube, and using a tube heater, the volume was reduced to 1 mL.

For samples with high levels of contamination and for the spiked blank or the matrix spike the following procedure was used:

Using a pipet, the AH/CH fraction was transferred to a GC vial labeled "AH/CH", in addition to the sample number. The vial was placed under a gentle stream of nitrogen gas and the solvent slowly evaporated to a volume of 160 µL.

Fifty microliters of CH GC I-std solution and 40 µL of Tissue AH GC I-std solution were added to the vial, and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

Three hundred microliters of CH GC I-std solution was added to each of the CH analyte-calibration solutions from the Extraction section, and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

One hundred sixty microliters of Tissue AH GC I-std solution was added to each of the AH analyte-calibration solutions from the Extraction section, and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

Approximately half of the fraction was transferred to a GC vial with an insert. The vial was labeled "AH" in addition to the sample number.

The remainder of the fraction was transferred to a GC vial with an insert. The vial was labeled "CH" in addition to the sample number.

The next procedure was GC/MS with the AH analyte-calibration solutions and AH fractions, while GC/ECD was used for the CH analyte-calibration solutions and the CH fractions.

For samples with low levels of contamination the following procedure was used:

Using a pipet, the AH/CH fraction was transferred to a GC vial labeled "AH/CH", in addition to the sample number. The vial was placed under a gentle stream of nitrogen gas, and the solvent slowly evaporated to a volume of 95 µL.

Thirty microliters of CH GC I-std solution and 25 µL of Tissue AH GC I-std solution were added to the vial, and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

Three hundred microliters of CH GC I-std solution was added to each of the CH analyte-calibration solutions from the Extraction section, and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

One hundred sixty microliters of Tissue AH GC I-std solution was added to each of the AH analyte-calibration solutions from the Extraction section, and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

The AH/CH fraction was transferred to a GC vial with an insert. The vial was labeled "AH/CH" in addition to the sample number.

The next procedure was GC/MS with the AH analyte-calibration solutions and AH fractions, while GC/ECD was used for the CH analyte-calibration solutions and the CH fractions.

9.3. Concentration of CH fractions from tissue

A boiling chip was added to the tube containing the CH fraction from SEC-HPLC CHROMATOGRAPHY, and, using a tube heater, the volume reduced to 1 mL.

Two milliliters of hexane was added to the tube, and the fraction volume reduced to 1 mL using a tube heater.

For samples with high levels of contamination and for the spiked blank or the matrix spike the following procedure was used:

Using a pipet, the CH fraction was transferred to a GC vial labeled "CH", in addition to the sample number. The vial was placed under a gentle stream of nitrogen gas, and the solvent slowly evaporated to a volume of 200 µL.

Fifty microliters of CH GC I-std solution was added to the vial, and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

Three hundred microliters of CH GC I-std solution was added to each of the CH analyte-calibration solutions from the Extraction section, and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

The fraction was transferred to a GC vial with an insert. The vial was labeled "CH" in addition to the sample number.

The next procedure was GC/ECD with the CH analyte-calibration solutions and the CH fractions.

For samples with low levels of contamination the following procedure was used:

Using a pipet, the CH fraction was transferred to a GC vial labeled "CH", in addition to the sample number. The vial was placed under a gentle stream of nitrogen gas, and the solvent slowly evaporated to a volume of 120 µL.

Thirty microliters of CH GC I-std solution was added to the vial, and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

Three hundred microliters of CH GC I-std solution was added to each of the CH analyte-calibration solutions from the Extraction section, and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

The fraction was transferred to a GC vial with an insert. The vial was labeled "CH" in addition to the sample number.

The next procedure was GC/ECD with the CH analyte-calibration solutions and the CH fractions.

9.4. Concentration of COP fractions from sediment samples

A boiling chip was added to the tube containing the COP fraction from the PAC-HPLC CHROMATOGRAPHY, and, using a tube heater, the fraction volume reduced to 1 mL.

Two milliliters of hexane was added to the tube, and the fraction volume reduced to 1 mL.

The fraction was transferred to a GC vial labeled "COP", in addition to the sample number. Twenty microliters of BSTFA with 1% TMCS solution from a previously unopened ampoule and 20 µL of pyridine were added, and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

Two hundred microliters of BSTFA with 1% TMCS solution and 200 µL of pyridine were added to the COP analyte calibration-solutions from the Extraction section, and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

The fraction and the COP analyte-calibration solutions were heated for 1.5 hr in a vial heating module brought to a temperature of 60°C. The vials were allowed to cool.

The cooled vials containing the COP fraction were placed under a gentle stream of nitrogen gas and the solvent slowly evaporated to a volume of 90 µL.

Ten microliters of COP GC I-std was added to the vial containing the COP fraction from the step immediately above, and the contents mixed on a Vortex Genie for 2 sec at setting 4-6

One hundred microliters of COP GC I-std was added to each of the COP analyte-calibration solutions, and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

The COP fraction was transferred to a GC vial with an insert. The vial was labeled "COP" in addition to the sample number.

The COP fractions were allowed to stand at least 24 hr before proceeding to GC/FID.

10. GAS CHROMATOGRAPHY

10.1. Instrument settings

GC analyses were performed using a Hewlett-Packard 5890 GC and 7673 autosampler with a 5970B mass selective detector (MSD) for aromatic hydrocarbons, a Hewlett-Packard 5890 GC and 7673 autosampler with an electron capture detector (ECD) for chlorinated hydrocarbons (i.e., PCBs and chlorinated pesticides), and a Hewlett-Packard 5880 GC and 7672 autosampler with a flame ionization detector (FID) for coprostanol. All gases were purified through molecular-sieve traps. Oxygen was removed from the helium and argon/methane mixture using oxygen traps. All gas-line fittings were free of leaks.

10.2. Operation of the GC/ECD

The instrument settings for the GC/ECD are listed in Table IV.12. The oven temperature profile is shown in Figure IV.14.

Table IV.12. Instrument description and operating conditions for analysis of chlorinated hydrocarbons using the GC/ECD.

Gas Chromatograph: Hewlett-Packard 5890 with a ^{63}Ni electron capture detector (ECD)

Instrument settings:		Temperature Program:	
Injection volume:	3 µL	Initial temp.:	1 min at 50°C
Inlet liner:	4-mm i.d. Vycor with 1 cm fused silica wool	First rate:	4°C/min to 155°C
		Isothermal pause:	0 min
		Second rate:	1°C/min to 210°C
Injection technique:	splitless	Isothermal pause:	0 min
Splitter closing time:	0.5 min	Third rate:	4°C/min to 315°C
Detector temp.:	320°C	Isothermal pause:	10 min at 315°C
Injector temp.:	300°C		
Recorder range:	(depends on ECD)	Column:	
Chart speed:	0.7 cm/min		
Carrier gas:	He	Material:	fused silica tubing
Linear gas velocity:	33 cm/sec at 300°C	Length:	30 m
		Int. Diameter:	0.25 mm
Carrier gas flow:	1.5 mL/min (varies with temperature)	Stationary phase:	DB-5
		Phase Composition:	5% phenyl, 95% methylpolysiloxane
Detector purge gas:	5% CH_4, 95% Ar		
Detector purge flow:	30 mL/min	Film Thickness:	0.25 µm
Septum purge flow:	2.5 mL/min		
Split vent flow:	52 mL/min		

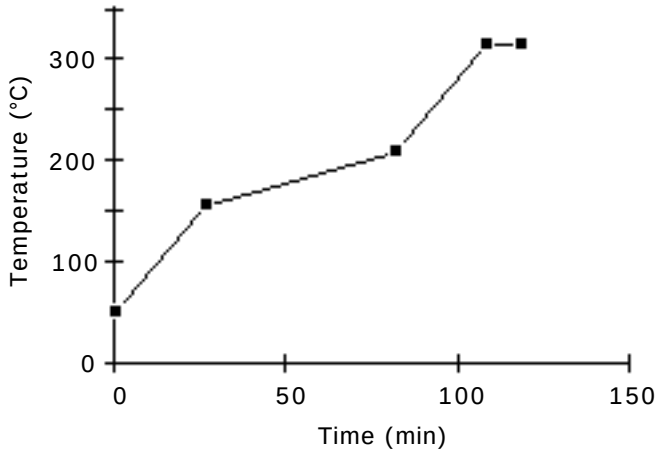

Figure IV.14. GC oven temperature profile for analysis of chlorinated hydrocarbons using the GC/ECD.

10.3. Operation of the GC/MSD

The instrument settings for the GC/MSD are listed in Table IV.13. The oven temperature profile is shown in Figure IV.15. Selected ions for PAH identification are shown in Table IV.14.

10.4. Operation of the GC/FID

The instrument settings for the GC/FID are listed in Table IV.15. The oven temperature profile is shown in Figure IV.16.

10.5. Injection of sample concentrates

The autosampler automatically injects the specified volume of sample into the GC inlet, eliminating operator error associated with this process. The sample vial sequence used during the GC analysis is listed in Table IV.16. The conditioning solution was a sample used as a warm-up for the system.

10.6. Verification of stable GC performance

Six vials of GC calibration-check solution level 4 were placed in the autosampler tray. The results of the calculations in Section 10.7 were used, substituting the GC calibration-check solutions for the AC vials.

10.7. Verification of GC reproducibility and the calibration mixture

To assess the reproducibility of analyte response, the results of the second analytical sample string of the first analyte-calibration (AC) solution vial were used as the reference for calculating the relative responses of the analytes from the other analyses of the first AC vial. For each analyte, the ratio of the response factor for the analysis of the AC vial to that for the reference analysis was calculated, and the result expressed as a percent. The response factor for an analyte in an individual analysis was defined as R_2/R_3, where

$$R_2 = \frac{\text{Analyte concentration in the AC vial (ng/µL)}}{\text{GC I-std concentration in the AC vial (ng/µL)}}$$

and

$$R_3 = \frac{\text{Analyte peak area from the analysis of the AC vial}}{\text{GC I-std peak area from the analysis of the AC vial}}$$

Table IV.13. Instrument description and operating conditions for analysis of aromatic hydrocarbons using the GC/MSD.

Gas Chromatograph: Hewlett-Packard 5890 with a mass selective detector (MSD)

Instrument settings:		Temperature Program:	
Injection volume:	3 µL	Initial temp.:	1.5 min at 60°C
Inlet liner:	4-mm i.d. Vycor with 1 cm fused silica wool	First rate:	4°C/min to 300°C
		Isothermal pause:	0 min
Injection technique:	splitless	Second rate:	none
Splitter closing time:	0.5 min	Isothermal pause:	0 min
Source temp.:	250°C	Third rate:	none
Transfer line temp.	300°C	Isothermal pause:	10 min at 300°C
Injector temp.:	300°C		
Recorder range:	not applicable	Column:	
Chart speed:	not applicable		
Carrier gas:	He	Material:	fused silica tubing
Linear gas velocity:	33 cm/sec at 300°C	Length:	30 m
Carrier gas flow:	1.5 mL/min (varies with temperature)	Int. Diameter:	0.25 mm
		Stationary phase:	DB-5
Detector purge gas:	not applicable	Phase Composition:	5% phenyl, 95% methyl polysiloxane
Detector purge flow:	not applicable		
Septum purge flow:	2.5 mL/min	Film Thickness:	0.25 µm
Split vent flow:	52 mL/min		
Source pressure:	5×10^{-5} Torr at 50°C oven temp.	Detection:	
		Acquisition delay:	5.0 min
		Full Scan (sediments):	60 to 300 amu, 2 cycles/sec
		Selected Ion Monitoring (for tissues):	See Table IV.14, 1 cycle/sec

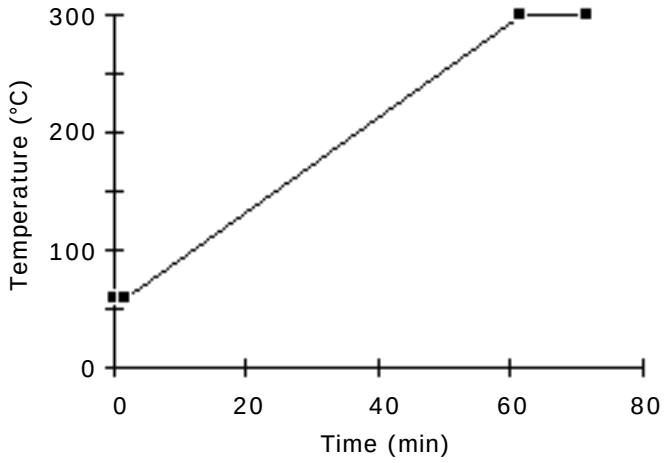

Figure IV.15. GC oven temperature profile for analysis of aromatic hydrocarbons using the GC/MSD.

Table IV.14. Selected ion monitoring for aromatic hydrocarbons in tissue samples.

Approximate Time Window	Compounds	Ions Monitored
10.0 to 18.5 min	Naphthalene	128
	Naphthalene-d_8	136
	2-Methylnaphthalene	142,141,127
	1-Methylnaphthalene	142,141,127
	Biphenyl	154
	Biphenyl-d_{10}	164
18.5 to 23.5 min	Hexamethylbenzene	147,162
	Biphenyl	154
	Biphenyl-d_{10}	164
	Acenaphthylene	152,151
	Acenaphthene	154,153,152
	Acenaphthene-d_{10}	164,162
	2,6-Dimethylnaphthalene	156,141
	1,6,7-Trimethylnaphthalene	170,155

Table IV.14. Selected ion monitoring for aromatic hydrocarbons in tissue samples (cont.).

23.5 to 32.2 min	Hexamethylbenzene	147,162
	1,6,7-Trimethylnaphthalene	170,155
	Fluorene	166,165
	Fluorene-d_{10}	176
	Acenaphthylene	152,151
	Acenaphthene	154,153,152
	Acenaphthene-d_{10}	164,162
	Phenanthrene	178,176
	Phenanthrene-d_{10}	188
	Dibenzothiophene	184,139
32.2 to 33.9 min	Phenanthrene	178,176
	Phenanthrene-d_{10}	188
	Dibenzothiophene	184,139
33.9 to 36.0 min	1-Methylphenanthrene	192,191
36.0 to 42.8 min	1-Methylphenanthrene	192,191
	Fluoranthene	202,101
	Pyrene	202,101
42.8 to 47.0 min	Benz[*a*]anthracene	228,226
	Chrysene	228,226
47.0 to 52.0 min	Benz[*a*]anthracene	228,226
	Chrysene	228,226
52.0 to 59.0 min	Benzo[*b*]fluoranthene	252,250
	Benzo[*k*]fluoranthene	252,250
	Benzo[*e*]pyrene	252,250
	Benzo[*a*]pyrene	252,250
	Perylene	252,250
	Benzo[*a*]pyrene-d_{12}	264,260
	Perylene-d_{12}	264,260
59.0 to 71.5 min	Indeno[1,2,3-*cd*]pyrene	276,138
	Benzo[*ghi*]perylene	276,138
	Dibenz[*a,h*]anthracene	278,139

Table IV.15. Instrument description and operating conditions for analysis of coprostanol using the GC/FID.

Gas Chromatograph: Hewlett-Packard 5880 with a flame ionization detector (FID)

Instrument settings:		Temperature Program:	
Injection volume:	3 µL	Initial temp.:	1 min at 50°C
Inlet liner:	2-mm i.d. Vycor with 1 cm fused silica wool	First rate:	4°C/min to 210°C
		Isothermal pause:	0 min
		Second rate:	2°C/min to 280°C
Injection technique:	splitless	Isothermal pause:	0 min
Splitter closing time:	0.5 min	Third rate:	8°C/min to 315°C
Detector temp.:	320°C	Isothermal pause:	10 min at 315°C
Injector temp.:	300°C		
Recorder range:	(depends on FID)	Column:	
Chart speed:	0.7 cm/min		
Carrier gas:	He	Material:	fused silica tubing
Linear gas velocity:	33 cm/sec at 300°C	Length:	30 m
		Int. Diameter:	0.25 mm
Carrier gas flow:	1.5 mL/min (varies with temperature)	Stationary phase:	DB-5
		Phase Composition:	5% phenyl, 95% methyl polysiloxane
Detector purge gas:	nitrogen		
Detector purge flow:	30 mL/min	Film Thickness:	0.25 µm
Septum purge flow:	10 mL/min		
Split vent flow:	40 mL/min		
Air flow:	240 mL/min		

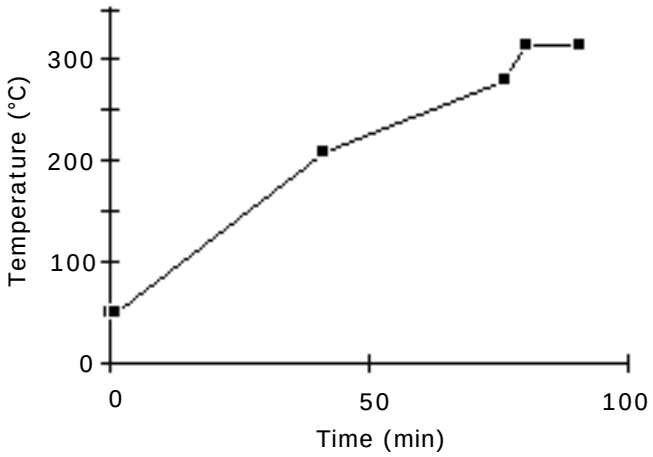

Figure IV.16. GC oven temperature profile for analysis of coprostanol using the GC/FID.

Table IV.16. Sample vial sequence used for GC analysis.

For the 5890 GCs, place the following vials in the slots of the autosampler tray and vials of 1:1 dichloromethane:hexane in the solvent vial slots of the injector turret. For the 5880 GC, place the following vials in the odd-numbered slots of the autosampler tray and vials of 1:1 dichloromethane:hexane in the even-numbered slots of the tray.

Conditioning solution	Sample
1st (of 2) analyte-calibration solns. (FIRST RUN)	Sample
Sample	Sample
Sample	Sample
Sample	2nd (of 2) analyte-calibration soln.
Sample	GC calibration-check solution level 1
Sample	GC calibration-check solution level 2
Sample	GC calibration-check solution level 3
Sample	GC calibration-check solution level 4
1st (of 2) analyte-calibration solns. (SECOND RUN)	GC calibration-check solution level 5
Sample	1st (of 2) analyte-calibration solns. (THIRD RUN)
Sample	1st (of 2) analyte-calibration solns. (FOURTH RUN)
Sample	

If the reference analysis of the AC was denoted with a "°", then the ratio of the response factors, expressed as a percent, reduced to:

$$\text{Ratio of response factors} = \frac{R_2^\circ \, R_3}{R_3^\circ \, R_2} \; 100\%$$

where the undenoted R_2 and R_3 represented the corresponding parameters of the AC analysis being compared to the AC reference analysis. A deviation of less than 5% from 100% indicated a problem with the GC system such as a leaking septum, a loose ferrule, or a worn out or dirty column. Such problems were rectified before proceeding with analyses of sample fractions.

To check the integrity of the solutions in the AC vials, for each analyte the ratio of the response factor for the GC calibration-check solution (level 4) to that for the last analysis of the AC vial was used. The same formula given above was used, except the last analysis of the AC vial took the place of the undenoted AC vial, and the GC calibration-check solution vial took the place of the AC reference analysis, with appropriate changes in the definitions of the R2° and R3° parameters (i.e., substitute "GC calibration-check solution" for "AC reference vial"). A deviation less than 5% from 100% indicated a problem with the solution in the last analysis of the AC vial, and perhaps with the other AC vials.

11. CALCULATION OF ANALYTE AND INTERNAL-STANDARD CONCENTRATIONS

11.1. Calculation of analyte concentrations

The analyte peaks in the chromatograms of the sample fractions were identified by matching their retention times with the retention times of the analyte peaks from the chromatogram of the AC reference vial. Samples analyzed by GC/ECD had PCB peaks in addition to those corresponding to the PCB peaks in the AC solution. Representative samples were analyzed by GC/MSD to identify these additional PCB peaks and to verify the presence of the other analytes identified by retention time matching.

The GC/MSD chromatograms were used to label the peaks in the GC/ECD chromatograms. Generally, the GC/MSD was not as sensitive as the GC/ECD, so the sample fraction was on occasion concentrated to as little as 20 µL for GC/MSD analysis. A ten-fold concentrated AC solution was analyzed for the chlorinated compounds listed in Table IV.10. The sum (∑) of the selected ion areas (A) and the total ion current (TIC) were determined for each analyte. The response ratio (RR) for each analyte standard was determined as follows:

$$RR = \frac{TIC}{\sum A}$$

For a multicomponent peak, the percent of each analyte comprising the peak was estimated by using the areas of the selected ions indicated in Table IV.17. For example, for a 2-component peak containing analytes x and y (peak x+y), the MS data system was set to determine the sum (∑) of the ion areas (A) for analyte x ($\sum A_x$) and analyte y ($\sum A_y$). The percentage of x (% x) in peak x+y was calculated by the equation:

$$\% \, x = \frac{(\sum A_x)\ (RR_x)}{(\sum A_x)\ (RR_x) + (\sum A_y)\ (RR_y)}$$

Percent y was then calculated by substituting $(\sum A_y)\ (RR_y)$ for $(\sum A_x)\ (RR_x)$ in the numerator.

The peak areas of each PCB congener (Table IV.10) with the same number of chlorine atoms (isomer set) were to be summed to give the total area for that set (e.g., the dichlorobiphenyls). Because the calibration standard contained only one isomer for each set, the response of that isomer was used as a surrogate standard to calculate the amounts of the other isomers in the set. In addition, the concentration of each calibration isomer in each sample was reported separately.

All extracted sulfur (S_8) was removed from the sample fractions before analysis for PCBs because the S_8 molecule interferes with the GC/ECD and GC/MSD responses.

The internal standards (I-stds) added to the sample at the beginning of the extraction were used to adjust for losses of analytes during sample preparation. Equation A was used to calculate analyte concentrations in an aquatic sediment or tissue sample, on a dry weight basis:

Equation A

$$\frac{\text{ng of the analyte}}{\text{g sample (dry wt)}} = \frac{R_1\ R_2}{R_3}\ \frac{\text{ng I-std added to sample}}{\text{sample wt.}}\ \frac{100\%}{\text{dry wt.}}$$

where

$$R_1 = \frac{\text{analyte peak area from the analysis of the extract fraction}}{\text{I-std peak area from the analysis of the extract fraction}},$$

$$R_2 = \frac{\text{analyte concentration in the AC reference vial (ng/µL)}}{\text{I-std concentration in the AC reference vial (ng/µL)}},$$

and

$$R_3 = \frac{\text{analyte peak area from the analysis of the AC reference vial}}{\text{I-std peak area from the analysis of the AC reference vial}}.$$

To calculate the concentrations of the chlorinated analytes, dibromooctafluorobiphenyl was used as the internal standard. To calculate the concentrations for the aromatic hydrocarbons, naphthalene-d_8 was used as the internal standard for naphthalene, 2-methylnaphthalene, and 1-methylnaphthalene. Perylene-d_{12} was used as the internal standard for benz[*a*]anthracene and the analytes below it in Table IV.10. All the other aromatic hydrocarbons were calculated using acenaphthene-d_{10} as the internal standard. Each aromatic hydrocarbon internal standard was used to calculate the analytes that elute in the same region of the chromatogram as the standard.

Equation B was used to calculate the percent recovery of each internal standard, based on the amount of the GC I-std (HMB and/or TCMX) added to the sample fraction just before it is transferred to the GC vial for analysis. When less than 50% of the internal standard was recovered, the unused portion of the sample was re-extract and re-analyze .

Equation B

$$\%\ \text{recovery of I-std} = \frac{R_1\ R_2}{R_3}\ \frac{\text{ng GC I-std added to the fraction}}{\text{ng I-std added to the sample}}\ 100\%$$

where

$$R_1 = \frac{\text{I-std peak area from the analysis of the extract fraction}}{\text{GC I-std peak area from the analysis of the extract fraction}},$$

$$R_2 = \frac{\text{I-std concentration in the AC reference vial (ng/µL)}}{\text{GC I-std concentration in the AC reference vial (ng/µL)}}$$

and

$$R_3 = \frac{\text{I-std peak area from the analysis of the AC reference vial}}{\text{GC I-std peak area from the analysis of the AC reference vial}}.$$

11.2. Calculation of spiked blanks

Analyte peaks were identified in the chromatogram of the spiked blank by matching their retention times with the retention times of the analytes from the chromatogram of the AC reference vial. The percent (%) recovery of the analytes added to the spiked blank was calculated by using the equation below. Calculation of I-std recovery was unchanged.

Table IV.17. Selected ions used for estimating proportions of analytes in multicomponent GC/MSD peaks.

Analytes:	Selected Ions (m/z)					
Dichlorobiphenyls (set)					222,	224
Trichlorobiphenyls (set)				256,	258,	260
Tetrachlorobiphenyls (set)			290,	292,	294,	296
Pentachlorobiphenyls (set)			324,	326,	328,	330
Hexachlorobiphenyls (set)		358,	360,	362,	364,	366
Heptachlorobiphenyls (set)		392,	394,	396,	398,	400
Octachlorobiphenyls (set)	426,	428,	430,	432,	434,	436
Nonachlorobiphenyls (set)	460,	462,	464,	466,	468,	470
DDE's (set)					246,	248
DDD's and DDT's (set)					235,	237
trans-Nonachlor		405,	407,	409,	411,	413
cis-Chlordane		371,	373,	375,	377,	379
Aldrin			261,	263,	265,	267
Dieldrin						79
Mirex			270,	272,	274,	276
Hexachlorobenzene			282,	284,	286,	288
gamma-HCH				181,	183,	185
Heptachlor						100
Heptachlor epoxide			351,	353,	355,	357
Internal Standards:						
Tetrachloro-m-xylene (TCMX)				242,	244,	246
Dibromooctafluorobiphenyl (DOB)				454,	456,	458
Tetrachloro-o-xylene				242,	244,	246

$$\% \text{ recovery of analyte} = \frac{R_1 R_2}{R_3} \frac{\text{ng I-std added to the spiked blank}}{\text{ng analyte added to the blank sample}} \times 100\%$$

where R_1, R_2, and R_3 correspond to the definitions given above.

11.3. Data management for aromatic hydrocarbons

Data from the GC/MSD were acquired and analyzed by the Hewlett-Packard HP-UX ChemStation data station and software. The analyte peaks were labeled and the areas quantified using this software by on-screen analysis, integration, and reporting. The areas under analyte and internal standard peaks detected by the GC/MSD were entered into Macintosh spreadsheets in Microsoft Excel to calculate percent recoveries of the internal standards and concentrations of the analytes. Naphthalene-d_8 was used as the internal standard for naphthalene, 2-methylnaphthalene, and 1-methylnaphthalene; acenaphthene-d_{10} was used as the internal standard for biphenyl through pyrene in the list of aromatic hydrocarbons in Table IV.10; and benzo[*a*]pyrene-d_{12} was used as the internal standard for benz[*a*]anthracene through benzo[*ghi*]perylene. The analyte concentrations and internal standard recoveries for all the samples were transferred into quality assurance tables and data tables in Microsoft Excel for

reporting. After reporting, the final data were stored in a database in Reflex Plus for retrieval as needed for statistical and graphical comparisons and analyses.

11.4. Data management for chlorinated hydrocarbons

Data from the GC/ECD were acquired and analyzed by the PE Nelson Turbochrom data station and software. The analyte peaks were labeled and the areas quantitated using this software by on-screen analysis, integration, and reporting. The areas were transferred via computer subroutines (macros) from the DOS-based Turbochrom system to Macintosh spreadsheets in Microsoft Excel to calculate percent recoveries of the internal standards and concentrations of the analytes. Dibromooctafluorobiphenyl was used as the internal standard for all analytes. The concentrations and internal standard recoveries for all samples are transferred into quality assurance tables and data tables in Microsoft Excel for reporting. Final data were stored in a database in Reflex Plus for retrieval as needed for statistical and graphical comparisons and analyses.

12. CONCLUSIONS

The analytical methods described here result from research, development, and application by chemists in the Environmental Chemistry Program (ECP) of the NWFSC's Environmental Conservation Division. These methods are continuously being upgraded for sensitivity, reproducibility, and cost-effectiveness. NOAA's National Status and Trends Program provides a unique opportunity to test and apply these state-of-the-art analytical methods developed in the ECP.

13. ACKNOWLEDGEMENTS

We are pleased to acknowledge the support and encouragement given by Dr. U. Varanasi, Division Director, Environmental Conservation Division NWFSC, and we thank ECP chemists Dr. M. Krahn, D. Burrows, K. Tilbury, S. Pierce, J. Bolton, R. Boyer, L. Moore, R. Bogar, and Dr. W. D. MacLeod, Jr. for their expert contributions.

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Standard Organic Analytical Procedures of the NOAA Southeast Fisheries Science Center
Extractable Toxic Organic Compounds

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ABSTRACT

This document describes changes implemented by the NOAA/NMFS Southeast Fisheries Science Center, Charleston, SC, to the methodologies developed by the NOAA/NMFS National Analytical Facility, Seattle, WA.

1. INTRODUCTION

The NOAA National Marine Fisheries Service (NMFS) Southeast Fisheries Science Center (SEFSC) laboratory in Charleston, SC, is one of the cooperating organizations of the NOAA National Status and Trends Program. This document describes the changes implemented by NMFS/SEFSC to the analytical procedure developed by MacLeod *et al.* described in detail elsewhere in this document. All changes are referenced to the appropriate sections of the revised MacLeod *et al.* document.

2. PROTOCOL DIFFERENCES AND CHANGES FOR SAMPLES COLLECTED IN 1984 AND 1985

[*Numbers refer to selected sections in MacLeod et al. (this document). Ed. Note.*]

2.2. Summary of analytical procedures

Excess water was removed from sediments only. Tissue samples were analyzed without decanting any fluid.

Figure 1. Tissue samples were homogenized using a TEKMAR Tissumizer. They are not tumbled as stated in the flow chart.

3.1.1. Gas chromatograph

The GC inlet was not modified. It was used as received from the manufacturer.

3.1.3. GC column and accessories

Supelco LB-2 Septa (11 mm) were used instead of Alltech Associates Blue septa. Supelco LB-2 septa have lower bleed characteristics and a higher maximum temperature. The increased diameter of the 11-mm septa versus that of the 9.5-mm septa decreases the incidence of failure due to repeated sample injections.

J&W ferrules are not designed to fit the proprietary fittings used by Hewlett-Packard, so Hewlett-Packard 0.5-mm graphite ferrules (part 5080-8853) were used.

3.1.2. Gas cylinders and accessories

NMFS/SEFSC used activated carbon traps as well as indicating oxygen traps.

[Indicating oxygen traps change color upon absorption of oxygen. Ed. Note.]

3.2.4. Air and gases

Helium, hydrogen, and nitrogen gases were grade 5.0. (99.999% purity).

3.4. Labware

The carboy used had a Teflon-lined cap, not a stopper.

Dichloromethane-rinsed aluminum foil (food grade) was used to cover glassware to prevent solvent contamination from moisture condensation.

The brand names of the glassware used at NMFS/SEFSC differ.

There are no windows in the NMFS/SEFSC laboratories. Light sources are either cool white fluorescent tubes with bug-free yellow sleeves, yellow fluorescent tubes, or incandescent "bug bulbs."

3.5.4.1. 6:4 Cyclohexane-methanol azeotrope preparation

The NMFS/SEFSC distillation apparatus was physically similar. The major difference was the use of a refrigeration unit for cooling tap water since tap water was too warm to provide adequate cooling.

3.5.4.2. Redistilled methanol

NMFS/SEFSC did not redistill the methanol.

3.5.5.1.2. Concentration

NMFS/SEFSC used a water bath instead of the Kontes tube heater. The water bath was based on a design obtained from Dr. Gadbois, NMFS/Gloucester Laboratory. The top consists of a 0.5-inch acrylic sheet with 8 openings large enough to accommodate Kuderna-Danish concentrators. Each opening has a set of support rings that allows different sections of the Kuderna-Danish concentrators to come in contact with the hot water as the level of sample extract in the concentrators decreases. The bath water was heated using two 1000-watt thermostatically-controlled immersion heaters. The water temperature was varied according to the solvent being evaporated from the sample extracts.

3.6.2. Column preparation

A 1.5-cm plug of anhydrous Na_2SO_4 was added to the top of the silica/alumina columns to prevent condensed water from entering the packing. The condensation on the glassware came from moist air present in the fume hoods, where dichloromethane and/or pentane were used. High humidity was an ambient condition during most of the

year at the Charleston, SC, location. Building air conditioning was unable to reduce humidity to acceptable levels.

4.2. Tissue composites

Due to small fish size, whole livers were composited. When a sufficient sample was available, the homogenized sample was divided into two portions prior to extraction. The second part was stored as a reserve.

5.2. Sample extraction

During the latter part of Cycle II (1985) sample analyses, 1 g copper and 20 g of acid-washed gravel was added to the sediment during the extraction process. The copper was added to reduce the amount of sulfur (S_8). The gravel was added to eliminate accretion of the sodium sulfate and sediment sample into large balls.

5.3. Extract concentration

A concentration procedure utilizing the water bath described in Section 3.5.5.1.2 above was used instead of the tube heater.

The concentration procedure for sediments diverged from that described in the second paragraph of section 5.3. The 25-mL tube was placed in the bath described above in an adapter which allows variation in the depth of penetration of the tube in the water bath. Glass chimneys were inserted in the top of the 25-mL tubes. The rest of procedure 5.3 was followed after concentration to 1 mL.

6.3. Extract concentration

The sediment extracts were concentrated initially in a steam bath in 500-mL Florence flasks with Kuderna-Danish condensers. When the volume of the solvent was reduced to approximately 10 mL, the contents were quantitatively transferred to 25-mL concentrator tubes. The flasks were rinsed twice with dichloromethane. The concentrator tubes were then placed in the Gloucester design water bath for further volume reduction. As the liquid volume decreased, upper pieces of glassware were rinsed with dichloromethane and removed. Finally, hexane was added as in Section 2.6.3.

8.1. Column preparation

Column packings were protected from moisture condensation by covering the column opening with dichloromethane-rinsed aluminum foil.

9.3.2. Concentration of fraction SA2-L2 from a clean tissue sample

The nitrogen gas was filtered through activated carbon.

10.1. Instrument settings

Injections are made using the Grob* splitless technique, as specified in MacLeod *et al.* (this document).

10.2. Table IV.2

NMFS/SEFSC heated the analytical column to 310°C for 10 min after each run.

GC/ECD runs were shortened by stopping the integrator after elution of PCB 209 and quickly ramping the oven temperature to the final desired temperature.

A septum purge gas flow of 1 mL/min was used.

The injection volume was 1 µL.

10.2. Table IV.3

NMFS/SEFSC heated the analytical column to 310°C for 10 min after each run.

A septum purge gas flow of 1 mL/min was used.

The injection volume was 1 µL.

The air flow was 430 mL/min.

The FID detector purge gas flow was set in the following manner. The column oven temperature was increased until column bleed was detected. The FID signal of column bleed was plotted at low attenuation. The detector purge gas flow was adjusted while monitoring the plotted detector response. The purge flow was optimized for maximum sensitivity when the plot reached maximum deflection.

3. PROTOCOL DIFFERENCES AND CHANGES FOR SAMPLES COLLECTED IN 1986 AND 1987

Sample collected in 1986 and 1987 were analyzed using protocols somewhat different than those used to analyze samples collected in 1984 and 1985. The MacLeod *et al.* (1985) protocols were revised as described in Krahn *et al*. (1988).

The Charleston Laboratory substituted a Water model M45 solvent pump, a Waters model 440 absorbance detector and a Fiatron CH460 oven for the HPLC described by Krahn *et al*. (1988). Procedures described in Section 2 were followed in the analysis for the 1986 and 1987 samples.

* The Grob splitless injection technique is commonly referred to as splitless injection, and is the same type of injection used by MacLeod *et al*. (this document). In general, the technique calls for the injection of a volatile solvent into an analytical column held at approximately 25°C below the boiling point of the solvent. This allows the injected sample to condense in a relatively narrow band at the head of the analytical column. The GC oven is then temperature programmed to elute the analytes of interest. Additional information can be found in: K. Grob and G. Grob (1972, Chromatographia, 5:3); K. Grob and K. Grob, Jr. (1974, J. Chromatography, 94:53); and K. Grob, Jr., and A. Romann (1981, J. Chromatography, 214:118).

4. GENERAL COMMENTS

NMFS/SEFSC modified the AH/PES analyte calibration (AC) solutions to raise the concentration of the deuterated internal standards (I-STD) to a level that, when integrated, produced areas greater than the parent analyte. This allowed automated identification of the analytes using the 5880A integrator in the GC internal computer. The mass of deuterated aromatic hydrocarbons (AH) I-STD added to the samples was increased for the same reason.

5. REFERENCES

Krahn, M. M., C. A. Wigren, R. W. Pearce, L. K. Moore, R. G. Bogar, W. D. MacLeod, Jr., S. - L. Chan, and D. W. Brown (1988) Standard analytical procedures for the NOAA National Analytical Facility, 1988: new HPLC cleanup and revised extraction procedures for organic contaminants. NOAA Tech. Memo. NMFS F/NWC-153. NOAA/NMFS/NWFSC, Seattle, WA. 52 pp.

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Standard Organic Analytical Procedures of the NOAA Northeast Fisheries Science Center
Extractable Toxic Organic Compounds

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ABSTRACT

This document provides minor changes implemented by the NOAA/NMFS Northeast Fisheries Science Center, Gloucester, MA, to the methodology developed by the NOAA/NMFS National Analytical Facility, Seattle, WA. The analytical procedures were used as part of the National Status and Trends Program.

1. INTRODUCTION

The NOAA National Marine Fisheries Service (NMFS) Northeast Fisheries Science Center (NEFSC) laboratory in Gloucester, MA, is one of the cooperating organizations of the NOAA National Status and Trends Program. This document describes slight changes implemented by NMFS/NEFSC to the analytical procedure developed by MacLeod *et al.* (this document). All changes are referenced to the appropriate sections of the revised MacLeod *et al.* document.

[*Minor changes and comments to the MacLeod et al. procedure (this document) are provided, and the numbers refer to selected sections. Sections with major changes and comments are included in this document. The modifications to the MacLeod et al. procedure are annoted or identified by underlining.*

[*One change seen throughout the document: Erlenmeyer flasks were replaced with Kuderna-Danish flasks and attached concentrator tubes. The advantage of this change is that the need for quantitative transfer steps was eliminated. Ed Note.*]

2. PROTOCOL DIFFERENCES AND CHANGES

[*Numbers refer to selected sections in MacLeod et al., (this document). Ed. Note.*]

2.2. Summary of analytical procedures

Figure 1. Tissue samples were macerated and then extracted (Figure IV.17).

3. Materials

3.1.2. Alternate Gas cylinders as listed below were used

Gas purifier, 450B. Matheson Gas Products. Gloucester, MA.
Gas purifier, for oxygen and water below 1 ppm, 6406. Propyoxs, Montgomery, PA.

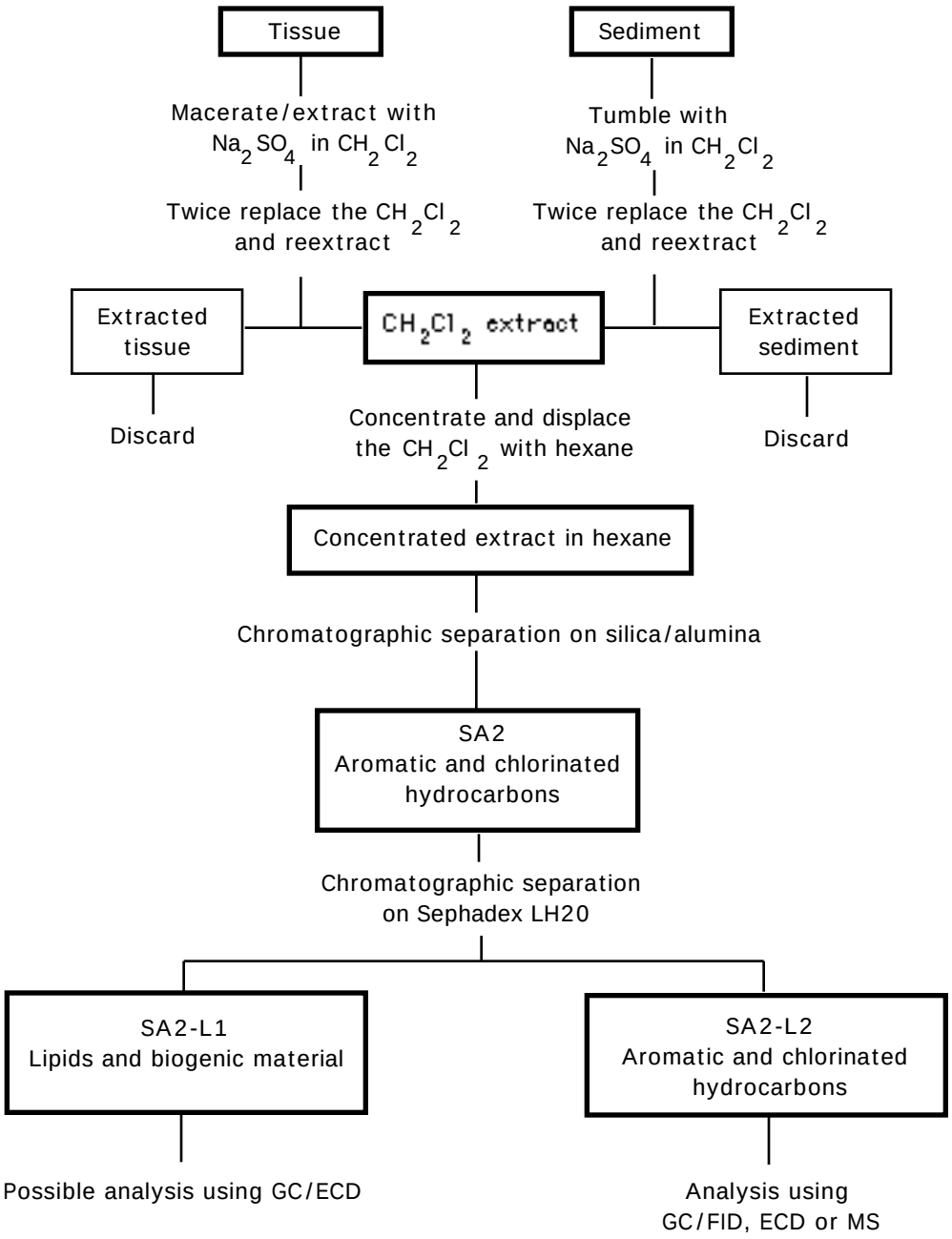

Figure IV.17. Schematic of NMFS/NEFSC analytical procedures.

3.1.3. GC column and accessories with modification shown below

Adapters and connectors, Swagelok, Solon, OH.
Conical vials, 100-µL, Viton septa and aluminum crimp caps, 5181-1212. Hewlett-Packard, Avondale, PA.
Copper tubbing, 1/8-in o.d.
DB-5 fused silica column, 30-m, 0.25 mm i.d., 0.25 µm film thickness, 122-5032. J & W Scientific, Inc., Folsom, CA.
Diamond-tip etcher
Ferrules, 0.4-mm graphite, 2004. J & W Scientific, Inc., Folsom, CA.
Glass wool, silanized. Analabs, Norwalk, CT.
Jeweler's loupe, 10x
Leak detector, Snoop. Nupro Co., Willoughby, OH.
O-ring, Viton 0.208-inch i.d., Parker Seal Co.
Septum, 9.5 mm, gray, 5080 - 8896. Hewlett-Packard, Avondale, PA.
Soap bubbles flow meters, 1, 10 and 100 mL, 0101-540, 0101-0030, and 0101-0113. Hewlett-Packard, Avondale, PA.
Syringe, 10-µL, Hamilton model 701N, part 80300
Vials, 2-mL, 5180 - 4197. Hewlett-Packard, Avondale, PA.

3.2. Chemicals

3.2.4. Air and gases

Helium, grade 5.0 (ultra pure 99.999%)
Nitrogen, grade 5.0 (ultra pure 99.999%)

3.3. Column packings

Sand, Ottawa, kiln dried, 30-40 mesh. Fisher Scientific, Pittsburg, PA.

3.4. Labware

Carboy, 20-L, with Teflon-lined stopper was not used
Concentration tubes, Kontes, 19/22-STJ, 25 mL, 570050-2525
Distiling column, Snyder, Kontes No. 503000-0121
Flask, 22-L, round bottom receiver, with a 45/50-STJ port (2)
Flasks, Kuderna-Danish, 24/40-STJ, top, 19/22-STJ, bottom
125 mL, 57001-0125
250 mL, 57001-0250
500 mL, 57001-500

[The Kuderna-Danish apparatus is shown in Figure IV.18. Ed. Note.]

Electrobalance, automatic, Cahn 29, limit ranges 25 mg, 250 mg, and 1250 mg, resolution of 0.1 µg
Balance, analytical top loading, Cahn TA 4200, limit 200 g, resolution of 0.1 mg
Balance, Mettler PE 1600, limit 1000 g, resolution of 1 mg

3.5. Internal standards, solutions, and solvents

3.5.4.3. Preparation of 6:4:3 cyclohexane:-methanol:dichloromethane solvent

Transfer the remaining 6:4 azeotrope into a 22-L round bottom flask in 2-L increments, noting the total volume. Multiply the total volume of the 6:4 azeotrope by 0.30. This is the volume of dichloromethane to be added to the 6:4 azeotrope to make the 6:4:3 solvent. Add the calculated amount of dichloromethane to the 22-L flask.

3.5.5.1. Methanol and redistilled methanol purity testing

3.5.5.1.1. Extraction *[The final step has been modified.]*

Repeat by adding 250 mL of carbon-filtered distilled water to the separatory funnel and shake vigorously. Allow the phases to separate. Use a clean 250-mL Kuderna-Danish flask with a 25-mL concentrator tube. Do not include the emulsion layer in the last step.

3.5.5.1.2. Concentration *[The first step has been modified.]*

Add 3-4 boiling chips to the assembly, and attach a Snyder column. Concentrate the extract in a 60°C water bath to between 10 and 15 mL.

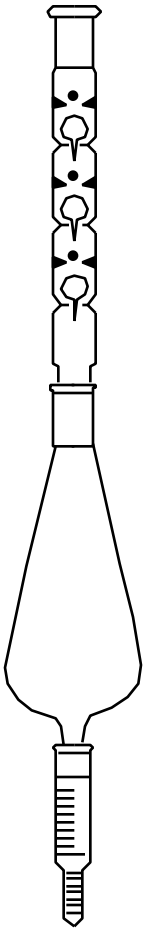

Figure IV.18. Kuderna-Danish apparatus

3.5.5.2. 6:4:3 Cyclohexane:methanol:dichloromethane solvent purity testing

3.5.5.2.1. Extraction *[The first two steps have been modified.]*

Transfer 100 mL of the 6:4:3 solvent to a 250-mL Kuderna-Danish flask with a 25-mL concentrator tube attached. Add 3-4 boiling chips and attach a Snyder column to the assembly.

Concentrate the sample in a 75°C water bath to between 10 and 15 mL. Do not wash flask with dichloromethane.

3.5.5.3. Dichloromethane *[The first three steps have been modified.]*

Attach a 25 mL concentrator tube to a 500 mL Kuderna-Danish flask and transfer 350 mL of dichloromethane. Add 3-4 Teflon boiling chips and attach a Snyder column to the flask.

Reduce the sample volume in a 60°C water bath to between 10 and 15 mL to concentrate any solvent impurities present. Do not wash flask with dichloromethane.

Add a boiling chip to the 25-mL concentrator tube and, using the tube heater, reduce the sample volume to between 0.9 and 1.0 mL.

3.5.5.4. Pentane *[The first two steps have been modified.]*

Attach a 25 mL concentrator tube to a 250 mL Kuderna-Danish flask and transfer 100 mL of pentane. Add 3-4 boiling chips and attach a Snyder column to the flask.

Reduce the sample volume in a 55°C water bath to between 10 and 15 mL. Do not wash flask with dichloromethane.

3.6. Lot testing and calibration of silica gel/alumina column packing

3.6.1.5. Silica-gel/alumina calibration extract

Calibration extracts were provided by the National Analytical Facility in Seattle, WA, so none were prepared.

3.6.3. Column calibration *[The final step has been modified.]*

Add 30 mL of 20% methanol in dichloromethane to the column and elute all of the solvent into a SA3-labeled 125-mL Kuderna-Danish flask with a 25-mL concentrator tube attached.

3.6.4. Fraction concentration

[The sample is not transferred into a concentrator tube in the first step.]

3.7. Sephadex LH-20 column preparation and calibration

3.7.1.2. Sediment/tissue calibration extract

The calibration extract was provided by the National Analytical Facility in Seattle, WA, so no additional calibration extract was prepared.

3.7.4. Column calibration with sediment/tissue calibration extract

Replace the last tube with a 50-mL graduated cylinder labeled L2.0, and collect 50 mL of eluate. Close the stopcock and transfer the eluate to a 125-mL Kuderna-Danish flask with a 25-mL concentrator tube attached labeled L2.0.

Wash down the graduated cylinder with 3-4 mL of dichloromethane and add the washings to the Kuderna-Danish flask. Repeat this step once. Set the Kuderna-Danish assembly aside for Section 3.7.5.

3.7.5. Fraction concentration

[A Kuderna-Danish assembly should replace the Erlenmeyer flask in the first step. There was no sample transfer to a concentrator tube.]

5. Sediment extraction

5.1.2. Field Blank

[No field blanks were analyzed as part of the analytical string.]

5.2. Sample extraction *[The final step has been modified.]*

Decant each extract into a labeled 500-mL Kuderna-Danish flask with a 25-mL concentrator tube attached and stopper flask with a 24/40 stopper. Add 100 mL of dichloromethane to each sediment or blank sample and repeat above steps, except roll each bottle for 6 hr (during the day instead of overnight). Decant the second extract into the flask from the first extraction.

5.3. Extract concentration *[The first step has been modified.]*

[Sample was not transferred to a concentrator tube.]

6. Tissue extraction

6.1.2. Field blank

[No field blanks were performed.]

6.2 Sample extraction *[The last three steps have been modified.]*

Wash down the probe with dichloromethane, collecting the washings in the centrifuge tube. Centrifuge the sample for 5 minutes at 2000 rpm. Decant the extract into a labeled 250-mL Kuderna-Danish flask with a 25-mL concentrator tube attached and stopper.

Add 35 mL of dichloromethane to the centrifuge tube. Extract as in previous step and combine with the previous extract.

Wash the Na_2S0_4 sample mixture by adding 10 mL of dichloromethane to the tube and mixing on the Vortex Genie for 5-10 sec at setting 5-6. Centrifuge and combine with the previous extracts.

6.3. Extract concentration *[The first step has been modified.]*

Add 3-4 Teflon boiling chips to the Kuderna-Danish assembly containing the combined dichloromethane extracts and attach a Snyder column. Concentrate the extract in a 60°C water bath to between 10 and 15 mL.

8.2. Column chromatography of extracts *[The last steps have been modified.]*

Replace the graduated cylinder with a concentrator tube labeled SA1. Partially open the stopcock and continue eluting until the volume of the SA1 fraction has been collected (18 mL), then close the stopcock.

Set aside the SA1-labeled tube for Section 8.2.2.

Place a 250 ml Kuderna-Danish apparatus with a 25-mL concentrator tube attached and labeled SA2 under the column. Lower to the packing top, then close the stopcock. Add the washings from the tube saved above to the top of the packing. Lower to the packing top, then close the stopcock.

Wash down the tube with 0.5 mL of the 1:1 dichloromethane:pentane and add the washings to the top of the packing. Add to the column the remaining 119 mL of 1:1 dichloromethane: pentane and partially open the stopcock. Lower to the packing top and close the stopcock.

8.2.1. Special instructions for sediment samples [The *third step has been modified. The silica gel/alumina columns were not recycled.]*

Discard the contents of the waste flask, and replace the flask with a 125 mL Kuderna Danish apparatus labeled SA3, which will be the coprostanol-containing fraction.

8.2.3. Concentration of fraction SA2 *[The first two steps have been modified.]*

Replace the Erlenmeyer flask with a Kuderna-Danish assembly and remove the associated Erlenmeyer flask using the wash-down procedure.

8.2.4. Concentration of fraction SA3 (sediment samples only) *[The last steps have been modified.]*

Replace the Erlenmeyer flask with a Kuderna-Danish assembly.

The coprostanol extract was stored at -20°C until analysis.

9.2. Column chromatography of extracts *[The last steps were modified.]*

Replace the cylinder with a 25-mL graduated tube labeled SA2-L1. Open the stopcock, collect the amount calibrated (14 mL) in Section 3.8 for fraction SA2-L1, then close the stopcock. Fraction SA2-L1 contains lipid and biogenic materials. It is not used in the NS&T Program analysis protocol.

Place a 100-mL graduated cylinder labeled SA2-L2 under the column. Open the stopcock, and collect the amount calibrated (100 mL) in Section 3.8 for fraction SA2-L2. Close the stopcock and transfer the eluate to a 250-mL Kuderna-Danish flask with a 25-mL concentrator tube attached and labeled SA2-L2.

Set the Kuderna-Danish assembly aside for Section 9.2.2, 9.2.3, or 9.2.4.

9.3. Concentration of fraction SA2-L2 *[The first three steps have been modified. Step two was deleted.]*

Add 3-4 Teflon boiling chips to the Kuderna-Danish assembly from Section 9.2 and attach a Snyder column. Concentrate the SA2-L2 fraction in a 75°C water bath to between 10 and 15 mL.

Add 1.0 mL of methanol and a Teflon boiling chip to the concentrator tube and using the tube heater reduce the volume to between 0.9 and 1.0 mL.

Table IV.18. Instrument description and conditions for tissue and sediment extract analysis using the ECD detector (*instrument conditions have been modified*).

Gas Chromatograph: Hewlett-Packard HP 5880 with ^{63}Ni electron capture detector (ECD)

Instrument settings:		Temperature Program:	
Injection volume:	1 µL	Initial temp.:	3 min at 50°C
Injection technique:	Splitless	First rate:	4°C/min to 170°C
Splitter closing time:	0.5 min	Isothermal pause:	0 min
Detector temp.:	320°C	Second rate:	1°C/min to 210°C
Injector temp.:	300°C	Isothermal pause:	0 min
Recorder range:	(Depends on ECD)	Third rate:	4°C/min to 300°C
Chart speed:	0.7 cm/min	Isothermal pause:	10 min at 300°C
Carrier gas:	He		
Linear gas velocity:	35 cm/sec at 300°C	Column:	
Carrier gas flow:	~1.5 mL/min (varies with temperature)	Material:	Fused silica tubing
		Length:	30 m
		Int. Diameter:	0.25 mm
Detector purge gas:	5% CH_4, 95% Ar	Stationary phase:	DB-5
Detector purge flow:	30 mL/min	Phase Composition:	5% phenyl, 95% methyl polysiloxane
Septum purge flow:	5 mL/min	Film Thickness:	0.25 µm
Split vent flow:	40 mL/min		

9.3.3. Concentration of fraction SA2-L2 from a contaminated tissue sample

[The GC analyses were performed using the reserve vials. This procedure was used to analyze high concentrations of PCB and DDT in tissue samples.]

10.6. GC reproducibility and calibration mixture verification

The definition for R_1 has been removed and the following definition for R_2 is applicable:

$$R_2 = \frac{\text{Analyte concentration in the ACC reference vial}}{\text{I-Std concentration in the ACC reference vial}}$$

$$R_3 = \frac{\text{Analyte peak area from the analysis of ACC vial}}{\text{I-Std peak area from the analysis of ACC vial}}$$

Table IV.19. Instrument description and conditions for tissue and sediment extract analysis using an FID detector (instrument conditions have been modified).

Gas Chromatograph: Hewlett-Packard HP 5880 with flame ionization detector (FID)

Instrument settings:

Injection volume:	1 µL
Injection technique:	Splitless
Splitter closing time:	0.5 min
Detector temp.:	320°C
Injector temp.:	300°C
Recorder range:	(Depends on FID)
Chart speed:	0.7 cm/min
Carrier gas:	He
Linear gas velocity:	35 cm/sec at 300°C
Carrier gas flow:	~1.5 mL/min (varies with temperature)
Detector purge gas:	Nitrogen
Detector purge flow:	30 mL/min
Septum purge flow:	5 mL/min
Split vent flow:	40 mL/min
Air flow:	240 mL/min

Temperature Program:

Initial temp.:	3 min at 50°C
First rate:	4°C/min to 300°C
Isothermal pause:	0 min
Second rate:	None
Isothermal pause:	0 min
Third rate:	None
Isothermal pause:	10 min at 300°C

Column:

Material:	Fused silica tubing
Length:	30 m
Int. Diameter:	0.25 mm
Stationary phase:	DB-5
Phase Composition:	5% phenyl, 95% methyl polysiloxane
Film Thickness:	0.25 µm

11.3. GC/MS analysis

The sample extract or blank was removed from storage and allowed to warm to ambient laboratory temperature if necessary. With a stream of dry, filtered nitrogen, the extract volume was reduced depending on anticipated analyte concentrations.

Mass spectral data was acquired using either full-range data acquisition conditions or SIM conditions, as appropriate, using the same data acquisition time and MS operating conditions previously used for all calibration solutions.

Ion profiles were extracted from SIM data files and integrated (Tables IV.20, IV.21 and IV.22). For all PCB candidates, the presence of an (M-70)+ ion was confirmed. For the tri-, tetra-, penta-, hexa-, and heptachlorobiphenyls congener groups, ICPs for the $(M+70)^+$ ion were also scanned for and, if found, would indicate a coeluting PCB congener containing two additional chlorines. For tri-, tetra-, penta-, hexa-, hepta and octachlorobiphenyl candidates, ICPs for the $(M=35)^+$ ion were also scanned for and, if found, would indicate a coeluting congener containing one additional chlorine. The ratio of the measured peak areas of the quantitation ion and confirmation ion(s) was calculated for each analyte and compared to the

Table IV.20. Ions for selected ion monitoring data acquisition for PAH's, surrogate compounds, and internal standards.

Analyte/Internal Std./ Surrogate Compound	Quant. Ion	Confirmation ions (Relative abundance)			
Naphthalene-d_8 *	136	137 (11)			
Naphthalene	128	129 (12)	127 (14)		
2-Methylnapthalene	142	141 (99)	143 (13)		
1-Methylnapthalene	142	141 (99)	143 (13)		
Biphenyl	154	153 (41)	152 (26)		
2,6-Dimethylnaphthalene	156	141 (79)	155 (39)		
Acenaphthalene	152	157 (26)	150 (16)		
Hexamethylbenzene	147	162 (47)			
Acenaphthene-d_{10}*	164	162 (100)	160 (42)		
Acenaphthene	153	154 (93)	152 (48)		
1,6,7-trimethylnaphthalene	170	155 (98)			
Fluorene	166	165 (100)			
Phenanthrene	178	176 (20)	179 (16)		
Anthracene	178	179 (17)	176 (17)		
Methylphenanthrene	192	191 (57)	189 (25)	193 (15)	
Fluoranthene	202	200 (22)	203 (17)		
Pyrene	202	200 (21)	203 (18)		
Benzo[*h*]anthracene	228	226 (27)	229 (22)		
Chrysene	228	226 (30)	224 (19)		
Benzo[*b*]fluoranthene	252	250 (20)	253 (22)	126 (21)	
Benzo[*k*]fluoranthene	252	250 (22)	253 (22)	126 (24)	
Benzo[*p*]pyrene	252	250 (32)	253 (21)	126 (22)	
Benzo[*a*]pyrene-d_{12}*	264	260 (25)	265 (22)	132 (30)	
Benzo[*a*]pyrene	252	250 (24)	253 (23)	126 (26)	
Perylene-d_{12}*	264	260 (22)	265 (24)	132 (34)	
Perylene	252	250 (32)	253 (27)	126 (24)	
Indeno[1,2,3-*cd*]perylene	276	277 (20)	274 (19)	138 (32)	
Dibenzo[*a,h*]anthracene	278	276 (34)	279 (23)	139 (33)	
Benzo[*ghi*]perylene	276	277 (24)	274 (23)	138 (32)	137 (33)

* Surrogate internal standard and ranges: naphthalene-d_8 (naphthalene to 1-methylnaphthalene, acenapthene-d_{10} (biphenyl to pyrene), and benzo[*a*]pyrene-d_{12} (dibenz[*a*]anthracene to benzo[*ghi*]perylene). Perylene-d_{12} was a surrogate internal standard during 1984 only.

Table IV.21. Ions for selected ion monitoring data acquisition for chlorinated pesticides.

Ion Analyte (MW)	Quant. Ion	Ions (Approx. Relative Abundance)		
Hexachlorobenzene (285)	284	284 (100)	286 (84)	282 (51)
gamma-HCH (288)	219	181 (100)	183 (90)	219 (75)
Heptachlor (370)	272	100 (100)	272 (60)	274 (40)
Aldrin (362)	263	66 (100)	263 (40)	265 (25)
Heptachlor epoxide (386)	353	81 (100)	353 (80)	355 (65)
alpha-chlordane (406)	373	373 (100)	375 (95)	
trans-Nonachlor (440)	409	409 (100)	407 (85)	
Dieldrin (378)	79	79 (100)	263 (10	108 (15)
2,4'- and 4,4'-DDE (316)	246	246 (100)	248 (65)	
2,4'- and 4,4'-DDD (318)	235	235 (100)	237 (65)	165 (65)
2,4'- and 4,4'-DDT (352)	235	235 (100)	237 (65)	165 (65)
Mirex	272	272 (100)	274 (75)	270 (54)
Dibromooctafluorobiphenyl*	456	454	296	

* The surrogate internal standard for chlorinated pesticides

Table IV.22. Quantitation, confirmation, and interference check ions for PCBs.

Analyte/ Intern. Stds.	Nominal MW	Quant. ion	Confirm. ion	Expected ratio	Accept ratio$^{\Delta}$	M-70 Confirm. ion	Interference Check Ions M+70	M+35
PCB chlorination group								
Mono-	188	188	190	3.0	2.5-3.5	152	256	222
Di-	222	222	224	1.5	1.3-1.7	152	292	256
Tri-	256	256	258	1.0	0.8-1.2	186	326	290
Tetra-	290	292	290	1.3	1.1-1.5	220	360	326
Penta-	324	326	324	1.6	1.4-1.8	254	394	360
Hexa-	358	360	362	1.2	1.0-1.4	288	430	394
Hepta-	392	394	396	1.0	0.8-1.2	322	464	430
Octa-	426	430	428	1.1	0.9-1.3	356	498	464
Nona-	460	464	466	1.3	1.1-1.5	390	-	498
Deca-	494	498	500	1.1	0.9-1.3	424	-	
DOB *	454	456	296					

PCB analytes monitored for each level of chlorination are: di-, PCB 8; tri, PCB 18, and 28; tetra-, PCB 44, 52, 66, and 77; penta-, PCB 87, 101, 105, 118, and 126; hexa-, PCB 128, 138, and 153; hepta-, PCB 170, 180, and 187; octa-, PCB 195; nona-, PCB 206; and deca-, PCB 209.

Δ Ratio of quantitation to confirmation ion.
* Dibromooctafluorobiphenyl is the surrogate internal standard for PCBs and chlorinated pesticides.

acceptable ratio in Table IV.22. If an acceptable ratio was not obtained, a coeluting or partially coeluting compound may have interfered. Absolute retention times of surrogate compounds and targeted analytes must be within 10 sec of that measured during the last acceptable calibration. The integrated ion current for each quantitation and confirmation ion must be at least three times background noise and must not have saturated the detector. The concentration of each PAH or pesticide or PCB compound was calculated using the formula

$$C_X = \frac{A_X \; Q_{IS}}{A_{IS} \; RF \; W}$$

where C_X is the concentration (ng/g) of an individual pesticide or PCB congener or PAH; A_X is the area of the quantitation ion for each PAH or pesticide or PCB congener; A_{IS} is the area of the surrogate internal standard quantitation ion; and Q_{IS} is the quantity (ng) of internal standard added to the extract before GC/MS analysis, and W (g) is the weight of sample extracted.

All samples were analyzed on a Hewlett-Packard Level 4 5880A gas chromatograph equipped with FID and ECD detectors and a 7672 auto sampler.

Samples and calibration solutions were put in the odd numbered slots in the autosampler tray. Fresh vials of hexane were put in the even numbered slots in the order prescribed by the method. To start a GC run, an analysis file and a modified copy of the basic program ROUTINE were called up on the 5880 terminal. This program controlled the actions of the GC and permit the identification of the chromatograms. After calling up and starting the program, the chemist gave his responses to the questions in the program. This program permitted uninterrupted operation of the GC for up to 25 samples.

The output for the run was generated on the 5880A terminal. This output consisted of three parts: a) a listing of the chromatographic conditions before the generation of the chromatogram, b) a plot of the chromatogram with retention times printed at the top of each peak, and c) a printout of retention times, peak areas, heights, and baseline type code plots. From the calibration solution, the retention times for each internal standard and analyte were known. The names, retention time values, and peak areas were transferred to a paper worksheet for each calibration chromatogram. The retention times were used to identify the analyte and internal standards in each sample, blank sample, and spiked blank (if present). If the difference between the retention times of the calibration peaks and unknown peaks in the sample were in the same interval of 0.10 min., that peak was identified as the analyte or internal standard corresponding to the calibration peak. Also, reference peaks and internal standard peaks were checked for changes in retention time positions from calibration to calibration throughout the run. These changes are accounted for before applying the above criteria. The peak area values for each internal standard and analyte were transferred to the paper worksheet.

After the peak areas of each analyte from the chromatograms were transferred to the paper worksheet, those areas were corrected for analyte impurities. This correction was done by taking the magnitude of the analyte areas in the blank and "projecting" it into the sample. This was done using the equations:

Table IV.23. Instrument description and conditions for chlorinated hydrocarbon and PAH analysis using GC/MS.

Gas Chromatograph: Hewlett-Packard HP 5980A GC Interfaced to HP's 5988A Mass Spectrometer

Instrument settings:

Injection volume:	1 µL
Inlet liner:	Splitless, 2 mm i.d., HP #18740-80220
Injection technique:	Splitless
Splitter closing time:	0.5 min
Source temp.:	200°C
Transfer line temp.	280°C
Injector temp.:	270°C
Recorder range:	Not Applicable
Chart speed:	Not Applicable
Carrier gas:	He
Linear gas velocity:	35 cm/sec at 300°C
Carrier gas flow:	~1.5 mL/min (varies with temperature)
Detector purge gas:	Not Applicable
Detector purge flow:	Not Applicable
Septum purge flow:	2 mL/min
Split vent flow:	40 mL/min
Source pressure:	~$5x10^{-6}$ Torr at 50°C oven temp.

Column:

Material:	Fused silica tubing
Length:	30 m
Int. Diameter:	0.25 mm
Stationary phase:	DB-5
Phase Composition:	5% phenyl, 95% methyl polysiloxane
Film Thickness:	0.25 µm

Temperature Program (chlorinated HCs):

Initial temp.:	3 min at 50°C
First rate:	4°C/min to 170°C
Isothermal pause:	0 min
Second rate:	170°C-210°C at 1°C/min
Isothermal pause:	0 min
Third rate:	210°C-300°C at 4°C/min
Isothermal pause:	10 min at 300°C

Temperature Program (PAHs):

Initial temp.:	3 min at 50°C
First rate:	4°C/min to 300°C
Isothermal pause:	10 min at 300°C

Detection:

Acquisition delay:	5.0 min
Full Scan (sediments):	60 to 300 amu, ~2 cycles/sec
Selected Ion Monitoring (tissues):	See table 4, ~1 cycle/sec
Electron Impact Mode:	70 eV

$$\begin{bmatrix}\text{Estimated Analyte}\\ \text{Area from}\\ \text{Sample Blank}\end{bmatrix} = \begin{bmatrix}\text{Analyte area}\\ \text{found in}\\ \text{the Blank}\end{bmatrix} \frac{\text{(TCMX Area) sample}}{\text{(TCMX Area) blank}}$$

$$\begin{bmatrix}\text{Corrected Analyte}\\ \text{Area in}\\ \text{the Sample}\end{bmatrix} = \begin{bmatrix}\text{Measured Analyte}\\ \text{Area in}\\ \text{the Sample}\end{bmatrix} - \begin{bmatrix}\text{Estimated Analyte}\\ \text{Area from Blank}\\ \text{in Sample}\end{bmatrix}$$

where TCMX Area-sample is the measured value of TCMX area in the sample, and TCMX area-blank is the measured value of TCMX area in the blank.

After this correction was done, these areas were transcribed to a Lotus 1-2-3 Master Worksheet to determine the analyte concentrations in ng/g.

Before transcribing data to a Lotus Worksheet, a special Master Worksheet was designed. The samples were arranged in columns with a place to type in the sample name and the analyte areas, the sample weight, and the dry weight. For each sample, all the necessary formulas to determine analyte concentration and internal standard recovery were also present. At the beginning of the worksheet, there was space for a report title, chemist's name, run number, and date. The concentrations for the analytes and internal standards for the ACC calibration solutions were stored in the worksheet until needed. By typing in the analyte and internal standard peak area for each sample and ACC-A2 plus the sample weight and dry weight, the concentrations and internal standard recoveries were determined. The worksheet was printed out in separate pages to generate the final report for a set of samples.

A Hewlett-Packard 9000 Workstation, equipped with Nelson Analytical Xtra Chrom software package and Nelson Analytical 760 Interface, was used for the purpose of collecting and archiving chromatographic data from the Hewlett-Packard 5880A GC. The interface can collect data from either the FID or ECD and store it until the chromatogram is done. Then the data is transferred to the workstation for storage. Each chromatogram creates a separate data file. The interface is started by switching on the 7672 Autosampler. The chromatographic data is transferred to magnetic tape for final storage. The Xtra Chrom Software can be used for data analysis as well, but we had no protocol to use Xtra Chrom for post analysis. For this reason, we limited the use of the software to data acquisition and storage for the years 1984, 1985, and 1986.

3. CONCLUSIONS

The Gloucester Laboratory participated in the National Benthic Surveillance Project of NOAA's National Status and Trends program during the years 1984 through 1986. Analyses of specific extractable toxic organic compounds in marine sediments and tissues (liver and stomach contents) were made on samples collected from Northeastern and Middle Atlantic coastal sites. No major changes were made in the MacLeod method. Minor changes were made in the concentration step. A 125/250/500 mL Kuderna flask attached with a 25 mL concentrator tube was used instead of an Erlenmeyer Flask. This negated the need to transfer the concentrated extract from an Erlenmeyer flask to a 25 mL concentrator tube with additional rinsings and transfers. The Gloucester Laboratory participated in NAF's Quality Assurance Program in 1984 and in NOAA's/NIST Quality Assurance Program in 1985 and 1986.

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GERG Trace Organics Contaminant Analytical Techniques

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ABSTRACT

This document describes the analytical methods used by the Geochemical and Environmental Research Group (GERG) for trace organic analyses of sediments and tissues. The method is GERG's modification of the methods developed by the NOAA/National Marine Fisheries Service/National Analytical Facility. The methods described here were used for the analyses of samples collected for the NOAA National Status and Trends Mussel Watch Program in the Gulf Coast of the United States from 1986 to 1992.

1. INTRODUCTION

Assessment of the environmental levels of aromatic and chlorinated hydrocarbons (pesticides and PCBs) requires their measurement in sediments and tissues at trace levels (parts per billion to parts per trillion). This report documents the analytical procedures used for trace organic analyses of marine sediments and tissue samples collected from the Gulf Coast of the United States as part of the Mussel Watch Project of NOAA's National Status and Trends Program. These procedures were used by the Geochemical and Environmental Research Group (GERG) at Texas A&M University to analyze samples collected from 1986 to 1992. These methods were based on those developed by MacLeod *et al.* (this document), and only the details of where the GERG methods differ are presented here.

2. TISSUES AND SEDIMENT ANALYSES

2.1. Sample collection, preservation, and storage

Sediment samples were collected with a box corer or by hand. The top 1 cm was collected in either case with a Teflon-coated scoop. Sediment samples were stored frozen in combusted mason jars with Teflon-lined screw caps. Oysters were collected by hand, with steel tongs, or with a steel dredge. Twenty oysters were processed for each station. The oyster shells were cleaned by scrubbing with sea water, opened under non-contaminating conditions, placed in clean mason jars, and frozen until analyses were performed. All tissue samples were homogenized mechanically by maceration prior to extraction. Sediment samples were homogenized by stirring and aliquots were removed for further processing.

2.2. Percent moisture determination

A separate 1 to 5 g aliquot of sediment or macerated tissue was placed in a tared beaker and weighed. The sample in the beaker was dried at 50°C in an oven or in a freeze dryer to a constant weight and reweighed. The percent moisture was then calculated based on the weight loss.

2.3 Summary of tissue and sediment methods

Two to 15 g (wet weight) of tissue were homogenized in a 200-mL centrifuge tube. Fifty grams of Na_2SO_4 was added and the tissue macerated in 100 mL of CH_2Cl_2. The extract was concentrated and purified using a silica gel/alumina column to remove matrix interferences. During the years 1986, 1987, and 1988, further purification was performed using Sephadex . High performance liquid chromatography (HPLC) was used during the years 1989 through 1992, to reduce matrix interferences. The extract was then concentrated for instrumental analysis by gas chromatography/mass spectroscopy (GC/MS) for aromatic hydrocarbons, and by GC/electron capture detector (GC/ECD) for chlorinated hydrocarbons.

Two methods were employed to extract sediment samples. The method used for the years 1986 through 1988 was a roller table method modified from the techniques of MacLeod *et al.* (this document). During the years 1989 through 1992, 10 g (dry weight) of freeze-dried sediment was Soxhlet-extracted with CH_2Cl_2. The extracts of either method were concentrated and purified using silica gel/alumina column purification to remove matrix interferences. The purified extract was analyzed for aromatic and chlorinated hydrocarbons by GC/MS or GC/ECD, respectively.

2.4. Interferences

Method interferences may be due to contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to false positives during instrumental analysis. All materials used in this method were routinely demonstrated to be free from interferences by processing procedural blanks identical to samples: one blank per 20 samples or each batch, whichever is more frequent. Care was taken during dissection of tissues from specimens to minimize contamination. Oyster shells were scrubbed to remove extraneous materials prior to shucking. Matrix interferences may be caused by compounds coextracted from the sample. Biogenic materials, especially excess lipids, that cause interferences in the analysis of tissue extracts were removed prior to analysis by silica/alumina and Sephadex or HPLC purification. Elemental sulfur can cause interferences in the analysis of sediment extracts. Silica gel/alumina cleanup with activated copper was used to remove sulfur from sediment samples prior to analysis.

2.5. Apparatus and materials

2.5.1. Glassware and labware

Glassware was cleaned using Micro detergent or equivalent cleaning solution, washed, and rinsed with tap water. The glassware was then combusted in a muffle furnace at 400°C for at least 4 hr. When determined to be appropriate by the analyst, the glassware underwent solvent rinses of acetone or methanol followed by methylene chloride instead of muffle furnace heating. After cooling, the glassware was sealed and stored in a clean environment to prevent the accumulation of dust or other contaminants. Stored glassware was maintained capped with combusted aluminum foil.

The following labware was needed to perform the tissue digestion and purification procedure:

Autosampler, Gilson Model 231-401. Gilson, Middleton, WI

Balance, top loading with an accuracy of 0.1 g

Boiling chips, Teflon, solvent extracted

Column, chromatography, 300 mm x 10 mm, with Pyrex glass wool plug at bottom and Teflon stopcock

Column, Kuderna-Danish, 3-ball column

Electrobalance, Cahn or equivalent, with an accuracy of 0.0001 mg
Flasks, Erlenmeyer, various sizes
Flasks, flat bottom, 100-mL and 500-mL capacity
Fraction collector, LKB 2211 Super Rac. Pharmacia LKB Biotechnology, Bromma, Sweden.
Glass wool, Pyrex, combusted at 400°C for 4 hr
Knife, stainless steel or shears for dissecting specimens
Nitrogen gas evaporation unit
Orbital shaker, Junior or equivalent
Pipettes, disposable glass Pasteur, 1-mL
Pump, ternary, HPLC, SP8000. Spectra-Physics, San Jose, CA.
Syringes, 1000-, 500-, 100-, 50-, and 10µL capacity
Tekmar Tissumizer, Polytron homogenizer, or equivalent
Tubes, concentrator, Kuderna-Danish, 25-mL, graduated with ground glass stoppers
Tubes, glass centrifuge, 200-mL capacity
Vessels, microreaction, 2-mL or 1-mL auto-sampler vials with crimp cap septa
Vials, with Teflon-lined caps, 1-mL to 7-mL capacity
Water bath, heated to 60-70°C

The following labware and equipment was needed to perform the sediment extraction and purification procedure:

Balance, analytical, capable of weighing to 0.0001 mg
Balance, analytical, capable of weighing to 0.1 g
Boiling chips, Teflon, solvent extracted
Bottles, amber glass, 500 mL
Chromatographic column, 300 mm x 10-mm ID, with Pyrex glass wool at bottom and Teflon stopcock
Concentrator tube, Kuderna-Danish, 25-mL, graduated, ground glass stoppers
Flasks, flat bottom, 250- and 500-mL
Flasks, Soxhlet extractor, 40-mm ID, and condenser
Glass funnels
Glass jars, 250-mL or 500-mL
Glass wool, Pyrex, combusted at 400°C for 4 hr
High performance liquid chromatography
    Spectra-Physics SP8000 ternary HPLC pump, two size-exclusion columns connected in series (22.5 x 250-mm Phenogel 100 Å columns), and a precolumn (8 x 50-mm Phenogel 100 Å) Autosampler (Gilson Model 231).
    UV absorbance detector (Model Water 440-Millipore).
    LKB Bromma 2211 fraction collector.
Micro reaction vessels, 2-mL or 1-mL autosampler vials with crimp cap septa
Nitrogen gas evaporation unit
Pipettes, disposable, glass, Pasteur, 1-mL
Roller table
Snyder column, Kuderna-Danish, 3-ball
Syringes, 10- or 25-µL
Thimbles, Alundum, medium or coarse, 44-mL round bottom
Vials, 1-mL to 7-mL glass vials with Teflon-lined caps
Water bath, heated to 60°-70°C

For chlorinated and aromatic hydrocarbon analyses:

    Gas chromatograph, 5890A or equivalent. Hewlett-Packard Co., Avondale, PA.
    Mass spectrometer, Hewlett-Packard MSD. Hewlett Packard Co., Avondale, PA.
    Fused silica capillary column (30-m long x 0.32-mm ID with DB-5 bonded phase). J&W Scientific, Folsom, CA.

For chlorinated hydrocarbon analyses:

    Gas chromatograph, 5890A or equivalent, equipped with an electron capture detector, G1223A. Hewlett-Packard Co., Avondale, PA.
    Fused silica capillary column (30-m long x 0.32-mm ID with DB-5 bonded phase). J&W Scientific, Folsom, CA.

2.5.2. Reagents and solvents

Alumina, neutral 80-325 MCB chromatographic grade or equivalent, combusted for 4 hr at 400°C and stored at 170°C until use. Burdick and Jackson, Muskegon, MI.

Copper (Cu) powder, activated with concentrated sulfuric acid. Burdick and Jackson, Muskegon, MI.

Cyclohexane (C_6H_6) [110-82-7], pesticide quality or equivalent. Burdick and Jackson, Muskegon, MI.

Hexane (C_6H_{14}) [110-54-3], pesticide quality or equivalent. Burdick and Jackson, Muskegon, MI.

Methanol (CH_3OH) [67-56-1], pesticide quality or equivalent. Burdick and Jackson, Muskegon, MI.

Methylene chloride (CH_2Cl_2) [75-09-2], pesticide quality or equivalent. Burdick and Jackson, Muskegon, MI.

MICRO detergent. International Product Corp., Burlington, NJ.

Pentane (C_5H_{12}) [109-66-0], pesticide quality or equivalent. Burdick and Jackson, Muskegon, MI.

Sand, combusted at 400°C for 4 hr.

Silica, grade 923, 100-200 mesh, Aldrich 21,447-7 or equivalent, dried overnight at 120°C prior to use. Aldrich, Milwaukee, WI.

Sodium sulfate (Na_2SO_4) [7757-82-6], ACS Granular, anhydrous (Purified by heating at 400°C for 4 hr in a shallow tray or by other suitable method.)

Water (Reagent water contains no analytes above the method detection limit.) Burdick and Jackson, Muskegon, MI.

2.5.3. Solutions

Solutions of the target compounds of interest (Table IV.24 and IV.25), the internal standard (IS), the matrix spike (MS), and the GC internal standard (GCIS) for aromatic and chlorinated hydrocarbon analysis, were made from standards of known purity (>95%). Solutions were made by weighing appropriate amounts of pure compounds into a volumetric flask and diluting to volume with CH_2Cl_2 or hexane. Certified standard reference materials from the National Institute of Standards and Technology (NIST) or equivalent materials were also used for this purpose. The calibration solution was comprised of, at a minimum, the PAHs listed in Table IV.26 plus the IS and GCIS. The compounds in the IS solution for PAH analyses (Table IV.24) were naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, and perylene-d_{12}. The PAH IS were added at a concentration of 40 ng/mL to the extract regardless of matrix. The calibration solution was comprised of, at a minimum, the chlorinated hydrocarbons marked with as asterisk in Table IV.25. The IS used for pesticide/PCB analyses was 4,4'-dibromooctafluorobiphenyl (DBOFB) for 1986 and 1987. The IS for the years 1988 through 1992 included DBOFB as well as PCB 103 and PCB 198. The GCIS for PAH analyses are fluorene-d_{10} and benzo[*a*]pyrene-d_{12}. The PAH GCIS is added at a concentration of approximately 40 ng/mL. The GCIS for pesticide/PCB analyses is tetrachloro-m-xylene (TCMX) and is added at a final concentration of approximately 100 ng/mL.

2.5.4. Matrix recovery standard spiking solution

A solution containing 2- to 5-ring PAH compounds was used to fortify matrix spike samples (Table IV.26). A certified solution purchased from NIST, SRM 1491, was diluted to the appropriate working concentration. The matrix spiking solution for chlorinated pesticides and PCBs contained the target compounds listed in Table IV.25. The matrix spike was added to samples at a concentration approximately 10 times the MDL.

Table IV.24. PAH target compounds, IS, and GCIS.

Compounds	GCIS	Internal Standard	Compounds	GCIS	Internal Standard
Naphthalene	A	1	Chrysene	B	4
C_1-Naphthalenes	A	1	C_1-Chrysene *	B	4
C_2-Naphthalenes	A	2	C_2-Chrysene *	B	4
C_3-Naphthalenes	A	2	C_3-Chrysene *	B	4
C_4-Naphthalenes *	A	2	C_4-Chrysene *	B	4
Biphenyl	A	2	Benzo[*b*]fluoranthene	B	4
Acenaphthylene	A	2	Benzo[*k*]fluoranthene	B	4
			Benzo[*e*]pyrene	B	4
Acenaphthene	A	2	Benzo[*a*]pyrene	B	4
			Perylene	B	5
Fluorene	A	2	Indeno[1,2,3-*cd*]pyrene	B	4
C_1-Fluorenes *	A	2	Dibenz[*a,h*]anthracene	B	4
C_2-Fluorenes *	A	2	Benzo[*ghi*]perylene	B	4
C_3-Fluorenes *	A	2			
Dibenzothiophene	A	3			
C_1-Dibenzothiophenes *	A	3	Specific Isomers		
C_2-Dibenzothiophenes *	A	3			
C_3-Dibenzothiophenes *	A	3	1-Methylnaphthalene	A	1
			2,6-Dimethylnaphthalene	A	2
Phenanthrene	A	3	1,6,7-Trimethylnapthalene	A	2
C_1-Phenanthrenes	A	3	1-Methylphenanthrene	A	3
C_2-Phenanthrenes *	A	3			
C_3-Phenanthrenes *	A	3			
C_4-Phenanthrenes *	A	3	Internal Standards		
Anthracene	A	3	Naphthalene-d_8	-1	
C_1-Anthracenes *	A	3	Acenaphthene-d_{10}	-2	
C_2-Anthracenes *	A	3	Phenanthrene-d_{10}	-3	
C_3-Anthracenes *	A	3	Chrysene-d_{12}	-4	
C_4-Anthracenes *	A	3	Perylene-d_{12}	-5	
Fluoranthene	B	3			
C_1-Fluoranthenes *	B	3			
			GC - Internal Standards		
Pyrene	B	3			
C_1-Pyrene	B	3	Fluorene-d_{10}	(A)	
			Benzo[*a*]pyrene-d_{12}	(B)	
Benz[*a*]anthracene	B	4			

* Alkylated homologues not included in the calibration solution.
NOTE: Alkylated phenanthrenes and anthracenes, and alkylated fluoranthenes and pyrenes were quantified together as total alkylated (Cx) phenanthrene/anthracenes and total alkylated (Cx) fluoranthenes/pyrenes. Only the parent compounds and specific isomers for which standards are available are reported as individual compounds.

Table IV.25. Chlorinated hydrocarbons target compounds.

Chlorinated pesticides	Polychlorinated biphenyls Δ	
Aldrin*	Dichlorobiphenyls	Heptachlorobiphenyls
alpha-Chlordane*	7, 8*, 15	178, 187/182*, 183, 185, 174, 177, 171, 172, 180*, 191, 170*, 189
alpha-HCH*		
beta-HCH*	Trichlorobiphenyls	
cis-Chlordane*	18*, 24, 16/32, 26, 25, 31, 28*, 33, 22, 37	Octachlorobiphenyls
delta-HCH*		202, 200, 201, 196, 195*, 194, 205
Dieldrin*		
Endrin*	Tetrachlorobiphenyls	
Heptachlor*	45, 46, 52*, 49, 47/48, 44*, 42, 41/64, 40, 74, 70, 66*, 60/56, 77	Nonachlorobiphenyls
Heptachlor epoxide*		208, 206*
Hexachlorobenzene*		
gamma-Chlordane*		
gamma-HCH*	Pentachlorobiphenyls	Decachlorobiphenyls
Mirex*	100, 88, 92, 84, 101*, 99, 83, 97, 87, 85, 110*, 82, 107/108, 118*, 114, 105*, 126*	209*
Oxychlordane*		
trans-Nonachlor*		
2,4'-DDT*		
4,4'-DDT*		
2,4'-DDD*		
4,4'-DDD*	Hexachlorobiphenyls	
2,4'-DDE*	136, 151, 144, 149, 146, 153*, 141, 137, 138*, 158, 129, 159, 128*, 167	
4,4'-DDE*		

Δ PCB numbers from Ballschmiter and Zell (1980).

Table IV.26. PAH matrix spike compounds in methylene chloride.

Compound	Spiking Solution concentration (µg/g)	Compound	Spiking Solution concentration (µg/g)
Naphthalene	10.30 ± 0.10	Fluoranthene	8.84 ± 0.06
1-Methylnaphthalene	12.4 ± 0.5	Pyrene	8.81 ± 0.08
2-Methylnaphthalene	11.8 ± 0.04	Benz[*a*]anthracene	7.85 ± 0.05
Biphenyl	10.46 ± 0.04	Chrysene	10.50 ± 0.06
2,6-Dimethylnaphthalene	10.8 ± 0.4	Benzo[*b*]fluoranthene	7.85 ± 0.05
Acenaphthylene	10.40 ± 0.07	Benzo[*k*]fluoranthene	8.33 ± 0.12
Acenaphthene	10.89 ± 0.15	Benzo[*e*]pyrene	8.40 ± 0.04
1,6,7-Trimethylnaphthalene	9.9 ± 0.4	Benzo[*a*]pyrene	10.14 ± 0.09
Fluorene	10.87 ± 0.08	Perylene	10.65 ± 0.06
Dibenzothiophene	10.00 ± 0.01*	Indeno[1,2,3-*cd*]pyrene	9.40 ± 0.07
Phenanthrene	10.48 ± 0.07	Dibenz[*a,h*]anthracene	7.74 ± 0.18
Anthracene	11.69 ± 0.06	Benzo[*ghi*]perylene	7.90 ± 0.13
1-Methylphenanthrene	10.4 ± 0.3		

*Added to NIST SRM 1491.

2.6. Procedures

2.6.1. Preparation of oyster samples

The bivalves were shucked and the tissues removed from the shell using a stainless steel knife. The tissue, after removing a portion for biological analyses, was pooled in a combusted mason jar and macerated using a Tissumizer. An aliquot of the macerated tissue was weighed into a 200 mL centrifuge tube (2-15 g wet weight) for extraction.

2.6.2. Oyster extraction

The appropriate amount of IS, 100 mL CH_2Cl_2, and 50 g Na_2SO_4 were added to each sample. The tissue was macerated for 3 min with the Tissumizer. The CH_2Cl_2 was decanted into a 500-mL flat bottom flask. When necessary, the extract was centrifuged at approximately 2000 rpm for 5 min before decanting. The extraction was repeated two more times with 100-mL aliquots of CH_2Cl_2 and the aliquots combined in a 500-mL flat bottom flask. A 20 mL aliquot was removed for lipid analyses. The aliquot was reduced to a volume of 1 mL and a 100-µL aliquot was weighed to determine percent lipid. The remaining extract was concentrated by attaching a 3-ball Snyder column to the 500-mL flat bottom flask and one clean boiling chip was added. The apparatus was placed in a hot water bath (60-70°C) and the volume reduced to 10-20 mL. The sample was transferred to a 25-mL concentrator tube. The 500-mL flat bottom flask was rinsed with 5-10 mL of hexane and the rinsing hexane was added to the concentrator tube. The extract was concentrated to 1 mL in a water bath (60-70°C).

2.6.3. Sediment extraction

Sediments from 1986 through 1988 were extracted on a roller table after addition of IS: once with 100 mL of methanol for 1 hr; once with 100 mL of 50:50 methanol:CH_2Cl_2 for 3 hr; and three times with 100 mL of CH_2Cl_2 for 16 hr, 3 hr, and 1 hr, respectively. After extraction, the combined solvents were partitioned into two phases by the addition of acidic NaCl solution. The organic phase was concentrated as described for tissues.

Sediments from 1989 through 1992 were extracted in a Soxhlet apparatus. An aliquot of 10 g dry weight of the sediment sample was placed in an extraction thimble and attached to an extraction flask containing 150 mL of CH_2Cl_2 and one or two boiling chips. The sediment in the thimble was wetted with CH_2Cl_2. The IS was added to the sediment in the thimble. The 250-mL flat bottom flask was heated and the sample extracted for 4-8 hrs. Cycling occurred every 4 min. When necessary, the extract was filtered through glass wool and dried with Na_2SO_4. The extract was concentrated as described for tissues.

2.6.4. Silica/Alumina column cleanup

A plug of glass wool and 2 cm of combusted sand were placed in the glass chromatographic column and the column filled with hexane. A slurry of 10 g of alumina, deactivated 1% with water, in CH_2Cl_2 was added to the column and allowed to settle. A slurry of 20 g of silica gel deactivated 5% with water in CH_2Cl_2 was added to the column and allowed to settle. One cm of combusted sand was added to the top of the packed column along with activated copper, for sediment analyses. The CH_2Cl_2 was drained to the top of the sand. Fifty milliliters of pentane were then added to the column and drained to the top of the sand. A 500-mL flat bottom flask was placed under the column. Using hexane, the sample extract was transferred to the column, which was then drained to the top of the sand. The concentrator tube was rinsed twice with 1 mL aliquots of 50:50 pentane:CH_2Cl_2, and added to the column. The column was drained to the

sand layer. Two hundred milliliters of 50:50 pentane:CH_2Cl_2 was added to the column, and eluted at 1 mL/min. This fraction contains the aromatic and chlorinated hydrocarbons. The eluents were concentrated as described above.

2.6.5. Sephadex cleanup

The Sephadex column cleanup was used for tissue samples from 1986 through 1988 and is described by MacLeod *et al.* (this document). The PAH/Pesticide/PCB fraction from silica gel/alumina columns was further purified by Sephadex LH-20 chromatography. The fraction was dissolved in 2 mL of 6:4:3 hexane:CH_3OH:CH_2Cl_2. The Sephadex columns were calibrated with standards before samples were applied. The extracts were transferred to the columns and 140 mL of 6:4:3 solvent mixture was added. The first 40 mL were discarded. The next 100 mL were collected. Then the columns were rinsed with 50 mL of solvent mix before the next sample was processed. The collected fraction was then concentrated to 1 mL for GC analyses.

2.6.6. HPLC cleanup

The HPLC column cleanup is described by MacLeod *et al.* (this document) and is only summarized here, including a description of the specific equipment used at GERG. Further purification of the tissue fraction from 1989 through 1992 was accomplished using high performance liquid chromatography with a Spectra-Physics SP8000 ternary HPLC pump, two size-exclusion columns connected in series (22.5 x 250-mm Phenogel 100 Å columns), and a precolumn (8 x 50-mm Phenogel 100 Å). Filtered (0.45 µm) CH_2Cl_2 was used at the mobile phase. The sample was injected into the columns with an autosampler (Gilson Model 231). The HPLC unit was equipped with a UV absorbance detector (Model Water 440-Millipore). The fractions containing the compounds of interest were collected into 50-mL vials using the LKB Bromma 2211 fraction collector. The time interval in which the desired fraction was collected was based on the retention times of DBOFB and perylene. Typically, these retention time markers were run in triplicate before each run to determine collection times. Collection of the sample fraction started 1.5 min before the elution of DBOFB and ended 2 min after the elution of perylene. Assuming a constant isocratic flow of the mobile phase of 7 mL/min, the total time needed to collect the fraction was approximately 7 min. At the end of every batch of ten samples, the marker standard mixture was run again to check retention times. After running a batch of 20 samples, the columns were flushed and the precolumn backflushed to remove sample matrix contamination from the system. On average, purification time was approximately 35 min.

2.6.7. Preparation for instrumental analysis

The extract solution was concentrated to 1 mL after silica gel/alumina chromatography for sediments and Sephadex or HPLC for tissues. The aromatic and chlorinated hydrocarbons were then analyzed by GC/MS or GC/ECD, respectively.

2.7. Quality control

Quality control samples were processed in a manner identical to actual samples. A minimum of one method blank was processed with every 20 samples. Blank levels were no more than 3 times the method detection limit (MDL). If blank levels for any component were above 3 times the MDL, samples analyzed in that sample set were reextracted and reanalyzed. Tissue and sediment reference materials were analyzed with each sample set for aromatic and chlorinated hydrocarbons.

Table IV.27. Measured retention times (MRT) and relative retention times to the internal standard (RRT-IS) used to quantify the analyte (min).*

Compound	MRT	Average RRT-IS	Compound	MRT	Average RRT-IS
Fluorene-d_{10} (GCIS)	11.29	1.000	Anthracene	13.84	1.014
Benzo[*a*]pyrene-d_{12} (GCIS)	23.34	1.000	1-Methylphenanthrene	15.26	1.118
Naphthalene-d_8 (IS)	5.87	1.000	Fluoranthene	16.67	1.221
Naphthalene	5.93	1.010	Pyrene	17.20	1.259
2-Methylnaphthalene	2.55	1.285	Chrysene-d_{12} (IS)	20.26	1.000
1-Methylnaphthalene	7.77	1.323	Benz[*a*]anthracene	20.25	1.000
Acenaphthene-d_{10} (IS)	10.01	1.000	Chrysene	20.33	1.003
Biphenyl	8.70	0.870	Benzo[*b*]fluoranthene	22.78	1.124
2,6-Dimethylnaphthalene	9.06	0.905	Benzo[*k*]fluoranthene	22.78	1.124
Acenaphthylene	9.65	0.964	Benzo[*e*]pyrene	23.31	1.150
Acenaphthene	10.09	1.008	Benzo[*a*]pyrene	23.41	1.156
1,6,7-Trimethylnaphthalene	11.10	1.109	Indeno[1,2,3-*cd*]pyrene	25.80	1.273
Fluorene	11.36	1.136	Dibenz[*a,h*]anthracene	25.89	1.278
Phenanthrene-d_{10} (IS)	13.65	1.000	Benzo[*ghi*]perylene	26.41	1.304
Dibenzothiophene	13.40	0.981	Perylene-d_{12} (IS)	23.51	1.000
Phenanthrene	13.72	1.005	Perylene	23.58	1.003

* This table is to serve as an example. Absolute retention times may vary depending on the length and condition of the GC column.

3. QUANTITATIVE DETERMINATION OF POLYNUCLEAR AROMATIC HYDROCARBONS BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS) - SELECTED ION MONITORING (SIM) MODE

3.1. Summary

The quantitative method described in this document determines polynuclear aromatic hydrocarbons (PAH) and their alkylated homologues in extracts of biological tissues and sediments. Quantitation was performed by GC/MS in the selected ion monitoring (SIM) mode. Target analytes are listed in Table IV.27.

3.2. Apparatus and materials

3.2.1. Gas chromatograph

The analytical systems included a temperature-programmable gas chromatograph and all accessories including syringes, analytical columns, and gases. The injection port was designed for split or splitless injection, though routine analyses were done in a splitless mode. A 30-m long x 0.32-mm ID fused silica capillary column with DB-5 bonded phase was used. The autosampler was capable of making 1-4 µL injections.

3.2.2. Mass Spectrometer

The mass spectrometer operated at 70 eV electron energy in the electron impact ionization mode and was tuned to maximize the sensitivity of the instrument based on manufacturer specifications. The GC capillary column was directly inserted into the ion source of the mass spectrometer.

The mass spectrometer computer system allowed continuous acquisition and storage of all data during the chromatographic analyses. Computer software allowed display of any GC/MS data file for ions of a specific mass and plotting of ion abundances versus time or scan number.

3.3. GC/MS calibrations

A five-point calibration was used to establish response factors for each analyte. The standard concentrations used were 20, 100, 250, 500, and 1000 ng/mL. The percent relative standard deviation for all calibrated analytes did not exceed ±15% with an r of 0.99 or higher for a linear fit of the data occurring.

After every 6-8 samples, the mass spectrometer response for each PAH relative to the internal standard was determined using check standards at concentrations of 250 ng/mL. Daily response factors for each compound were compared to the initial calibration curve. If the average daily response factors for all analytes was within ±15% of the calibration value, analyses proceeded. If, for any analyte, the daily response factor exceeded ±35% of calibration value, a five-point calibration was repeated prior to the analysis of additional samples and samples analyzed while the detector was out of calibration were reanalyzed.

Qualitative identification of target compounds was based on relative retention time to the internal standard (RRT-IS) used to quantify an analyte (Table IV.27). Retention time windows for alkyl homologues were based on analysis of a reference oil, because standards for all the alkylated PAHs are not available.

3.4. Daily GC/MS performance tests

The mass spectrometer was tuned to perfluorotributylamine (PFTBA) criteria, as specified by the manufacturer.

3.5. GC/MS analyses

Just prior to analysis, the GCIS was added to the sample extract, producing a final concentration of approximately 40 ng/mL. Representative aliquots were injected into the capillary column of the gas chromatograph. The instrument conditions are listed in Table IV.28.

The effluent from the GC capillary column was routed directly into the ion source of the mass spectrometer. The MS was operated in the SIM mode using appropriate windows to include the quantitation and confirmation masses for the PAHs listed in Table IV.29. For all compounds detected at a concentration above the MDL, the confirmation ion was checked to confirm its presence. The total alkylated PAH for each compound (i.e. C_1, C_2, C_3 fluorenes) were only measured from 1990 through 1992.

Table IV.28. Instrument description and conditions for PAH analyses.

Injector Temp:	300°C
Transfer Line Temp:	280°C
Initial Oven Temp:	40°C
Initial Hold Time:	0 min.
Ramp Rate:	10°C
Final Temperature:	300°C
Final Time:	4 min.

Reporting limits were defined as 3 times the procedural blank, and were used from 1986 to 1989. From 1990-1992, compounds identified and quantified below the MDL were reported. If the concentration of any target compound in a sample exceeded the linear range defined by the standards above, the extract was diluted, more IS was added, and the sample was reinjected.

3.6. Calculations

3.6.1. Qualitative identification

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

The characteristic masses of each parameter of interest maximized in the same or within one scan of each other. The retention time fell within ±30 sec of the retention time of the authentic compound or alkyl homologue grouping determined by analysis of reference oil. The alkylated PAH homologue groupings (e.g., C_3 naphthalene) appeared as clusters of isomers. The pattern of each cluster and the retention time window for the cluster was established by analysis of a reference crude oil. The cluster was integrated in its entirety and the total response used for quantification.

The relative peak heights of the primary ion compared to the confirmation or secondary ion masses fell within ±30% of the relative intensities of these masses in a mass spectrum of the pure compound. In some instances, a compound that does not meet secondary ion confirmation criteria was still determined to be present in a sample after close inspection of the data by the mass spectroscopist. Supportive data included the presence of the secondary ion, but the ratio was greater than ±30% of the primary ion which may have been caused by an interference with the secondary ion. Data not meeting these criteria was reported but appropriately qualified in the database.

3.6.2. Quantitation

The relative response factors (RR) of the internal standard relative to each of the calibration standards was calculated using the following formula:.

$$RR = \frac{A_S \, C_{IS}}{A_{IS} \, C_S}$$

Table IV.29. Parameters for target analytes.

Analyte ions	Quantitation ion	Confirmation ions	Percent relative abundance of confirmation ions
Naphthalene-d_8 (IS)	136	134	15
Naphthalene	128	127	15
C_1-Naphthalenes (including isomers)	142	141	80
C_2- Naphthalenes	156	141	N D
C_3-Naphthalenes	170	155	N D
C_4-Naphthalenes	184	169, 141	N D
Acenaphthene-d_{10} (IS)	164	162	95
Acenaphthylene	152	153	15
Biphenyl	154	152	30
Acenaphthene	154	153	98
Fluorene-d_{10} (GCIS)	176	174	85
Fluorene	166	165	95
C_1- Fluorenes	180	165	100
C_2-Fluorenes	194	179	25
C_3-Fluorenes	208	193	N D
Phenanthrene-d_{10} (IS)	188	184	N D
Phenanthrene	178	176	20
Anthracene	178	176	20
C_1-Phenanthrenes/anthracenes	192	191	60
C_2-Phenanthrenes/anthracenes	206	191	N D
C_3-Phenanthrenes/anthracenes	220	205	N D
C_4-Phenanthrenes/anthracenes	234	219, 191	N D
Dibenzothiophene	184	152, 139	15
C_1-Dibenzothiophenes	198	184, 197	25
C_2-Dibenzothiophenes	212	197	N D
C_3-Dibenzothiophenes	226	211	N D
Fluoranthene	202	101	15
Chrysene-d_{12} (IS)	240	236	N D
Pyrene	202	101	15
C_1-Fluoranthenes/pyrenes	216	215	60
Benz[*a*]anthracene	228	226	20
Chrysene	228	226	30
C_1-Chrysenes	242	241	N D
C_2-Chrysenes	256	241	N D
C_3-Chrysenes	270	255	N D
C_4-Chrysenes	284	269, 241	N D
Benzo[*a*]pyrene-d_{12} (GCIS)	264	260	20
Benzo[*b*]fluoranthene	252	253, 125	30, 10
Benzo[*k*]fluoranthene	252	253, 125	30, 10
Benzo[*e*]pyrene	252	253	30, 10
Perylene	264	253	20
Perylene-d_{12} (IS)	264	260	N D
Benzo[*a*]pyrene	252	253, 125	30, 10

Table IV.29. Parameters for target analytes (cont.).

Analyte ions	Quantitation ion	Confirmation ions	Percent relative abundance of confirmation ions
Indeno[1,2,3-*cd*]pyrene	276	277, 138	25, 30
Dibenz[*a,h*] anthracene	278	279, 139	25, 20
Benzo[*ghi*]perylene	276	277, 138	25, 20

ND = Not determined

where A_S is the area of the characteristic ion for the parameter to be measured, A_{IS} is the area of the characteristic ion for the internal standard, C_{IS} is the concentration of the internal standard in calibration solution (ng/mL), and C_S is the concentration of the parameter to be measured (ng/mL).

The response factor of alkyl homologues was assumed to be equal to that of respective unsubstituted compounds. Based on these response factors, sample extract concentrations for each PAH and alkyl homologue grouping was calculated using the following formula:

$$C_E = \frac{A_S \; IS_A}{A_{IS} \; RR_A \; S_{DW}}$$

where C_E is the example concentration (ng/g), A_S is the area of the characteristic ion for the parameter to be measured, A_{IS} is the area of the characteristic ion for the internal standard, RR_A is the average relative response factor from five-point calibration, IS_A is the amount of internal standard added to each extract (ng/mL), and S_{DW} is the sample dry weight (g).

Alkyl homologues were reported as total C-1, total C-2, etc. Specific isomers were also reported as detailed in Table IV.24.

3.7. GC/MS initial and continuing calibration

A five-point response factor calibration curve was established demonstrating the linear range of the analysis. After every 8-10 analyses, the mass spectrometer relative response factor (RR) for each PAH of interest relative to the internal standard was determined. The daily response factors for each compound were compared to the initial calibration curve. The percent difference was calculated using the following equation:

$$\text{Percent difference} = \frac{(RR_A - RR_C) \; 100}{RR_A}$$

where RR_A is the average relative response factor from initial calibration, and RR_C is the response factor from the current verification check standard.

If the average daily response factors were within ±15% of the calibration value, the analysis proceeded. If, for any individual analyte, the daily response factor is not within ±25% of the

corresponding calibration curve value, a five-point calibration curve was repeated for that compound prior to the analysis of samples.

3.7.1. Internal standard recoveries

The laboratory spiked all samples and quality control samples with deuterated PAH IS compounds. The IS compounds were spiked into the sample prior to extraction and are used to measure sample matrix effects associated with sample preparation and analysis. They included naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, and perylene-d_{12}. The recovery of these IS was monitored in each sample using the response of the GCIS that was added to the final extract.

$$\text{Percent IS recovery} = \frac{(A_{IS}\ C_{GCIS})\ 100}{C_{IS}\ A_{GCIS}\ RR_{IS}}$$

where A_{IS} is the area of the characteristic ion for the IS, C_{GCIS} is the concentration (ng) of GCIS added to the sample extract, C_{IS} is the concentration (ng) of IS added to the sample, A_{GCIS} is the area of the characteristic ion for the appropriate GCIS, and RR_S is the relative response factor for the IS.

The laboratory took the following corrective actions whenever the recovery for any IS was less than 20% or greater than 130%:

- The calculations were checked to assure there were no errors.
- The IS and GCIS solutions were checked for degradation, contamination, etc., and the instrument performance checked.
- If the IS recovery was outside the control limits, the secondary ion was used to check the quantitation of the IS. If the secondary ion was within the control limits, this recovery was appropriately annotated.
- If the upper control limit was exceeded for an IS, and the instrument calibration and IS standard concentration were in control, it was concluded that an interference specific to the IS was present which resulted in high recovery, and that this interference did not affect the quantitation of other target compounds. The presence of this type of interference was confirmed by evaluation of chromatographic peak shapes.
- The sample extract was reanalyzed if the steps above failed to correct a problem. If reanalysis of the extract yielded IS recoveries within the stated limits, then the reanalysis data was reported. If reanalyses did not yield acceptable recoveries, the samples were reextracted.

3.7.2. Matrix spike analysis

Laboratory spikes and a matrix spike were sometimes processed with a sample set. A sample was randomly chosen, split into two subsamples, and one subsample was fortified with the matrix spike (Table IV.26). The acceptable matrix spike recovery criteria for sediment and tissue analysis was that the average recoveries for all 25 compounds must fall between 70 and 120%.

Table IV.30. Analytical system for chlorinated hydrocarbon analyses.

Instrument:	Hewlett-Packard 5880A or Varian 3500 Series
Features:	Split/splitless capillary inlet system, HP-1000 LAS 3357 data acquisition system
Inlet:	Splitless
Detector:	Electron Capture
Column:	0.25-mm ID x 30-m DB-5 fused silica capillary column
Gases:	
Carrier:	Helium 1 mL/min
Make-Up:	Argon/methane (95/5) or nitrogen, 20 mL/min.
Confirmation Column:	0.20 mm ID x 25-m HP-17 fused silica capillary column
Temperatures:	
Injection port:	275°C
Detector:	325°C
Oven Program:	100°C for 1 min., then 5°C/min. to 140°C, hold 1 min.; 1.5°C/min to 250°C, hold 1 min.; 10°C/min to 300°C, hold 5 min.
The GC oven temperature program may be modified to improve resolution.	
Calibration:	Four-point calibration (5, 20 or 40, 80, and 200 ng/mL)
Quantification:	Internal standard/calibration

3.7.3. Reference materials

A standard reference material (SRM) was analyzed with each batch of samples. The average percent difference for the target compounds did not exceed 20% of the mean of all previous values, and no single compound/isomer grouping should deviate by more than 35% of the mean value of all previous determinations.

3.7.4. Method detection limit

The analytical method detection limit (MDL) was calculated following procedures outlined in the Code of Federal Regulations (1990).

4. QUANTITATIVE DETERMINATION OF CHLORINATED HYDROCARBONS

4.1. Summary

The quantitative method described in this section was used to determine chlorinated hydrocarbons (e.g., chlorinated pesticides and PCBs) in extracts of biological tissues and sediments. The method is based on high resolution, capillary gas chromatography using electron capture detection (GC/ECD).

4.2. Apparatus and materials

A gas chromatograph with a split/splitless injection system, capillary column capability, and an electron capture detector (ECD) was utilized (Table IV.30).

A 30-m x 0.25-mm ID fused silica capillary column with DB-5 bonded phase was used. The column provided good resolution of chlorinated hydrocarbons, surrogates, and internal standards.

4.2.1. Calibration

Pesticide/PCB calibration was done as part of the analytical run. The four calibration mixtures were interspersed with actual samples during the GC/ECD analyses. The calibration curve was then based on these four standards. If the calibration curve had an r^2 of 0.995 or higher for all analytes present in the samples it was accepted; if not, the calibration standards as well as all the samples were reanalyzed by GC/ECD. This procedure is superior to the procedure where the instrument is initially calibrated at four points and then mid-level standards are run during the analytical run. This latter calibration only insures that mid-level samples remain in calibration. Since the ECD detector is nonlinear, a one point check on its calibration is not as rigorous as calibration during the GC/ECD run.

4.2.2. Sample analysis

Calibration mixture, actual samples, and QA samples such as blanks, matrix spikes, and SRMs, were analyzed as one analytical sequence. Sample injections of 1 to 4 µL were made with an autosampling device.

If the response for any peak exceeds the highest calibration solution, the extract was diluted, more IS solution added, and the sample reanalyzed for those analytes that exceeded the calibration range.

4.2.3. Calculations

Concentrations in samples were based on IS added. All analyte concentrations were normally calculated from PCB-103 IS. The GCIS was used to calculate IS recovery. In selected cases, DBOFB and/or PCB-198 were used to calculate selected analyte concentrations if it was demonstrated that they produce more reliable data (i.e., if matrix interference occurred with PCB-103) based on percent recoveries in spiked blanks, matrix spikes, or reference materials.

5. QUALITY ASSURANCE/QUALITY CONTROL (QA/QC) REQUIREMENTS

5.1. Calibration checks

A four-point calibration curve established the response of the detector. The calibration curve was prepared using a non-linear calibration equation of the form:

$$Y = A\,(x)^B$$

$$Y = \frac{C_A}{C_{IS}} = A\left(\frac{A_A}{A_{IS}}\right)^B$$

where A is the slope of the line fit, B is the polynomial coefficient for the line fit, C_A is the concentration (ng/mL) of the analyte measured, C_S is the concentration of the IS (ng/mL) (PCB 103), A_A is the area for the analyte measured, and A_S is the area for the IS standard (PCB 103).

The calibration solutions that were analyzed as part of the analytical GC/ECD run were preceded by no more than six samples and no more than six samples were run between calibration mixtures.

5.2. Method blank analysis

An acceptable method blank analysis did not contain any target compound at a concentration 3 times greater than the MDL. If the method blank did not meet these criteria, the analytical system was out of control and the source of the contamination was investigated and corrective measures taken and documented before further sample analysis proceeded.

5.3. IS analysis

All samples and quality control samples were spiked with DBOFB, PCB 103, and PCB 198. The IS solution was spiked into the sample prior to extraction in an attempt to compensate for individual sample matrix effects associated with sample preparation and analysis.

The laboratory took corrective action whenever the recovery of the IS used to quantitate was outside of the 40 to 130% range.

The following corrective action was taken when an out of control event occurred:

Calculations were checked to assure that no errors were made.

The IS solutions were checked for degradation, contamination, and other problems, and instrument performance was checked.

If the IS could not be measured because the sample required dilution or only a portion of the sample was analyzed, or matrix interference occurred with only one IS, no corrective action was required. The IS recovery was properly annotated.

If the steps above failed to reveal a problem, the sample or extract was reanalyzed. If reanalysis of the extract yielded IS recoveries within the stated limits, then the reanalysis data was reported. If QA criteria were still violated upon reinjection, the sample was submitted for re-extraction if sufficient sample was available. If the sample was completely consumed, the data was reported but designated as outside the QA criteria.

5.4. Matrix spike analysis

Matrix spikes were occasionally analyzed with sample sets. A sample was randomly chosen, split into subsamples and the subsamples were fortified with the matrix spike. The acceptable matrix spike recovery criteria were:

- The average recoveries for all compounds except HCB and beta-HCH must fall between 40 and 120%.

- Recoveries of HCB and beta-HCH are acceptable if they are greater than 10%.

If the matrix spike criteria were not met, the matrix spike was reinjected on the GC. If the reinjected matrix spike analysis met the criteria, then the reanalyses data was reported. If none of the analytes that were in violation were present in the sample, the violation was noted but no action was required. If analytes that were present in the sample were in violation, the entire batch of samples was submitted for re-extraction. If the sample was completely consumed the data was reported but designated as outside the QA criteria.

5.5. Method detection limit

The method detection limit was determined following the procedures outlined in the Code of Federal Regulations (1990).

5.6. GC resolution

The target compounds, IS, and GCIS were resolved from one another and from interfering compounds. When they were not, coelutions were documented.

5.7. Reference material analysis

SRMs or laboratory reference materials were analyzed for chlorinated hydrocarbons. One sample was analyzed per batch of samples. The result should agree within ±3 standard deviations of the mean of the previously reported data for laboratory reference material. For SRM, the results should agree within 75 to 125% of certified values or ± 35% of reference values.

6. CALCULATIONS

6.1. Chlorinated hydrocarbon calculations

All calculations were based on the IS added before extraction and purification. The actual sample concentration for each compound was calculated using the following formula:

$$Y = A \left(\frac{A_A}{A_{IS}}\right)^B \left(\frac{I_{IS}}{S_{DW}}\right)$$

where A is the slope of the curve fit, B is the polynomial coefficient for the line fit, A_A is the area for the analyte measured, A_{IS} is the area for the IS (PCB 103), I_{IS} is the amount of IS added to the sample, and S_{DW} is the sample dry weight.

6.2. Calculation notes

To each sample, a specific amount of IS was added. The recovery of these compounds was monitored in each sample using the response of TCMX, the GCIS was added to the final extract just prior to GC/ECD analyses.

$$\text{Percent IS recovery} = (R_1\ R_2\ R_3\ R_4)\ (100\%)$$

where R_1 is the IS peak area/GCIS peak area the in sample, R_2 is the IS concentration/GCIS concentration in one of the calibration mixtures, R_3 is the GCIS peak area/IS peak area in one of the calibration mixtures, and R_4 is the amount of GCIS added to the sample just prior to GC analysis or the amount of IS added to the sample just prior to sample extraction.

7. CONCLUSIONS

The methods described in this document have been validated and produce data that is of acceptable quality for the purpose of the NOAA NS&T Program. There are differences in these methods and those of other NS&T laboratories. The changes made to our methods were done to improve the efficiency of the analyses. Before method changes were instituted, we documented that they produced results comparable to the original methods. It is important to note that many different methods can provide reliable results, but require adequately trained personnel.

8. ACKNOWLEDGEMENTS

We wish to thank the GERG NS&T field crews that collected the samples, because that is where the quality of our data begins. We acknowledge financial support by the National Oceanic and Atmospheric Administration Contract No. 50-DGNC-00262, through the Texas A&M Research Foundation, Texas A&M University.

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Code of Federal Regulations (1990) 40 CFR, Ch. 1, Part 136, Appendix B.

Analytical Procedures Followed by Battelle Ocean Sciences and Science Applications International Corporation to Quantify Organic Contaminants

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ABSTRACT

This document describes the analytical methods for the measurement of trace organic compounds in sediments and tissues initially developed by the NOAA/National Marine Fisheries Service/National Analytical Facility, and modified for use by Battelle Ocean Sciences and their subcontractor, Science Applications International Corporation. These methods were used in many NOAA projects and formed the basis of the NOAA National Status and Trends Program trace organic analytical methodology.

1. INTRODUCTION

This document summarizes the methods used by Battelle and its subcontractor Science Applications International Corporation (SAIC) for the preparation and analysis of bivalve and sediment samples for trace organic contaminants for the National Oceanic and Atmospheric Administration's (NOAA) National Status and Trends Program Mussel Watch Project. These methods are based on those developed by MacLeod *et al.* (1985 and this document), with the modifications included in the detailed descriptions below. All equipment, apparatus, standards, and reagents necessary to perform the following procedures are listed in MacLeod *et al.* (this document), except where noted. For the most part, these modifications were the result of the natural evolution in laboratory equipment and instrumentation and our efforts to incorporate these new technologies into the Mussel Watch Project methods.

The Mussel Watch Project began in 1986. From 1986 to 1987, Battelle performed the collection and analysis of bivalves and sediments on the East Coast, Oregon, Washington and Alaska, while SAIC performed the collection and analysis in California and Hawaii. In 1988, Battelle continued to perform the collection and analysis on the East Coast, Oregon, and Washington, and SAIC in California. Alaska and Hawaii were not sampled. During 1989, Washington and Alaska were again sampled by Battelle and collection and analysis responsibilities remained the same as 1988. After 1990, Battelle performed all collection and analysis.

2. EQUIPMENT AND REAGENTS (MATERIALS REQUIRED FOR YEAR 4, 1989)

2.1. Tissue Extraction

2.1.1. Labware

Balance, Electrobalance 25 or equivalent, accuracy of 0.0001 mg. Cahn, Cerritos, CA.

Balance, top-loading, minimum accuracy of 0.01 g. Ohaus, Mettler, or equivalent.

Boiling chips, solvent rinsed.

Bottles, Teflon, 250-mL

Calipers

Centrifuge, capable of holding 250-mL Teflon centrifuge bottles and maintaining 2000 rpm

Chromatography columns, 19-mm ID, with 200-mL reservoir and Teflon stopcock

Desiccator

Dishwasher, automatic, Fury 2000 model. Forma Scientific, Marietta, OH.

Drying oven maintained at 105°C, Blue M, Model SW17TA or equivalent. Blue M, Blue Island, MN.

Erlenmeyer flasks, 250- and 500-mL

Foil, aluminum

Forceps, stainless steel

Glass wool, pesticide grade, 2-0409. Supelco, Bellefonte, PA.

Graduated cylinders, glass

Kuderna-Danish apparatus: 500-mL reservoir, 10- or 20-mL concentrator tube, 3-ball macro Snyder column

Nitrogen evaporation apparatus, N-Evap or equivalent

Pasteur pipettes, glass, disposable, rubber bulbs. VWR Scientific, West Chester, PA.

Pipette bulb, three-way

Pipettes, 10-mL glass, Class "A"

Stainless steel shucking knives

Stainless steel spatula

Syringes, microliter. Hamilton, Reno, NV.

Tissumizer with probes, Polytron or equivalent. Tekmar, Cincinnati, OH.

Vials, 4-mL with Teflon lined caps. Wheaton, Millville, NJ.

Water bath capable of reaching 100°C, in fume hood

Weighing pans, aluminum

2.1.2. Reagents

Alumina, Sigma-F20 or equivalent, 80-200 mesh, activated by heating to 400°C for 4 hr. Deactivated with water, 1:1 v:v. Stored in sealed container at 150°C. Sigma Chemical Co., St. Louis, MO.

Dichloromethane (CH_2Cl_2) [75-09-2], pesticide grade or equivalent

Hexane (C_6H_{14}) [110-54-3], pesticide grade or equivalent

Internal standard and calibration solutions. NIST, Gaithersburg, MD, or Supelco, Bellefonte, PA.

Sodium sulfate, anhydrous (Na_2S0_4) [7757-82-6], reagent grade, heated to 400°C for 4 hr, cooled and stored in sealed container at ambient temperature

Water, Milli-Q or equivalent. Millipore Corp., Bedford, MA.

2.2. Sediment Extraction

2.2.1. Labware

Balance, Cahn Electrobalance 25 or equivalent, accuracy of 0.0001 mg. Cahn, Cerritos, CA.

Balance, top-loading, minimum accuracy of 0.01 g. Ohaus, Mettler, or equivalent

Boiling chips, solvent rinsed

Bottles, Teflon, 250-mL

Centrifuge, capable of holding 250-mL Teflon centrifuge bottles and maintaining 2000 rpm

Chromatography columns, 19-mm ID, with 200-mL reservoir and Teflon stopcock

Desiccator

Dishwasher, automatic, Fury 2000 model. Forma Scientific, Marietta, OH.

Drying oven maintained at 105°C, Blue M Model SW17TA or equivalent. Blue M, Blue Island, MN.
Erlenmeyer flasks, 250- and 500-mL
Glass wool
Graduated cylinders, glass
Kuderna-Danish apparatus: 500-mL reservoir, 10- or 20-mL concentrator tube, 3-ball macro Snyder column
Nitrogen evaporation apparatus, N-Evap or equivalent
Pasteur pipettes, glass, disposable, rubber bulbs
Pipette bulb, three-way
Pipette, 10-mL glass, Class "A"
Shaker table
Spatula, Teflon
Stainless steel spatula
Syringes, microliter. Hamilton, Reno, NV.
Tissumizer with probes, Polytron or equivalent. Tekmar, Cincinnati, OH.
Vials, 4-mL, with Teflon lined caps
Vortex Genie. American Scientific Products, McGaw Park, IL.
Water bath, capable of reaching 100°C, in fume hood
Weighing pans, aluminum

2.2.2. Reagents

Acetone (CH_3COCH_3) [67-64-1], reagent grade or equivalent
Alumina, Sigma-F20 or equivalent, 80-200 mesh, activated by heating to 400°C for 4 hr. Deactivated with water, 1:1 v:v. Stored in sealed container at 150°C. Sigma Chemical Co., St. Louis, MO.
Copper [744-50-8], granular, 99% pure or equivalent. Aldrich, Milwaukee, WI.
Dichloromethane (CH_2Cl_2) [75-09-2], pesticide grade or equivalent
Hexane (C_6H_{14}) [110-54-3], pesticide grade or equivalent
Internal standard and calibration solutions. NIST, Gaithersburg, MD, or Supelco, Bellefonte, PA.
Methanol (CH_3OH) [67-56-1], pesticide grade or equivalent
Sodium sulfate, anhydrous (Na_2S0_4) [7757-82-6], reagent grade, heated to 400°C for 4 hr, cooled and stored in sealed container at ambient temperature
Water, Milli-Q or equivalent. Millipore Corp., Bedford, MA.

2.3. Tissue Extraction, Butyltins

2.3.1. Labware

Centrifuge
Chromatography column, 22 mm i.d. x 300 mm
Erlenmeyer flasks, 500-mL
Flasks, pear shaped, 25-mL
Flasks, round bottom, 500-mL
Funnels, Pyrex
Graduated cylinders, 10- and 100-mL
Jars, Teflon, 500-mL
Kuderna-Danish apparatus: 500-mL reservoir, 10- or 20-mL concentrator tube, 3-ball macro Snyder column
Nitrogen evaporation apparatus, N-Evap or equivalent
Pasteur pipettes, glass, disposable, rubber bulbs
Pipette bulb, three-way
Pipette, 10-mL glass, Class "A"
Rotary evaporator with hot water bath and water aspirator vacuum system
Separatory funnel, 250-mL
Syringes, microliter. Hamilton, Reno, NV.
Tissumizer with probes, Polytron or equivalent. Tekmar, Cincinnati, OH.
Vials, 4- and 40-mL with Teflon lined caps
Volumetric flasks, 20-, 25-, 100-mL
Water bath capable of reaching 100°C, in fume hood

2.3.2. Reagents

Florisil, PR-grade or equivalent activated at 130°C for a minimum of 5 hr. Aldrich, Milwaukee, WI.

Hexane (C_6H_{14}) [110-54-3], pesticide grade

Hydrobromic acid (HBr) [10035-10-6], 48% ACS reagent grade or better

Internal standards and spiking solutions. Alfa Products, Ward Hill, MA.

n-Pentymagnesium bromide [$CH_3(CH_2)_4MgBr$] [693-25-4], 1.9 M in diethyl ether. Alfa Products, Ward Hill, MA.

Silica gel, 1% deactivated. Aldrich, Milwaukee, WI.

Sodium sulfate, anhydrous (Na_2S0_4) [7757-82-6], reagent grade, heated to 400°C for 4 hr, cooled and stored in sealed container at ambient temperature

Sulfuric acid (H_2S0_4) [7664-93-9], 10 N, ACS reagent grade or better

Toluene ($C_6H_5CH_3$) [108-88-3], pesticide grade

Tropolone (2-hydroxy-2,4,6-cycloheptatrienone) [533-75-5]. Alfa Products Inc., Ward Hill, MA.

Water, Milli-Q or equivalent. Millipore Corp., Bedford, MA.

2.4. HPLC Accessories

Autosampler, 231/401. Gilson, Worthington, OH.

Columns, two Phenogel 22.5 x 250 mm 100 Å size. Phenomenex exclusion columns in series; one Phenogel 100 Å 7.8 x 50 mm precolumn. Phenomenex, Rancho Palos Verdes, CA.

Detector, UV/VIS, 8450. Spectra-Physics, San Jose, CA.

Filtration apparatus for dichloromethane (mobile phase), 47-mm Teflon disks

Fraction collector, programmable, Foxy 200. Isco, Inc., Lincoln, NE.

HPLC pump, 8800. Spectra-Physics, San Jose, CA.

Integrator, CR-3A. Shimadzu , Columbia, MD.

2.5. GC Accessories

Adapters and connectors as needed. Swagelok, Solon, OH.

Autosampler, 7673. Hewlett-Packard, Avondale, PA.

Diamond tip column cutter. Supelco, Bellefonte, PA.

Ferrules, graphite, 0.5-mm, Hewlett-Packard 5080-8853. Hewlett-Packard, Avondale, PA.

Gas chromatographs, Hewlett-Packard Model 5890 Series II. Hewlett-Packard, Avondale, PA.

GC vials, 0.25- and 1.8-mL, Hewlett-Packard or equivalent. Hewlett-Packard, Avondale, PA.

Injection port liners. Hewlett-Packard, Avondale, PA.

O-rings, 5180-4182. Hewlett-Packard, Avondale, PA.

Septa, 11 mm, 7813. Alltech, Deerfield, IL.

Syringes, 10 µL. Hamilton, Reno, NV.

2.6. Gases

Argon/methane (95/5), 99.999% pure
Helium, 99.999% pure
Hydrogen, 99.9999% pure

3. SUMMARY OF ANALYTICAL PROCEDURES, 1986

3.1. Bivalve sample preparation and extraction

3.1.1. Bivalve shell-length determination

The shell size of representative bivalves from each site were measured and recorded. To meet this objective, all bivalves collected for analysis of trace organic contaminants were measured prior to shucking.

All sample handling was carried out in a clean hood. Shell length measurements were performed with microcalipers and recorded to the nearest millimeter. The measurements were made from the bivalve hinge to the edge of the valves opposite the hinge. Samples were measured while frozen, with minimal thawing permitted.

3.1.2. Bivalve sample extraction

All glassware was washed with soap and water, rinsed with methanol, and dried at 200°C for a minimum of 4 hr. Prior to use, all glassware was rinsed with pesticide-grade dichloromethane or heated in a muffle furnace at 400°C for 1 hr.

The bivalve shells were rinsed with deionized water to remove loose external debris. Fouling organisms were not removed, however. Care was taken to ensure that these organisms were not included in the sample ultimately used for organic analysis. Byssal threads were not included in the samples. Shucking was done on a non-contaminating surface using a clean, dichloromethane-rinsed stainless steel knife.

Tissue from all 20-30 bivalves comprising one station's sample were shucked into a 500-mL pre-cleaned glass jar. All bivalve fluids accumulated during shucking were considered part of the sample. Tissue was not rinsed in any way. All tissue from one station was homogenized with a Polytron blender, Tissumizer, or equivalent for a minimum of 5 min. Tissue sample homogenates were either stored frozen at -20°C, or aliquots were prepared for analysis. Any excess tissue was frozen and archived.

If the tissue was frozen, it was allowed to thaw, mixed thoroughly with a Teflon or stainless steel spatula, and an aliquot of 15 g wet weight (weighed to the nearest 0.01 g) transferred to a cleaned container (e.g., screw-top Teflon centrifuge tube) for extraction. Simultaneously, a 1 g aliquot was also removed for dry weight determination (see Section 2.5). To each sample, 75 mL of dichloromethane, 50 g of sodium sulfate, and the surrogate internal standards (20 µL AH I-Std (internal standard), and 20 µL PES I-Std) were added. The internal standards were supplied by the National Institute of Standards and Technology (NIST).

The sample was macerated/extracted in the extraction container for 1 min with the Tissumizer at setting 100, and then for 2 min at setting 50. After homogenization, the probe was washed with dichloromethane, collecting the washings in the extraction container. The sample was centrifuged for 5 min at approximately 2000 rpm, and the extract decanted into a labeled flask. The extraction procedure was repeated with 75 mL of dichloromethane two more times, combining the extracts from the same sample in the same labeled Erlenmeyer flask. After the third extraction, 50 mL of dichloromethane were added to the extraction container and shaken vigorously for 30 sec. The sample was centrifuged for 5 min at approximately 2000 rpm and the supernate added to the combined extracts.

Proceed to Section 3.3 for extract concentration.

3.2. Sediment sample preparation and extraction

Sediment samples arrived in the laboratory frozen in Teflon jars. The sediment samples were thawed and the excess water decanted. The sediment was stirred with a spatula to homogenize the entire sample. Pebbles, seaweed, wood, and other extraneous materials were removed. Using the spatula, 50 g of sediment were removed and placed in a tared 250-mL Teflon extraction bottle. The bottle was weighed to the nearest 0.01 g. Ten grams of the homogenized sediment was removed from the original sample jar and placed in an aluminum weighing pan for dry weight determination (see Section 3.5). The remaining sample was frozen for archival.

3.2.1. Battelle

Approximately 60 g sodium sulfate, 100 mL 1:1 dichloromethane:acetone, 100 µL of AH I-Std solution, 100 µL PES I-Std solution, and 100 µL COP I-Std solution were added to the sediment sample. The I-Std solutions were added into the solvent. Each bottle was shaken manually until contents were loose. The bottles were shaken on a shaker table for 12 hr.

The sample bottles were centrifuged at approximately 1500 rpm for 5 min and the extract decanted into a labeled Erlenmeyer flask. One hundred milliliters of 1:1 dichloromethane:acetone was added to the bottle and again shaken for 4 hr. The centrifugation was repeated and the extracts combined in the flask. Another 100 mL of 1:1 dichloromethane:acetone was added to the bottle and again shaken for 12 hr. The centrifugation was repeated and the extracts combined in the flask. Proceed to Section 3.3.

3.2.2. SAIC

Approximately 100 mL methanol, 50 µL of AH I-Std solution, 50 µL PES I-Std solution, and 50 µL COP I-Std solution were added to each sample and the sample bottle manually shaken to loosen the contents. The bottle was placed on a tumbler and rolled for 1 hr. The bottle was centrifuged for 5 min at approximately 1500 rpm and the extract decanted into a round bottom flask.

Approximately 100 mL of 1:1 dichloromethane:methanol was added to the sample. The tumbler extraction was repeated for one hour, followed by centrifugation, and combination of extracts in the flask. Another 100 mL of dichloromethane was added to each sample and again tumbled for 16 hr, centrifuged and the extracts combined. Another aliquot of 100 mL of dichloromethane was added to the bottle and tumbled for 3 hr. The bottle was centrifuged and the extract held for the back extraction step.

The combined extracts were transferred to a 1000 mL separatory funnel and 300 mL of 3% sodium chloride in distilled water was added. The separatory funnel was gently shaken for 2 min and the layers allowed to separate. The lower dichloromethane layer was drained through sodium sulfate into a Kuderna-Danish concentration apparatus. The 100 mL of dichloromethane from the last sample extraction was added to the separatory funnel, shaken for 2 min, and passed through the sodium sulfate into the Kuderna-Danish apparatus. The sodium sulfate was rinsed with approximately 10 mL of dichloromethane and the dichloromethane rinse was combined with the extracts. Proceed to Section 3.3.

3.3. Extract concentration

The combined extracts were transferred to a 250-mL Kuderna-Danish apparatus fitted with a three-ball Snyder column. Three or 4 boiling chips were added to the solvent and the Snyder column was attached to the flask. The extract was concentrated to between 10 and 15 mL in a 60°C water bath, and then transferred to a 25 mL concentrator tube using dichloromethane

rinses of 3 - 4 mL (The SAIC tissue extract concentration procedure is described in Section 3.4.2.). A boiling chip was added to the tube and the extract was concentrated to 0.9 - 1.0 mL using a tube heater block.

Seven milliliters of hexane was added to the sediment extract tube only, and the volume reduced to 1 mL. Proceed to Step 3.6.

3.4. Tissue lipid weight determination

3.4.1. Battelle

The total volume of the concentrated tissue extract was measured using a 1 or 2 mL glass syringe, and the volume recorded to the nearest 0.05 mL. Using a 50 µL syringe, a 30 µL aliquot of the sample was obtained and placed on a tared aluminum weighing pan on an electrobalance. Sufficient time was allowed for the solvent to evaporate, determined by attaining of constant weight on the balance, and the weight recorded to the nearest 0.001 mg. For quality control purposes, the lipid weight determination was performed in triplicate on one sample per analytical sample string.

The total extractable lipid weight was calculated, and the total extractable lipid weight normalized to sample dry weight. The tissue extract solvent was exchanged with 7 mL of hexane and the entire sample was concentrated to 1 mL using the tube heater block.

3.4.2. SAIC

The volume of the extract was adjusted to 12 mL with the addition of hexane. Four milliliters was removed for lipid weight determination. Sufficient time was allowed for the solvent to evaporate, and the sample weighed to the nearest 0.001 mg. The total extractable lipid weight was calculated, and the total extractable lipid weight normalized to sample dry weight.

After removing the aliquot for lipid weight determination, a boiling chip was added to the remaining extract and the volume reduced to between 0.9 to 1.0 mL using the tube heater.

Proceed to Section 3.6, Silica Gel/Alumina Chromatography.

3.5. Dry weight determination

3.5.1. Tissue

One gram of tissue sample aliquot was placed in a pre-weighed, labeled aluminum weighing pan, and spread evenly over the bottom of the pan with a spatula. Forceps were used to handle the pan at all times. The pan containing the tissue was weighed to the nearest 0.01 g and the weight recorded. The pan was covered loosely with foil, and placed in a drying oven at 120°C overnight. The covered sample was allowed to cool in a desiccator for 30 min, and weighed to the nearest 0.01 g. The average dry weight of the two measurements was determined. The percent dry weight was calculated as follows

$$\text{Dry Wt. \%} = \frac{\text{Dry weight - Tare weight}}{\text{Wet weight - Tare weight}}\ 100\%.$$

3.5.1. Sediment

Ten grams of homogeneous sediment sample aliquot were placed in a pre-weighed, labeled aluminum weighing pan. Forceps were used to handle the pan at all times. The pan containing the sediment was weighed to the nearest 0.01 g and the weight recorded. The pan was covered loosely with foil, and placed in a drying oven at 120°C for 24 hr. The covered sample was allowed to cool in a desiccator for 30 min, and weighed to the nearest 0.01 g. The samples were placed back in the oven for 24 hr, removed, allowed to cool as before, and reweighed. The percent dry weight was calculated as follows

$$\text{Dry Wt. \%} = \frac{\text{Average dry weight - Tare weight}}{\text{Wet weight - Tare weight}} 100\%.$$

3.6. Silica gel/alumina chromatography of tissue and sediment extracts

(The procedures in *italicized* text mark the differences between the tissue and *sediment* procedures).

The extract was carefully transferred to the top of the packing using a Pasteur pipette. The column was drained until the sample was flush with the packing top. The vial that contained the extract was rinsed two times with 0.5 mL hexane, and the rinsates were added to the column. The column was drained to the packing top. One half milliliter of 1:1 dichloromethane:hexane was placed in the empty extract vial and the vial set aside until needed. Twenty milliliters of hexane was added to the column and eluted to the top of the packing at approximately 3 mL/min, discarding the eluate. The tip of the column was washed with dichloromethane and the waste container was replaced with a 20-mL vial labeled with the sample identification number and the notation "SA1." This fraction was archived. Fifteen milliliters of hexane was added to the column and eluted to the packing top at 3 mL/min. The "SA1" vial was replaced with a flask labeled with the sample identification number and the notation "SA2." The washings from the original extract vial were added to the column (0.5 mL 1:1 dichloromethane:hexane) and drained to the packing top. One hundred and fifty milliliters of 1:1 dichloromethane:hexane were added to the column and slowly drained to the packing top at approximately 3 mL/min.

A waste flask was placed under the column, 50 mL of dichloromethane was added to the column, and the column drained to the packing top of the packing at 3 mL/min. Twenty five milliliters of 10% methanol in dichloromethane was added to the column and drained to the packing top at 2 mL/min. The column tip was rinsed and the waste flask was replaced with a flask labeled with the sample identification number and the notation "SA3." Thirty milliliters of 20% methanol in dichloromethane was added to the column and all of the solvent eluted into the "SA3" flask. Forty milliliters of methanol was added to the column and the entire volume eluted into a vial labeled with the sample identification number and the notation "SA4" for archival.

The extracts were transferred from the 250-mL “SA2” flask to a Kuderna-Danish apparatus equipped with a 3-ball Snyder column. The fraction was concentrated to between 5 and 10 mL using a 80°C water bath, and transferred to a concentrator tube with two 1-mL dichloromethane rinses. Two or three boiling chips were added to the tube and the extract was concentrated on the heater block until the volume was approximately 1 mL. Seven milliliters of hexane was added to the tube and again the extract concentrated to 1 mL. The sample at this point was solely in hexane. Appropriate amounts of methanol and dichloromethane were added to make the sample solvent approximately 2.3 mL of a solution of 6:4:3 hexane:methanol:dichloromethane by volume.

Proceed to Section 3.7, Sephadex Chromatography.

Two or three boiling chips were added to the "SA3" flask and a Snyder column was attached. The "SA3" fraction was concentrated to between 5 and 10 mL using a 80°C water bath, and transferred to a concentrator tube with two 1-mL dichloromethane rinses. Two or three boiling chips were added to the tube and the extract was concentrated on the heater block until the volume was approximately 1 mL. Seven milliliters of dichloromethane and a new boiling chip were added and the extract concentrated to 1 mL. Four milliliters of dichloromethane and a new boiling chip were added to the extract again and concentrated to 1 mL. Seven milliliters of hexane and a new boiling chip were added and the extract concentrated to 1 mL. Another 4 mL of hexane were added and the volume again reduced to 1 mL. The 1-mL extract was transferred to a 4-mL vial using 2 to 3 hexane rinses, and concentrated gently under nitrogen to 1 mL. Fifty microliters of the HMB GC/I-std solution were added to the vial and mixed for 2 sec on the Vortex Genie. This final extract was concentrated under a gentle stream of nitrogen down to the injection volume, and transferred to a labeled GC vial. The "SA3" fraction was ready for GC/FID analysis for coprostanol.

3.7. Sephadex chromatography

The Sephadex column preparation is described in MacLeod *et al.*, (this document).

3.7.1. Chromatographic clean-up

The less than 2.3-mL "SA2" sample extract was mixed thoroughly so that any layers in the solvent mix were eliminated. The excess solvent was removed from the top of the Sephadex column with a Pasteur pipette without disturbing the packing material. Ten milliliters of the 6:4:3 solvent was added to the column and drained to the packing top. The sample extract was added to the column using a Pasteur pipette, dispensing it slowly down the column wall so as not to disturb the Sephadex packing. The column was drained to the packing top. The vial which contained the 2-mL sample extract was rinsed with approximately 0.5 mL of the solvent mixture two times, each time adding the rinses to the column and draining it to the top of the packing. Twenty milliliters of the 6:4:3 solvent were added to the column and eluted to the packing top. The waste elute container was replaced with a 20-mL vial labeled with the sample identification number and the notation "SA2-L1." Twenty milliliters of the 6:4:3 solvent was added and drained into the vial until the solvent was again flush with the packing material. The 20-mL vial was replaced with a 100-mL pear-shaped flask labeled with the sample identification number and the notation "SA2-L2." The "SA2-L1" fraction was archived. Ninety milliliters of the 6:4:3 solvent was added to the column and drained to the top of the packing. The "SA2-L2" fraction was concentrated for analysis. To prepare the Sephadex for the next sample, the column was eluted with 50 mL of the solvent mixture prior to adding a new sample extract to the column.

3.7.2. Sample concentration

Three or four boiling chips were added to the flask containing the "SA2-L2" fraction, a 3-ball Snyder column was attached, and the fraction concentrated to between 5 and 10 mL using a 90-95°C water bath. This reduced extract was transferred to a concentrator tube with two dichloromethane rinses of 1 to 2 mL. Two or three boiling chips were added, and the volume reduced to 1 mL using a heater block. Seven milliliters of dichloromethane was added to the extract and the volume reduced to 1 mL. Four milliliters of dichloromethane was added and the volume again reduced to 1 mL. Seven milliliters of hexane was added and the volume reduced to 1 mL. Four milliliters of hexane was added and the volume again reduced to 1 mL. The final concentrated extract was transferred to a 4-mL vial, using two hexane rinses. The volume was reduced to 1 mL using a gentle stream of nitrogen.

A few grains of activated copper were added to the final extract until no discoloration occurred. The vial was capped and refrigerated overnight. The extract was transferred to a clean vial with 2 to 3 hexane rinses, and concentrated to 1 mL under a gentle stream of nitrogen.

Fifty microliters of HMB GC/I-Std solution and 50 µL of TCMX GC/I-Std solution were added to the final extract, and the solution mixed using the Vortex Genie for 2 sec. The sample was concentrated under nitrogen blow down to its injection volume of approximately 500 µL for tissue and 900 µL for sediment. The sample was transferred to a labeled GC vial for analysis.

3.8. Instrumental analysis

The SA2L2 fraction was analyzed using gas chromatography with ^{63}Ni electron capture detection (GC/ECD) for PCBs and pesticides, and gas chromatography with flame ionization detection (GC/FID) for polycyclic aromatic hydrocarbons (PAHs). The SA3 fraction was analyzed by GC/FID for coprostanol. The target analytes are listed in Table IV.31.

3.8.1. Instruments and instrumental conditions

Chromatographic and temperature conditions for the instrumental analyses are listed in Table IV.32.

3.8.2. Calibration and quantification

Prior to sample analysis, the gas chromatography systems were calibrated using stock calibration solutions at three concentration levels for all analytes. The concentration of analytes in the standards ranged from near the method detection limits to near the highest degree of contamination expected in the field samples. Once the system was calibrated, the linearity of the detector was verified once daily, at a minimum, while samples were being analyzed.

The three-point initial calibration curves were analyzed prior to each analytical sample string. Initial calibration response factors (RF) were calculated for each analyte relative to the appropriate surrogate internal standard (SIS) using the formula

$$RF = \frac{A_x \ C_{IS}}{A_{IS} \ C_x}$$

where A_x is the peak area of the analyte, A_{IS} is the peak area of the SIS, C_{IS} is the concentration of the SIS, and C_x is the concentration of the analyte.

The SIS and the analytes they are used to quantify are summarized in Table IV.33.

The average RF and its relative standard deviation (RSD) for each analyte from the three-level calibration were calculated. The RSD for each analyte had to be 25% or less, or the initial calibration was redone. At a minimum, every 24 hr a calibration check was performed where a mid-range calibration standard was analyzed, RFs generated, and the percent difference between these new RFs and the average RF from the initial calibration calculated. The calibration was considered acceptable if this percent difference was $\leq$25%. If the check

Table IV.31. Organic chemicals determined as part of the NOAA National Status and Trends Program [PCB congener nomenclature using method of Ballschmitter and Zell (1980)].

PAHs
Naphthalene
2-Methylnaphthalene
1-Methylnaphthalene
Biphenyl
2,6-Dimethylnaphthalene
Acenaphthylene★
Acenaphthene
1,6,7-Trimethylnaphthalene
Fluorene
Phenanthrene
Anthracene
1-Methylphenanthrene
Fluoranthene
Pyrene
Benz[*a*]anthracene
Chyrsene
Benzo[*b*]fluoranthene★
Benzo[*k*]fluoranthene★
Benzo[*e*]pyrene
Benzo[*a*]pyrene
Perylene
Indeno[1,2,3-*cd*]pyrene★
Dibenz[*a,h*]anthracene
Benzo[*ghi*]perylene★

Pesticides
2,4'-DDD
2,4'-DDE
2,4'-DDT
4,4'-DDD
4,4'-DDE
4,4'-DDT
Aldrin
cis-Chlordane
Dieldrin
Endrin◆
gamma-HCH
Heptachlor
Heptachlorepoxide
Hexachlorobenzene
Mirex
trans-Nonachlor

PCBs▼
PCB 8, PCB 18, PCB 28, PCB 44, PCB 52, PCB 66, PCB 77×, PCB 101, PCB 105, PCB 118, PCB 126×, PCB 128, PCB 138, PCB 153, PCB 170, PCB 180, PCB 187, PCB 195, PCB 206, PCB 209

Auxiliary Parameters
Gonadal index (selected bivalves)
Lipid content
Dry weight
Shell size

Organotins ⌘
Tributyltin (TBT)
Dibutyltin (DBT)
Monobutyltin (MBT)
Tetrabutyltin (TTBT)

★ PAH analytes added in 1988.

◆ Added to analyte list in 1990.

× Included in calibration solution prepared by NIST, not a target analyte until 1990.

▼ Congener method of quantification used by Battelle beginning in 1987, and by SAIC beginning in 1988.

⌘ Organotins added to analyte list in 1988.

Table IV.32. Instrument description and conditions for tissue and sediment extract analysis using electron capture (ECD) and flame ionization (FID) detectors for 1986.

GC/ECD	
Column:	30-m, 0.25-mm i.d., and 0.25 µm film thickness of DB-5
Carrier gas:	He, 28-32 mL/sec
Detector make-up gas:	95% Ar: 5% methane, 30 mL/min
Injection:	Splitless, 1-2 µL
GC Oven Program:	Initial oven temp: 50°C
	Hold: 3 min
	Ramp 1: 4°C/min to 170°C
	Ramp 2: 1°C/min to 210°C
	Ramp 3: 4°C/min to 300°C
	Hold: 10 min
Injector temp:	275°C
Detector temp:	325°C
GC/FID	
Column:	30-m, 0.25-mm i.d., and 0.25 µm film thickness DB-5 (PAH) or DB-17 (coprostanol)
Carrier gas:	He, 28-32 cm/sec
Injection:	Splitless, 1-2 µL
GC Oven Program:	Initial oven temp: 60°C (50°C for coprostanol analysis)
	Hold: 3 min
	Ramp 1: 4°C/min to 300°C for PAH analysis (7°C/min to 290°C for coprostanol analysis)
	Hold: 10 min
Injector temp:	275°C
Detector temp:	325°C

Table IV.33. Surrogate internal standards (SIS) and the analytes they are used to quantify.

SIS	Analyte Class	Analytes
Naphthalene-d_8	PAHs	Naphthalene to biphenyl
Acenaphthene-d_{10}	PAHs	Dimethylnaphthalene to pyrene
Perylene-d_{12}	PAHs	Benz[*a*]anthracene to dibenz[*a*,*h*]anthracene
Androstanol	Sterols	Coprostanol
Dibromooctafluorobiphenyl (DBOFB)	PCBs and pesticides	All PCBs and pesticides

standard was out of range, remedial maintenance was performed and the calibration solution reanalyzed. All samples had to be bracketed by two calibrations which met acceptance criteria. The %RSD was calculated using the relationship

$$\%RSD = \frac{\sigma}{X} 100\%,$$

where X is the mean of the three RF values, and σ is the standard deviation of the mean. The average RF generated from the initial calibration was used for quantitation of the samples associated with that calibration. The following formula was used to calculate C, the amount of analyte in each sample

$$C = \frac{A_x \text{ (amount SIS added to sample)}}{A_{IS} \text{ (Avg. RF of analyte from Init. Cal.) (Sample dry wt.)}}.$$

PCBs were quantitated as levels of chlorination. The individual PCB congeners were identified in the chromatogram as having a specific number of chlorine atoms. Each peak that had the same number of chlorine atoms was quantified using the RF from a specific PCB congener in the calibration solution that had the same number of chlorine atoms (e. g., PCB 47 in the calibration solution was used to quantitate all the tetrachlorobiphenyls). The specific congeners included PCB 7 (2 chlorine atoms), PCB 31 (3 chlorine atoms), PCB 47 (4 chlorine atoms), PCB 101 (5 chlorine atoms), PCB 153 (6 chlorine atoms), PCB 187 (7 chlorine atoms), PCB 194 (8 chlorine atoms), and PCB 206 (9 chlorine atoms). Concentrations of these congeners were also reported individually. The concentrations of all the congeners containing the same number of chlorine atoms were summed and reported (e. g., the concentrations of all the tetrachlorobiphenyls were summed).

The recovery of the SISs were calculated using the recovery internal standards (RIS) added to the samples at the completion of the extraction procedure. These RIS [hexamethylbenzene (HMB) for PAH and coprostanol analyses, tetrachloro-m-xylene (TCMX) for PCB/pesticide analyses] were used to determine the RFs of the SIS compounds. SIS percent recoveries were calculated as follows

$$\text{SIS RF} = \frac{A_{SIS}}{A_{RIS}} \frac{C_{RIS}}{C_{SIS}}$$

$$\%\text{ Recovery SIS} = \frac{\text{(ng RIS added to sample)}}{\text{(ng SIS added to sample) (area RIS in sample) (SIS RF)}} \frac{\text{(area SIS in sample)}}{} 100\%.$$

4. SUMMARY OF ANALYTICAL PROCEDURES, BATTELLE AND SAIC, 1987

The methods for tissue and sediment sample preparation and analysis performed by Battelle and SAIC changed very little between 1986 and 1987 of the Mussel Watch Project. The following sections summarize the salient changes.

4.1. Tissue extraction

Twice the amount of the RIS HMB was added to each sample after extraction and final extract concentration. This step was taken in order to make the internal standard amount in the sample more similar in concentration to the PAH analytes found in most of the samples.

4.2. Sediment extraction

The following modifications were made to the sediment extraction procedure:

Approximately 20 g of activated copper was added to the extraction bottle at the start of extraction.

No copper was added to the silica gel/alumina column.

Twice the amount of the internal standard HMB was added to each sample after extraction and final concentration.

4.3. Sample analysis

PAH analysis was performed by Battelle using gas chromatography with mass spectrometry (GC/MS) in the full scan mode. Battelle's subcontractor, SAIC, continued to use GC/FID for PAH analysis. The GC/MS conditions ultimately used by Battelle for PAH analysis of 1987 samples are listed in Table IV.34.

5. SUMMARY OF ANALYTICAL PROCEDURES, BATTELLE AND SAIC, 1988

The following section summarizes the changes made to sample preparation and analysis by Battelle and SAIC in 1988 of the Mussel Watch Project. In addition to procedural changes, five additional PAHs were added to the target analyte list in 1988 (Table IV.31). The addition of these five analytes did not necessitate changes in the generic sample extraction scheme. Coprostanol analyses were not performed after 1987.

Table IV.34. Instrument conditions for tissue and sediment extract analysis using gas chromatography with mass spectrometry (GC/MS) in the full scan mode for 1987.

Mass Spectrometer: Hewlett-Packard 5970B quadrupole (additional equipment)	
Interface:	Capillary-direct
Ionization Mode:	70 ev, positive ion
Scan rate:	4 scan/sec minimum
Scan Range:	50-500 a.m.u.
Interface temp.:	280°C
Source temp.:	280°C
GC	
Column:	30-m, 0.25-mm i.d., 25 µm film thickness of DB-5
Carrier gas:	He, 28-32 cm/sec
Injector temp:	250°C
Injection:	Splitless, 1-2 µL
GC Oven Program:	Initial oven temp: 40°C
	Hold: 1 min
	Ramp 1: 6°C/min to 290°C
	Hold: 25 min

5.1. Sample extraction

The automated high performance liquid chromatography (HPLC) size-exclusion chromatography cleanup method of Krahn *et al.* (1988) was tested by Battelle during 1988 as an alternative to the conventional silica gel/alumina and Sephadex column cleanup procedure. This method was ultimately adopted and has been used since 1989 for sample processing. The details of the HPLC instrumentation, calibration, and operation are summarized in Table IV.35.

The HPLC system was calibrated by injecting a solution containing the first and last eluting analytes of interest (DBOFB and perylene, respectively), and primary contaminants [lipid (corn oil) and sulfur]. Concentrations were: DBOFB, 20 µg/mL; perylene, 20 µg/mL; corn oil, 20 mg/mL; and elemental sulfur, 40 µg/mL.

The size-exclusion HPLC cleanup procedure was performed on the concentrated sample extract after silica gel/alumina column chromatography. The extract volume was reduced to between 900 and 1000 µL, and the volume measured exactly with a syringe. The extract was transferred to an autosampler vial, and 600 µL of the extract was injected into the HPLC system. The fraction collector was programmed to collect the portion of the sample that eluted between 1 min before the retention time (RT) of DBOFB, and 2 min after the RT of perylene. The sample passed through 2 Phenomonex Phenogel columns at 5 mL/min (dichloromethane mobile phase).

The fraction collected, approximately 40 mL, was concentrated under a gentle stream of nitrogen to approximately 1 mL, and spiked with the appropriate recovery internal standards (RIS). The sample solvent was exchanged with hexane and analyzed for organic contaminants by GC/MS and GC/ECD.

5.2. Sample analysis

In 1988, Battelle modified its approach for PAH analysis by using GC/MS with selected ion monitoring (SIM) rather than GC/MS full scan. This step was taken to improve sensitivity of the GC/MS method, since many of the PAH compounds found in bivalve tissues were very near the GC/MS full-scan detection limit. The instrumental conditions for GC/MS with selected ion monitoring are listed in Table IV.36. The primary and secondary ions and the acceptance criteria for the ratio between these ions for each of the PAH analytes is given in Table IV.37.

Table IV.35. HPLC conditions used for 1989 analyses.

Pump:	Isocratic pump delivering 5 mL/min
Column:	2 Phenomonex Phenogel 250 mm x 22.5 mm i.d., 10 µm pore size columns plumbed in series
Detector:	UV/VIS at 254 nm for qualitative calibration
Injector:	Autosampler capable of injecting 600 µL aliquot

Table IV.36. Instrument conditions for tissue and sediment extract analysis using gas chromatography with mass spectrometry (GC/MS) in the full scan mode for 1989 analyses

Mass Spectrometer	
Mass spectrometer:	Hewlett-Packard 5970B quadrupole
Interface:	Capillary, direct
Ionization mode:	70 ev, positive ion
Scan range:	Selected Ion Monitoring
Scan rate:	4 scan/sec
Interface temperature:	280°C
Source temperature:	280°C
Gas Chromatograph	
Column:	30-m, 0.25-mm i.d., and 0.25 µm film thickness of DB-5
Carrier gas:	He, 28-32 cm/sec
Injector temp:	300°C
Injection:	Splitless, 1-2 µL
GC oven program:	Initial oven temp: 40°C
	Hold: 1 min
	Ramp 1: 6°C/min to 290°C
	Hold: 20 min

5.3. Tributyltin analysis

In 1988, bivalves from 23 sites were analyzed for the antifouling agent tributyltin (TBT), its degradation products dibutyltin (DBT) and monobutyltin (MBT), and the TBT manufacturing impurity tetrabutyltin (TTBT). This section summarizes the methods used for sample extraction and instrumental analysis for these organometallic compounds. The methods described here are modifications of Unger *et al.* (1986), Uhler *et al.* (1989), and Uhler and Durell (1989).

5.3.1. Sample extraction

Approximately 30 g (wet weight) of the shucked and homogenized bivalve organics sample was spiked with 1 µg of the surrogate internal standard (SIS) tripropyltin chloride (TPT), desiccated with 30 g of anhydrous sodium sulfate, and serially extracted three times with 60 mL of 0.05% tropolone in hexane. Simultaneously, a 5-g aliquot of the homogenate was obtained for percent dry weight determination. The hexane extract was concentrated to approximately 10 mL using a rotary evaporator with the water bath set to approximately 60°C and a water aspirator to reduce the pressure. The extracted butyltins and the SIS were converted into the chromatographically-inert n-pentyl derivatives using a Grignard reaction by adding 1 mL of 1.9 M n-pentylmagnesium bromide. The mixture was allowed to stand 15 min and was purified by passing through a combined 16-g Florisil/5-g silica gel chromatography column. The column was eluted with 100-mL of hexane and the eluate concentrated to approximately 0.5 mL in a 60°C waterbath using a 250-mL Kuderna-Danish apparatus fitted with a 3-ball Snyder column. After concentration, approximately 1 µg of the recovery internal standard (RIS) dipropyldipentyltin (DPT) was added. DPT is used to measure the recovery of the SIS TPT on a sample-by-sample basis and provided a quality control check on the effectiveness of the analytical method.

Table IV.37. Selected PAH quantification and confirmation ions for GC/MS full-scan and SIM analysis.

Analyte	Quantification Ion (AMU)	Confirmation Ions (AMU) [% of Base Peak]			
Acenaphthene	154	153	[98]	152	
Acenaphthalene	152	151	[20]	153	
Anthracene	178	179	[20]	176	[20]
Benz[*a*]anthracene	228	226	[30]	229	
Benzo[*b*]fluoranthene	252	253	[30]	125	
Benzo[*k*]fluoranthene	252	253	[30]	125	
Benzo[*ghi*]perylene	276	277	[25]	138	[20]
Benzo[*a*]pyrene	252	253	[20]	125	
Benzo[*e*]pyrene	252	253	[20]	125	
Biphenyl	154	152	[30]		
Chrysene	228	226	[30]	229	
Dibenz[*a,h*]anthracene	278	279	[25]	139	[20]
2,6-Dimethylnaphthalene	156	155	[30]		
Fluoranthene	202	101	[15]	203	
Fluorene	166	165	[95]	167	
Hexamethylbenzene	162	147			
Indeno[1,2,3-*cd*]pyrene	276	277	[25]	138	[20]
1-Methylphenanthrene	192	191	[60]		
1-Methylnaphthalene	142	141	[90]	115	[25]
2-Methylnaphthalene	142	141	[90]	115	[25]
Naphthalene	128	127	[15]	129	
Perylene	252	235	[20]	125	
Phenanthrene	178	179	[20]	176	[20]
Pyrene	202	101	[15]	203	
1,6,7-Trimethylnaphthalene	170	155	[95]		
Acenaphthene-d_{10}	164	162	160		
Benzo[*a*]pyrene-d_{12}	264	260	265		
Chrysene-d_{12}	240	120	236		
Naphthalene-d_8	136	68			
Perylene-d_{12}	264	260	265		

The method detection limit (MDL) for the TBT, DBT, MBT, and TTBT using this method was approximately 0.020 µg/g, on a dry weight basis. Butyltin levels approximately 10 times lower could be detected but not accurately quantified. Butyltin data was expressed on a tissue dry weight basis and in terms of compound cation weight following the recommendations of a NOAA-led Federal interagency TBT work group [Landy *et al.*, 1986].

5.3.2. Instrumental analysis

Sample extracts were analyzed for butyltins using capillary gas chromatography with flame photometric detection (GC/FPD). The instrumental conditions are listed in Table IV.38.

Table IV.38. Instrument conditions for butyltins using capillary gas chromatography with flame photometric detection (GC/FPD)

Detector:	Flame Photometric
Detector temp:	250°C
FPD Filter:	610 nm cutoff
Hydrogen:	30 mL/min
Air:	100 mL/min
Gas Chromatograph	
Column:	30-m, 0.25-mm i.d. 0.25-µm film thickness
Carrier gas:	He, 28-32 cm/sec
Injection:	1-2 µL splitless
Injector temp:	250°C
GC oven program:	Initial oven temp: 60°C
	Hold: 1 min
	Ramp : 20°C/min to 250°C
	Hold: 4 min

Butyltins were quantified using the method of internal standards. A three-point calibration was performed for each butyltin compound relative to the SIS TPT. Concentrations of the butyltins in the standards were approximately 0.04 ng/µL (LOW standard), 0.1 ng/µL (MID standard), and 1 ng/µL (HIGH standard). The concentration of the SIS and RIS was approximately 0.1 ng/µL in all three standards. A three-point calibration was prepared just prior to analysis of samples. A calibration check standard (MID-level standard) was analyzed after every 12 samples to ensure that the GC/FPD system remained in calibration over the course of sample analysis.

The response factors for each butyltin compound in the LOW, MID, and HIGH standards relative to the SIS TPT were calculated using the equation

$$RF = \frac{H_i \; C_{is}}{H_{is} \; C_i},$$

where H_i is the peak height of the analyte in the standard solution, H_{is} is the peak height of the internal standard in the standard solution, C_i is the concentration (or amount) of analyte in the standard solution, and C_{is} is the concentration (or amount) of internal standard in the standard solution.

The calibration was considered acceptable if the percent relative standard deviation (%RSD) among the three RFs was within 30%. If the %RSD exceeded 30%, a new calibration was performed.

A MID level check calibration standard was analyzed after every 12 samples. The original calibration was considered valid if the RF from this analysis was within ±30% of the original mean RF. If the check MID met the acceptance criterion, the next 12 samples were quantified using the original RF. If the check MID differed from the original average RF by more than 30%, a new three-point calibration was performed.

5.4. Additional analyses

Because the identification of PCB and pesticide analytes relies entirely on comparison of retention behavior with standards, an analyte confirmation study was conducted during 1988 to examine the adequacy of the current analytical methodology. PCBs and pesticides were initially identified and quantified using GC/ECD with a 30-m DB-5 capillary column. For the confirmation study, 68 East Coast bivalve samples were also analyzed by GC/ECD using a 30-m DB-17 capillary column.

6. SUMMARY OF ANALYTICAL PROCEDURES, BATTELLE AND SAIC, 1989

The following changes were incorporated into the analytical methods in 1989.

6.1. Tissue sample extraction

6.1.1. Battelle

During 1989, the HPLC method of sample clean-up tested during 1988 was incorporated into the standard sample extraction scheme by Battelle for tissues and sediments. The changes in sample preparation procedures initiated during 1989 are summarized below.

Approximately 25 g (wet weight) from the shucked and homogenized bivalve sample was placed in a tared 250 mL Teflon extraction container. Simultaneously, a 5-g aliquot of the homogenate was removed for percent dry-weight determination. SIS's, 50 g of sodium sulfate, and 75 mL of dichloromethane were added to the sample homogenate. The sample was homogenized using a Tissumizer for 2 min, centrifuged for 5 min at 2000 rpm, and decanted into a labeled Erlenmeyer flask. The extraction was repeated with another 75 mL of dichloromethane, centrifuged, and decanted. After the second extraction, 50 mL of dichloromethane was added to the sample container and hand shaken for 1 min. The sample was centrifuged and the extract added to the flask. The combined extracts were mixed by gentle swirling of the flask. The total volume was determined using the graduation marks on the flask. Using a clean class “A” pipette, 10 mL of the extract was removed and placed in a pre-weighed aluminum weighing pan. The pan was covered with foil and the aliquot allowed to air dry overnight. After drying, the pan was weighed and the weight recorded. The lipid weight was calculated using the following formula

$$\text{Total lipid wt. (g)} = \frac{\text{Vol. of combined extracts (mL)}}{\text{Aliquot vol. (mL)}} \text{ (Aliquot dry wt (g) - Tare wt. (g))}$$

$$\text{Sample lipid content (g/g)} = \frac{\text{Total lipid wt. (g)}}{\text{Sample dry wt. (g)}} .$$

After removing the aliquot for lipid weight determination, 20-50 g sodium sulfate was added to the Erlenmeyer and mixed. After approximately 30 min, the sample was decanted through a 20-g 2% deactivated F-20 alumina column and the eluant collected in a clean 500 mL flask. When the extract reached the top of the column, 50 mL of dichloromethane was added to the column and the solvent completely drained into the flask. The sample extract was transferred to a Kuderna-Danish apparatus, rinsing the flask with approximately 5 mL of dichloromethane. Boiling chips were added to the Kuderna-Danish receiver tube, a Snyder column was attached, and the extracts concentrated to between 5 to 10 mL on a 65 - 85°C water bath. Concentration continued under a gentle stream of nitrogen to a volume between 0.9 to 1.0 mL. The volume of

the concentrated extract was measured exactly using a syringe. HPLC fractionation procedures were then followed (Section 4.1).

The fraction collected, approximately 40 mL, was concentrated under a gentle stream of nitrogen to approximately 500 µL and spiked with the appropriate RIS. Half of the final extract was solvent exchanged with hexane and analyzed for PCBs and pesticides by GC/ECD. The other half was analyzed for PAHs by GC/MS.

6.1.2. SAIC

The tissue extraction methodology used by SAIC during 1989 was similar to that used in previous years, with the following modification. After the samples were homogenized using the Tissumizer, the extract was decanted through sodium sulfate contained on fluted filter paper in a powder funnel and into a labeled 500 mL round bottom flask.

6.2. Tributyltin

The quantity of the internal standards added to the tissue samples prior to extraction (SIS) and just before instrumental analysis (RIS) were reduced to approximately 0.25 µg. This step was taken because the concentrations of TBT and its degradation products was found to be decreasing dramatically from earlier years. These reductions in environmental levels of butyltins are presumably due to the effects of the 1988 Organotin Paint Control Act which severely curtailed the inputs of butyltins to the aquatic environment.

6.3. Sediment extraction

Approximately 10 g of well-mixed sediment were weighed into a pre-weighed aluminum weighing pan for dry weight determination. Simultaneously, approximately 50 g of wet, well-mixed sediment were weighed into a 250-mL Teflon jar to the nearest 0.01 g and the weight recorded. One hundred milliliters of 1:1 dichloromethane:acetone, 60 g of sodium sulfate, 20 g of activated granular copper, and the appropriate SIS were added. The contents of the bottle were shaken on a shaker table for approximately 12 hr. The sample was centrifuged for 5 min at approximately 1500 rpm and the extract decanted into an Erlenmeyer flask. The extraction was repeated twice more using 100 mL dichloromethane:acetone each time, and shaking for 4 hr and 0.5 hr respectively. The centrifuged extracts were combined in the flask. Five to 10 g of sodium sulfate was added to the flask. If clumping of the sodium sulfate occurred, more was added until it remained granular. The combined extracts were decanted through a 5-g 2% deactivated F-20 alumina column at approximately 2 mL/min into a clean Erlenmeyer flask until the sample was flush with the top of the packing. Twenty milliliters of dichloromethane was added to the column and drained into the flask.

The extracts were transferred to a Kuderna-Danish apparatus using dichloromethane rinses. Boiling chips were added to the Kuderna-Danish receiver tube, a Snyder column was attached, and the extracts were concentrated to between 5 to 10 mL using a 65 - 85°C water bath. The extract volume was further reduced to between 0.9 and 1.0 mL under a gentle stream of nitrogen. Five-hundred microliters of the extract was injected into the HPLC for further processing.

The fraction collected, approximately 40 mL, was concentrated under a gentle stream of nitrogen to approximately 900 µL and spiked with the appropriate RIS. Half of the final extract was solvent exchanged with hexane and analyzed for PCB/pesticides using GC/ECD. When necessary, activated copper was added to the extracts to complete sulfur removal if determined to be present during GC/ECD analysis. The remaining half of the extract was analyzed for PAHs by GC/MS.

6.4. Sample analysis

Due to the success of using GC/MS SIM for PAH analysis in 1988, both Battelle and SAIC changed to this method for analysis of all samples.

6.5 Additional analyses

As requested by NOAA, Battelle undertook two additional tasks. In one task, Battelle assessed the comparability of two PCB quantitation methods. Samples from three highly PCB-contaminated sites were analyzed following both the usual methods (GC/ECD capillary, isomer-specific analysis) and EPA Method 608 (1982) which provides a description of the analysis of samples for Aroclor formulations using GC/ECD equipped with a packed column. The second task included the analysis of specified tissue extracts for pesticides not included in the target analyte list, including toxaphene, endosulfan I, endosulfan II, atrazine, propanil, methyl parathion, carbaryl, and alachlor.

7. SUMMARY OF ANALYTICAL PROCEDURES, BATTELLE, 1990 - 1992

Several procedural and operational changes were made to the analytical program beginning in 1990, and remain in effect to date. These changes are summarized below. A schematic diagram of the extraction methods currently being used by Battelle for Mussel Watch Project sample processing is shown in Figure IV.19.

Method detection limits (MDL) for organic contaminants in sediment and tissues were determined in accordance with EPA's statistical approach for estimating MDLs (Federal Register, 1984).

An analyte list was finalized to include those compounds shown in Table IV.31.

A 5-g (wet weight) tissue aliquot was taken for dry weight determination.

Operational changes were made to the GC/ECD analysis of chlorinated pesticides and PCBs in order to improve resolution and detection of the compounds (described below).

PCBs were quantified and reported on an individual congener basis.

The PAH RIS used during 1990 through 1992 included biphenyl-d_{10}, phenanthrene-d_{10}, and benzo[*e*]pyrene-d_{12}.

In order to maximize the efficiency of the SIS, modifications have been made to the surrogate list and are described in Table IV.39.

Internal standards (SIS and RIS) were added to samples in amounts that would result in concentrations in the final extract approximately equal to similar analytes in the mid-range calibration solution.

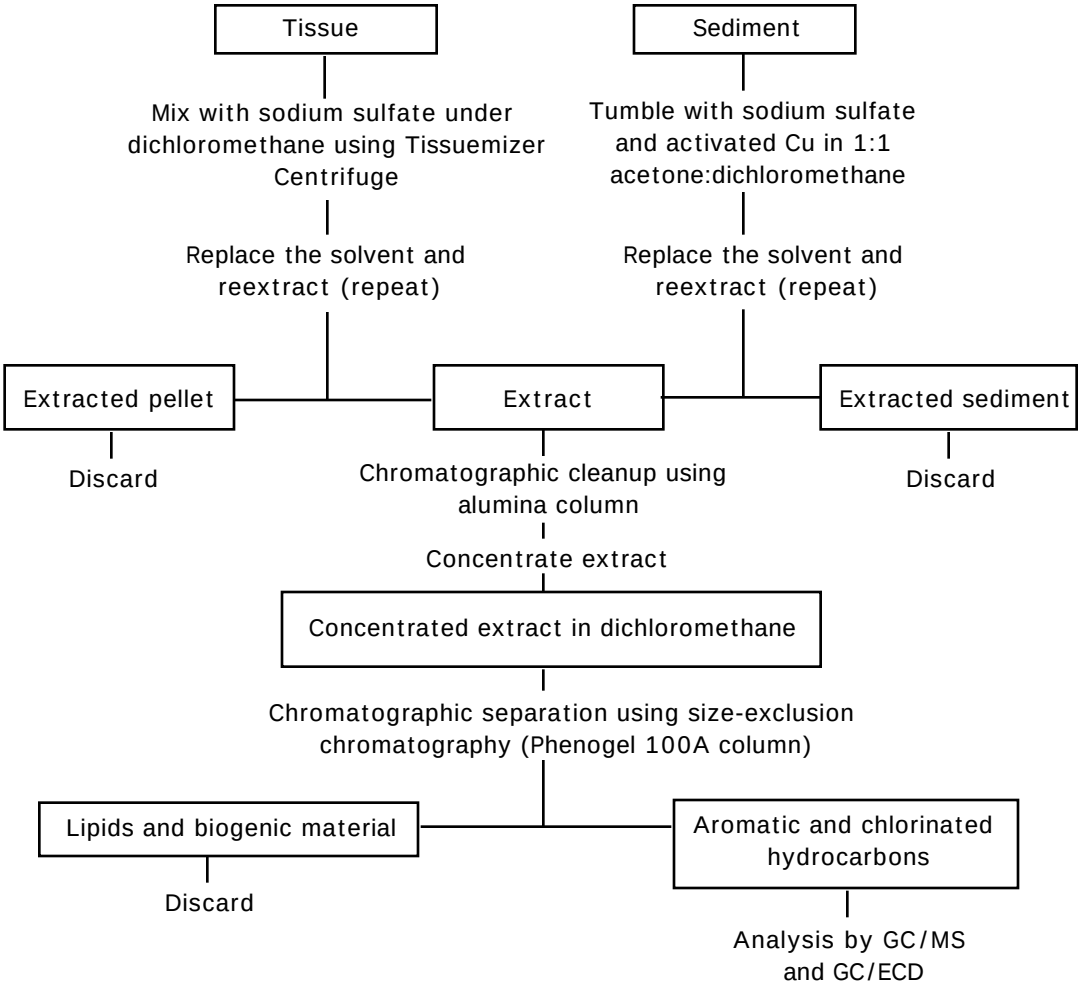

Figure IV.19. Extraction methods currently used by Battelle for Mussel Watch sample processing.

Beginning in 1990, hydrogen was substituted for helium as the carrier gas for GC/ECD analysis in order to improve chromatographic resolution and minimize analysis time. Revised instrumental and analytical conditions for chlorinated pesticide and PCB analysis are listed in Table IV.40.

8. CONCLUSIONS

The organic sample preparation and analysis methods followed by Battelle (1986-1992) and SAIC (1986-1989) for the NS&T Mussel Watch Project are presented above. These methods are constantly being reviewed and modified in order to incorporate improvements, with the ultimate aim to produce data of the highest quality.

Table IV.39. Modification made to the SIS list in years 1990 - 1992.

Year	Analyte Group	SIS
1990	PAHs	Naphthalene-d_9 Acenaphthene-d_{10} Perylene-d_{12} Dibenz[*a,h*]anthracene-d_{14}
1991	PAHs	Naphthalene-d_9 Acenaphthene-d_{10} Chrysene-d_{12} Benzo[*a*]pyrene-d_{12}
	PCBs and pesticides	DBOFB Tetrachloronaphthalene (TCN)
1992	PAHs	Naphthalene-d_8 Acenaphthene-d_{10} Benzo[*a*]pyrene-d_{12}
	PCBs and pesticides	DBOFB PCB 112 (5 chlorine atoms)

Table IV.40. Revised instrumental and analytical conditions for chlorinated pesticide and PCB analysis using GC/ECD.

Column:	30-m, 0.25-mm i.d., 0.25 µm film thickness of DB-5
Carrier gas:	Hydrogen, 38-40 cm/sec
Detector make-up gas:	95% Ar: 5% methane at 30-50 mL/min
Injection:	Splitless, 1-2 µL
GC Oven Program:	Initial oven temp: 60°C
	Hold: 1 min
	Ramp 1: 15°C/min to 150°C
	Ramp 2: 1°C/min to 210°C
	Ramp 3: 10°C/min to 280°C
	Hold: 10 min
Injector temp:	280°C
Detector temp:	325°C

9. ACKNOWLEDGEMENTS

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NIST Methods for Certification of SRM 1941 and SRM 1974

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ABSTRACT

A marine sediment and a mussel tissue Standard Reference Material (SRM) have been prepared and analyzed for the determination of trace organic constituents. SRM 1941, Organics in Marine Sediment, has been certified for concentrations of 11 PAHs. Non-certified concentrations for 24 additional PAHs, 15 PCB congeners, and 7 chlorinated pesticides are also reported. SRM 1974, Organics in Mussel Tissue (*Mytilus edulis*), is a frozen mussel tissue homogenate that has been certified for the concentrations of 9 PAHs. Non-certified concentrations for 19 additional PAHs, 13 PCB congeners, and 9 chlorinated pesticides are also reported. The preparation and certification analyses are described in this chapter.

1. INTRODUCTION

The National Institute of Standards and Technology (NIST) has been involved in two elements of the National Status and Trends (NS&T) Program: the Specimen Banking and the Quality Assurance Projects. The specimen banking component was incorporated in 1985 as part of the National Biomonitoring Specimen Bank at NIST. During the annual collection of specimens for the NS&T Program, samples from selected sites are also collected specifically for specimen banking. This archive of well-preserved environmental specimens provides the opportunity for retrospective analyses.

The Quality Assurance element was established to assess the quality and comparability of the analytical results from the different laboratories performing analytical measurements for the NS&T monitoring effort. Two natural matrix Standard Reference Materials (SRMs), SRM 1941 (Organics in Marine Sediment) (Schantz *et al.*, 1990) and SRM 1974 [Organics in Mussel Tissue (*Mytilus edulis*)] (Wise *et al.*, 1991), have been developed to assist in the validation of analytical procedures.

The analytical methods used at NIST for the quantification of polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyl (PCB) congeners, and chlorinated pesticides in these SRMs are described here. Certified concentrations of 11 PAHs in SRM 1941 are based on the combination of measurements by gas chromatography with flame ionization detection (GC-FID), gas chromatography-mass spectrometry (GC-MS), and reversed-phase liquid chromatography with fluorescence detection (LC-FL). Certified concentrations of nine PAHs in SRM 1974 are based on a combination of measurements by GC-MS and LC-FL. Noncertified concentrations of selected PCB congeners and chlorinated pesticides are based on measurements by GC with electron capture detection (GC-ECD) for SRM 1941 and on a combination of measurements by GC-ECD and GC-MS for SRM 1974. Even though these materials were developed primarily for organic constituents, noncertified concentrations of 32 and 36 trace elements are reported for SRM 1941 and SRM 1974, respectively.

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2. SRM 1941, ORGANICS IN MARINE SEDIMENT

2.1. Summary

SRM 1941 is intended for use in validating analytical methods for the determination of trace levels of selected PAHs in marine sediments. Noncertified concentrations of additional PAHs, PCB congeners, chlorinated pesticides, and inorganic constituents are also provided.

2.2. Collection and preparation

The marine sediment used to prepare this SRM was collected in the Chesapeake Bay at the mouth of the Baltimore Harbor, MD, near the Francis Scott Key Bridge, using a Kynar-coated, modified Van Veen grab sampler. The wet sediment was transported to the laboratory where it was air dried, pulverized, sieved, homogenized in a cone blender, and subsampled. The fraction of particles less than 150 µm in size was used for the SRM. Approximately 70 g of sediment was dispensed into each amber glass bottle with Teflon-lined screw caps. The material was radiation-sterilized (^{60}Co) at an estimated minimum dose of 3.2 megarads.

2.3. Moisture determination

The amount of moisture in SRM 1941 was determined by measuring the weight loss after freeze drying or oven drying. For the freeze drying studies, duplicate subsamples (2-3 g) of the sediment from 15 different bottles of SRM 1941 were dried in five batches at different times. The samples were dried for 3-5 days at 1 Pa with a -10°C shelf temperature and a -50 °C condenser temperature. For the oven drying studies, duplicate subsamples of approximately 2 g of sediment from eight of the same bottles used in the freeze drying study were dried in two different batches in an oven at 90 °C for 18 hr and the weight loss determined. Based on these studies, the water content in SRM 1941 at the time of the certification analyses was determined to be 3.98 ± 0.57% (95% confidence limits). Analytical results were determined on an "as received" basis and then converted to a dry weight basis by using the correction factor for water loss of 1.04.

2.4. Polycyclic aromatic hydrocarbons

This SRM was analyzed for selected PAHs using GC-FID, GC-MS, and LC-FL. For GC-FID analyses, 7 to 25 g subsamples of sediment from 12 randomly selected bottles were Soxhlet extracted for 16-20 hr using dichloromethane. A silica solid phase extraction column was used to remove the polar interferences from the sediment extract. Finely divided copper was then added to the extract to remove elemental sulfur. The PAH fraction was isolated from the sediment extract by normal-phase liquid chromatography using a semi-preparative aminopropylsilane column (Wise *et al.*, 1977). For the normal-phase LC fractionation, 2% (v/v) dichloromethane in hexane was used as the mobile phase for the isolation of the PAH fraction. The GC-FID analyses were performed using a 0.25 mm x 60 m fused silica capillary column with a 5% phenyl-substituted polysiloxane phase (0.25 µm film thickness). Automatic splitless injections of 2 µL were made with the splitter closed for 1 min and then opened at a split flow of 80 mL/min helium. The injector and FID temperatures were maintained at 300°C and 350°C, respectively. Helium was used as the carrier gas at an inlet pressure of 280 kPa (40 psi) and as the detector make-up gas at a flow rate of 30 mL/min. The column temperature program is listed in Table IV.41.

For the GC-MS analyses, approximately 10 g subsamples of sediment from four randomly selected bottles were Soxhlet extracted for 16-20 hr using dichloromethane. The extract was filtered through a 0.45-µm fluoropolymer filter prior to quantitative analysis by GC-MS

Table IV.41. GC temperature programs used during analyses of SRM 1941.

Temperature program for PAHs GC/FID analysis using a fused silica capillary column with a 5% phenyl-substituted methyl= polysiloxane phase:

Initial temp.:	1 min at 50°C
First rate:	50°C/min to 150°C
Isothermal pause:	5 min at 150°C
Second rate:	1.5°C/min to 280°C
Isothermal pause:	30 min at 280°C

Temperature program for PAHs (Method A) GC/MS analysis using a fused silica capillary column with a 5% phenyl-substituted methyl= polysiloxane phase:

Initial temp.:	1 min at 50°C
First rate:	50°C/min to 100°C
Isothermal pause:	2 min at 100°C
Second rate:	4°C/min to 268°C

Temperature program for PAHs (Method B) GC/MS analysis using a fused silica capillary column with a 5% phenyl-substituted methyl= polysiloxane phase:

Initial temp.:	2 min at 120°C
First rate:	4°C/min to 300°C
Isothermal pause:	40 min at 300°C

Temperature program for PCBs and 4,4'-DDE fraction GC/ECD analysis using a 5% phenyl-substituted methylpolysiloxane phase:

Initial temp.:	30 min at 200°C
First rate:	2°C/min to 270°C
Isothermal pause:	15 min at 270°C

Temperature program for pesticide fraction GC/ECD analysis using a 5% phenyl-substituted methylpolysiloxane phase:

Initial temp.:	50 min at 190°C
First rate:	1.5°C/min to 215°C
Second rate:	45°C/min to 270°C
Isothermal pause:	15 min at 270°C

using a column identical to that employed for the GC-FID analyses. The GC-MS analyses were performed without removing the precipitated elemental sulfur. Two different GC-MS procedures were used for the determination of PAHs in SRM 1941. Eight of the more volatile PAHs (naphthalene, 1-methyl- and 2-methylnaphthalenes, 2,6-dimethylnaphthalene, biphenyl, acenaphthylene, acenaphthene, and fluorene) were determined using Method A (see below); whereas, the other 25 species were measured using Method B. The injector and GC-MS interface temperatures were maintained at 300°C. Helium was used as the carrier gas with an inlet pressure of 175 kPa (25 psi) and a split flow of 6 and 30 mL/min for Methods A and B, respectively. The column temperature programs used for Method A and B are listed in Table IV.41. The following masses were monitored for Method A: 128, 136, 124, 152, 154, 156, 155, 164, 170, 166, 178, and 188 amu. The following masses were monitored for Method B: 178, 188, 192, 206, 202, 212, 226, 228, 240, 252, 264, 276, and 288 amu.

Table IV.42. LC-FL fluorescence detector excitation and emission wavelengths, used for the determination of PAHs in SRM 1941.

Wavelength Change	Excitation (nm)	Emission (nm)	PAH determined
Initial	249	362	Phenanthrene-d_{10}
			Phenanthrene
1	250	400	Anthracene
2	285	450	Fluoranthene-d_{10}
			Fluoranthene
3	333	390	Pyrene
4	285	385	Benz[*a*]anthracene
			Chrysene
5	406	440	Perylene-d_{12}
			Perylene
6	296	405	Benzo[*a*]pyrene
			Benzo[*k*]fluoranthene
			Benzo[*ghi*]perylene
7	300	500	Indeno[1,2,3-*cd*]pyrene

For the LC-FL analyses, approximately 11 g subsamples of sediment from three randomly selected bottles were Soxhlet extracted using hexane:acetone (1:1 v/v). An aminopropylsilane solid phase extraction column was used to remove the more polar interferences from the sediment extract. The extracts were then analyzed by reversed-phase LC using a polymeric octadecylsilane (C18) column (4.6 mm i.d. x 25 cm, 5 µm particle size) with wavelength programmed fluorescence detection (May and Wise, 1984; Kline *et al.*, 1985; Wise *et al.*, 1988; and Schantz *et al.*, 1990). The chromatographic conditions were as follows: linear gradient from 50% (v/v) acetonitrile in water to 100% acetonitrile in 50 min at 1.5 mL/min; held at 100% acetonitrile for 5 min. The fluorescence detector excitation bandpass was 10 nm and the emission bandpass was 2.5 nm. The fluorescence detector excitation and emission wavelengths used are listed in Table IV.42.

In order to quantify several PAHs that have low fluorescence sensitivity or that are subject to interferences from the matrix, a portion of the sediment extract was fractionated on a semi-preparative aminopropylsilane column to isolate isomeric PAH fractions as described previously (May and Wise 1984; Kline *et al.*, 1985; and Wise *et al.*, 1988). These isomeric PAH fractions were then analyzed by reversed-phase LC-FL on the same octadecylsilane column. Triphenylene was determined in the four aromatic ring fraction which contains triphenylene, chrysene, and benz[*a*]anthracene. The same C18 column as above and the following conditions were used for the analysis: 60% (v/v) acetonitrile in water for 15 min, then linear gradient to 100% acetonitrile in 5 min; flow rate of 1.5 mL/min. The fluorescence detection conditions were set to excitation/emission 252/352 nm (10/2.5 nm bandpass) to determine triphenylene-d_{12} and triphenylene; then, after the elution of triphenylene, the fluorescence detection wavelengths were changed to excitation/emission 285/385 nm to determine benz[*a*]anthracene-d_{12}, benz[*a*]anthracene, and chrysene.

Benzo[*ghi*]perylene and indeno[1,2,3-*cd*]pyrene were determined in the six aromatic ring fraction using the same C18 column as above and the following conditions: 80% (v/v)

acetonitrile in water for 5 min, then linear gradient to 100% acetonitrile in 15 min at a flow rate of 1.5 mL/min. The fluorescence detection conditions were set to excitation/emission 380/405 nm (10/5 nm bandpass) to monitor benzo[*ghi*]perylene-d_{12} and benzo[*ghi*]perylene, then changed to 300/500 nm after the elution of benzo[*ghi*]perylene to monitor indeno[1,2,3-*cd*]pyrene.

The five aromatic ring fraction was analyzed to obtain additional results for perylene, benzo[*k*]fluoranthene, benzo[*b*]fluoranthene, and benzo[*a*]pyrene. The same C18 column as above was used with an isocratic mobile phase of 75% (v/v) acetonitrile in water at 1.5 mL/min. Fluorescence conditions were set at excitation/emission 406/440 nm (10/5 nm bandpass) to determine perylene-d_{12} and perylene, then changed to excitation/emission 296/405 nm to determine benzo[*k*]fluoranthene and benzo[*a*]pyrene.

The internal standards used for quantification purposes are listed in Table IV.43. For the GC-FID method, two PAHs not significantly present in the sediment were utilized as internal standards. For the GC-MS and LC-FL methods, selected perdeuterated PAHs were utilized as internal standards. The internal standards were added to the sediment samples immediately prior to extraction. Calibration response factors for the analytes relative to the internal standards were determined by analyzing SRM 1491, Aromatic Hydrocarbons in Hexane/Toluene, in the case of GC-FID and GC-MS analyses. For LC-FL analyses response factors were determined with the use of SRM 1647a, Priority Pollutant PAHs (in Acetonitrile).

2.5. Polychlorinated biphenyl congeners and chlorinated pesticides

This SRM was analyzed for selected PCB congeners and chlorinated pesticides using GC-ECD. Subsamples of approximately 11 g of sediment from four bottles were Soxhlet extracted for 16-20 hr using dichloromethane. A silica solid phase extraction column was used to remove the polar interferences from the sediment extract. Additional analytical interferences were removed from this extract on a semi-preparative aminopropylsilane liquid chromatographic column. For this normal-phase LC fractionation, hexane was used as the mobile phase for the isolation of the PCB and 4,4'-DDE fraction; and 5% (v/v) dichloromethane in hexane was used for the isolation of the pesticide fraction.

For the GC-ECD analyses, a similar column and the same carrier gas conditions were used as for the GC-FID analyses. Manual split injections of 2 µL were made with a split flow of 25 mL/min helium. The ECD temperature was 320°C, and nitrogen was used as the detector make-up gas at a flow rate of 30 mL/min. For the PCB and 4,4'-DDE analyses, the injector temperature was 280°C. The column temperature program used is listed in Table IV.41. For the pesticide fraction, the injector temperature was 250°C.

The internal standards used for quantification purposes are listed in Table IV.43. The internal standards were added to the sediment samples immediately prior to extraction. Calibration response factors for the analytes relative to the internal standards were determined by fractionating and analyzing fractions of gravimetrically prepared calibration solutions of the analytes of interest and the internal standards.

2.6. Certified and noncertified concentrations

The results from at least two independent analytical procedures are used at NIST to determine the "certified" concentrations of the analytes in environmental SRMs. When only one analytical technique is used, then the concentrations are reported as "noncertified or information" values rather than "certified" values. SRM 1941 has been certified for concentrations of 11 PAHs

Table IV.43. Internal standards used in the analysis of SRM 1941 for the determination of organic constituents.

Technique *	Internal Standard	Analytes Determined
GC-FID	1-Butylpyrene	Phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, and benzofluoranthene isomers
	m-Tetraphenyl	Benzo[*e*]pyrene, benzo[*a*]pyrene, perylene, benzo[*ghi*]perylene, and indeno[1,2,3–*cd*]= pyrene
GC-ECD	PCB 103	PCB 18, 28, 52, 66, and 101
	PCB 198	PCB 118, 153, 105, 138, 187, 180, 170, 195, 206, 209, and 4,4'-DDE
	Endrin	Heptachlor epoxide, *cis*-chlordane, *trans*-nonachlor, dieldrin, 4,4'-DDD, and 4,4'-DDT
LC-FL (Total)	Phenanthrene-d_{10}	Phenanthrene and anthracene
	Fluoranthene-d_{10}	Fluoranthene, pyrene, benz[*a*]anthracene, and chrysene
	Perylene-d_{12}	Perylene, benzo[*k*]fluoranthene, benzo[*a*]= pyrene, benzo[*ghi*]perylene, and indeno[1,2,3-*cd*]pyrene
LC-FL (Fraction)	Triphenylene-d_{12}	Triphenylene, benz[*a*]anthracene, and chrysene
	Perylene-d_{12}	Perylene, benzo[*k*]fluoranthene, and benzo[*a*]= pyrene
	Benzo[*ghi*]perylene-d_{12}	Benzo[*ghi*]perylene and indeno[1,2,3-*cd*]pyrene
GC-MS (Method A)	Naphthalene-d_8	Naphthalene, methyl/dimethylnaphthalenes, and biphenyl
	Acenaphthene-d_{10}	Acenaphthylene and acenaphthene
	Phenanthrene-d_{10}	Fluorene
GC-MS (Method B)	Phenanthrene-d_{10}	Phenanthrene, anthracene, and methyl/dimethylphenanthrenes
	Pyrene-d_{10}	Fluoranthene and pyrene
	Benz[*a*]anthracene-d_{12}	Benz[*a*]anthracene and chrysene/triphenylene
	Benzo[*e*]pyrene-d_{12}	Benzofluoranthene isomers, benzo[*e*]pyrene, benzo[*a*]pyrene, and perylene
	Benzo[*ghi*]perylene-d_{12}	Benzo[*ghi*]perylene and indeno[1,2,3-*cd*]pyrene

* See Experimental Section for description of the techniques used for the analyses.

(Table IV.44), and noncertified values are also reported for 24 additional PAHs (Table IV.45). Gas chromatography with electron capture detection was used to provide noncertified concentrations for 15 PCB congeners and 7 chlorinated pesticides (Table IV.46). Recently, these noncertified concentrations were confirmed using GC-MS and GC-ECD on a C-18 column (see discussion below for SRM 1974) (Schantz *et al.*, 1993). In addition to the organic contaminants, concentrations of 32 major and trace elements were determined using neutron activation analysis, and the sulfur content was also determined using isotope dilution thermal ionization mass spectrometry (Schantz *et al.* 1990).

3. SRM 1974, ORGANICS IN MUSSEL TISSUE

3.1. Summary

SRM 1974 is intended for use in validating analytical methods for the determination of trace levels of selected PAHs in marine tissue samples. Noncertified concentrations of additional PAHs, PCB congeners, chlorinated pesticides, and inorganic constituents are also provided.

3.2. Collection and preparation

The mussels (*Mytilus edulis*) used for the preparation of SRM 1974 were collected from Dorchester Bay within Boston Harbor, MA. Approximately 2400 individual mussels were collected by hand at low tide. The mussels were rinsed in a tank supplied with pumped sea water and then placed in insulated, Teflon-lined wooden containers, frozen and transported to NIST on dry ice. At NIST, the mussels were transferred to Teflon bags and stored in a liquid nitrogen vapor freezer (-120°C) until they were shucked.

For shucking, the mussels were allowed to warm up to about 0°C to avoid breaking the shell. The tissue was placed in Teflon bags and immediately returned to the liquid nitrogen freezer. The frozen mussel tissue was pulverized in batches of ~150 g each using a cryogenic grinding procedure described previously by Zeisler *et al.* (1983). The frozen pulverized material was then combined in an aluminum mixing drum (59 cm x 73 cm). The drum was placed inside a liquid nitrogen vapor freezer and motor-driven to rotate at 20 rpm about its horizontal axis. Interior vanes at an angle to the axis of rotation provided a longitudinal component of mixing. After mixing for 2 hr, subsamples (15-20 g) of the mussel tissue homogenate were aliquoted into clean, pre-cooled glass bottles. All of the subsampling manipulations were performed using Teflon implements to avoid contamination and in the liquid nitrogen freezer to avoid warming of the samples or moisture condensation on the frozen material. The bottles of SRM 1974 have been stored since preparation at -80°C.

3.3. Moisture determination

The amount of moisture in the frozen mussel homogenate was determined by measuring the weight loss after freeze drying. Twenty bottles of SRM 1974 were selected according to a stratified randomization scheme for the drying study. The entire contents of each bottle were transferred to a Teflon jar and dried for 5 days at 1 Pa with a -10°C shelf temperature and a –50°C condenser temperature. Based on these studies, a 95% prediction interval for the moisture content of a previously unopened bottle of SRM 1974 is 87.7 ± 0.2%. Analytical results for organic and inorganic constituents are reported on a dry weight basis for the convenience of the users. The results for the organic constituents were determined on a wet weight basis; they were converted to a dry weight basis by dividing by the conversion factor of 0.1235.

Table IV.44. Summary of analytical results of the determination of PAHs in SRM 1941, Organics in Marine Sediment (ng/g dry weight).

Compound	GC-FID	GC-MS	LC-FL (Direct)	LC-FL (Fraction)	Certified Value
Phenanthrene	597 (4) Δ	603(10)	531(12)		577 ± 59
Anthracene	202 (6)	228(12)	174 (8)		202 ± 42
Fluoranthene	1116(20)	1401(41)	1135(10)		1220 ±240
Pyrene	1008(16)	1238(18)	989(34)		1080 ± 00
Benz[*a*]anthracene	538(12)	599(14)	516 (7)	521 (11) ❖	550 ± 78
Chrysene	577(12) ▼	702(16) ▼	425(42)	473 (5) ❖	
Triphenylene				192 (3) ❖	
Benzo[*b*]fluoranthene	635(17)	864(28)	839(14)	843	780 ±190
Benzo[*j*]fluoranthene	351(14)				
Benzo[*k*]fluoranthene	439(19)	857(25) ◊	456 (6) ◆ 441 (8) ◆	443(16)	444 ± 49
Benzo[*e*]pyrene	472(25)	672(24)			
Benzo[*a*]pyrene	566(12)	754(49)	674(12)	690(25)	670 ±130
Perylene	415 (8)	437(27)	411 (6)	426 (5)	442 ± 33
Benzo[*ghi*]perylene	478(14)	566(26)		504 (7)	516 ± 83
Indeno[1,2,3-*cd*]pyrene	572(28)	559(19)	573(20)	575 (8)	569 ± 40

The certified values are weighted means of results from two or more analytical techniques. Each uncertainty is obtained from a 95% prediction interval plus an allowance for systematic error among the methods used. The allowance for systematic error is equal to the greatest difference between the weighted mean (certified value) and the component means for the analytical methods used. In the absence of systematic error, the resulting uncertainty limits will cover the concentration of approximately 95% of samples of this SRM having a minimum sample size of approximately 5 g.

Δ Uncertainties (values in parentheses) for GC-FID, LC-FL, and GC-MS measurements are one standard deviation of a single measurement; for GC-FID measurements, 12 samples analyzed in triplicate; for LC measurements, three samples analyzed in triplicate; for GC-MS measurements, four samples analyzed in duplicate.

▼ Value is for chrysene and triphenylene.

❖ Determined using triphenylene-d_{12} as internal standard.

◆ Benzo[*k*]fluoranthene was determined at different times, i.e., during initial analyses of total PAH fraction and during benzo[*b*]fluoranthene analyses.

◊ Value is for benzo[*k*]fluoranthene and benzo[*j*]fluoranthene.

Table IV.45. Noncertified concentrations of additional PAHs in SRM 1941, Organics in Marine Sediment (ng/g dry weight).

Compound Δ	Concentration*	
Naphthalene	1322	(14)
2-Methylnaphthalene	406	(36)
1-Methylnaphthalene	229	(19)
Biphenyl	115	(15)
2,6-Dimethylnaphthalene	198	(23)
Acenaphthylene	115	(10)
Acenaphthene	52	(2)
Fluorene	104	(5)
3-Methylphenanthrene	50	(5)
2-Methylphenanthrene	190	(6)
2-Methylanthracene	66	(7)
9-Methyl and 4-Methylphenanthrene ◆	145	(8)
1-Methylphenanthrene	109	(6)
2,6-Dimethylphenanthrene	68	(4)
2,7-Dimethylphenanthrene	52	(4)
1,3-, 2,10-, 3,9-, and 3,10- Dimethylphenanthrene ◆	161	(11)
1,6- and 2,9-Dimethylphenanthrene ◆	93	(6)
1,7-Dimethylphenanthrene	62	(4)
2,3-Dimethylphenanthrene	36	(3)
Benzo[*a*]fluoranthene	146	(4)
Triphenylene ▼	192	(3)
Chrysene ▼	449	
Benzo[*j*]fluoranthene ❖	351	(14)
Benzo[*e*]pyrene ❖	573	

Δ Naphthalene through fluorene determined using GC/MS Method A; remaining PAHs determined using GC/MS Method B.

* Concentrations reported on dry weight basis; material as received contains residual moisture; four sediment extracts were analyzed in duplicate; uncertainties (values in parenthesis) are one standard deviation of a single measurement.

◆ Represents co-elution of two or more compounds.

▼Triphenylene and chrysene were determined by LC-fluorescence; value for chrysene is the mean value of results obtained by the two LC-fluorescence procedures.

❖ Benzo[*j*]fluoranthene and benzo[*e*]pyrene were determined by GC-FID; value for benzo[*e*]pyrene is the mean value of the results obtained by GC-FID and GC-MS.

Table IV.46. Noncertified concentrations of selected PCB congeners and chlorinated pesticides in SRM 1941 as determined by GC-ECD (ng/g dry weight).

		Concentration*	
Polychlorinated biphenyls[Δ]			
PCB 18	2,2',5-Trichlorobiphenyl	9.90	(0.25)
15	4,4'-Dichlorobiphenyl		
PCB 28	2,4,4'-Trichlorobiphenyl	16.1	(0.4)
PCB 52	2,2',5,5'-Tetrachlorobiphenyl	10.4	(0.4)
PCB 66	2,3',4,4'-Tetrachlorobiphenyl	22.4	(0.7)
95	2,2',3,5',6-Pentachlorobiphenyl		
PCB 101	2,2',4,5,5'-Pentachlorobiphenyl	22.0	(0.7)
90	2,2',3,4',5-Pentachlorobiphenyl		
PCB 105	2,3,3',4,4'-Pentachlorobiphenyl	5.76	(0.23)
PCB 118	2,3',4,4',5-Pentachlorobiphenyl	15.2	(0.7)
PCB 138	2,2',3,4,4',5'-Hexachlorobiphenyl	24.9	(1.8)
163	2,3,3',4',5,6-Hexachlorobiphenyl		
164	2,3,3',4',5',6-Hexachlorobiphenyl		
PCB 153	2,2',4,4',5,5'-Hexachlorobiphenyl	22.0	(1.4)
PCB 170	2,2',3,3',4,4',5-Heptachlorobiphenyl	7.29	(0.26)
190	2,3,3',4,4',5,6-Heptachlorobiphenyl		
PCB 180	2,2',3,4,4',5,5'-Heptachlorobiphenyl	14.3	(0.3)
PCB 187	2,2',3,4',5,5'6-Heptachlorobiphenyl	12.5	(0.6)
159	2,3,3',4,5,5'-Hexachlorobiphenyl		
182	2,2'3',4,4',5,6'-Heptachlorobiphenyl		
PCB 195	2,2',3,3',4,4'5,6'-Octachlorobiphenyl	1.51	(0.10)
208	2,2'3,3',4,5,5',6,6'-Nonachlorobiphenyl		
PCB 206	2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	4.81	(0.15)
PCB 209	Decachlorobiphenyl	8.35	(0.21)
Chlorinated pesticides			
Heptachlor epoxide		0.23	(0.02)
cis-Chlordane		2.06	(0.05)
trans-Nonachlor		0.97	(0.03)
Dieldrin		0.63	(0.03)
4,4'-DDE		9.71	(0.17)
4,4'-DDD		10.3	(0.1)
4,4'-DDT		1.11	(0.05)

[Δ] PCBs are numbered according to Ballschmiter and Zell (1980); PCB congener listed first is the major component; additional PCB congeners listed may be present as minor components.

* Concentrations reported on dry weight basis; material as received contains approximately 4% moisture. Four extracts were analyzed in triplicate; uncertainties are one standard deviation of a single measurement.

3.4. Polycyclic aromatic hydrocarbons

This SRM was analyzed for selected PAHs using GC-MS and LC-FL. For the GC-MS analyses, 13-26 g (wet weight portions) of the mussel homogenate from 12 randomly selected bottles were each mixed with approximately 100 g of pre-extracted anhydrous sodium sulfate. These mixtures were then placed in glass extraction thimbles, spiked with an internal standard solution, and Soxhlet extracted for 18 hr with 250 mL of dichloromethane. Gel-permeation chromatography (GPC) on a preparative divinylbenzene-polystyrene column (10 µm particle size, 100 Å pore size, 2.5 cm i.d. x 60 cm) was used to remove the majority of the lipid and biogenic materials from the concentrated extract. The GPC separation was performed using dichloromethane at a flow rate of 9.9 mL/min. The majority of the lipid and biogenic material which elutes immediately after the void volume of the column was discarded, and the fraction containing the PAHs (as well as the PCBs and pesticides) which are retained longer was then collected. The collected eluant was concentrated to ~0.3 mL and then placed on a silica SPE cartridge and eluted with 12 mL of 10% (v/v) dichloromethane in *n*-pentane as the final cleanup step prior to GC-MS analysis.

GC-MS analyses were performed using a 0.25 mm x 60 m fused silica capillary column with a 5% phenyl-substituted methylpolysiloxane phase (0.25 µm film thickness). The column temperature program is listed in Table IV.47. Selected ions were monitored during the run for the analytes of interest and the internal standards. For these GC-MS measurements, the following perdeuterated PAHs were utilized as the internal standards: naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, pyrene-d_{10}, benz[*a*]anthracene-d_{12}, benzo[*e*]pyrene-d_{12}, and benzo[*ghi*]perylene-d_{14}. Calibration response factors for the analytes relative to the internal standards were determined by analyzing SRM 1491, Aromatic Hydrocarbons in Hexane/Toluene.

For the LC-FL analyses, 14-18 g (wet weight portions) of mussel homogenate from six randomly selected bottles were mixed with approximately 100 g of pre-extracted sodium sulfate; these mixtures were then placed in glass extraction thimbles, spiked with an internal standard solution, and Soxhlet extracted for 18 hr using 250 mL of hexane:acetone (1:1 v/v). The extracts were concentrated to approximately 1 mL. About 0.5 mL of dichloromethane was added to the extract to redissolve some of the hexane insoluble material and the extract was then passed through a pre-cleaned aminosilane solid phase extraction (SPE) cartridge and eluted with 15 mL of 10% (v/v) dichloromethane in *n*-hexane. The eluant from the SPE cartridge was concentrated, and the SPE procedure was repeated a second and third time. After the third SPE cleanup, the eluant was concentrated and injected onto a semipreparative aminopropylsilane column (9 mm i.d. x 30 cm) to isolate the total PAH fraction by normal-phase LC using 2% (v/v) dichloromethane in *n*-hexane at 5 mL/min (May and Wise, 1984). The isolated PAH fraction was then concentrated, and the solvent changed to acetonitrile for the reversed-phase LC analysis. Reversed-phase LC analysis of the PAH fraction was performed on a polymeric octadecylsilane (C18) column (4.6 mm i.d. x 25 cm, 5-µm particle size) using gradient elution from 50% (v/v) acetonitrile in water to 100% acetonitrile at 1%/min with a flow rate of 1.5 mL/min. Fluorescence detection with wavelength programming, as described in section 2.4 above for SRM 1941, was used to monitor the LC separation. For LC-FL measurements, perdeuterated PAHs (phenanthrene-d_{10}, fluoranthene-d_{10}, and perylene-d_{12}) were utilized as the internal standards. Calibration response factors for the analytes relative to the internal standards were determined by analyzing SRM 1647a, Priority Pollutant PAH (in Acetonitrile).

Table IV.47. GC temperature programs used during analyses of SRM 1974.

Temperature program for PAHs GC/MS analysis using a fused silica capillary column with a 5% phenyl-substituted methyl= polysiloxane phase:

Initial temp.:	37°C
First rate:	30°C/min to 150°C
Second rate:	2°C/min to 300°C
Isothermal pause:	32 min at 300°C

Temperature program for PCBs and pesticides GC/ECD analysis using a fused silica capillary column with a 5% phenyl-substituted methylpolysiloxane phase:

Initial temp.:	30 min at 200°C
Rate:	2°C/min to 270°C
Isothermal pause:	10 min at 270°C

Temperature program for PCBs and pesticides GC/ECD analysis using a CP SIL 8 (SE-54) plus 10% methyl-C18 incorporated:

Initial temp.:	3 min at 60°C
First rate:	20°C/min to 170°C
Second rate:	1.5°C/min to 270°C
Isothermal pause:	5 min at 270°C

Temperature program for PCBs and lower polarity pesticides GC/MS analysis using a 5% phenyl-substituted methylpolysiloxane phase:

Initial temp.:	2 min at 50°C
First rate:	40°C/min to 170°C
Second rate:	1.5°C/min to 290°C
Isothermal pause:	5 min at 290°C

Temperature program for higher polarity pesticides GC/MS analysis using a 5% phenyl-substituted methylpolysiloxane phase:

Initial temp.:	1 min at 68°C
First rate:	40°C/min to 200°C
Isothermal phase:	30 min at 200°C
Second rate:	2°C/min to 270°C
Isothermal pause:	10 min at 270°C

3.5. Polychlorinated biphenyl congeners and chlorinated pesticides

Subsamples from five bottles of SRM 1974 were extracted, and the extract processed through the GPC as described above for the GC-MS analysis. Following the GPC, normal-phase LC on the semipreparative aminopropylsilane column was used to isolate two fractions containing: (1) the PCBs and lower polarity chlorinated pesticides, and (2) the more polar chlorinated pesticides. For the normal-phase LC fractionation, n-hexane was used as the mobile phase for the isolation of the PCBs and lower polarity pesticides, and 5% (v/v) dichloromethane in *n*-hexane was used for the isolation of the second fraction. GC-ECD and GC-MS analyses were performed on a column similar to the one used for the GC-MS determination of the PAHs. The column temperature program is listed in Table IV.47. PCB 103, PCB 198, and 4,4'-DDT-d_8 were used as internal standards. GC-ECD analyses of the two chlorinated compound fractions were also performed using a second column, a 0.25 mm x 50 m fused silica capillary column coated with a 0.2 µm thick film of CP SIL 8 (SE-54) plus 10% methyl-C18 incorporated. The column temperature program is listed in Table IV.47. For measurement of the PCBs, selected ions were monitored for each of the 10 degrees of chlorination (two ions per degree of chlorination, the molecular ion and the M+2 ion). For the chlorinated pesticides, the molecular ion was monitored.

Table IV.48. Summary of analytical results and certified concentrations for PAHs in SRM 1974, Organics in Mussel Tissue (*Mytilus edulis*).

Compound	Concentrations (ng/g dry weight)				Certified Concentrations	
	LC/Fluorescence		GC-MS		(ng/g dry weight)	(ng/g wet weight)
Phenanthrene	44.6	(2.7) Δ	45.3	(7.3)	45 ± 11	5.6 ± 1.4
Anthracene	5.97	(0.52)	6.14	(0.72)	6.1 ± 1.7	0.75 ± 0.21
Fluoranthene	289	(10)	255	(21)	272 ± 47	33.6 ± 5.8
Pyrene	294	(10)	259	(12)	276 ± 30	34.1 ± 3.7
Perylene	8.56	(0.35)	8.5	(1.7)	8.5 ± 2.4	1.05 ± 0.29
Benzo[*b*]fluoranthene	55.9	(2.2)	48.7	(5.2)	52.3 ± 9.4	6.5 ± 1.2
Benzo[*a*]pyrene	20.1	(2.3)	17.1	(2.2)	18.6 ± 3.8	2.29 ± 0.47
Benzo[*ghi*]perylene	19.6	(1.4)	20.3	(2.3)	20.0 ± 2.3	2.47 ± 0.28
Indeno[1,2,3-*cd*]pyrene	15.6	(1.4)	13.6	(1.4)	14.6 ± 2.7	1.80 ± 0.33

Certified values were determined on a wet weight basis; concentrations were converted to a dry weight basis for user convenience. The certified values are equally weighted means of results from two analytical techniques. The uncertainty is obtained from a 95% prediction interval plus an allowance for systematic error between the methods used. In the absence of systematic error, the resulting uncertainty limits will cover the concentration of approximately 95% of samples of this SRM having a minimum sample size of 15 g (wet weight).

Δ Uncertainties are one standard deviation of a single measurement treating all measurements as statistically independent and identically distributed.

For the GC-ECD and GC-MS analyses, calibration response factors for the analytes relative to the internal standards were determined by processing diluted solutions of SRM 2261 and 2262 and the internal standards. Two subsamples of SRM 1588, Organics in Cod Liver Oil, were processed as extracts and analyzed with the mussel samples as control materials.

3.6. Certified and noncertified concentrations

SRM 1974 has been certified for concentrations of nine PAHs (Table IV.48). Noncertified concentrations are available for 19 additional PAHs (Table IV.49), and 13 polychlorinated biphenyl congeners and nine chlorinated pesticides (Table IV.50). Representative GC-ECD chromatograms using the DB-5 column (see discussion above) from the PCB and lower polarity pesticide fraction and from the more polar pesticide fraction are shown in Figures IV.20 and IV.21, respectively. In addition to the organic contaminants, noncertified concentrations for 36 trace elements were determined primarily by instrumental neutron activation analysis (Wise *et al.*, 1991.)

4. CONCLUSIONS

SRM 1941 is a dry sediment which has concentrations of the organic contaminants typical of an urban harbor environment. SRM 1974 is the first frozen mussel tissue SRM for environmental measurements of organic constituents. It has concentrations of the organic contaminants which are typical of mussels harvested in an urban area.

Table IV.49 Noncertified concentrations of additional PAHs in SRM 1974, Organics in Mussel Tissue (*Mytilus edulis*) (ng/g dry weight).

Compound	Concentration*	
2-Methylnaphthalene ❖ Δ	17	(4)
1-Methylnaphthalene ❖ Δ	9	(2)
Fluorene ❖ Δ	12	(2)
9-Methyl- and 4-methylphenanthrene ❖ Δ ◆	22	(5)
1-Methylphenanthrene ❖ Δ	19	(5)
2- and 9-Ethylphenanthrenes and 3,6-Dimethylphenanthrene ❖ ◆	34	(8)
2,6-Dimethylphenanthrene ❖	37	(7)
2,7-Dimethylphenanthrene ❖	35	(9)
1,3-, 2,10-, 3,9-, and 3,10-Dimethyl-phenanthrenes ❖ Δ	91	(17)
1,6- and 2,9-Dimethylphenanthrenes ❖ ◆	47	(11)
1,7-Dimethylphenanthrene ❖	42	(9)
Benz[*a*]anthracene ❖	37	(3)
Chrysene/Triphenylene ❖ ◆	124	(11)
Benzo[*a*]fluoranthene ❖	4.1	(1.2)
Benzo[*j*]fluoranthene/benzo[*k*]fluoranthene ❖ ◆	35	(6)
Benzo[*k*]fluoranthene ▼	24	(1)
Benzo[*e*]pyrene ❖	81	(6)
Indeno[1,2,3-*cd*]fluoranthene ❖	3.9	(0.6)
Dibenz[*a,h*]anthracene ▼	2.8	(0.1)

* Uncertainties (values in parenthesis) are one standard deviation of a single measurement treating all measurements as statistically independent and identically distributed. Results reported in dry weight may be converted to wet weight by multiplying by 0.1235.

❖ Concentration was determined by GC-MS.

Δ Three aliquots were analyzed for these compounds; nine to twelve aliquots were analyzed for all other compounds determined by GC-MS; six aliquots were analyzed for compounds determined by LC-FL.

◆Represents the coelution of two or more compounds.

▼Concentration was determined by LC-FL.

Table IV.50. Noncertified concentrations of selected PCB congeners and chlorinated pesticides in SRM 1974, as determined by GC-ECD [PCBs numbered according to Ballschmiter and Zell (1980). PCB congener listed first is the major component; additional PCB congeners listed may be present as minor components.] (ng/g dry weight).

Polychlorinated biphenyls			Concentration* GC-ECD (DB-5) ‡	GC-ECD (C-18) ‡	GC-MS (DB-5) ‡	Noncertified Values ▲
PCB	18	2,2',5-Trichlorobiphenyl	22 (5)	28 (4)	23 (1)	24 ± 9
PCB	28	2,4,4'-Trichlorobiphenyl	61 (5)	62 (5)	159(14)⁘	62 ± 3
PCB	44	2,2'3,5'-Tetrachlorobiphenyl	62 (5)	58 (5)	76 (3)	65 ± 23
PCB	52	2,2',5,5'-Tetrachlorobiphenyl	85 (7)	95 (8)	115 (7)	98 ± 39
PCB	66	2,3',4,4'-Tetrachlorobiphenyl	133 (9) ▼	107 (8)	113 (4)	110 ± 5 ▼
PCB	101	2,2',4,5,5'-Pentachlorobiphenyl	132 (9)	105 (9) ◆	126 (6)	105 ± 11 ◆
	90	2,2',3,4',5-Pentachlorobiphenyl				
PCB	105	2,3,3',4,4'-Pentachlorobiphenyl	46 (3)	45 (3)	44 (3)	45 ± 3
PCB	118	2,3',4,4',5-Pentachlorobiphenyl	110(10)	107 (9)	112 (6)	110 ± 5
PCB	128	2,2',3,3',4,4'-Hexachlorobiphenyl	15 (2)	12 (2)	18 (5)	15 ± 2
PCB	138	2,2',3,4,4',5'-Hexachlorobiphenyl	124(10)	118 (9)	110 (9) ◊	110 ± 11 ◊
	163	2,3,3',4',5,6-Hexachlorobiphenyl				
	164	2,3,3',4',5',6-Hexachlorobiphenyl				
PCB	153	2,2',4,4',5,5'-Hexachlorobiphenyl	147(12)	154(16)	135(11)	145 ± 8
PCB	180	2,2',3,4,4',5,5'-Heptachlorobiphenyl	13 (2)	14 (2)	13 (3)	13 ± 1
PCB	187	2,2',3,4',5,5'6-Heptachlorobiphenyl	30 (2)	29 (3)	31 (2)	30 ± 1
	182	2,2',3',4,4',5,6'-Heptachlorobiphenyl				
Chlorinated pesticides						
2,4'-DDD			23 (3)	17 (2)	20 (2)	20 ± 7
2,4'-DDE			5.6(0.2)	5.8(0.2)	6.0(0.4)	5.8 ± 0.6
2,4'-DDT			3.1(0.4)	4.1(0.3)	3.4(0.4)	4 ± 1
4,4'-DDD			70 (6)	69 (6)	64 (6)	68 ± 3
4,4'-DDE			49 (4)	48 (2)	47 (3)	48 ± 2
4,4'-DDT			2.0(0.2)	3.7(0.2)	2.3(0.3)	3 ± 2
cis-Chlordane			26 (3)	25 (2)	26 (2)	26 ± 1
Dieldrin			10 (2)	7.7(0.8)	7.1(0.9)	8 ± 4
trans-Nonachlor			22 (1)	19 (1)	21 (2)	21 ± 5

* Results reported in dry weight may be converted to wet weight by multiplying by 0.1235.

‡ Uncertainties (values in parenthesis) are one standard deviation of a single measurement treating all measurements as statistically independent and identically distributed. Samples from five bottles were extracted; each extract was analyzed in triplicate for the GC-ECD analyses on both columns and analyzed once for the GC-MS analyses. Results reported in dry weight may be converted to wet weight by multiplying by 0.1235.

▲ Noncertified concentrations are means of the values from the three methods with uncertainties expressed as 95% confidence intervals.

⁘ PCB 31 coeluted with PCB 28 in the GC-MS analyses. These results were not included in the noncertified value.

▼PCB 95 coeluted with PCB 66 in the GC-ECD analyses using the DB-5 column. The GC-ECD (DB-5) results were not included in the noncertified value for PCB 66.

◆ PCB 90 was separated from PCB 101 using the C-18 column; only the results from the C-18 column were used for the noncertified value for PCB 101.

◊ PCB 163 was separated from PCB 138 in the GC-MS results; only these results were used for the noncertified value.

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Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are the best available for the purpose.

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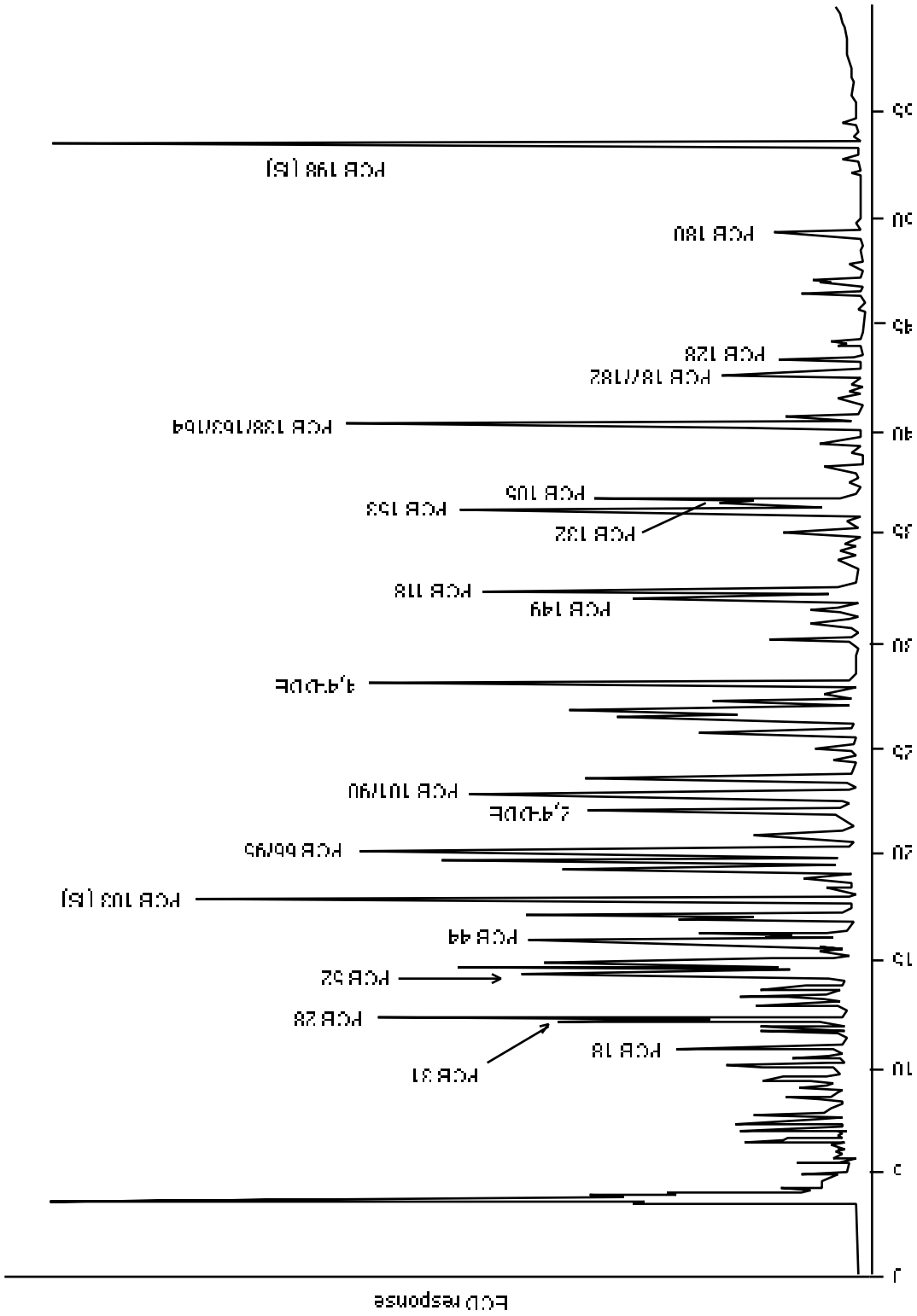

Figure IV.20. GC-ECD analysis using DB-5 column of PCBs and lower polarity pestiide fraction isolated from SRM 1974 (IS - internal standard)

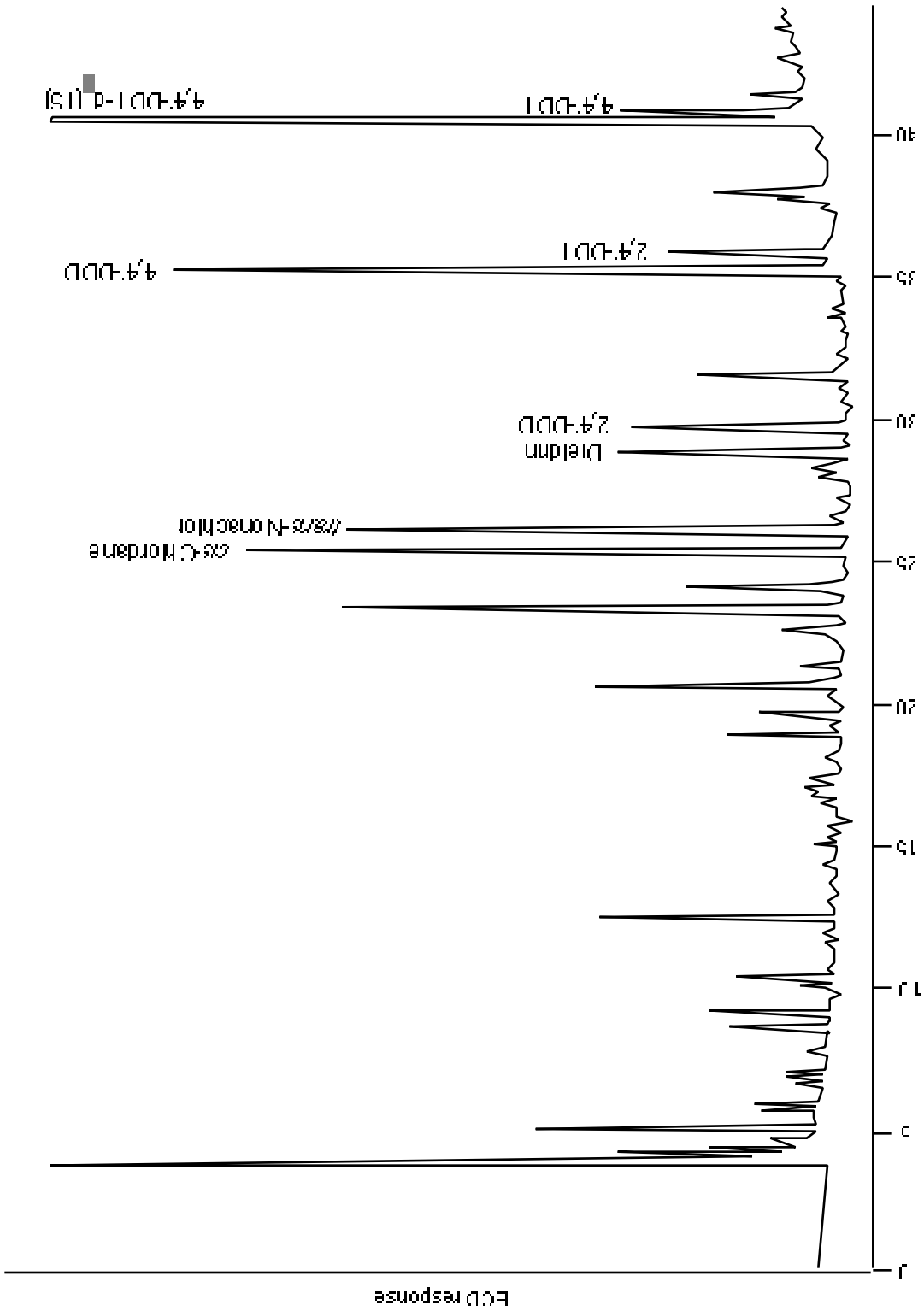

Figure IV.21. GC-ECD analysis using DB-5 column of higher polarity pesticide fraction isolated from SRM 1974 (IS - internal standard).