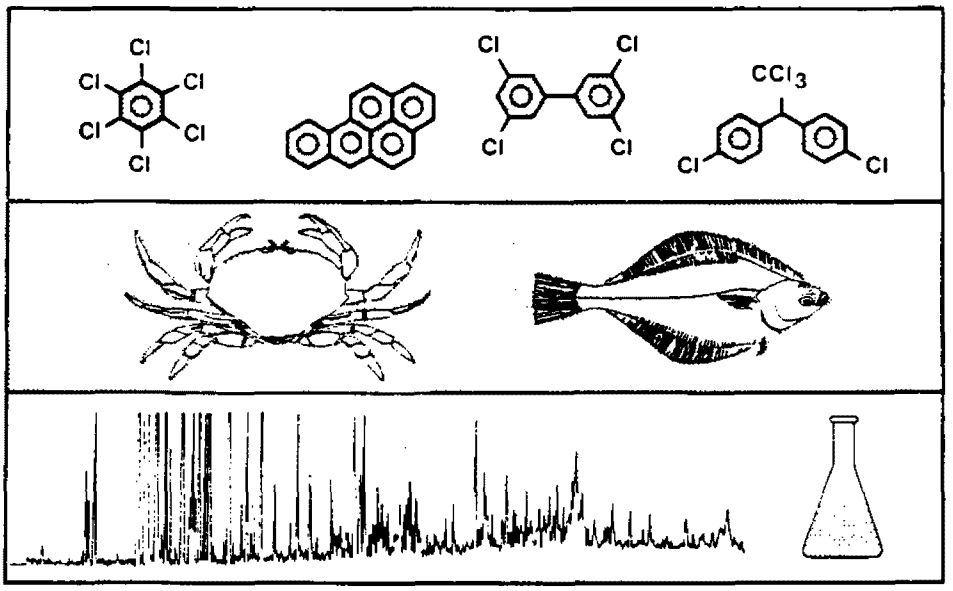
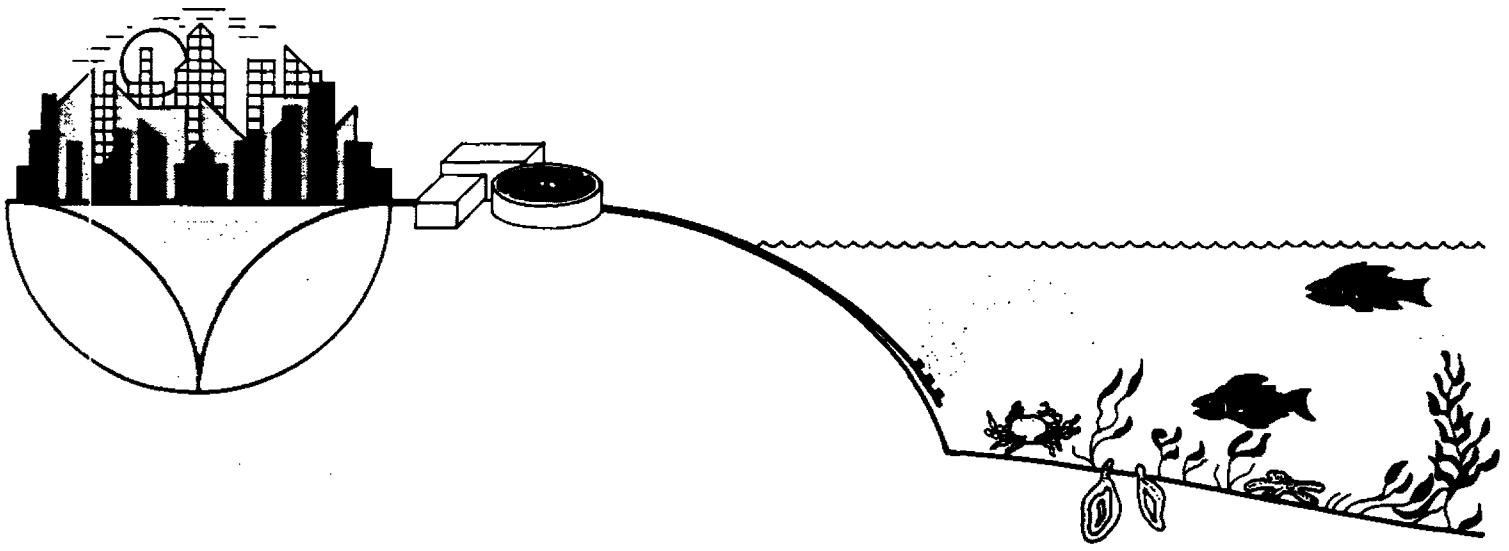
United States Environmental Protection Agency | Office of Water Washington, D.C. 20460 | EPA 503/6-90-002 May 1986

EPA

BIOACCUMULATION MONITORING GUIDANCE:

4. ANALYTICAL METHODS FOR U.S. EPA PRIORITY POLLUTANTS AND 301(h) PESTICIDES IN TISSUES FROM ESTUARINE AND MARINE ORGANISMS





EPA 503/6-90-002
May 1986

BIOACCUMULATION MONITORING GUIDANCE:

ANALYTICAL METHODS FOR U.S. EPA PRIORITY POLLUTANTS AND 301(h) PESTICIDES IN TISSUE FROM ESTUARINE AND MARINE ORGANISMS

Prepared by:
Tetra Tech, Inc.
11820 Northup Way, Suite 100
Bellevue, Washington 98005

Prepared for:
Marine Operations Division: 301(h) Program
Office of Marine and Estuarine Protection
U.S. Environmental Protection Agency
401 M Street SW
Washington, D.C. 20460

PREFACE

This report is one element of the Bioaccumulation Monitoring Guidance Series. The purpose of this series is to provide guidance for monitoring of priority pollutant residues in tissues of resident marine organisms. These guidance documents were prepared for the sewage discharge program of Section 301(h) of the Clean Water Act under the U.S. EPA Office of Marine and Estuarine Protection, Marine Operations Division. Two kinds of monitoring guidance are provided in this series; recommendations for sampling and analysis designs, and aids for interpretation of monitoring data.

Some basic assumptions were made in developing the guidance presented in these documents: 1) each bioaccumulation monitoring program will be designed to meet the requirements of the 301(h) regulations, 2) tissue samples will be collected from appropriate locations near the sewage discharge and from an unpolluted reference site, 3) the initial chemicals of concern are the U.S. EPA priority pollutants and 301(h) pesticides, and 4) the monitoring data should be suitable for a meaningful evaluation of the potential hazards to living marine resources as well as human health. It should be recognized that the design of a monitoring program reflects the site-specific characteristics of the pollutant discharge and the receiving environment. Thus, site-specific considerations may lead to a modification of the generic recommendations herein. Finally, although these guidance documents were prepared specifically for monitoring of sewage discharges under the 301(h) program, their potential use extends to assessment and monitoring of bioaccumulation resulting from other kinds of pollutant discharges into marine and estuarine environments.

CONTENTS

FIGURES

TABLES

ACKNOWLEDGEMENTS

This document has been reviewed by the 301(h) Task Force of the Environmental Protection Agency, which includes representatives from the Water Management Divisions of U.S. EPA Regions I, II, III, IV, IX, and X; the Office of Research and Development - Environmental Research Laboratory - Narragansett (located in Narragansett, RI and Newport, OR), and the Marine Operations Division in the Office of Marine and Estuarine Protection, Office of Water.

This technical guidance document was produced for the U.S. Environmental Protection Agency under the 301(h) post-decision technical support contract No. 68-01-6938, Allison J. Duryee, Project Officer. This report was prepared by Tetra Tech, Inc., under the direction of Dr. Thomas C. Ginn.

SECTION I

The primary authors were Mr. Robert C. Barrick and Mr. Harry R. Beller. The assistance of Mr. Raleigh C. Farlow is appreciated.

Existing U.S. EPA analytical methods were incorporated into Section I whenever possible. Specifically, many sections were adapted from the Contract Laboratory Program for Organics Analysis (Section I, reference 2) and U.S. EPA Method 1625 Revision B (Section I, reference 3), which was developed by the Industrial Technology Division of the Office of Water Regulation and Standards.

Validation data presented in Section I (Precision and Accuracy) were generated by California Analytical Laboratories and Weyerhaeuser Technology Center.

SECTION II

The primary authors were Mr. Robert C. Barrick and Mr. Harry R. Beller. The assistance of Mr. Raleigh C. Farlow is appreciated.

The procedure described in Section II is largely a compilation of methods developed by U.S. EPA. Specifically, the methods were developed by the Environmental Monitoring Systems Laboratory (EMSL) in Las Vegas (Section II, references 1 and 2) and the Industrial Technology Division of the Office of Water Regulation and Standards (Section II, reference 3). Dr. M. Hiatt (Analytical Technologies, Inc., National City, CA, previously at EMSL Las Vegas) was a valuable source of technical information presented in this document.

SECTION III

The primary authors were Mr. Robert C. Barrick, Mr. Harry R. Beller, and Mr. Robert W. Deverall. The assistance of Dr. Charles R. Lytle is appreciated.

Validation data presented in Section III (Precision and Accuracy) were generated by Analytical Service Laboratories, Ltd.

Mention of trade names or commercial products herein does not constitute endorsement for use by U.S. EPA or Tetra Tech, Inc.

INTRODUCTION

The three analytical methods in this document have been designed to be consistent with probable uses of 301(h) bioaccumulation monitoring data. Comparison of tissue contaminant concentrations from contaminated and relatively uncontaminated areas and estimation of the potential health effects of bioaccumulated substances often require sensitive analytical techniques for a wide range of chemically diverse pollutants. The recommended 301(h) procedures allow for sensitive analyses of the target compounds with a reasonable amount of laboratory effort. Organophosphate 301(h) pesticides have not yet been tested with the recommended techniques (i.e., Malathion, Parathion, Demeton, Guthion). Analyses for 2,3,7,8-TCDD with appropriate detection limits will require the dedicated U.S. EPA Contract Laboratory Program procedure for dioxin analysis (9/15/83), which involves selected ion monitoring (SIM) GC/MS analysis.

There are currently no formally approved U.S. EPA procedures for analyzing priority pollutants and 301(h) pesticides in biological tissue. However, various U.S. EPA procedures were reviewed during development of this report [e.g., Interim Methods for the Sampling and Analysis of Priority Pollutants in Sediments and Fish Tissue (1977, revised 1980); Contract Laboratory Program procedures for organics analysis and inorganics analysis]. Consequently, the recommended 301(h) procedures include portions of U.S. EPA analytical and quality assurance procedures that were considered appropriate for sensitive, full-scan analyses (e.g., at the low parts per billion level for organics analysis). The 301(h) methods have been assembled according to guidelines for EMSL (Environmental Monitoring and Support Laboratory, Cincinnati) analytical methods (as specified in EPA-600/8-83-020).

SECTION I

ANALYSIS OF EXTRACTABLE ORGANIC COMPOUNDS IN ESTUARINE AND MARINE TISSUES

CONTENTS

ANALYSIS OF EXTRACTABLE ORGANIC COMPOUNDS IN ESTUARINE AND MARINE TISSUES

1.0 SCOPE AND APPLICATION

1.1 This method is designed to determine the semivolatile priority pollutants (Table I-1) associated with the Clean Water Act Section 301(h) regulation [40 CFR 125.58(k) and (v)]. Additional compounds amenable to extraction and analysis by capillary column gas chromatography-mass spectrometry (GC/MS) and/or gas chromatography-electron capture detection (GC/ECD) may be suitable for analysis, subject to testing.

These procedures are applicable when low part per billion analyses are required to monitor differences between body burdens in organisms from relatively uncontaminated reference areas and contaminated estuarine and marine environments. In addition, the procedures are applicable when low detection limits are required for the estimation of potential health effects of bioaccumulated substances.

Two GC/MS options included in the methods are analyses by isotope dilution GC/MS (preferred) or by a GC/MS internal standard technique (minimum required). In both cases, the laboratory procedures for sample extraction and concentration of the resulting extract are identical. Compound-specific recovery corrections used in the isotope dilution technique are designed to increase the accuracy of the analysis and the comparability of results among laboratories. In addition, use of the multiple recovery standards in each analysis increases confidence in the validity of detection limits reported for undetected target compounds. By forcing a search for every recovery standard in the sample extract (over 50 are available), the technique also increases the efficiency of detection and reporting frequency of target compounds that otherwise may be overlooked in complex extracts.

1.2 The compounds listed in Table I-1 include pesticides subject to regulation under Section 301(h) of the Clean Water Act. However, the applicability of this method to non-chlorinated organopnosphorous pesticides (Malathion, Parathion, Demeton, and Guthion) has not been demonstrated. Chemists at the Food and Drug Administration have recently published a technique for determining organophosphate pesticides of wide ranging polarity in matrices including fatty animal tissue (J.J. Blaha and P.J. Jackson, J. Assoc. Anal. Chem., Vol. 68, pp. 1095-1099, 1985). The technique involves liquid-liquid partitioning and gel permeation chromatography [both are included in this recommended 301(h) procedure] as well as N/P alkali thermionic or flame photometric detection. Further work is required to determine the suitability of the recommended 301(h) procedure for organophosphorous pesticides.

1.3 The detection limit of this method is usually dependent on the level of interferences rather than instrumental limitations. The limits listed in Table I-1 represent the minimum quantity that can be detected with no interferences present.

Lower limits of detection (LLD) are established by analysts based on their experience with the instrumentation and with interferences in the sample matrix being analyzed. LLD are greater than the instrumental detection limits in Table I-1 because they take into account sample interferences. To estimate LLD, the noise level should be determined in the retention window for the quantitation mass of representative analytes. These determinations should be made for at least three field samples in the sample set under analysis. The signal required to exceed the average noise level by at least a factor of two should then be estimated. This signal is the minimum response required to identify a potential signal for quantification. The LLD is the concentration corresponding to the level of this signal based on calibrated response factors. Based on best professional judgment, this LLD would then be applied to samples in the set with comparable or lower interference. Samples with much higher interferences (e.g., at least a factor of two higher) should be assigned LLD at a multiple of the original LLD.

These LLD values may be less than the rigorously defined method detection limits specified in the revised "Guidelines Establishing Test Procedures for the Analysis of Pollutants" (40 CFR Part 136, 10/26/84). This latter procedure requires the analysis of seven replicate samples and a statistical determination of the method detection limit with 99 percent confidence. Data quantified between the LLD and the rigorous method detection limit are valid and useful in environmental investigations of low-level contamination, but have a lower statistical confidence associated with them than data quantified above the method detection limit.

LLD for the described method on a wet weight basis are 10 ug/kg for aromatic hydrocarbons and phthalates (GC/MS analysis) and 10-20 ug/kg for chlorinated hydrocarbons and halogenated ethers (GC/MS analysis). Detection limits for GC/MS analyses of pesticides are 50 ug/kg (wet weight); the GC/ECD detection limits for pesticides are 0.1-5 ug/kg. A method detection limit of 20 ug/kg is attainable for GC/ECD analysis of total PCBs.

1.4 The GC/MS portions of this method are for use only by analysts experienced with GC/MS or under the close supervision of such qualified persons. Laboratories unfamiliar with the analyses of environmental samples by GC/MS should run the performance tests in reference 1 before beginning.

1.5 This procedure has been designed to analyze for a large number of organic compounds with wide-ranging chemical properties (e.g., polarity, molecular weight) while minimizing procedural complexity. The accuracy and sensitivity that could be attained by conducting a dedicated analysis for only one of the compounds in Table I-1 cannot be attained in such a comprehensive analysis.

1.6 Several analytes are particularly susceptible to decomposition during analysis. Benzidine can be subject to oxidative losses during solvent extraction. Hexachlorocyclopentadiene is subject to thermal decomposition in the inlet of the gas chromatograph, chemical reaction in acetone solution, and photochemical decomposition. The polycyclic aromatic hydrocarbons are also photosensitive, especially benzo(a)pyrene.

2.0 SUMMARY OF METHOD

2.1 A macerated homogenized 20 to 25 g tissue sample is Soxhlet extracted and the extract is dried over sodium sulfate. Biological macromolecules are removed from the extract by gel permeation chromatography (GPC) with Bio Beads S-X3 (or equivalent) (reference 2). A portion of the extract (20 percent) is subjected to alumina chromatography to separate polar compounds from pesticides and PCBs prior to capillary column GC/ECD analysis (reference 2). The remaining 80 percent of the extract is analyzed for acid, base, and neutral compounds by capillary column GC/MS. An isotope dilution technique (EPA Method 1625 Revision B, reference 3) is recommended for all compounds analyzed by GC/MS. This technique involves spiking the homogenized tissue sample with the stable isotope labeled analogs of most of the pollutants to be analyzed by GC/MS. The advantage of isotope dilution is that reliable recovery corrections can be made for each analyte with a labeled analog or a chemically similar analog.

2.1.1 Much of the text of EPA Method 1625 Revision B has been incorporated into this method in modified form. The modifications were necessary because, in relation to Method 1625 Revision B, the present method involves different sample matrices (biological tissues), different calibration requirements, and additional analytes (pesticides and PCBs, both requiring GC/ECD analysis).

2.2 Identification of compounds is performed by comparing the GC retention times and background-corrected characteristic spectral masses with those of authentic standards. Tentative identifications of low levels of pesticides and PCBs are made by comparing GC retention times to standards. The identities of pesticides and PCBs are confirmed by GC/ECD analysis on an alternative column phase or by GC/MS when sufficient concentrations occur.

2.3 Quantitative analysis is performed by GC/MS using extracted ion current profile (EICP) areas. Isotope dilution, with labeled analogs of pollutants acting as recovery standards, is the method of quantification when labeled compounds are available. When the isotope dilution technique is used and

certain labeled compounds are unavailable, the nearest eluting, most chemically similar labeled compound is used as a recovery standard. Pesticides and PCBs are quantified by an internal standard method. Concentrations of compounds quantified by GC/MS are reported after correcting for method recoveries when the isotope dilution technique is used.

3.0 INTERFERENCES

3.1 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or elevated baselines, causing misinterpretation of chromatograms and spectra. All materials should be demonstrated to be free from interferences under the conditions of the analysis by running method blanks initially and with each sample lot (Sect. 9.4). Specific selection of reagents and purification of solvents by distillation in all-glass systems are required. High-purity, distilled-in-glass solvents are commercially available (e.g., Burdick and Jackson Laboratories, Muskegon, MI). An effective way of cleaning laboratory glassware is covering with aluminum foil, heating at 450° C for several hours, and rinsing with polar and non-polar solvents before use. Note that heating without subsequent solvent rinsing may not eliminate laboratory residues of PCBs and certain other chlorinated hydrocarbons.

3.2 Phtnalates are common laboratory contaminants that are used widely as plasticizers. Phthalates can derive from plastic labware, plastic tubing, plastic gloves, plastic coated glassware clamps, and have been found as a contaminant in Na_2SO_4. Polytetrafluoroethylene (PTFE) can be used instead of polypropylene or polyethylene to minimize this potential source of contamination. However, use of PTFE labware will not necessarily preclude all phthalate contamination.

3.3 Interferences coextracted from tissue samples limit the method detection and quantitation limits. For this reason, sample extract cleanup is necessary to yield reproducible and reliable analyses of low level contaminants in tissue samples.

4.0 SAFETY

4.1 The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of data handling sheets should also be made available to all personnel involved in these analyses. Additional information on laboratory safety can be found in references 4-6.

4.2 The following compounds covered by this method have been tentatively classified as known or suspected human or mammalian carcinogens: benzene, benzo(a)anthracene, 3,3'-dichlorobenzidine, benzo(a)pyrene, dibenzo(a,h)anthracene, N-nitrosodimethylamine, 4,4'-DDT, alpha-, beta-, delta-, and gamma-hexachlorocyclohexane, and PCBs. Primary standards of these compounds should be prepared in a hood, and a NIOSH/MESA approved toxic gas respirator should be worn when high concentrations are handled.

5.0 APPARATUS AND EQUIPMENT

5.1 Soxhlet Extractor - 50 mL extractor (Corning 3740-S, or equivalent), with 250 mL flask (Corning 4320-250, or equivalent) and condenser with 34/45 joint. Cellulose thimbles of the appropriate size should be cleaned with the extraction solvent mixture for at least 30 cycles.

5.2 Drying Column - 30 cm x 2 cm borosilicate glass chromatography column with glass wool plug. Glass wool is extracted with the appropriate solvents and allowed to dry before use.

5.3 Kuderna-Danish (K-D) Apparatus -

5.3.1 Concentrator tube - 10 mL, graduated (Kontes K-570050-1025, or equivalent. A ground glass stopper (19/22 joint) is used to prevent evaporation of extracts.

5.3.2 Evaporation flask - 500 mL (Kontes K-570050-0500, or equivalent), attached to concentrator tube with springs (Kontes K-662750-0012).

5.3.3 Snyder column - three ball macro (Kontes K-503000-0232, or equivalent).

5.3.4 Snyder column - two ball micro (Kontes K-469002-0219, or equivalent).

5.3.5 Silicon carbide boiling chips - approximately 10/40 mesh, extracted with methylene chloride and heated at 450° C for 1 h minimum. Note that boiling chips can be a significant source of contamination if not properly cleaned.

5.4 Separatory Funnel - 500 mL, borosilicate glass with PTFE stopcock.

5.5 Borosilicate Glass Beaker - 400 mL and 100 mL.

5.6 Water Bath - heated, with concentric ring cover, capable of temperature control ($\pm 2^{\circ}$ C), installed in a fume hood.

5.7 Sample Vials - amber glass, 2-5 mL with PTFE-lined screw cap.

5.8 Analytical Balance - capable of weighing 0.1 mg.

5.9 Micro-grinder (e.g., Tekmar Tissuemizer, Tekmar Company, Cincinnati, OH).

5.10 Nitrogen evaporation device equipped with a water bath that can be maintained at 35-40° C. The N-Evap by Organomation Associates, Inc., South Berlin, MA is suitable.

5.11 Balance - capable of 100 g to the nearest 0.01 g.

5.12 Disposable Pasteur Pipets - sealed with aluminum foil and annealed at 450° C for several h, and rinsed with solvents before use.

5.13 Drying Oven.

5.14 Annealing Oven - capable of reaching 450° C.

5.15 Dessicator.

5.16 Chromatography Column for Alumina - 5 mL disposable borosilicate glass serological pipet with borosilicate glass wool plug. (Glass wool must be extracted with the appropriate solvents and allowed to dry before use.)

5.17 Gel Permeation Chromatography Cleanup Device -

5.17.1 Automated system: gel permeation chromatograph (GPC), e.g., Analytical Biochemical Labs, Inc. GPC Autoprep 1002, including

- 25 mm ID x 600-700 mm glass column packed with 70 g of Bio Beads S-X3.
- Syringe, 10 mL with Luer Lok fitting.
- Syringe Filter Holder and Fitters - stainless steel and TFE, Gelman 4310 or equivalent.

5.17.2 Manual system assembled from parts (Wise, R.H., D.F. Bishop, R.I. Williams, and B.M. Austern. Gel permeation chromatography in the

GC/MS analysis of organics in sludges. U.S. EPA, Municipal Environmental Research Laboratory, Cincinnati, OH. 45268) (see reference 2, p. D-35).

5.18 Gas Chromatograph - 1) one equipped with electron capture detector (ECD) and 2) one interfaced to the mass spectrometer (Sect. 5.19). Both should have splitless injection ports for capillary column, temperature programs with 300° C hold, and should meet all the performance specifications in Sect. 9.9.

5.18.1 Column - 30± 5 m x 0.25 ± 0.02 mm i.d. 5 percent phenyl, 94 percent methyl, 1 percent vinyl silicone bonded phase (0.25 um film thickness) fused silica capillary column (J & W DB-5, or equivalent).

5.19 Mass Spectrometer - 70 eV electron impact ionization, should repeatedly scan from 35 to 450 amu in 0.95 to 1.00 second and should produce a unit resolution (valleys between m/z 441-442 less than 10 percent of the height of the 441 peak), background-corrected mass spectrum from 20 ng decafluorotriphenylphosphine (DFTPP) introduced through the GC inlet. The spectrum should meet the mass-intensity criteria in Table I-2 (reference 7). The mass spectrometer should be coupled with the GC such that the end of the capillary column terminates within one centimeter of the ion source but does not intercept the electron or ion beams. All portions of the column which connect the GC to the ion source should remain at or above the column temperature during analysis to preclude condensation of less volatile compounds.

5.20 Data System - should collect and record MS data, store mass intensity data in spectral libraries, process GC/MS data, generate reports, and should compute and record response factors.

5.20.1 Data acquisition - mass spectra should be collected continuously throughout the analysis and stored on a mass storage device.

5.20.2 Mass spectral libraries - user created libraries containing mass spectra obtained from analysis of authentic standards should be employed to reverse search GC/MS runs for the compounds of interest (Sect. 8.2).

5.20.3 Data processing - the data system should be used to search, locate, identify, and quantify the compounds of interest in each GC/MS analysis. Software routines should be employed to compute retention times and peak areas. Displays of spectra, mass chromatograms, and library comparisons are required to verify results.

5.20.4 Response factors and multipoint calibrations - the data system should be used to record and maintain lists of response factors (response ratios for isotope dilution) and multipoint calibration curves (Sect. 8). Computations of relative standard deviation (coefficient of variation) are useful for testing calibration linearity.

6.0 REAGENTS AND CONSUMABLE MATERIALS (partially adapted from references 2 and 3)

6.1 Reagents

6.1.1 Acetic acid, acetone, benzene, n-hexane, isooctane, methanol, and methylene chloride (CH_2Cl_2) (pesticide quality, distilled-in-glass).

6.1.2 Alumina - neutral, super Woelm or equivalent (Universal Scientific, Atlanta, GA). Extract alumina with methylene chloride for 30-40 cycles in a Soxhlet extractor to remove contamination. Allow solvent to evaporate. Prepare activity III alumina by adding 7 percent (v/w) reagent water to neutral alumina that has been activated at approximately 225° C for at least 2 h or preferably overnight. Store in tightly sealed, clean glass container.

6.1.3 Hydrochloric acid - concentrated, make 2N HCl with reagent water. Solvent clean in a separatory funnel with methylene chloride.

6.1.4 Potassium hydroxide - reagent grade, 6N in reagent water. Solvent clean in a separatory funnel with methylene chloride.

6.1.5 Sodium sulfate - reagent grade, granular anhydrous, rinsed with CH_2Cl_2 (20 mL/g) and conditioned at 450° C for 1 h minimum.

6.1.6 Reagent water - water in which the compounds of interest and interfering compounds are not detected by this method.

6.2 GPC Calibration Solutions:

6.2.1 Corn oil - 200 mg/mL in CH_2Cl_2.

6.2.2 Bis(2-ethylhexyl)phthalate and pentachlorophenol - 4 mg/mL in CH_2Cl_2.

6.3 Stock Standard Solutions - purchased as solutions or mixtures with certification to their purity, concentration, and authenticity, or prepared from materials of known purity and composition. If the compound purity is 96 percent or greater, the weight may be used without correction to compute the concentration of the standard. When not being used, standards are stored in the dark at -20 to -10° C in screwcapped vials with PTFE-lined lids. A mark is placed on the vial at the level of the solution so that solvent evaporation loss can be detected. The vials are brought to room temperature prior to use. Any precipitate is redissolved and solvent is added if solvent loss has occurred.

6.3.1 Preparation of stock solutions - prepare in methylene chloride, benzene, isooctane, or a mixture of these solvents according to the steps below. Observe the safety precautions given in Sect. 4. The large number of labeled and unlabeled acid and base/neutral compounds used for combined calibration (Sect. 8) and calibration verification (9.9.1.3) require high concentrations (approximately 40 mg/mL) when individual stock solutions are prepared, so that dilutions of mixtures will permit calibration with all compounds in a single set of solutions. The working range for most compounds is 1-50 ug/mL. Compounds with a reduced MS response may be prepared at higher concentrations.

Standards for GC/ECD have lower working ranges (e.g., 0.04 to 2.0 ug/mL for single component pesticides) than GC/MS standards. However, GC/ECD stock solutions should be prepared with at least 10 mg of the pure material (e.g., in 10 mL of solvent) to reduce potential weighing error.

6.3.2 Dissolve an appropriate amount of assayed reference material in a suitable solvent. For example, weigh 400 mg naphthalene in a 10 mL ground glass stoppered volumetric flask and fill to the mark with benzene. After the naphthalene is completely dissolved, transfer the solution to a 15 mL vial with PTFE-lined cap.

6.3.3 Stock standard solutions should be checked for signs of degradation prior to the preparation of calibration or performance test standards. Quality control check samples that can be used to determine the accuracy of calibration standards are available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

6.3.4 Stock standard solutions should be replaced after 6 mo, or sooner if comparison with quality control check samples indicates a change in concentration.

6.4 Injection Internal Standard Solutions

6.4.1 GC/MS internal standard solution - prepare 2,2'-difluorobiphenyl (DFB) at a concentration of 2 mg/mL in benzene.

6.4.2 GC/ECD internal standard solution - prepare decafluorobenzophenone (DFBP) at a concentration of 2.5 ug/mL in isooctane.

6.5 GC/MS Secondary Dilution Standards - using stock solutions (Sect. 6.3), prepare a secondary standard containing each of the unlabeled priority pollutants in Table I-1 at a concentration of 100 ug/mL, or at a higher concentration appropriate to the MS response of the compounds.

6.6 Labeled Compound Spiking Solution - prepare a spiking solution from stock standard solutions prepared as in Sect. 6.3, or from mixtures, at a concentration of 100 ug/mL, or at a concentration appropriate to the MS response of each compound. The deuterium and ^{13}C-labeled compounds listed in Table I-1 are commercially available individually or as mixtures (e.g., Merck Sharp & Dohme/Isotopes, Montreal, Canada).

6.7 Solutions for obtaining authentic mass spectra (Sect. 8.2) - prepare mixtures of labeled and unlabeled compounds at concentrations that will assure that authentic spectra are obtained for storage in libraries.

6.8 Calibration Solutions - the concentrations of calibration solutions suggested in the following sections are intended to bracket concentrations that will be encountered during sample analysis without overloading GC columns or saturating detection systems.

6.8.1 GC/MS calibration solutions - combine 0.1 mL of the spiking solution (Sect. 6.6) with 10, 50, 100, 200, and 500 uL of the secondary dilution solution (Sect. 6.5) and bring to 1.00 mL total volume each. This will produce calibration solutions of nominal 1, 5, 10, 20, and 50 ug/mL of the pollutants and a constant nominal 10 ug/mL of the labeled compounds. Spike each solution with 10 uL of the GC/MS internal standard solution, yielding 20 ug/mL.

6.8.2 PCB calibration solutions -

6.8.2.1 Aroclor stock solution for GC/MS - prepare a solution in hexane with 250 ng/uL of each of three PCB mixtures, Aroclor 1016, Aroclor 1254, and Aroclor 1260.

6.8.2.2 Aroclor standard solution for GC/ECD - dilute the stock solution (Sect. 6.8.2.1) to one-tenth its original concentration. It is essential that this solution be prepared directly from the batch used for Sect. 6.8.2.1. Combine 20, 50, 250, 500, and 1,000 uL of the diluted standard

with 100 uL of the GC/ECD internal standard solution and bring each solution to a final volume of 5.0 mL.

This will produce calibration solutions of nominal concentrations of 100, 250, 1,250, 2,500, and 5,000 ng/mL of the 1:1:1 Aroclor mixture and a constant nominal concentration of 50 ng/mL of internal standard.

6.8.3 Pesticide calibration solution - combine 20 uL of the GC/ECD internal standard solution with 2, 5, 10, 50, and 100 uL of a 20 ug/mL stock solution of all chlorinated pesticides listed in Table I-1 (except toxaphene) and bring to a 1.0 mL total volume. This will produce calibration solutions of 40, 100, 200, 1,000, and 2,000 ng/mL of each pesticide and a constant internal standard concentration of 50 ng/mL.

6.8.4 Toxaphene calibration solution - prepare toxaphene solutions of 100, 250, 1,250, 2,500, and 5,000 ng/mL with constant internal standard concentration of 50 ng/mL.

6.8.5 DFTPP solution - prepare at 20 ug/mL in acetone.

6.9 Stability of Solutions - all standard solutions (Sect. 6.4-6.8.4) should be analyzed within 48 h of preparation and on a monthly basis thereafter for signs of degradation. Standards will remain acceptable if response factors relative to the internal standard correspond within $\pm$15 percent to those obtained in the initial analysis of the standard.

7.0 SAMPLE COLLECTION, PREPARATION, AND STORAGE

7.1 In the field, sources of contamination include sampling gear, grease from ship winches or cables, ship engine exhaust, dust, and ice used for cooling. Efforts should be made to minimize handling and to avoid sources of contamination. This will usually require that resection (i.e., surgical removal) of tissue be performed in a controlled environment (e.g., a laboratory). For example, to avoid contamination from ice, the samples should be wrapped in aluminum foil, placed in watertight plastic bags, and immediately

cooled in a covered ice chest. Organisms should not be frozen prior to resection if analyses will only be conducted on selected tissues (e.g., internal organs), because freezing may cause internal organs to rupture and contaminate other tissue. If organisms are eviscerated on board the survey vessel, the remaining tissue may be wrapped as described above and frozen.

7.2 To avoid cross-contamination, all equipment used in sample handling should be thoroughly cleaned before each sample is processed. All instruments must be of a material that can be easily cleaned (e.g., stainless steel, anodized aluminum, or borosilicate glass). Before the next sample is processed, instruments should be washed with a detergent solution, rinsed with tap water, soaked in high-purity acetone and methylene chloride, and finally rinsed with reagent water. Work surfaces should be cleaned with 95 percent ethanol and allowed to dry completely.

7.3 Resection should be carried out by or under the supervision of a competent biologist. Each organism should be handled with clean stainless steel or quartz instruments (except for external surfaces). The specimens should come into contact with precleaned glass surfaces only. Polypropylene and polyethylene surfaces are a potential source of contamination and should not be used. To control contamination when resecting tissue, separate sets of utensils should be used for removing outer tissue and for resecting tissue for analysis. For fish samples, special care must be taken to avoid contaminating target tissues (especially muscle) with slime and/or adhering sediment from the fish exterior (skin) during resection. The incision "troughs" are subject to such contamination; thus, they should not be included in the sample. In the case of muscle, a "core" of tissue is taken from within the area boarded by the incision troughs, without contacting them. Unless specifically sought as a sample, the dark muscle tissue that may exist in the vicinity of the lateral line should not be mixed with the light muscle tissue that constitutes the rest of the muscle tissue mass.

7.4 The resected tissue sample should be placed in a clean glass or TFE container which has been washed with detergent, rinsed twice with tap water,

rinsed once with distilled water, rinsed with acetone, and, finally, rinsed with high-purity methylene chloride. Firing of the glass jar at 450° C may be substituted for the final solvent rinse, but precautions must be taken to avoid contamination as the container is cooled after drying.

7.5 The U.S. EPA and other federal agencies (e.g., National Bureau of Standards) have not yet provided specific guidance regarding holding times and temperatures for tissue samples to be analyzed for semi-volatile organic compounds. Until U.S. EPA develops definitive guidance, the following holding conditions should be observed. Resected tissue samples should be maintained at -20° C and extracted as soon as possible, but within 10 days of sample receipt. Complete analyses should be performed within 40 days. These holding times are based on the Contract Laboratory Program requirements for sediments (reference 10). Liquid associated with the tissue sample must be maintained as part of the sample (the liquid will contain lipid material).

8.0 CALIBRATION AND STANDARDIZATION (adapted from reference 3)

8.1 Establish the GC/MS operating conditions in Table I-1. Analyze standards per the procedure in Sect. 10.2 to demonstrate that the analytical system meets the detection limits in Table I-1 and the mass-intensity criteria in Table I-2 for 20 ng DFTPP.

8.2 Mass Spectral Libraries - detection and identification of compounds of interest are dependent upon spectra stored in user created libraries.

8.2.1 Obtain a mass spectrum of each pollutant, labeled compound, and the internal standard by analyzing an authentic standard either singly or as part of a mixture in which there is not interference between closely eluting components. Confirmation that only a single compound is present is attained by examination of the spectrum. Fragments not attributable to the compound under study indicate the presence of an interfering compound.

8.2.2 Adjust the analytical conditions and scan rate (for this test only) to produce an undistorted spectrum at GC peak maximum. An undistorted spectrum will be obtained if five complete spectra are collected across the upper half of the GC peak. Software algorithms designed to "enhance" the spectrum may eliminate distortion, but may also eliminate authentic masses or introduce other distortion.

8.2.3 The authentic reference spectrum is obtained under DFTPP tuning conditions (Sect. 8.1 and Table I-2) to normalize it to spectra from other instruments.

8.2.4 The spectrum is edited for entry in the library by saving the five most intense mass spectral peaks and all other mass spectral peaks greater than 10 percent of the base peak. This edited spectrum is stored for reverse search and for compound confirmation.

8.3 Polar Compound Detection - demonstrate that unlabeled pentachlorophenol and benzidine are detectable at the 10 ug/mL level (per all criteria in Sect. 10.4). The 10 ug/mL calibration standard (Sect. 6.8.1) can be used to demonstrate this performance.

8.4 Calibration with the isotope dilution technique - isotope dilution technique is used when labeled compounds are available and interferences do not preclude its use. If either of these conditions precludes isotope dilution, the internal standard method (Sect. 8.5) is used and noted as such in the report.

8.4.1 A calibration curve encompassing the concentration range is prepared for each compound determined. The relative response (pollutant to labeled) vs. concentration in standard solutions is plotted or computed using a linear regression. The example in Figure I-1 shows a calibration curve for an unlabeled compound and its labeled analog. Also shown are the $\pm$10 percent error limits (dotted lines). Relative Response (RR) is determined according to the procedures described below. A minimum of five data points are employed for calibration.

8.4.2 The relative response of a pollutant to its labeled analog is determined from isotope ratio values computed from acquired data. Three isotope ratios are used in this process:

R_x = the isotope ratio measured for the pure pollutant
R_y = the isotope ratio measured for the labeled compound
R_m = the isotope ratio of an analytical mixture of pollutant and labeled compounds.

The m/zs are selected such that $R_x > R_y$. If R_m is not between 2 R_y and 0.5R_x, the method does not apply and the sample is analyzed by the internal standard method (Sect. 8.5).

8.4.3 Capillary columns usually separate the pollutant-labeled pair, with the labeled compound eluting first (Figure I-2). For this case,

$$R_x = (\text{area } m_1/z)/1$$

at the retention time of the pollutant (RT_2) and

$$R_y = 1/(\text{area } m_2/z)$$

at the retention time of the labeled compound (RT_1). Also,

$$R_m = [\text{area } m_1/z \text{ (at } RT_2)]/[\text{area } m_2/z \text{ (at } RT_1)]$$

as measured in the mixture of the pollutant and labeled compounds (Figure I-2), and RR = R_m.

8.4.4 Special precautions are taken when the pollutant and its labeled analog are not chromatographically separated and have overlapping spectra, or when another labeled compound with interfering spectral masses overlaps the pollutant (a case that can occur with isomeric compounds). In such cases, it is necessary to determine the respective contributions of the

pollutant and labeled compounds to the respective EICP areas. If the peaks are separated well enough to permit the data system or operator to remove the contributions of the compounds to each other, the equations in Sect. 8.4.3 apply. This usually occurs when the height of the valley between the two GC peaks at the same m/z is less than 10 percent of the height of the shorter of the two peaks. If significant chromatographic and spectral overlap occur, RR is computed using the following equation:

$$RR = (R_y - R_m)(R_x + 1)/((R_m - R_x)(R_y + 1)$$

where R_x is measured as shown in Figure I-3A, R_y is measured as shown in Figure I-3B, and R_m is measured as shown in Figure I-3C. For the example,

$$R_x = 46100/4780 = 9.644$$

$$Ry = 2650/43600 = 0.0608$$

$$R_m = 49200/48300 = 1.019$$

8.4.5 To calibrate the analytical system by isotope dilution, analyze a 1.0 uL aliquot of each of the GC/MS calibration standards (Sect. 6.8.1) using the procedure in Sect. 10.2. Compute the RR at each concentration.

8.4.6 Linearity - if the ratio of relative response to concentration for any compound is constant (less than 20 percent coefficient of variation) over the five point calibration range, an averaged relative response/concentration ratio may be used for that compound; otherwise, the complete calibration curve for that compound should be used over the five point calibration range.

8.5 Calibration by Internal Standard - used when criteria for isotope dilution (Sect. 8.4) cannot be met. The internal standard to be used for both acid and base/neutral analyses is 2,2'-difluorobiphenyl. The internal standard for pesticide and PCB analysis by GC/ECD is decafluorobenzophenone. The internal standard method is also used to determine compounds having

no labeled analog, and to measure labeled compounds for intralaboratory statistics (Sect. 9.5.1).

8.5.1 Response factors - calibration requires the determination of response factors (RF) which are defined by the following equation:

$$RF = (A_s \times C_{is})/(A_{is} \times C_s)$$

where:

A_s = the area of the target peak in the daily standard
A_{is} = the area of the internal standard peak
C_{is} = the concentration of the internal standard (ug/mL)
C_s = the concentration of the compound in the daily standard (ug/mL).

8.5.1.1 The response factor is determined over the range of concentrations described in Sect. 6.8.1, 6.8.2, 6.8.3, and 6.8.4. The amount of internal standard added to each extract is the same so that C_{is} remains constant. The RF is plotted versus concentration for each compound (or class of compounds in the case of toxaphene) in the standard (C_s) to produce a calibration curve.

8.5.1.2 Linearity - if the response factor (RF) for any compound is constant (less than 35 percent coefficient of variation) over the calibration range, an averaged response factor may be used for that compound; otherwise, the complete calibration curve for that compound should be used over the range.

8.6 Combined Calibration - by using calibration solutions (Sect. 6.8.1) containing the pollutants, labeled compounds, and the internal standard, a single set of analyses can be used to produce calibration curves for the isotope dilution and internal standard methods. These curves are verified each shift by analyzing the 10 ug/mL calibration standard (Sect. 9.9.1). Pesticide and PCB calibration standards must be analyzed separately by

GC/ECD. Recalibration is required only if calibration verification (Sect. 9.9.1.3) criteria cannot be met.

8.7 Ongoing Calibration (see Sect. 9.9)

9.0 QUALITY ASSURANCE/QUALITY CONTROL [For further guidance, see Quality Assurance/Quality Control (QA/QC) for 301(n) Monitoring Programs: Guidance on Field and Laboratory Methods (Tetra Tech 1986).]

9.1 Each laboratory that uses this method is required to operate a formal quality assurance program. The requirements of this program consist of an initial demonstration of laboratory capability, analyses of replicates and matrix spikes used to evaluate and document data quality, and analysis of standards and blanks used to test continued performance.

9.2 Initial Demonstration of GC/MS Capability - the analyst should make an initial demonstration of the ability to generate acceptable accuracy and precision with the GC/MS component of this method. This ability is established as described in reference 1.

9.3 The analyst is permitted to modify this method to improve separations or lower the costs of measurements, provided that the new method is demonstrated to perform comparably to the present method (i.e., with comparable spike recoveries and precision).

9.4 Blanks - method blanks should be analyzed by GC/MS and GC/ECD to demonstrate freedom from contamination.

9.4.1 At least one method blank must be included with each batch of samples; method blanks must constitute at least 5 percent of all samples analyzed.

9.4.2 Method blank concentrations of compounds of interest and of potentially interfering compounds should be less than 5 percent of the expected values for the corresponding analytes in samples and below the LLD, if possible. It is recommended that if blank concentrations of compounds

of interest (except phthalates) are greater than 30 percent of the corresponding analyte concentrations in samples, sample analysis should be halted until the contamination source is eliminated.

9.5 Spiked samples are required to assess method performance on the sample matrix.

9.5.1 For samples analyzed by isotope dilution, the percent recovery (p) of labeled compounds can be computed by the internal standard method (Sect. 8.5) and serves as an indication of analytical accuracy (but not necessarily of extraction efficiency). After the analysis of five samples, compute the average percent recovery (P) and the standard deviation of the percent recovery (s_p) for the labeled compounds only. Express the accuracy assessment as a percent recovery interval from $P - 2s_p$ to $P + 2s_p$. For example, if P = 90 percent and s_p = 10 percent, the accuracy interval is expressed as 70-110 percent. Update the accuracy assessment for each compound on a regular basis (e.g., after each 5-10 new accuracy measurements).

9.5.2 Laboratories unable to use isotope dilution must analyze matrix spikes of pollutants (other than pesticides and PCBs) at a frequency of 5 percent of all samples analyzed or once with each sample set, whichever is more frequent. Compounds should be added at concentrations one to five times those in the sample.

9.5.3 All laboratories are required to spike samples with PCBs and/or pesticides at a frequency of 5 percent of all samples analyzed or once per sample set, whichever is more frequent. The spike can be a standard pesticide mixture or an Aroclor mixture, whichever is considered to be more representative of the sample. The mixture should be added at one to five times the sample concentration of these compounds.

9.6 Replicates (i.e., analyses of at least two separate aliquots from a tissue homogenate) must be analyzed by GC/MS and GC/ECD to monitor the precision of laboratory analyses. At a minimum, 5 percent of the analyses should be laboratory replicates. A triplicate analysis should be performed with each sample batch of over 40 samples.

9.7 The laboratory should maintain records to define the quality of data that is generated. These records include documentation of blanks and reports of labeled compound recovery (Sect. 9.5.1), if the latter is applicable.

9.8 The laboratory should, on an ongoing basis, demonstrate through calibration verification that the analysis system is in control (Sect. 9.9.1.3).

9.9 System and Laboratory Performance

9.9.1 At the beginning and end of each 12-h shift during which analyses are performed, GC/MS system performance and calibration are verified for all pollutants and labeled compounds. For these tests, analysis of the 10 ug/mL calibration standards (Sect. 6.8.1) should be used to verify all performance criteria. The GC/ECD performance is checked at the beginning and end of each shift or at least every 6 h by analyses of 250 and 100 ug/mL solutions of the PCB and pesticide standards (Sect. 6.8.2.2 and 6.8.3).

9.9.1.1 Retention times - the absolute GC/MS retention time of 2,2'-difluorobiphenyl should be within the range of 1078 to 1248 sec. The absolute GC/ECD retention time of 4,4'-DDT should be within the range of 1050 and 1200 sec.

9.9.1.2 GC resolution for GC/MS analysis - the valley height between anthracene and phenanthrene at m/z 178 (or the analogs at m/z 188) should not exceed 10 percent of the taller of the two peaks.

GC resolution for GC/ECD analysis - the valley height between two peaks should not exceed 25 percent of the taller of the two peaks for the following pairs: beta- and delta-HCH, dieldrin and 4,4'-DDE, 4,4'-DDD and endrin aldehyde, and endosulfan sulfate and 4,4'-DDT.

9.9.1.3 Ongoing calibration verification - compute the concentration of each pollutant (Table I-1) by isotope dilution (Sect. 8.4) for those compounds that have labeled analogs. Compute the concentration of each pollutant

that has no labeled analog with the nearest eluting labeled standard. Compute the concentration of the labeled compounds by the internal standard method. Also compute individual pesticide concentrations, total PCB and toxaphene concentrations by the internal standard method (GC/ECD). These concentrations are computed based on the calibration data determined in Sect. 8. Preparations of new calibration standards or revisions of calibration curves are required if observed responses of analytes vary from predicted responses by more than ±20 percent. Samples and blanks may be run only after calibration performance meets this control limit.

9.9.1.4 Multiple peaks - each compound injected should give a single, distinct GC peak.

9.9.2 DFTPP spectrum validity - inject 1 uL of the DFTPP solution (Sect. 6.8.5) either separately or within a few seconds of injection of the standard (Sect. 9.9.1) analyzed at the beginning of each shift. The criteria in Table I-2 should be met.

10.0 PROCEDURE (see Figure I-4)

10.1 Sample Processing and Extraction

10.1.1 Mince tissue sample with a scalpel and homogenize the sample to a uniform consistency with a micro-grinder. Care must be taken to ensure that the micro-grinder is thoroughly cleaned after each use. This usually entails disassembly of the unit. Devices with large surface areas (e.g., blenders, meat grinders) should be avoided, as they are difficult to clean and a small sample is difficult to remove after grinding.

10.1.2 Dry weight determination - if sample size permits and dry weight concentrations are required, dry weight determinations may be performed as follows: transfer an aliquot of approximately 3 g (weighed to the nearest 0.1 g) to a preweighed dish. Allow the sample to dry in a 105° C oven overnight, and determine the solid residue weight (to the nearest 0.1 g). Calculate and report the percent solids (T_S) as:

T_S = [dry residue wt (g)]/[wet sample wt (g)] x 100%

10.1.3 Weigh a 20 to 25-g (wet weight) tissue aliquot to the nearest 0.1 g. Add any excess liquid from the sample directly to the round bottom flask to be used for Soxhlet extraction; this will help to avoid saturating the cellulose extraction thimble with tissue fluids, which can lessen extraction efficiency. Also add approximately 50 mL methanol and 140 mL CH_2Cl_2 to the flask.

Spike the tissue aliquot with 10 ug of each stable isotope labeled base/neutral compound and 15 ug of each labeled acid compound. Mix the spiked, homogenized tissue sample with approximately 30 g precleaned Na_2SO_4 in a beaker to improve the texture of the sample. Place the mixture into a precleaned Soxhlet thimble for extraction. Add 20 mL of methanol directly to the thimble and stir to enhance the removal of water from the sample. Cover the sample with a thin layer of solvent-cleaned glass wool.

10.1.3.1 An as yet unvalidated procedure may enhance extraction efficiency for phenolic materials. This procedure entails adding 20 mL of acetic acid directly to the thimble (rather than methanol, as specified in 10.1.3). The purpose of adding acetic acid is to reduce the extraction pH and thus to increase the solvent solubility of phenols. Acetic acid, like methanol, would facilitate water removal. The acetic acid step is recommended only if laboratory validation data is made available.

10.1.4 Soxhlet extract the tissue/Na_2SO_4 mixture with CH_2Cl_2/MeOH (2/1) for 24 h (60-90 cycles). Stir the sample in the thimble at least twice (after the first cycle and after approximately 12 h) to prevent solvent channeling (replace the glass wool cover). The Soxhlet apparatus should be wrapped up to the condensor with aluminum foil to ensure even heating during cycling.

10.1.6 Liquid-liquid extraction -

10.1.6.1 After Soxhlet extraction, transfer the extract to a 500 mL separatory funnel. Rinse the Soxhlet flask with clean extraction solvent and add this rinse to the extract in the separatory funnel. Wash the solvent extract with approximately 100 mL of pH 2, 50 percent saturated Na_2SO_4 organic-free water; the pH of the water should be adjusted with solvent-cleaned HCl. Collect and store the CH_2Cl_2 layer. The purpose of washing the extract with an acidic, aqueous solution is to remove water and methanol from the CH_2Cl_2 and to enhance the partitioning of acidic, organic compounds into the CH_2Cl_2 layer. Re-extract the acidic, aqueous phase twice with 60 mL of clean CH_2Cl_2 and add both extracts to the initial CH_2Cl_2 fraction.

10.1.6.2 Adjust the pH of the aqueous phase to $\geq$12 with solvent-cleaned, 6 N KOH. Back-extract the base compounds three times with 60 mL CH_2Cl_2. The pH adjustment to alkaline conditions ehances the partitioning of basic compounds into the CH_2Cl_2 layer. Combine all CH_2Cl_2 layers from Sect. 10.1.6.1 and 10.1.6.2.

10.1.6.3 Formations of emulsions or precipitates during liquid-liquid extraction should be noted and considered when reviewing results. The addition of Na_2SO_4 may reduce emulsions. However, if the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must emloy mechanical techniques to complete the phase separation. The optimum technique depends on the sample and may include stirring, filtration of the emulsion through pre-cleaned glass wool, centrifugation, or other physical methods (reference 2).

10.1.7 Dry the total combined solvent extract by pouring it through an anhydrous Na_2SO_4 drying column (approximately 30 cm x 2 cm). Use approximately 30 mL of CH_2Cl_2 to rinse the drying column and combine this with the dried extract. Collect the extract in a Kuderna-Danish (K-D) concentrator containing 1 to 2 clean boiling chips.

10.1.8 Attach a 3-ball macro Snyder column to the K-D concentrator and concentrate the extract on an 80° C water bath. Place the K-D apparatus on the hot water bath so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed with hot vapor. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume reaches 5 mL, remove the K-D apparatus from the water bath and rinse the flask with 3 mL CH_2Cl_2 draining into the concentrator tube. Reduce the contents of the concentrator tube to 3 mL using a stream of purified N_2 gas, never allowing the extract to go to dryness.

10.1.9 Alternative methods of extract concentration may be used if evidence of acceptable performance [i.e., retention of more volatile compounds (e.g., naphthalene) comparable to that of K-D concentration] is provided.

10.1.10 Accurately measure the extract volume, V_T, remove a small aliquot (100 uL) and apply directly to a preweighed microbalance weighing boat. Allow the solvent to evaporate on a hotplate at low temperature (less than 40° C). Quickly remove the boat when it is dry and weigh the residue and boat to the nearest 0.1 mg. Calculate the total extracted residue, R_T, as:

$$R_T \text{ (mg)} = [V_t \text{ (mL)} \times W \text{ (mg)} \times 10^3]/V_a \text{ (uL)}$$

where:

V_T = the extract volume (3 mL) in mL
V_a = the aliquot volume transferred to the weighing boat in uL
W = the residue weight in mg as determined with the analytical microbalance.

Report the total extracted residue as R_T (mg)/sample wet weight (g).

10.1.11 Extract cleanup - GPC cleanup is required to separate biological macromolecules from the analytes.

10.1.11.1 GPC setup and calibration (reference 2).

10.1.11.1.1 Place 70 g of Bio Beads S-X3 in a 400-mL beaker. Cover the beads with CH_2Cl_2. Allow the beads to swell overnight. Transfer the swelled beads to the column and start pumping solvent through the column, from bottom to top, at 5 mL/min. After 1 h, adjust the pressure on the column to 7-10 psi and pump for an additional 4 h to remove air from the column. Adjust the column pressure as required to maintain 7-10 psi.

10.1.11.1.2 Calibration of the column - Load 5 mL of the corn oil solution into sample loop No. 1 and 5 mL of the phthalate-PCP solution into loop No. 2. Inject the corn oil solution and collect 10 mL fractions for 36 min. Determine the corn oil elution pattern by evaporation of each fraction to dryness followed by a gravimetric determination of the residue. Analyze the phthalate PCP fractions by GC/FID, a UV-spectrophotometer at 254 nm, or a GC/MS system. Plot the concentration of each component in each fraction versus total eluant volume. Choose a "dumptime" that allows $\geq$85 percent removal of the corn oil and $\geq$85 percent recovery of the phthalate. Select the "collect time" to extend at least 10 min after the elution of pentachlorophenol. Wash the column at least 15 min between samples. Typical parameters are: dumptime, 30 min (150 mL); collect time, 36 min (180 mL); and wash time, 15 min (75 mL). The S-X3 Bio Beads column may be reused for several months, but should be checked by frequent system recalibration for every 20 extracts loaded onto the GPC.

10.1.11.2 Extract cleanup - Prefilter or load all extracts via the filter holder to avoid particulates that might cause system blockage. Load the 3-mL extract onto the GPC column. Do not apply excessive pressure when loading the GPC. Purge the sample loading tubing thoroughly with solvent between extracts. Process the extracts using the dump, collect, and wash parameters as selected from the calibration and collect the cleaned extracts in 400-mL beakers.

10.1.11.3 Rerun the phthalate-PCP calibration solution to check and recalibrate the system once for every 20 extracts loaded onto the GPC. The recoveries and elution profiles are reported as deliverables.

10.1.12 Transfer the extract to a Kuderna-Danish (K-D) concentrator consisting of a 10-mL concentrator tube, a 500-mL evaporative flask, boiling chips, and a Snyder column. Carefully concentrate the extract to 2.5 mL using methods previously described and the N_2 gas blowdown technique. Nitrogen blowdown should be performed at approximately 350 C. A gentle stream of clean, dry N_2 (filtered through a column of activated carbon) should be used. The inside walls of the tube containing the extract should be rinsed down with the appropriate solvent several times during concentration. The extract must not be allowed to go to dryness.

10.1.13 Use a 20 percent aliquot (500 uL) of the extract for alumina column cleanup and subsequent GC/ECD analysis for pesticides and PCBs. Use the remaining 80 percent (2 mL) for GC/MS analysis. Carefully reduce the 2 mL extract to 400 uL using the N_2 blowdown technique.

10.1.13.1 Solvent exchange of extract for alumina cleanup (reference 2) - transfer 0.5 mL of the extract to a separate concentrator tube. Add 5 mL of hexane and a boiling chip and mix using a vortex mixer. Attach a two-ball micro-Snyder column. Pre-wet the Snyder column by adding 0.5 mL of hexane to the top of the column. Place the K-D apparatus on a hot water bath (80-900 C) so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 5 to 10 min. Concentrate the extract to an apparent volume of approximately 1 mL. Use N_2 blowdown to reduce the volume to 0.5 mL. Dilute to 1 mL by adding 0.5 mL of acetone. Proceed with alumina column cleanup.

10.1.14 Alumina column setup and use - The alumina column cleanup is required to remove polar interferences prior to GC/ECD analysis of pesticides and PCBs (reference 2).

10.1.14.1 Add 3 g of activity III neutral alumina to the clean 5 mL disposable serological pipet (with glass wool plug). Tap the column to settle the alumina. Do not pre-wet the alumina with solvent.

10.1.14.2 Transfer the 1.0 mL hexane/acetone extract (Sect. 10.1.13.1) to the top of the alumina column with a disposable Pasteur pipet. Collect the eluate in a 10 mL K-D concentrating tube. Add 1 mL of hexane to the original extract concentrator tube to rinse it. Transfer these rinsings to the alumina column. Elute the column with an additional 9 mL of hexane. Do not allow the column to go dry during the addition and elution of the sample.

10.1.14.3 Note that batches of alumina may differ and storage may alter the water content of deactivated alumina. Thus, column performance must be checked regularly and for each batch of alumina. PCB and pesticide standards (e.g., from Sect. 6.8.2.2, 6.8.3) and a suitable model polar compound (e.g., tribromophenol) should be used to determine the appropriate elution volumes for these pollutants. Recovery of single PCB or pesticide components should be greater than 85 percent and the tribromophenol should not be detected.

10.1.14.4 Concentrate the eluate to 500 uL using a micro-Snyder column and the N_2 blowdown technique (e.g., Sect. 10.1.13.1).

10.1.14.5 Care must be taken to allow the N_2 gas to create only a small dimple on the surface of the solvent and prevent blowdown to dryness. Submit extract for GC/ECD analyses.

10.2 GC/MS Analysis

10.2.1 Establish the following operating conditions for the GC (Table I-1): 5 min at 30° C; 30-280° C at 8° C/min; isothermal at 280° C until benzo(g,h,i)perylene) elutes. Make certain that the concentrated extract or standard is at room temperature and make note of any precipitate that does not redissolve.

10.2.2 Add 5 uL of the GC/MS internal standard solution to the 400 uL extract to yield a 10 ug spike. Add the solution immediately prior to injection to minimize the possibility of loss by evaporation, adsorption, or reaction. Mix thoroughly.

10.2.2.1 It is advised that a late eluting internal injection standard (e.g., 5-alpha-cholestane) be used in addition to DFB. The use of early eluting (DFB) and late eluting injection standards will allow the analyst to detect and compensate for problems in the GC injection port related to differential loading of analytes onto the GC column.

10.2.3 Inject 1.0-1.5 uL and start the GC column initial isothermal hold. Start MS data collection after the solvent peak elutes. Stop data collection after the benzo(g,h,i)perylene elutes.

10.2.3.1 Dilution and re-injection are required for samples that exceed the upper concentration limit of the calibration standards. Data for compounds within the calibration range should be acquired in the initial run. Data for compounds exceeding the calibration range should be acquired after dilution. Respike the sample with labeled compounds and assume 100 percent recovery. This assumption is not unreasonable considering the high concentrations of native compounds involved when dilution is necessary.

10.3 GC/ECD Analysis

10.3.1 The recommended GC conditions are modified from those specified in reference 2:

Helium carrier gas: 4 mL/min at 280° C and 25 psi
Septum purge: 15 mL/min
Split vent: none
Initial temperature: 60° C, initial hold - 2 min
Program at 25° C/min to 160° C

Program at 5° C/min from 160° C
Final temperature: 270° C;
hold until decachlorobiphenyl elutes
Injection port temperature: 225° C

10.3.2 Add 10 uL of the GC/ECD internal standard solution to the 500 uL extract to yield a 25 ng spike. Add the solution immediately prior to injection to minimize the possibility of loss by evaporation, adsorption, or reaction. Mix thoroughly. Inject 1.0-1.5 uL.

10.3.3 Dilution and re-injection are required for samples that exceed the upper concentration limit of the calibration standards. Data for compounds within the calibration range should be retained from the initial run. Data for compounds exceeding the calibration range should be acquired after dilution.

Column overloading can result in abnormal peak shape, which can reduce the accuracy of quantification. It may also result in a marked increase in the retention time of the peak maximum, which can displace an analyte from the retention time window established with standards at lower concentrations.

10.4 Qualitative Determination

10.4.1 Qualitative determination is accomplished by comparison of data from analysis of a sample or blank with data from analysis of the shift standard (Sect. 9.9.1) and, for GC/MS analyses, with data stored in the spectral libraries (Sect. 8.2.4). Identification is confirmed when spectra and retention times agree per the criteria below.

10.4.2 Labeled compounds and pollutants having no labeled analog:

10.4.2.1 The signals for all characteristic masses stored in the spectral library (Sect. 8.2.4) should be present and should maximize within the same two consecutive scans.

10.4.2.2 Either 1) the background corrected extracted ion current profile (EICP) areas, or 2) the corrected relative intensities of the mass spectral peaks at the GC peak maximum should agree within a factor of two (0.5 to 2 times) for all masses stored in the spectral library.

10.4.2.3 The retention time difference between an analyte and the nearest eluting internal standard during sample analysis should be within $\pm$5 scans or $\pm$5 sec (whichever is greater) of this difference in the shift standard (Sect. 9.9.1).

10.5 Pollutants Having a Labeled Analog:

10.5.1 The signals for all characteristic masses stored in the spectral library (Sect. 8.2.4) should be present and should maximize within the same two consecutive scans.

10.5.2 Either 1) the background corrected EICP areas, or 2) the corrected relative intensities of the mass spectral peaks at the GC peak maximum should agree within a factor of two for all masses stored in the spectral library.

10.5.3 The retention time difference between the pollutant and its labeled analog should agree within $\pm$2 scans or $\pm$2 sec (whichever is greater) of this difference in the shift standard (Sect. 9.9.1).

10.6 If an experimental mass spectrum contains masses that are not present in the reference mass spectrum, an experienced spectrometrist is to determine the presence or absence of the compound.

10.7 Chlorinated Pesticides and PCBs

10.7.1 Single component chlorinated pesticides are tentatively identified by comparison of sample peak relative retention times to those of authentic standards (Sect. 6.8.3). Three times the standard deviation of relative

retention times established from calibration standards (Sect. 6.8.3) can be used to calculate relative retention time window boundaries. Confirm the identities of pesticides by comparing the relative retention times of sample and standard peaks on another column phase (e.g., 86% dimethyl-[14%]-cyanopropyl phenyl polysiloxane or J&W DB-1701). Confirmation by GC/MS is required when concentrations are sufficient.

10.7.2 Peaks of multi-component mixtures (PCBs and toxaphene) are tentatively identified in samples by comparison of relative retention times to those of authentic standards (Sect. 6.8.2.2 and 6.8.4). Three times the standard deviation of relative retention times established from standards can be used to calculate relative retention time window boundaries. Choose as many peaks as possible while avoiding those with potential interferences (e.g., PCBs co-eluting with DDT and DDE isomers). Label on all sample chromatograms the peaks identified as PCB and toxaphene congeners. All GC/ECD chromatograms are part of the deliverables. Interpretation of chromatograms requires the attention of an experienced analyst. Confirm the identities of all selected congeners by injection on an alternate column phase (e.g., J&W DB-1701). Confirmation by GC/MS is required if concentrations are sufficient.

10.8 Tentatively Identified Compounds (GC/MS Analysis) - The ten non-target peaks of greatest area in the RIC (reconstructed ion chromatogram) should be identified and quantified, if possible.

10.8.1 Guidelines for making tentative identification (reference 10):

1) Tentative identifications should be based on a forward search of the EPA/NIH mass spectral library. Sample spectra should be visually compared with the most similar library match.

2) Relative intensities of major ions in the reference spectrum (ions greater than 10 percent of the most abundant ion) should be present in the sample spectrum.

3) The relative intensities of the major ions should agree within ±20 percent. (Example: For an ion with an abundance of 50 percent in the standard spectra, the corresponding sample ion abundance must be between 30 and 70 percent.)

4) Molecular ions present in reference spectrum should be present in sample spectrum.

5) Ions present in the reference spectrum but not in the sample spectrum should be reviewed for possible subtraction from the sample spectrum because of background contamination or co-eluting compounds. Data system library reduction programs can sometimes create these discrepancies.

10.8.1.1 If, in the opinon of the mass spectral specialist, no valid tentative identification can be made, the compound should be reported as unknown. The mass spectral specialist should give additional classification of the unknown compound if possible (e.g., unknown phthalate, unknown hydrocarbon, unknown aromatic compound, unknown chlorinated compound). If probable molecular weights can be distinguished, include them.

10.8.2 Tentative quantification - quantification of TICs will be based on the internal standard technique and an assumed response factor of one (in the absence of data from authentic standards). The uncertain nature of this quantification should be clearly noted in the data report.

11.0 QUANTITATIVE DETERMINATION (CALCULATIONS)

11.1 Isotope Dilution - by adding a known amount of a labeled compound to every sample prior to extraction, correction for losses of the pollutant during the analysis can be made because the pollutant and its labeled analog exhibit similar behavior during extraction, concentration, and gas chromatography. Note that pollutants and their labeled analogs are not always retained identically by complex matrices, so their behavior during the extraction step may differ.

11.1.1 Relative response (RR) values for sample mixtures are used in conjunction with calibration curves described in Sect. 9.4 to determine concentrations directly, so long as labeled compound spiking levels are constant.

11.1.2 Specifically, the concentration, C(in ug/kg), can be determined as:

$$C(ug/kg) = \frac{C_A(ug/kg) \times RR \times n}{\sum_{i=1}^{n} \frac{RR \times Z_{Ai}}{Z_i}}$$

where:

C_A = the concentration of the stable isotope-labeled compound as spiked into the sample, wet weight

RR = relative response of unlabeled pollutant to isotope labeled surrogate in the sample

RR_i = relative response of i^{th} point in calibration

Z_i = absolute amount of unlabeled compound of i^{th} point in calibration

Z_{Ai} = absolute amount of labeled compound of i^{th} point in calibration

n = number of calibration points.

11.2 Internal Standard - all data reported as determined by this method are uncorrected for method recoveries.

11.2.1 GC/MS internal standards method - Compute the concentration in the sample, C (in ug/kg), using the response factor, RF, determined from calibration data, and the following equation:

$$C(ug/kg) = (A_x \times Z_{is} \times 1.25 \times 10^3)/(S \times A_{is} \times RF)$$

where:

A_x = the area at the characteristic mass for the compound in the sample

A_{is} = the area of the characteristic mass for the internal standard

Z_{is} = the absolute amount, in ug, of the GC/MS internal standard added to the final extract prior to instrumental analysis

S = sample wet weight (g) that was extracted.

11.2.1.1 The stable isotope labeled compound recovery, X, is determined and reported for each sample in the following manner:

$$X = [C(ug/kg)/C_A(ug/kg)] \times 100\%$$

where:

C_A = the concentration of the stable isotope labeled compound as spiked into the sample

11.2.2 GC/ECD internal standard method -

11.2.2.1 Pesticides - compute the concentration in the sample, C (in ug/kg), using the response factor (RF, determined from calibration data) and the following equation:

$$C(ug/kg) = (A_x \times Z_{is} \times 5 \times 10^3)/(S \times A_{is} \times RF)$$

where:

A_x = the area of the integrated GC peak for the compound in the sample (A_x represents the summation of areas for a group of GC peaks if toxaphene is being quantified)

A_{is} = the area of the integrated GC peak for the internal standard

Z_{is} = the absolute amount, in ug, of the GC/ECD internal standard added to the final extract prior to instrumental analysis

S = the sample wet weight (g) that was extracted.

11.2.2.2 Accurate PCB quantification is difficult to achieve in routine full-scan analyses. It has been common practice to quantify PCBs with packed-column GC/ECD by comparing several selected peaks in samples to corresponding peaks in commercial Aroclor formulations that most closely resemble the sample. Shortcomings of this technique have been described elsewhere (e.g., references 8 and 9). The critical difficulties with this procedure relate to two factors: (1) environmental PCB assemblages often differ considerably from commercial Aroclor mixtures because of the variable properties of PCB congeners (e.g., aqueous solubility, volatility, susceptibility to biodegradation) and (2) the ECD has a markedly variable response to the 209 PCB congeners depending on the number and position of chlorine atoms on the biphenyl nucleus (e.g., reference 10).

It has been suggested that "the least systematic error [in PCB quantification] will be given by the summation of all or at least nearly all areas of PCB peaks corrected by their individual ECD-response factor and their biphenyl content" (reference 11). Another alternative is to use GC/MS instead of GC/ECD. However, GC/MS analysis is relatively insensitive unless selected ion monitoring (SIM) is used, which can involve considerable effort and expense.

The quantification technique recommended in this 301(h) document relies on high resolution (capillary column) GC/ECD and a determination of response factors for resolvable PCB peaks (as suggested in the previous paragraph). The technique is modified from the Webb and McCall technique (reference 12), which has been widely used for packed-column PCB quantification. Briefly, the resolved peaks in a PCB standard (Sect. 6.8.2.1, 6.8.2.2) are quantified by GC/MS and GC/ECD. The GC/MS results are used to correct for the variability of ECD response. Samples are analyzed and quantified by GC/ECD. Total PCBs are calculated as the sum of all resolved, response factor-corrected PCB peaks.

11.2.2.2.1 GC/MS analysis of PCB standard - Each resolvable peak in a PCB calibration standard (Sect. 6.8.2.1) is quantified by GC/MS, which can identify the chlorine content of biphenyls in each peak and can quantify PCBs based on their chlorine content. This quantification does not require that the analyst know the exact identity of the congeners constituting a peak, only the chlorine content must be determined.

Another GC/MS calibration standard is necessary to perform this quantification. An MS response factor standard consisting of representatives of all the congener groups (mono- through decachlorobiphenyl) is necessary to convert areas of peaks in ion plots to the appropriate masses of chlorobiphenyls. A standard solution should be made with approximately 10 ng/uL of each of the following congeners (see reference 13 for an explanation of these choices):

2
2,3
2,4,5
2,2',4,6
2,2',3,4,5'
2,2',4,4',5,6'
2,2',3,4',5,6,6'
2,2',3,3',4,5',6,6'
2,2',3,3',4,4',5,5',6,6' - (used for nona- and deca-congeners).

These congeners are available from Ultra Scientific, Inc. (Hope, RI) except the heptachloro-congener, which is available from Wellington Environmental Consultants, Inc. (Guelph, Ontario, Canada). The primary quantification ions used for mono- through deca-chlorobiphenyl are: 188, 222, 256, 292, 326, 360, 394, 430, 464, and 498. The spectrum for each peak should be manually confirmed at least once for the Aroclor standard.

Relative amounts of co-eluting congeners of different chlorine content in a given peak in the standard can be determined during GC/MS analysis. Co-elution can be accounted for with appropriate response factors. For example, if a peak is composed of tetrachloro- and pentachloro-isomers as determined by ion plots of m/z 292 and 326, the 2,2',4,6 response factor is used for the m/z 292 area and the 2,2',3,4,5' response factor for the m/z 326 area. Care must be taken to ensure that M-70 ions are not interpreted as M+ ions if congeners differing by two chlorine atoms co-elute.

11.2.2.2.2 GC/ECD analysis of PCB standard (Sect. 6.8.2.2) -

Each resolvable peak in the PCB calibration standard is quantified by GC/ECD according to the internal standard technique (Sect. 8.5). The GC/ECD analysis is performed with the same GC column phase and temperature program used for GC/MS analysis of the standard. An ECD response factor (RF) is established for each peak based on the GC/MS analysis of the PCB standard using the equation defined in Section 8.5.1

where:

C_s = the mean concentration of the peak in the PCB standard as determined by GC/MS (determined with at least three replicate analyses).

11.2.2.2.3 GC/ECD PCB quantification in samples - Total PCBs are calculated as the sum of all PCB peaks identified in a sample (Sect. 10.7.2):

$$C \text{ (ug/kg, wet wt)} = \sum_{i=1}^{n} [(A_x \times Z_{is} \times 5 \times 500)/(S \times A_{is} \times RF)]_i$$

where:

i = each identified PCB peak, with n total peaks
A_x = area of the integrated GC peak for the compound in the sample
A_{is} = area of the integrated GC peak for the internal standard
Z_{is} = the absolute amount, in ug, of the GC/ECD internal standard added to the final extract prior to instrumental analysis
S = the sample wet weight (g) extracted
RF = calibration response factor (Sect. 8.5.1).

11.2.2.2.4 This quantification method involves two noteworthy limitations:

(1) Interferences can be a significant problem in ECD analyses. PCB peaks co-eluting with interferents may be neglected or quantified, in either case resulting in a decrease in accuracy. It is essential that experienced analysts evaluate chromatograms to determine the presence of suspected interferents. Interferents suspected of overwhelming PCB peaks should be neglected. The alumina column cleanup step is designed to preclude major interferences. High resolution capillary columns also reduce the potential for co-eluting interferences.

(2) When two or more congeners have identical retention times on a given column phase, it is impossible to determine their relative concentrations in a peak when using GC/ECD. Thus, it is not possible to determine whether sample peaks are composed of the same relative combination of congeners as corresponding standard peaks. Thus, the response factor for a peak may be different during calibration and sample analysis. The potential error in assigning appropriate reponse factors has been minimized in this technique by the use of high resolution capillary columns.

11.2.2.2.5 Alternative techniques of detection [e.g., Hall electrolytic conductivity detector (HECD) or MS (with selected ion monitoring)] can provide comparable or superior PCB identification and quantification relative to ECD (e.g., references 13 and 14) and are acceptable substitutes for ECD detection. Although ECD is widely available and is more sensitive for PCBs than HECD or MS, HECD has a linear response to chlorine content and is more specific to chlorinated compounds, and MS offers more definitive compound identification than ECD.

11.2.2.3 Quantify PCBs by summing the response factor-corrected areas of the characteristic PCB peaks identified in Sect. 10.7.2. Report the results as total PCBs.

11.3 Report results for all pollutants and labeled compounds found in all standards and samples, in ug/kg, to two significant figures. Note in the report all compounds that have not been recovery corrected. Report results for blanks as total ng/sample.

12.0 PRECISION AND ACCURACY

Multiple laboratory comparison studies of the precision and accuracy attainable with this technique are not available. Limited precision data for environmental samples are available; they derive from replicate analyses of English sole muscle and liver tissue (reference 15). Mean coefficients of variation for total PCBs were 11 percent for muscle tissue (calculated from six duplicates) and 15 percent for liver tissue (calculated from one duplicate and one triplicate). These PCB results were generated by packed column analysis and comparisons of sample chromatograms to Aroclor standards. The accuracy of these PCB analyses was not assessed. Validation data using the PCB quantification procedure in this 301(h) document has not yet been generated.

A method test was recently conducted with spiked blanks. Replicate blanks were spiked with known amounts of labeled and unlabeled compounds.

The amounts of the unlabeled compounds were calculated using the isotope dilution technique (i.e., the calculated amounts of the unlabeled compounds were adjusted for the recovery of the labeled compounds). The ratio of the calculated amount of the unlabeled compounds relative to their actual spiked amount (expressed as percent) is given in Table I-3. The procedure for this method test included two additional cleanup steps (metallic mercury and reverse phase column chromatography) that are not part of this 301(h) tissue protocol. Therefore results for the 301(h) tissue procedure should be comparable or superior to those of the method test.

13.0 REFERENCES

1. "Performance Tests for the Evaluation of Computerized Gas Chromatography/-Mass Spectrometry Equipment and Laboratories," USEPA, EMSL/Cincinnati, OH 45268, EPA-600/4-80-025 (April 1980).

2. U.S. Environmental Protection Agency. 1984 (revised January, 1985). U.S. EPA Contract Laboratory Program - statement of work for organics analysis, multi-media, multi-concentration. IFB WA 85-J176, J177, J178.

3. Fed. Register, Vol. 49, No. 209, October 26, 1984, pp. 43416-43429.

4. "Carcinogens - Working with Carcinogens," DHEW, PHS, CDC, NIOSH, Publication 77-206 (Aug 1977).

5. "OSHA Safety and Health Standards, General Industry," OSHA 2206, 29 CFR 1910 (revised Jan 1976).

6. "Safety in Academic Chemistry Laboratories," ACS Publications, Committee on Chemical Safety, 3rd Edition (1979).

7. Eichelberger, J.W., L.E. Harris, and W.L. Budde, "Reference compound to calibrate ion abundance measurement in gas chromatography-mass spectrometry," Anal. Chem. Vol. 47, 1975, pp. 995-1000.

8. Duinker, J.C., M.T.J. Hillebrand, K.H. Palmork, and S. Wilhelmsen, "An evaluation of existing methods for quantitation of PCBs in environmental samples and suggestions for an improved method based on measurement of individual components," Bull. Environm. Contam. Toxicol. Vol. 25, 1980, pp. 956-964.

9. Alford-Stevens, A.L., W.L. Budde, and T.A. Bellar, "Interlaboratory study on determination of PCBs in environmentally contaminated sediments," Anal. Chem. Vol. 57, 1985, pp. 2452-2457.

10. Mullin, M.D., C.M. Pochini, S. McCrindle, M. Romkes, S.H. Safe, and L.M. Safe, "High-resolution PCB analysis: synthesis and chromatographic properties of all 209 PCB congeners," Environ. Sci. Technol. Vol. 18, 1984, pp. 468-476.

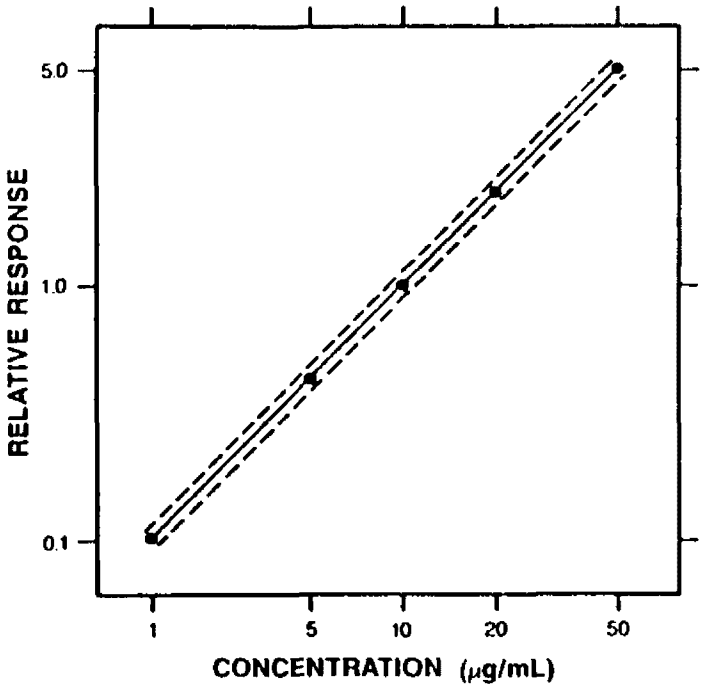
11. Ballschmiter, K., and M. Zell, "Analysis of PCB by glass capillary gas chromatography," Fresenius Z. Anal. Chem. Vol. 302, 1980, pp. 20-31.

12. Webb, R.G., and A.C. McCall, "Quantitative PCB standards for electron capture gas chromatography," J. Chromatographic Science, Vol. 11, 1973, pp. 366-373.

13. Gebhart, J.E., T.L. Hayes, A.L. Alford-Stevens, and W.L. Budde, "Mass spectrometric determination of polychlorinated biphenyls as isomer groups," Anal. Chem. Vol. 57, 1985, pp. 2458-2463.

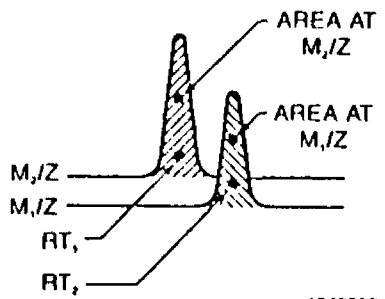
14. Sonchik, S., D. Madeleine, P. Macek, and J. Longbottom, "Evaluation of sample preparation techniques for the analysis of PCBs in oil," J. Chromatographic Science, Vol. 22, 1984, pp. 265-271.

15. Tetra Tech, Inc. Commencement Bay nearshore/tideflats remedial investigation. Vol. 1. Final report prepared for the Washington State Department of Ecology and U.S. Environmental Protection Agency. 1985.



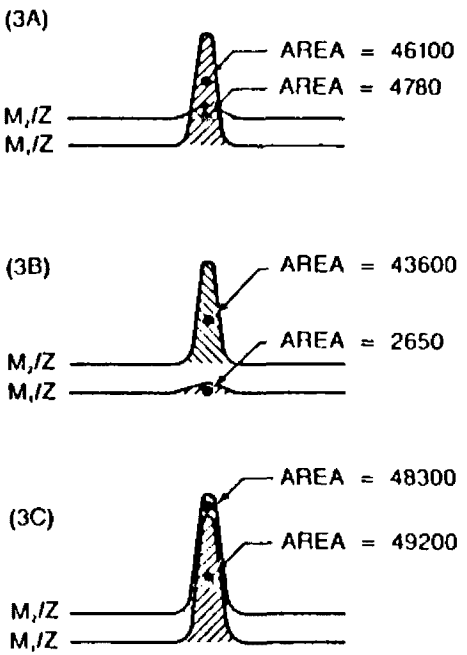
ADAPTED FROM REFERENCE 3

1.



ADAPTED FROM REFERENCE 3

2.



ADAPTED FROM REFERENCE 3

3.

Figure I-1. Relative response calibration curve.
Figure I-2. Extracted ion current profiles for chromatographically resolved labeled (m_2/z) and unlabeled (m_1/z) pairs.
Figure I-3. Extracted ion current profiles for (3A) unlabeled compound, (3B) labeled compound, and (3C) equal mixture of unlabeled and labeled compounds.

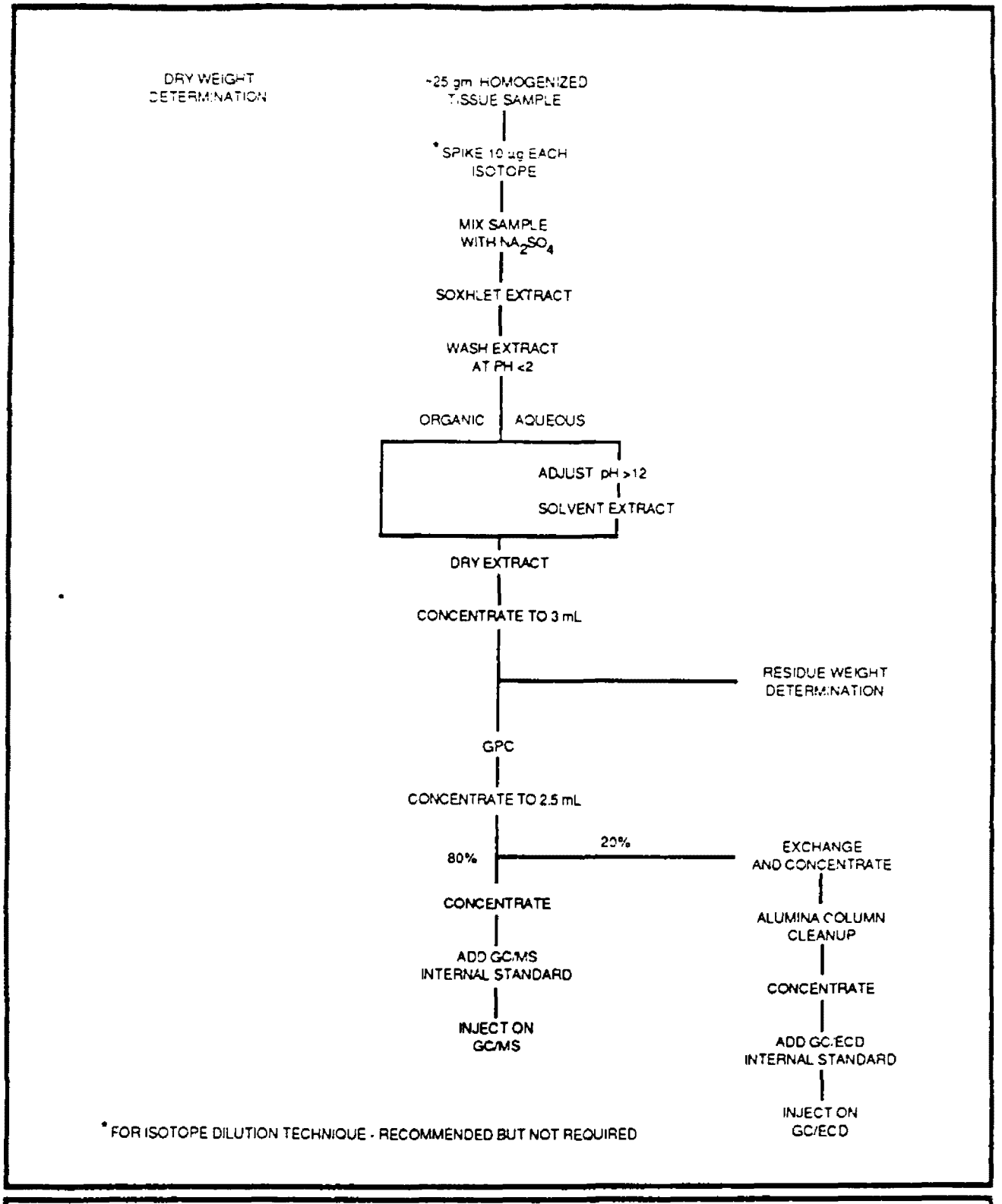


Figure I-4. Flow chart for sample preparation.

TABLE I-1. GAS CHROMATOGRAPHY OF EXTRACTABLE COMPOUNDS

Compound	Retention Time Sec	Retention Time Relative[a]	Quantitation (Primary) m/z	Instrumental Sensitivity (ng) GC/MS	ODES	CASRN
2,2'-difluorobiphenyl (DFB)	1163	1.000	190	1		
N-nitrosodimethylamine	385	0.330	74	5	NNOMA	62-75-9
phenol-d_5[(4)]	696	0.600	71	1		
phenol	700	1.003	94	1	PHENOL	108-95-2
bis(2-chloroethyl)ether-d_8	696	0.596	101	1		
bis(2-chloroethyl)ether	704	1.012	93	1	BCEE	111-44-4
1,3-dichlorobenzene-d_4	722	0.621	152	1		
1,3-dichlorobenzene	724	1.003	146	1	13-2CLBNZ	541-73-1
1,4-dichlorobenzene-d_4	737	0.634	152	1		
1,4-dichlorobenzene	740	1.004	146	1	14-2CLBNZ	106-46-7
1,2-dichlorobenzene-d_4	758	0.652	152	1		
1,2-dichlorobenzene	760	1.003	146	1	12-2CLBNZ	95-50-1
bis(2-chloroisopropyl)ether-d_{12}	788	0.678	131	1		
bis(2-chloroisopropyl)ether	799	1.014	121	1	B2CIE	108-60-1
hexachloroethane-^{13}C[(2)]	819	0.704	204	1		
hexachloroethane	823	1.005	201	1	6CLETH	67-72-1
N-nitrosodi-n-propylamine	830	0.714	70	2	NNONPRA	621-64-7
nitrobenzene-d_5	845	0.726	128	1		
nitrobenzene	849	1.005	123	1	NBNZ	98-95-3
isophorone-d_8	881	0.757	88	1		
isophorone	889	1.009	82	1	ISOPHORONE	78-59-1
2,4-dimethylphenol-d_3	921	0.792	125	1		
2,4-dimethylphenol	924	1.003	122	1	24-2MPHN	105-67-9
bis(2-chloroethoxy)methane	939	0.807	93	1	BCEOM	111-91-1
1,2,4-trichlorobenzene-d_3	955	0.821	183	1		
1,2,4-trichlorobenzene	958	1.003	180	1	124-3CLBNZ	120-82-1
naphthalene-d_8	963	0.828	136	1		
naphthalene	967	1.004	128	1	NAPHTHALENE	91-20-3
hexachlorobutadiene-$^{13}C_4$	1005	0.864	231	1		
hexachlorobutadiene	1006	1.000	225	1	6CLBUTAD	87-68-3
hexachlorocyclopentadiene-$^{13}C_4$	1147	0.986	241	3		
hexachlorocyclopentadiene	1142	0.996	237	3	6CLCYCPEN	77-47-4
2-chloronaphthalene-d_7	1185	1.019	169	1		
2-chloronaphthalene	1200	1.013	162	1	2-CLNAP	91-58-7
biphenyl-d_{10}	1205	1.036	164	1		
biphenyl	1211	1.005	154	1	BIPHENYL	92-52-4
acenaphthylene-d_8	1245	1.071	160	1		
acenaphthylene	1247	1.002	152	1	ACENAPTYLE	208-96-8
dimethylphthalate-d_4	1269	1.091	167	1		
dimethylphthalate	1273	1.003	163	1	DMP	131-11-3
2,6-dinitrotoluene-d_3	1283	1.103	167	2		
2,6-dinitrotoluene	1300	1.013	165	2	26-2NTOL	606-20-2
acenaphthene-d_{10}	1298	1.116	164	1		
acenaphthene	1304	1.005	154	1	ACENAPE	83-32-9
dibenzofuran-d_8	1331	1.144	176	1		
dibenzofuran	1335	1.003	168	1	DIBNZFURAN	132-64-9
fluorene-d_{10}	1395	1.119	176	1		
fluorene	1401	1.004	166	1	FLUORENE	86-73-7
4-chlorophenylphenyl ether-d_5	1406	1.209	209	1		
4-chlorophenylphenyl ether	1409	1.002	204	1	4CPPE	7005-72-3
diethyl phthalate-d_4	1409	1.211	153	1		
diethyl phthalate	1414	1.004	149	1	DEP	84-66-2
2,4-dinitrotoluene-d_3	1344	1.156	168	2		
2,4-dinitrotoluene	1359	1.011	165	2	24-2NTOL	121-14-2
1,2-diphenylhydrazine-d_8	1433	1.232	82	1		
1,2-diphenylhydrazine[(3)]	1439	1.004	77	1	12-2PHHYZ	122-66-7

TABLE I-1. (Continued)

diphenylamine-d_{10}	1437	1.236	179	1		
diphenylamine	1439	1.001	169	1	DPA	122-39-4
N-nitrosodiphenylamine-d_6	1438	1.236	175	1		
N-nitrosodiphenylamine[4]	1439	1.001	169	1	NNP	86-30-6
4-bromophenyl phenyl ether	1498	1.288	248	1	4-BPPE	101-55-3
hexachlorobenzene-$^{13}C_6$	1521	1.308	292	1		
hexachlorobenzene	1522	1.000	284	1	6CLBNZ	118-74-1
phenanthrene-d_{10}	1578	1.357	188	1		
phenanthrene	1583	1.003	178	1	PHENANTHRN	85-01-8
anthracene-d_{10}	1586	1.365	188	1		
anthracene	1592	1.003	178	1	ANTHRACENE	120-12-7
dibenzothiophene-d_8	1559	1.340	192	1		
dibenzothiophene	1564	1.003	184	1	DIBNZTHIO	132-75-0
carbazole	1650	1.419	167	3	CARBAZOLE	86-74-2
di-n-butyl phthalate-d_4	1719	1.478	153	1		
di-n-butyl phthalate	1723	1.002	149	1	DINBP	84-74-2
fluoranthene-d_{10}	1813	1.559	212	1		
fluoranthene	1817	1.002	202	1	FLUORANTHN	206-44-0
pyrene-d_{10}	1844	1.586	212	1		
pyrene	1852	1.004	202	1	PYRENE	129-00-0
benzidine-d_8	1854	1.594	192	5		
benzidine	1855	1.000	184	5	BZID	92-87-5
butylbenzyl phthalate	2060	1.771	149	1	BUTBNZPHT	85-68-7
chrysene-d_{12}	2081	1.789	240	1		
chrysene	2083	1.001	228	1	CHRYSENE	218-01-9
benzo(a)anthracene-d_{12}	2082	1.790	240	1		
benzo(a)anthracene	2090	1.004	228	1	BAA	56-55-3
3,3'-dichlorobenzidine-d_6	2086	1.794	258	5		
3,3'-dichlorobenzidine	2088	1.001	252	5	33-2CLBZID	91-94-1
bis(2-ethylhexyl)phthalate-d_4	2123	1.825	153	1		
bis(2-ethylhexyl)phthalate	2124	1.000	149	1	B2ETHXPHTH	117-81-7
di-n-octyl phthalate-d_4	2239	1.925	153	1		
di-n-octyl phthalate	2240	1.000	149	1	2NOCTP	117-84-0
benzo(b)fluoranthene-d_{12}	2281	1.961	264	1		
benzo(b)fluoranthene	2286	1.002	252	1	BBF	205-99-2
benzo(k)fluoranthene-d_{12}	2287	1.966	264	1		
benzo(k)fluoranthene	2293	1.003	252	1	BKF	207-08-9
benzo(a)pyrene-d_{12}	2350	2.021	264	1		
benzo(a)pyrene	2352	1.001	252	1	BAP	50-32-8
benzo(g,h,i)perylene-d_{12}	2741	2.357	288	2		
benzo(g,h,i)perylene	2750	1.003	276	2	BGHIP	191-24-2
indeno(1,2,3-c,d)pyrene	2650	0.967	276	2	INDENO-PYR	193-39-5
dibenzo(a,h)anthracene	2660	0.970	278	2	DBAHA	53-70-3
2-chlorophenol-d_4	701	0.603	132	1		
2-chlorophenol	705	1.006	128	1	2-CLPHN	95-57-8
2-nitrophenol-d_4	898	0.772	143	2		
2-nitrophenol	900	1.002	139	2	2-NPHN	88-75-5
2,4-dichlorophenol-d_3	944	0.812	167	1		
2,4-dichlorophenol	947	1.003	162	1	24-2CLPHN	120-83-2
4-chloro-3-methylphenol-d_2	1086	0.934	109	2		
4-chloro-3-methylphenol	1091	1.005	107	2	4-CL2-MPHN	59-50-7
2,4,6-trichlorophenol-d_2	1162	0.999	200	2		
2,4,6-trichlorophenol	1165	1.003	196	2	246-3CLPHN	88-06-2
2,4,5-trichlorophenol	1170	1.007	196	2		
2,3,6-trichlorophenol	1195	1.028	196	2	236-3CLPHN	93-37-55
2,4-dinitrophenol-d_3	1323	1.138	187	20		
2,4-dinitrophenol	1325	1.002	184	20	24-2NPHN	51-28-5
4-nitrophenol-d_4	1349	1.160	143	6		
4-nitrophenol	1354	1.004	139	6	4-NPHN	100-02-7
2-methyl-4,6-dinitrophenol-d_2	1433	1.232	200	13		
2-methyl-4,6-dinitrophenol	1435	1.001	198	13	46-2NOCRES	534-52-1
pentachlorophenol-$^{13}C_6$	1559	1.340	272	5		
pentachlorophenol	1561	1.001	266	5	5CLPHN	87-86-5

a Relative retention times for labeled compounds are referenced to DFB. Relative retention times for unlabeled compounds are referenced to their labeled analogs or to the most chemically similar, most closely eluting labeled compounds if labeled analogs are not listed.

TABLE I-1. (Continued)

Compound	Relative Retention Time to DFB	Quantitation (Primary) m/z	GC/MS (ng)	GC/ECD (pg)	ODES	CASRN
decafluorobenzophenone (DFBP)	0.736	-	-	5		
toxaphene mixture	1.2-1.9	231, 233	10	100	TOXAPHENE	8001-35-2
Aroclor 1242 (PCB mixture)			10	100	PCBS	53469-21-9
Aroclor 1254 (PCB mixture)			10	100	PCBS	11097-69-1
Aroclor 1260 (PCB mixture)			10	100	PCBS	11096-82-5
α-HCH	1.32	183, 181	2	20	6CL-CHX-A	319-84-6
β-HCH	1.36	183, 181	3	5	6CL-CHX-B	319-84-7
γ-HCH	1.41	183, 181	2	5	LINDANE	319-86-8
δ-HCH	1.43	183, 181	3	20	6CL-CHX-D	58-89-9
aldrin	1.64	263, 265	2	5	ALDRIN	309-00-2
heptachlor	1.70	100, 272	2	5	HEPTACHLOR	76-44-8
heptachlor epoxide	1.83	353, 355	2	15	HEPCL EPOX	1024-57-3
γ-chlordane	1.85	373, 375	2	10		
α-endosulfan	1.88	195, 207	2	5	ENDOSULFAN	115-29-7
α-chlordane	1.91	373, 375	2	5		
dieldrin	1.98	241, 263	2	5	DIELDRIN	60-57-1
4,4'-DDE	2.00	246, 248	2	30	DDE	72-55-9
β-endosulfan	2.02	207, 195	2	5		115-29-7
endrin	2.02	263, 277	2	5	ENDRIN	72-20-8
endrin aldehyde	2.07			30	ENDRIN-ALD	7421-93-4
4,4'-DDD	2.10	235, 165	2	30	DDD	72-54-8
endosulfan sulfate	2.13	272, 387	5	30	ENDOSLFN-S	1031-07-8
γ-chlordene	2.13	336, 338	2	10		
4,4'-DDT	2.17	235, 237	2	-(5)	DDT	50-29-3
2,3,7,8-TCDD(6)	2.01	320, 322	3	40	DIOXIN	1746-01-6
Additional 301(h) Pesticides:						
Demeton					SYSTOX	8065-48-3
Guthion					GUTHION	86-50-0
Malathion	1.19	127, 99, 174			MALATHION	121-75-5
Parathion	1.19	291, 109, 139			PARATHION	56-38-2
Methoxychlor	1.51	238, 227, 274		50	METHOXYCL	72-43-5
Mirex	1.52	272, 237, 274		100	MIREX	2385-85-5

[1] Deuterium labeled recovery (surrogate) standard; isotopically labeled surrogates do not have ODES codes.

[2] ^{13}C-labeled recovery (surrogate) standard; isotopically labeled surrogates do not have ODES codes.

[3] Detected as azobenzene.

[4] Detected as diphenylamine.

[5] Low level amounts (<2 ng) of DDT are dehydrohalogenated and converted to DDE at variable rates on the GC system.

[6] Acceptable detection limits will be attainable with the U.S. EPA Contract Laboratory Program Dioxin Analysis procedure [Soil/Sediment Matrix, Multi-Concentration, Selected Ion Monitoring (SIM) GC/MS Analysis; 9/15/83].

Column: 30 m x 0.25 mm i.d., 94% methyl, 4% phenyl, 1% vinyl bonded phase fused silica capillary (J&W DB-5, or equivalent)

Temperature program (GC/MS): 5 min at 30° C; 30-280° C at 8° C per min; isothermal at 280° C until benzo(g, h,i)perylene elutes.

Carrier gas linear velocity: 30 cm/sec, helium.

TABLE I-2. DFTPP MASS-INTENSITY SPECIFICATION

Mass	Intensity Required
51	30-60% of mass 198
68	Less than 2% of mass 69
70	Less than 2% of mass 69
127	40-60% of mass 198
197	Less than 1% of mass 198
198	Base peak, 100% relative abundance
199	5-9% of mass 198
275	10-30% of mass 198
365	1% of mass 198
441	Less than mass 443
442	Greater than 40% of mass 198
443	17-23% of mass 442

TABLE I-3. PRECISION AND ACCURACY OF METHOD BLANKS

EPA Priority Pollutants	Percent Recovery[a] Blank 1	Blank 2	Blank 3	Blank 4	Mean	Coeff. of Variation
Phenols						
phenol	96	102	91	96	96	4.7
2,4-dimethylphenol	92	120	110	97	105	5.0
2-chlorophenol	100	102	110	104	104	4.2
2,4-dichlorophenol	98	101	100	110	102	5.2
4-chloro-3-methylphenol	99	97	110	100	102	5.7
2,4,6-trichlorophenol	98	100	140	100	110	18.6
pentachlorophenol	110	110	120	110	113	4.4
2-nitrophenol	96	99	120	110	106	10.3
4-nitrophenol	110	110	110	110	110	0.0
2,4-dinitrophenol	99	100	98	110	102	5.5
4,6-dinitro-2-methylphenol	110	100	110	110	108	4.7
Aromatic Hydrocarbons						
naphthalene	110	110	120	120	115	5.0
acenaphthene	120	110	120	120	118	4.3
acenaphthylene	110	110	120	120	115	5.0
fluorene	120	120	130	120	123	4.1
phenanthrene	120	120	120	130	123	4.1
anthracene	120	120	110	130	120	6.8
fluoranthene	120	120	130	130	125	4.6
pyrene	120	110	150	130	129	13.4
benz(a)anthracene	120	110	95	110	109	9.5
chrysene	120	120	150	130	130	10.9
benzo(b)fluoranthene	100	102	110	110	106	5.0
benzo(k)fluoranthene	110	113	120	110	113	4.2
benzo(a)pyrene	130	125	130	130	129	1.9
indeno(1,2,3-cd)pyrene	160	161	170	160	163	3.0
dibenzo(a,h)anthracene	150	189	180	190	177	10.6
benzo(ghi)perylene	120	120	120	120	120	0.0
Chlorinated Hydrocarbons						
1,2-dichlorobenzene	100	110	110	110	108	4.7
1,3-dichlorobenzene	87	120	94	120	105	16.4
1,4-dichlorobenzene	110	120	120	130	120	6.8
1,2,4-trichlorobenzene	93	98	130	100	105	15.9
2-chloronaphthalene	110	110	120	120	115	5.0
hexachlorobenzene	160	110	120	110	125	19.0
hexachloroethane	73	104	69	77	81	19.6
hexachlorobutadiene	98	105	130	110	111	12.4
hexachlorocyclopentadiene	23	25	24	25	24	3.9

TABLE I-3. (Continued)

Phthalates						
bis(2-ethylhexyl)phthalate	120	130	120	130	125	4.6
benzylbutylphthalate[b]	270	298	120	310	250	35.2
di-n-butylphthalate	120	120	130	120	123	4.1
di-n-octylphthalate	120	120	120	120	120	0.0
diethylphthalate	120	120	130	120	123	4.1
dimethylphthalate	110	120	140	120	123	10.3
Halogenated Ethers						
bis(2-chloroethyl)ether	91	93	91	100	94	4.6
bis(2-chloroisopropyl)ether	c	c	c	61	---	---
bis(2-chloroethoxy)methane	200	110	190	140	160	26.5
4-chlorophenylphenylether	120	120	140	120	125	8.0
4-bromophenylphenylether	150	140	120	150	140	10.1
Organonitrogen Compounds						
nitrobenzene	37	21	19	c	19	38.4
N-nitrosodipropylamine	26	135	150	73	96	59.7
N-nitrosodimethylamine	110	120	110	120	115	5.0
N-nitrosodiphenylamine	76	89	173	77	104	44.9
2,4-dinitrotoluene	120	120	120	100	115	8.7
2,6-dinitrotoluene	130	74	130	100	109	24.9
benzidine	0	0	0	0		
3,3'-dichlorobenzidine	120	170	140	120	138	17.2
1,2-diphenylhydrazine	120	110	140	100	118	14.5
Miscellaneous						
isophorone	130	120	72	120	111	23.6

[a] Method blanks were processed after spiking with known amounts of unlabeled and labeled compounds. Recovery-corrected concentration of unlabeled priority pollutants was calculated using the recovery of labeled analogs for each compound. The final percent recovery for the unlabeled compounds was then computed as the ratio of the calculated concentration to the known spike level of each compound.

[b] Benzylbutylphthalate results are anomolously high because of laboratory contamination traced to mercury used in the sulfur-removal step of the procedure. The contamination was subsequently eliminated.

[c] Spectral interferences precluded quantification.

SECTION II

ANALYSIS OF VOLATILE ORGANIC COMPOUNDS IN ESTUARINE AND MARINE TISSUES

CONTENTS

ANALYSIS OF VOLATILE ORGANIC COMPOUNDS IN ESTUARINE AND MARINE TISSUES

1.0 SCOPE AND APPLICATION

1.1 This method is designed to determine the volatile toxic organic pollutants associated with Clean Water Act Section 301(h) regulation [40 CFR 125.58(k) and (v)] and additional compounds amenable to purge and trap gas chromatography-mass spectrometry (GC/MS) (Table II-1).

1.2 The chemical compounds listed in Table II-1 can be determined in biological tissue samples collected from estuarine and marine environments by this method.

1.3 The detection limit of this method is usually dependent on the level of interferences rather than instrumental limitations.

Lower limits of detection (LLD) are established by analysts based on their experience with the instrumentation and with interferences in the sample matrix being analyzed. LLD are greater than instrumental detection limits because they take into account sample interferences. To estimate LLD, the noise level should be determined in the retention window for the quantitation mass of representative analytes. These determinations should be made for at least three field samples in the sample set under analysis. The signal required to exceed the average noise level by at least a factor of two should then be estimated. This signal is the minimum response required to identify a potential signal for quantification. The LLD is the concentration corresponding to the level of this signal based on calibrated response factors. Based on best professional judgment, this LLD would then be applied to samples in the set with comparable or lower interference. Samples with much higher interferences (e.g., at least a factor of two higher) should be assigned LLD at a multiple of the original LLD.

These LLD values may be less than the rigorously defined method detection limits specified in the revised "Guidelines Establishing Test Procedures for the Analysis of Pollutants" (40 CFR Part 136, 10/26/84). This latter procedure requires the analysis of seven replicate samples and a statistical determination of th emethod detection limit with 99 percent confidence. Data quantified between the LLD and the rigorous method detection limit are valid and useful in environmental investigations of low-level contamination, but have a lower statistical confidence associated with them than data quantified above the method detection limit.

The LLD are roughly 5-10 ppb (wet weight) with the exception of acrolein and acrylonitrile, which have not been thoroughly tested on tissue matrices with this method.

2.0 SUMMARY OF METHOD

2.1 Volatile organic compounds are vacuum extracted from a macerated, 5-g (wet wt) tissue sample and concentrated in a cryogenic trap cooled with liquid nitrogen (references 1 and 2). The cryogenic trap is then transferred to a conventional purge-and-trap device. The extract is diluted to a 5 mL volume with water and treated as an aqueous sample. In the purge-and-trap device, the volatile organic compounds are purged from the aqueous phase into a gaseous phase with an inert carrier gas. The volatile compounds are passed into a sorbent column and trapped. After purging is completed, the trap is backflushed and heated rapidly to desorb the compounds into a gas chromatograph (GC). The compounds are separated by GC and detected with a mass spectrometer (MS).

Analysis is carried out by GC/MS either according to the isotope dilution technique (U.S. EPA Method 1624 Revision B; reference 3) or U.S. EPA Method 624 (reference 4). Both of these methods were developed for water/wastewater sample matrices. The isotope dilution technique, which requires spiking the sample with a mixture of stable isotope labeled analogs of the analytes, is the technique of choice because it provides reliable recovery data for

each analyte. Method 624 requires spiking samples with only three surrogate compounds and does not allow for recovery corrections. If uniformly high recoveries can be attained with Method 624, then addition of numerous labeled compounds (Method 1624B) and recovery corrections are unnecessary. However, until such performance can be demonstrated, Method 1624B provides a detailed and valuable assessment of analytical performance.

Hiatt (reference 5) proposed another vacuum distillation procedure that did not include a purge-and-trap device. In this technique, volatile organic compounds are transferred directly from a cryogenically cooled trap to a fused-silica capillary column for GC/MS analysis. This capillary column technique allows for optimum resolution and rapid conditioning between samples. However, the performance of the technique has not been thoroughly tested (reference 6). A potential problem is that water can enter the capillary column and cause chromatographic problems or freeze, effectively plugging the column. Thus, Hiatt's original procedure (references 1 and 2), which has been tested more thoroughly, is recommended here.

Vacuum distillation is recommended rather than direct purge-and-trap extraction (i.e., without vacuum distillation) because the former technique has been demonstrated to allow for better recoveries of spiked compounds than the latter technique (reference 1; comparisons based on similar spiking levels).

2.2 Laboratories may use alternative analytical procedures provided that evidence of performance comparable to the recommended procedure is provided.

3.0 INTERFERENCES

3.1 Impurities in the purge gas, organic compounds out-gassing from the plumbing upstream of the trap, and solvent vapors in the laboratory account for the majority of contamination problems. The analytical system is demonstrated to be free from interferences under conditions of the analysis by analyzing blanks initially and with each sample lot (samples analyzed

on the same 8 h shift), as described in Sect. 9.3. Common laboratory solvents (e.g., methylene chloride) are often contaminants in volatiles analyses.

3.1.1 Newly packed traps should be conditioned overnight at 170°-180° C by backflushing with an inert gas at a flow rate of 20-30 mL/min. Traps must be conditioned daily for a minimum of 10 min before use.

3.2 There is potential for ambient contamination of samples and extracts when using vacuum and cryogenic concentration techniques. Care must be taken to eliminate any leaks in the vacuum extraction and concentration device. A critical source of potential contamination is pump oil vapor and exhaust from the vacuum pump; this should not be a problem if the system is properly sealed. A cold trap is placed between the vacuum pump and concentration trap to prevent condensation of pump oil vapors in the concentration trap (Figure II-1). All materials in the vacuum extraction and concentration device that contact the sample and its vapors must be made of stainless steel and/or borosilicate glass. All connections and seals must be free of elastomers or grease that either outgas or allow penetration of ambient contaminant vapors.

3.3 Samples can be contaminated by diffusion of volatile organic compounds (particularly methylene chloride) through the bottle seal during shipment and storage. A field blank prepared from reagent water and carried through the sampling and handling protocol serves as a check on such contamination.

3.4 Contamination by carry-over can occur when high level and low level samples are analyzed sequentially. When an unusually contaminated sample is analyzed, it should be followed by analysis of a reagent water blank to check for carry-over. Because the transfer lines, trap, and other parts of the system can retain contaminants and interferences, frequent bakeout and purging of the entire system may be required.

4.0 SAFETY

4.1 The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined; however, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of data handling sheets should also be made available to all personnel involved in these analyses. Additional information on laboratory safety can be found in references 7-9.

4.2 The following compounds covered by this method have been tentatively classified as known or suspected human or mammalian carcinogens: benzene, carbon tetrachloride, chloroform, and vinyl chloride. Primary standards of these toxic compounds should be prepared in a hood, and a NIOSH/MESA-approved toxic gas respirator should be worn when high concentrations are handled.

4.3 The following safety measures must be employed when handling cryogenic and vacuum systems:

4.3.1 Liquid nitrogen (LN_2) must not be allowed to contact flesh since it will cause extreme frostbite and deaden (kill) tissues.

4.3.2 The concentrator and cold traps must never be closed off or sealed after allowing any concentration of liquid air. The liquid air will vaporize, resulting in tremendous pressure build up and explosive damage to the vacuum system. Always vent any vessel immediately after removing the cryogenic or LN_2 bath. Wear safety goggles when working with cryogenic and vacuum systems.

5.0 APPARATUS AND EQUIPMENT

5.1 Sample Handling Equipment

5.1.1 Stainless steel spatula, rinsed with methanol and oven-dried at 150° C.

5.1.2 Sample vessel - Pyrex flask with 15 mm O-ring connector, washed with detergent and rinsed with distilled water and oven-dried at 450° C.

5.1.3 O-ring, Buna N, sonicated with 50 percent methanol/water then dried by vacuum at 60° C.

5.1.4 Tissue homogenizer (e.g., Tekmar Tissuemizer, Tekmar Co., Cincinnati, OH) - must be free of volatiles and solvents before use.

5.2 Apparatus for Vacuum Distillation and Cryogenic Concentration (Figure II-1).

5.2.1 Vacuum pump, capable of achieving 10^{-3} Torr and 25 L/min.

5.2.2 Vaccum/pressure gauge - with a range of subatmospheric pressure to 10 psi.

5.2.3 Concentrator trap or purge flask, 25 mL capacity (Tekmar Part No. 14-0957-024 or equivalent) modified with 9 mm O-ring connectors.

5.2.4 Cold trap - glass trap (easily produced by glassblowing, Figure II-1) with O-ring fittings (e.g., Kontes 671750-009).

5.2.5 Transfer line, 1/4 in o.d. glass-lined stainless steel tubing. Lines should be kept as short as possible to minimize sample carryover.

5.2.6 Vacuum valves, Nupro B-4BKT or equivalent.

5.2.7 Dewar flasks, 665 mL or 1,000 mL, for liquid nitrogen bath.

5.2.8 Assorted compression fittings and graphite ferrules (Figure II-1).

5.2.9 Ultrasonic bath, Branisonic 12 or equivalent.

5.2.10 Heater tape - to wrap around stainless steel lines and valve bodies to maintain a temperature of 60° C.

5.2.11 Pinch clamps, Thomas - to secure O-ring connections.

5.3 Purge-and-Trap Device - capable of meeting specifications listed in U.S. EPA Method 1624 B (see below). Complete devices consisting of a purging device (the concentrator trap, Sect. 5.2.3), a Tenax/silica trap, and a desorber are commercially available (e.g., Tekmar Model LSC-2, Tekmar Co., Cincinnati, OH).

5.3.1 Trap - 25 to 30 cm x 2.5 mm i.d. minimum, containing the following:

5.3.1.1 Methyl silicone packing - one ±0.2 cm, 3 percent OV-1 on 60/80 mesh Chromosorb W, or equivalent.

5.3.1.2 Porous polymer - 15 ±1.0 cm, Tenax GC (2,6-diphenylene oxide polymer), 60/80 mesh, chromatographic grade, or equivalent.

5.3.1.3 Silica gel - 8 ±1.0 cm, Davison Chemical, 35/60 mesh, grade 15, or equivalent.

5.3.2 Desorber - should heat the trap to 175 ±5° C in 45 sec or less. The polymer section of the trap should not exceed 180° C, and the remaining sections should not exceed 220° C.

5.3.3 Commercial purge and trap devices are easily coupled to GC systems.

5.4 GC/MS (Gas Chromatograph-Mass Spectrometer) System.

5.4.1 GC - should be linearly temperature programmable with initial and final temperature holds.

5.4.2 GC column - 6 ft long x 0.1 in i.d. (stainless steel or glass) packed with 1 percent SP-1000 on Carbopak B, 60/80 mesh or equivalent.

5.4.3 MS - 70 eV electron impact ionization; capable of repeatedly scanning from 20 to 250 amu every 2 to 3 sec.

5.4.4 GC/MS interface - GC to MS interfaces constructed of all-glass or glass-lined materials are recommended. Glass can be deactivated by silanizing with dichloro-dimethyl silane.

5.5 Data System - should collect and record MS data, store mass intensity data in spectral libraries, process GC/MS data and generate reports, and should calculate and record response factors.

5.5.1 Data acquisition - mass spectra should be collected continuously throughout the analysis and stored on a mass storage device.

5.5.2 Mass spectral libraries - user created libraries containing mass spectra obtained from analysis of authentic standards should be employed to reverse search GC/MS runs for the compounds of interest.

5.5.3 Data processing - the data system should be used to search, locate, identify, and quantify the compounds of interest in each GC/MS analysis. Software routines should be employed to compute retention times and extracted ion current profile (EICP) areas. Displays of spectra, mass chromatograms, and library comparisons are required to verify results.

5.5.4 Response factors and multipoint calibrations -- the data system should be used to record and maintain lists of response factors (response ratios for isotope dilution) and generate multi-point calibration curves.

Computations of relative standard deviation (coefficient of variation) are useful for testing calibration linearity.

5.6 Other Materials

5.6.1 Syringe, 10 uL ± 1 percent of volume.

5.6.2 Syringe, 50 uL ± 1 percent of volume.

5.6.3 Syringe, 5 mL ± 1 percent of volume, gas-tight with shut-off.

5.6.4 Bubble flowmeter.

6.0 REAGENTS AND CONSUMABLE MATERIALS

6.1 Reagent Water

6.1.1 Reagent water is defined as water free of interferences (i.e., interferents are not observed at the detection limits of the compounds of interest).

6.1.2 Prepare water by boiling 1 L of freshly distilled water down to 900 mL and transferring the water to a 1-L volumetric flask that has been modified by replacing the ground glass joint with a 15-mm i.d., Buna-N O-ring connector.

6.1.3 Connect the flask to the distillation apparatus at the sample chamber site and evacuate for 15 min while continuously agitating the flask in an ultrasonic cleaner.

6.1.4 After evacuation, release an inert gas (N_2 or He can be used) into the flask until equilibrium is obtained, then seal with a cap made from a Buna-N O-ring connector.

6.2 Methanol - pesticide quality or equivalent.

6.3 Stancard Solutions - purchased as solutions or mixtures with certification as to their purity, concentration, and authenticity, or prepared from materials of known purity and composition. If compound purity is 96 percent or greater, the weight may be used without correction to calculate the concentration of the standard.

6.4 Preparation of Stock Solutions - prepare in methanol using liquid or gaseous standards per the steps below. Observe the safety precautions given in Sect. 4.

6.4.1 Place approximately 9.8 mL of methanol in a 10 mL ground glass stoppered volumetric flask. Allow the flask to stand unstoppered for approximately 10 min or until all methanol-wetted surfaces have dried. In each case, weigh the flask, immediately add the compound, then immediately reweigh to prevent evaporation losses from affecting the measurement.

6.4.1.1 Liquids - using a 100 uL syringe, permit two drops of liquid to fall into the methanol without contacting the neck of the flask. Alternatively, inject a known volume of the compound into the methanol in the flask using a micro-syringe. With the exception of 2-chloroethylvinyl ether, stock standards of compounds that boil above room temperature are generally stable for at least 4 wk when stored at 4° C.

6.4.1.2 Gases (chloromethane, bromomethane, chloroethane, vinyl chloride) - fill a valved 5 mL gas-tight syringe with the compound. Lower the needle to approximately 5 mm above the methanol meniscus. Slowly introduce the compound above the surface of the meniscus. The gas will dissolve rapidly in the methanol.

6.4.2 Fill the flask to volume, stopper, then mix by inverting several times. Calculate the concentration in mg/mL (ug/uL) from the weight gain (or density if a known volume was injected).

6.4.3 Transfer the stock solution to a Teflon sealed screw-cap bottle. Store, with minimal headspace, in the dark at -10 to -20° C.

6.4.4 Prepare fresh standards weekly for the gases and 2-chloroethylvinyl ether. All other standards are replaced after 1 mo, or sooner if comparison with check standards indicates a change in concentration of over 10 percent. Quality control check standards that can be used to determine the accuracy of calibration standards are available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

6.5 Labeled Compound Spiking Solution - from stock standard solutions prepared as above, or from mixtures, prepare the spiking solution to contain a concentration such that a 5-10 uL spike into each 5 mL sample "extract", blank, or aqueous standard analyzed will result in a concentration of 10 ng/mL of each labeled compound. For the gases and for the water soluble compounds (acrolein, acrylonitrile), a concentration of 50 ng/mL may be used. Include the internal standards (Sect. 8.1.2) in this solution so that a concentration of 10 ng/mL in each sample, blank, or aqueous standard will be produced.

6.6 Secondary Standards - using stock solutions, prepare a secondary standard in methanol to contain each pollutant at a concentration of 250 ug/mL. For the gases and water soluble compounds (Sect. 6.5), a concentration of 1.25 mg/mL may be used.

6.7 Aqueous Calibration Standards - the concentrations of calibration solutions suggested in this section are intended to bracket concentrations that will be encountered during sample analysis that will not overload the analytical system. Use sufficient amounts of the secondary standard (Sect. 6.6) and reagent water to produce concentrations of 5, 10, 20, 50, and 100 ug/L in the aqueous calibration standards. The concentrations of gases and water soluble compounds will be higher (i.e., 25, 50, 100, 250, and 500 ug/L). Analysts may use a wider range of standard concentrations if linearity can be demonstrated.

6.8 Aqueous Performance Standard - an aqueous standard containing all pollutants, internal standards, labeled compounds, and BFB (4-bromofluorobenzene) is prepared daily, and analyzed each shift to demonstrate performance (Sect. 11). This standard should contain either 10 or 50 ug/L of the labeled and pollutant gases and water soluble compounds, 5 ug/L of BFB, and 10 ug/L of all other pollutants, labeled compounds, and internal standards. It may be the nominal 10 ug/L aqueous calibration standard (Sect. 6.7).

6.9 A methanolic standard containing all pollutants and internal standards is prepared to demonstrate recovery of these compounds when syringe injection and purge-and-trap analyses are compared. This standard should contain either 10 ug/mL or 50 ug/mL of the gases and water soluble compounds, and 10 ug/mL of the remaining pollutants and internal standards (consistent with the amounts in the aqueous performance standard in Sect. 6.8).

6.10 Other standards that may be needed are those for testing of BFB performance (Sect. 8.2.1) and for collecting mass spectra for storage in spectral libraries (Sect. 8.1.4).

6.11 High Purity Helium - 99.999 percent.

6.12 Liquid Nitrogen (LN_2).

7.0 SAMPLE COLLECTION, PREPARATION, AND STORAGE

7.1 In the field, sources of contamination include sampling gear, grease from ship winches or cables, ship engine exhaust, dust, and ice used for cooling. Efforts should be made to minimize handling and to avoid sources of contamination. This will usually require that resection (i.e., surgical removal) of tissue be performed in a controlled environment (e.g., a laboratory). For example to avoid contamination from ice, the samples should be wrapped in aluminum foil, placed in watertight plastic bags and immediately iced in a covered ice chest. Aluminum foil should be cleaned by heating at over 105° C before use. Solvent cleaning is unacceptable unless heating is performed afterward. Organisms should not be frozen prior to resection

if analyses will only be conducted on selected tissues, because freezing may cause internal organs to rupture and contaminate other tissue (e.g., muscle). If organisms are eviscerated on board the survey vessel, the remaining tissue may be wrapped as described above and frozen.

7.2 To avoid cross-contamination, all equipment used in sample handling should be thoroughly cleaned before each sample is processed. All instruments must be of a material that can be easily cleaned (e.g., stainless steel, anodized aluminum, or borosilicate glass). Before the next sample is processed, instruments should be washed with a detergent solution, rinsed with tap water, soaked in high-purity acetone and methylene chloride, and finally rinsed with reagent water.

7.3 Resection should be carried out by or under the supervision of a competent biologist. Each organism should be handled with clean stainless steel or quartz instruments (except for external surfaces). The specimens should come into contact with precleaned glass surfaces only. Polypropylene and polyethylene surfaces are a potential source of contaminatin and should not be used. To control contamination when resecting tissue, separate sets of utensil should be used for removing outer tissue and for dissecting tissue for analysis. For fish samples, special care must be taken to avoid contaminating target tissues (especially muscle) with slime and/or adhering sediment from the fish exterior (skin) during resection. The incision "troughs" are subject to such contamination; thus, they should not be included in the sample. In the case of muscle, a "core" of tissue is taken from within the area boarded by the incision troughs, without contacting them. Unless specifically sought as a sample, the dark muscle tissue that may exist in the vicinity of the lateral line should not be mixed with the light muscle tissue that constitutes the rest of the muscle tissue mass.

7.4 The resected tissue sample should be placed in a clean glass or TFE container which has been washed with detergent, rinsed twice with tap water, rinsed once with distilled water, and heated at 105° C for several hours. Jars should be heated at 105° C and allowed to cool immediately before use.

7.5 The U.S. EPA and other federal agencies (e.g., National Bureau of Standards) have not yet provided specific guidance regarding holding times and temperatures for tissue samples to be analyzed for volatile organic compounds. Until U.S. EPA develops definitive guidance, the following holding conditions should be observed. Resected tissue samples should be maintained at -20° C and analyzed as soon as possible, but within 10 days of sample receipt. The 10 day holding time is based on the Contract Laboratory Program regulations for sediments to be analyzed for volatiles (reference 10).

8.0 CALIBRATION AND STANDARDIZATION

8.1 Initial Calibration

8.1.1 Calibration by the isotope dilution technique -- the isotope dilution technique is used for the purgeable organic compounds when appropriate labeled compounds are available and when interferences do not preclude the analysis. If labeled compounds are not available or interferences are present, the internal standard technique (Sect. 8.1.2) is used. A calibration curve encompassing the concentration range of interest is prepared for each compound determined. The relative response (RR) vs. concentration (ug/L) is plotted or computed using a linear regression. An example of a calibration curve for a pollutant and its labeled analog is given in Figure II-2. Also shown are the ±10 percent error limits (dotted lines). Relative response is determined according to the procedures described below. A minimum of five data points is required for calibration (Sect. 6.7).

8.1.1.1 The relative response (RR) of pollutant to labeled compound is determined from isotope ratio values calculated from acquired data. Three isotope ratios are used in this process:

R_x = the isotope ratio measured in the pure pollutant (Figure II-3A)
R_y = the isotope ratio of pure labeled compound (Figure II-3B)
R_m = the isotope ratio measured in the analytical mixture of the pollutant and labeled compounds (Figure II-3C).

The correct way to calculate RR is:

$$RR = (R_y - R_m)(R_x + 1)/(R_m - R_x)(R_y + 1).$$

If R_m is not between $2R_y$ and $0.5R_x$, the method does not apply and the sample is analyzed by the internal standard technique (Sect. 8.1.2).

8.1.1.2 In most cases, the retention times of the pollutant and labeled compound are the same and isotope ratios (R's) can be calculated from the EICP areas, where:

$$R = (\text{area at } m_1/z)/(\text{area at } m_2/z)$$

If either of the areas is zero, it is assigned a value of one in the calculations; that is, if: area of m_1/z=50,721, and area of m_2/z=0, then R=50721/1= 50720. The m/z's are always selected such that $R_x > R_y$. When there is a difference in retention times (RT) between the pollutant and labeled compounds, special precautions are required to determine the isotope ratios.

R_x, R_y, and R_m are defined as follows:

$$R_x = [\text{area } m_1/z \text{ (at } RT_2)]/1$$
$$R_y = 1/[\text{area } m_2/z \text{ (at } RT_1)]$$
$$R_m = [\text{area } m_1/z \text{ (at } RT_2)]/[\text{area } m_2/z \text{ (at } RT_1)].$$

8.1.1.3 An example of the above calculations can be taken from the data plotted in Figure II-3 for a pollutant and its labeled analog. For these data, R_x=168920/1= 168920, R_y=1/60960=0.00001640, and R_m=96868/82508 =1.174. The RR for the above data is then calculated using the equation given in Sect. 8.1.1.1. For the example, RR=1.174. Note: Not all labeled compounds elute before their pollutant analogs.

8.1.1.4 To calibrate the analytical system by isotope dilution, analyze a 5 mL aliquot of each of the aqueous calibration standards (Sect. 6.7)

spiked with an appropriate constant amount of the labeled compound spiking solution (Sect. 6.5), using the purge and trap procedure in Sect. 10. Compute the RR at each concentration.

8.1.1.5 Linearity - if the ratio of relative response to concentration for any compound is constant (less than 20 percent coefficient of variation) over the five point calibration range, an averaged relative response/concentration ratio may be used for that compound; otherwise, the complete calibration curve for that compound should be used over the 5 point calibration range.

8.1.2 Calibration by internal standard - used when criteria for isotope dilution (Sect. 8.1.1) cannot be met. The method is applied to pollutants having no labeled analog and to the labeled compounds themselves. The internal standards used for volatiles analyses are bromochloromethane, 2-bromo-1-chloropropane, and 1,4-dichlorobutane. Concentrations of the labeled compounds and pollutants without labeled analogs are computed relative to the nearest eluted internal standard.

8.1.2.1 Response factors - calibration requires the determination of response factors (RF), which are defined by the following equation:

$$RF = (A_s \times C_{is})/(A_{is} \times C_s)$$

where:

A_s = the EICP area at the characteristic m/z for the compound in the daily standard

A_{is} = the EICP area at the characteristic m/z for the internal standard

C_{is} = the concentration (ug/L) of the internal standard

C_s = the concentration of the pollutant in the daily standard.

8.1.2.2 The response factor is determined at 5, 10, 20, 50, and 100 ug/L for the pollutants (optionally at five times these concentrations for gases and water soluble pollutants - see Sect. 6.6 and 6.7), in a way

analogous to that for calibration by isotope dilution (Sect. 8.1.1.4). The RF is plotted against concentration for each compound in the standard (C_S) to produce a calibration curve.

8.1.2.3 Linearity - if the response factor (RF) for any compound is constant (less than 35 percent coefficient of variation) over the five point calibration range, an averaged response factor may be used for that compound; otherwise, the complete calibration curve for that compound should be used over the five point range.

8.1.3 Combined calibration - by adding the isotopically labeled compounds and internal standards (Sect. 6.5) to the aqueous calibration standards (Sect. 6.7), a single set of analyses can be used to produce calibration curves for the isotope dilution and internal standard methods.

8.1.4 Mass spectral libraries - detection and identification of the compound of interest during calibration and sample analysis are dependent upon the spectra stored in user created libraries.

8.1.4.1 Obtain a mass spectrum of each pollutant and labeled compound and each internal standard by analyzing an authentic standard either singly or as part of a mixture in which there is no interference between closely eluted components. That only a single compound is present is determined by examination of the spectrum. Fragments not attributable to the compound under study indicate the presence of an interfering compound. Adjust the analytical conditions and scan rate (for this test only) to produce an undistorted spectrum at the GC peak maximum. An undistorted spectrum will usually be obtained if five complete spectra are collected across the upper half of the GC peak. Software algorithms designed to "enhance" the spectrum may eliminate distortion, but may also eliminate authentic ions or introduce other distortion.

8.1.4.2 Obtain the authentic reference spectrum under BFB tuning conditions (Table II-2) to normalize it to spectra from other instruments.

8.1.4.3 The spectrum is edited by saving the five most intense mass spectral peaks and all other mass spectral peaks greater than 10 percent of the base peak. This spectrum is stored for reverse search and for compound confirmation.

8.2 Ongoing Calibration

8.2.1 The BFB standard must be analyzed at the beginning of each 8-h shift. The tuning criteria in Table II-2 must be met before blanks and samples may be analyzed.

8.2.2 At the beginning and end of each 8-h shift, system calibration should be verified by purging the aqueous performance standard (Sect. 6.8).

8.2.2.1 Calibration is tested by computing the concentration of unlabeled compounds by the isotope dilution technique (Sect. 8.1.1) for compounds with labeled analogs. Concentrations of unlabeled compounds without labeled analogs are calculated according to the internal standard technique (Sect. 8.1.2).

A complete (five-point) recalibration should be performed when results vary from predicted concentrations by more than $\pm$25 percent. The last sample analyzed before failing criteria should then be reanalyzed. If the results differ by more than $\pm$20 percent (i.e., twice the median reproducibility for replicate analysis of tissue samples, Table II-3), then it is to be assumed that the instrument was out of control during the original analysis and the earlier data should be rejected. Reanalysis of samples should progress in reverse order until it is determined that there is $\leq$20 percent difference between initial and reanalysis results.

9.0 QUALITY CONTROL [For further guidance, see Quality Assurance/Quality Control (QA/QC) for 301(h) Monitoring Programs: Guidance on Field and Laboratory Methods (Tetra Tech 1986).]

9.1 Each laboratory that uses this method is required to operate a formal quality assurance program. The minimum requirements of this program consist

of an initial demonstration of laboratory capability, analysis of samples spiked with labeled compounds to evaluate and document data quality, and analysis of standards and blanks as tests of continued performance.

9.2 Initial Demonstration of Laboratory Capability

9.2.1 Analyze the aqueous performance standard (Sect. 6.8) according to the purge-and-trap procedure in Sect. 10. Compute the area at the primary m/z (Table II-1) for each compound. Compare these areas to those obtained by injecting one uL of the methanolic standard (Sect. 6.9) to determine compound recovery. The recovery should be greater than 20 percent for the water soluble compounds (acrolein and acrylonitrile), and 60-110 percent for all other compounds. This recovery should be demonstrated initially for each purge-and-trap GC/MS system. The test should be repeated only if the purge and trap or GC/MS systems are modified in any way that might result in a change in recovery.

9.3 Blanks

9.3.1 Reagent water blanks must be analyzed to demonstrate freedom from carry-over (Sect. 3) and contamination.

9.3.1.1 The level at which the purge-and-trap system will carry greater than 5 ug/L of a pollutant of interest into a succeeding blank should be determined by analyzing successively larger concentrations of these compounds. When a sample contains this concentration or more, a blank should be analyzed immediately following this sample to demonstrate no carry-over at the 5 ug/L level.

9.3.1.2 With each sample lot (samples analyzed on the same 8-h shift), a blank should be analyzed immediately after analysis of the aqueous performance standard (Sect. 8.2.2) to demonstrate freedom from contamination. If any of the compounds of interest, except common laboratory contaminants (e.g., methylene chloride and toluene), or any potentially interfering compound is found in a blank at greater than 10 ug/L (assuming a response

factor of 1 relative to the nearest eluted internal standard for compounds not listed in Table II-1), analysis of samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination at this level. This control action also applies if methylene chloride or toluene is detected in a blank at greater than 50 ug/L.

9.4 Sample Spiking

9.4.1 The laboratory should spike all samples with labeled compounds to assess method performance on the sample matrix.

9.4.2 Spike and analyze each sample according to the method beginning in Sect. 10.

9.4.3 Compute the percent recovery (P) of the labeled compounds using the internal standard technique (Sect. 8.1.2).

9.5 Replicates

9.5.1 Replicate analyses (i.e., analyses of two subsamples from the same tissue homogenate) must be performed to monitor laboratory precision.

9.5.2 At least one laboratory duplicate should be run for cases of up to 20 samples. For cases of over 20 samples, one blind triplicate and additional duplicates must be run for a minimum of 5 percent replication overall.

10.0 PROCEDURE

10.1 Sample Processing

10.1.1 Mince tissue sample with a scalpel and homogenize the sample to a uniform consistency with a micro-grinder. Care must be taken to ensure that the micro-grinder is thoroughly cleaned after each use. This usually entails disassembly of the unit. Devices with large surface areas (e.g., blenders, meat grinders) should not be used, as they are difficult to clean

and a small sample is difficult to remove after grinding. Liquid associated with the sample should be retained throughout the procedure.

10.1.2 Dry weight determination - if sample size permits and dry-weight concentrations are required, dry weight determinations may be performed as follows: transfer an aliquot of approximately 3 g (weighed to the nearest 0.1 g) to a preweighed dish. Allow the sample to dry in an oven at 105° C overnight and determine the solid residue weight to the nearest 0.1 g. The percent total solids is calculated as:

$$T_S = [\text{dry residue wt (g)}]/[\text{wet sample wt (g)}]$$

Dry weight determinations should not be made at the cost of having insufficient sample for volatiles analysis. Significant decreases in the size of samples used for extraction will decrease attainable detection limits.

10.1.3 Immediately after homogenization, use a stainless steel spatula to transfer a 5-g aliquot to a preweighed sample vessel (Sect. 5.1.2). Weigh the transferred portion to the nearest 0.1 g.

10.1.4 Spike 50 nanograms of each labeled compound (or 250 nanograms of gaseous and water soluble compounds) into 2 mL of reagent water and add to the sample matrix. Seal the sample vessel with an O-ring connector and clamp and sonicate for 10 min. After sonication, store the sample contained in the sample vessel overnight in a refrigerator/freezer and analyze the next day.

10.2 Vaccum Distillation and Concentration (Reference 2)

10.2.1 The vacuum extractor must be airtight and free of moisture before an extraction can be started.

10.2.2 A clean 100 mL pyrex flask is connected to the vacuum distillation apparatus at the sample vessel site (see Figure II-1), the vacuum pump started, and V_2-V_4 opened to evacuate the apparatus. Line condensation

is prevented by warming the transfer lines while evacuating the system. Heating tape is effective in creating even transfer line temperatures and can be used continuously during the procedure.

10.2.3 The vacuum apparatus is pressurized with helium by closing V_3 and opening V_1. The apparatus is tested for leaks with a helium leak detector or Snoop , and appropriate adjustments are made as necessary. When the apparatus has been found to be airtight, close V_1, open V_3 and then heat the transfer lines and concentrator trap to 100° C for 5 min to eliminate any residual contamination.

10.2.4 The flask containing the sample should be immersed in liquid nitrogen, before the flask is uncapped. To begin the distillation, close V_2 (with V_3 and V_4 remaining open), cool the concentrator trap with a liquid nitrogen bath, and replace the empty sample vessel with the cooled sample flask. Disconnect the vacuum source by closing V_3. Open V_2 to permit vapors from the sample vessel to reach the concentrator trap. Immerse the sample vessel in a 50° C water bath and sonicate for 5 min.

10.2.5 Connect the vacuum source to the sample vessel by opening V_3. The lower pressure hastens the transfer of volatile compounds from the sample to the cooled concentrator trap. After 15 min of vacuum, close V_3 and open V_1 to fill the system with helium to atmospheric pressure. Close V_1 and V_2 to isolate the condensate. The distillation is now completed and the condensate is ready for transfer to a purge-and-trap device. The condensate can be held in the liquid nitrogen bath for up to 1 h prior to analysis. Care should be taken to ensure that moisture does not freeze in the narrow glass tubing in the concentrator trap. Careful drying of the system prior to analysis and maintenance of an airtight system will preclude this problem.

10.2.6 Disconnect the sample concentrator trap from the vacuum apparatus and connect it to the purge-and-trap device. Some outgassing is observed when the sample condensate is melted; therefore, the condensate should be kept frozen until the concentrator trap is attached tc the purge-and-

trap device. After attachment, warm the concentrator trap walls to loosen the condensate and allow the ring of ice formed during condensation to drop to the bottom of the trap. To this partially melted extract add 3 mL of reagent water containing 50 ng of each of the internal standards (bromochloromethane, 2-bromo-1-chloropropane, and 1,4-dichlorobutane). The internal stancards are added after vacuum extraction to allow the analyst tne assess analytical losses of labeled compounds during the extraction/ concentration procedure.

10.3 Purge-and-Trap Procedure

10.3.1 Because commercial purge flasks must be slightly modified (with O-ring fittings) to be attached to the vacuum distillation apparatus, a simple O-ring adapter is necessary to connect the purge flask to the commercial device for which it was designed. The modified purge flask (Sect. 5.2.3) used in this procedure can be fitted to a commercial purge-and-trap device (e.g., a Tekmar ALS interfaced with a Tekmar LSC-2) with 9 mm O-ring fittings fused to short sections of glass tubing. Commercial purge-and-trap devices are almost entirely automated and are easy to operate with manufacturer's instructions.

10.3.2 Purge the extract solution with the concentrator trap immersed in an ice-water bath for 5 min followed by immersion in a 55° C-water bath for an additional 7 min. This provides conditions for reproducibly melting the frozen extracts in order to obtain reproducible purging efficiencies.

10.3.3 The GC conditions for analysis are as follows:

Injector zone temp.	225° C
Initial GC oven temp.	60° C
Final GC temp.	175° C
Initial hold time	3 min
Ramp rate	8° C/min
Final hold time	24 min
Jet separator oven temp.	225° C

10.4 Qualitative Determination - accomplisned by comparison of data from analysis of a sample or blank with data from analysis of the shift standard (Sect. 8.2.2). Identification is confirmed when spectra and retention times agree according to the criteria below.

10.4.1 Labeled compounds and pollutants having no labeled analog:

10.4.1.1 The signals for all characteristic masses stored in the spectral library (Sect. 8.1.4.3) should be present and should maximize within the same two consecutive scans.

10.4.1.2 Either 1) the background corrected EICP areas or 2) the corrected relative intensities of the mass spectral peaks at the GC peak maximum should agree within a factor of two (0.5 to 2 times) for all masses stored in the library.

10.4.1.3 The retention time relative to the nearest eluted internal standard should be within $\pm$7 scans or $\pm$20 sec, whichever is greater, of this difference in the shift standard.

10.4.2 Pollutants having a labeled analog:

10.4.2.1 The signals for all characteristic masses stored in the spectral library should be present and should maximize within the same two consecutive scans.

10.4.2.2 Either 1) the background corrected EICP areas or 2) the corrected relative intensities of the mass spectral peaks at the GC peak maximum should agree within a factor of two for all masses stored in the spectral library.

10.4.2.3 The retention time difference between the pollutant and its labeled analog should agree within $\pm$2 scans or $\pm$6 sec, whichever is greater, of this difference in the shift standard.

10.4.2.4 If the experimental mass spectrum contains masses that are not present in the reference spectrum, an experienced spectrometrist is to determine the presence or absence of the compound.

10.5 Tentatively Identified Compounds (GC/MS Analysis) - The ten non-target peaks of greatest area in the RIC (reconstructed ion chromatogram) should be identified and quantified, if possible.

10.5.1 Guidelines for making tentative identification (reference 10):

(1) Tentative identifications should be based on a forward search of the EPA/NIH mass spectral library. Sample spectra should be visually compared with the most similar library match.

(2) Relative intensities of major ions in the reference spectrum (ions greater than 10 percent of the most abundant ion) should be present in the sample spectrum.

(3) The relative intensities of the major ions should agree within ±20 percent. (Example: For an ion with an abundance of 50 percent in the standard spectra, the corresponding sample ion abundance must be between 30 and 70 percent.)

(4) Molecular ions present in reference spectrum should be present in sample spectrum.

(5) Ions present in the reference spectrum but not in the sample spectrum should be reviewed for possible subtraction from the sample spectrum because of background contamination or co-eluting compounds. Data system library reduction programs can sometimes create these discrepancies.

10.5.1.1 If, in the opinion of the mass spectral specialist, no valid tentative identification can be made, the compound should be reported

as unknown. The mass spectral specialist should give additional classification of the unknown compound if possible (e.g., unknown hydrocarbon, unknown aromatic compound, unknown chlorinated compound). If probable molecular weights can be distinguished, include them.

10.5.2 Tentative quantification - quantification of TIOs will be based on the internal standard technique and an assumed response factor of one (in the absence of data from authentic standards). The uncertain nature of this quantification should be clearly noted in the data report.

11.0 QUANTITATIVE DETERMINATION (CALCULATIONS)

11.1 Isotope Dilution - by adding a known amount of a labeled compound to every sample prior to vacuum distillation, correction for recovery of the pollutant can be made because the pollutant and its labeled analog exhibit similar behavior during purging, desorption, and gas chromatography. Note that pollutants and their labeled analogs are not always retained identically by complex matrices, so their behavior during the extraction step may differ. Use of this technique is to enable correction for analytical losses after extraction, not for matrix recovery.

11.1.1 Relative response (RR) values for sample mixtures are used in conjunction with calibration curves described in Sect. 8.1.1 to determine concentrations directly, so long as labeled compound spiking levels are constant.

11.1.2 For the isotope dilution technique, concentration is calculated as follows:

$$C \text{ (ug/kg, wet wt tissue)} = \frac{C_A \text{ (ug/kg)} \times RR \times n}{\sum_{i=1}^{n} \frac{RR_i \times Z_{Ai}}{Z_i}}$$

where

C_A = the concentration of the stable isotope labeled compound as spiked into the sample

RR = relative response of unlabeled pollutant to isotope labeled surrogate in the sample

RR_i = relative response at i^{th} point in calibration

Z_i = absolute amount of unlabeled compound at i^{th} point of calibration

Z_{Ai} = absolute amount of labeled compound at i^{th} point in calibration

n = number of calibration points.

11.2 Internal Standard - calculate the concentration using the response factor determined from calibration data (Sect. 8.1.2) and the following equation:

Concentration = $(A_S \times C_{iS})/(A_{iS} \times RF)$ where the terms are as defined in Sect. 8.1.2.1, except that C_{iS} is in ug/kg (wet tissue) and A_S is the EICP area at the characteristic m/z for the analyte in the sample.

11.3 If the EICP area at the quantitation mass for any compound exceeds the calibration range of the system, a smaller sample aliquot should be analyzed if possible. However, sample sizes of less than 0.5 g are not recommended because such small samples may not be representative.

11.4 Report results for all pollutants and labeled compounds found in samples, in ug/kg (wet weight, unless dry weight is required) to three significant figures. Pollutants and labeled compounds in blanks should be reported in ng/sample.

12.0 PRECISION AND ACCURACY

12.1 Recoveries from replicate spiked water and tissue analyses are presented in Table II-3 (references 1 and 2). These analyses were not performed with the isotope dilution technique and recovery results are uncorrected.

13.0 REFERENCES

1. Hiatt, M.H., "Analysis of Fish and Sediment for Volatile Priority Pollutants," Anal. Chem. Vol. 53, 1981, pp. 1541-1543.

2. Hiatt, M.H., and T.L. Jones. Isolation of Purgeable Organics from Solid Matrices by Vacuum Distillation. U.S. Environmental Protection Agency, Region IX, Las Vegas Laboratory, 1984.

3. Fed. Register, Volume 49, No. 209, October 26, 1984, pp. 43407-43415.

4. Fed. Register, Volume 49, No. 209, October 26, 1984, pp. 43373-43384.

5. Hiatt, M.H., "Determination of Volatile Organic Compounds in Fish Samples by Vacuum Distillation and Fused Silica Capillary Gas Chromatography/Mass Spectrometry," Anal. Chem. Vol. 55, 1983, pp. 506-516.

6. Hiatt, M.H. 4 November 1985. Personal Communication (phone by Mr. Harry Beller). Analytical Technologies, Incorporated, National City, CA.

7. "Working with Carcinogens," DHEW, PHS, NIOSH, Publication 77-206 (1977).

8. "OSHA Safety and Health Standards, General Industry," 29 CFR 1910, OSHA 2206, (1976).

9. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety (1979).

10. U.S. Environmental Protection Agency. 1984 (revised January, 1985). U.S. EPA Contract Laboratory Program - statement of work for organics analysis, multi-media, multi-concentration. IFB WA 85-J176, J177, J178.

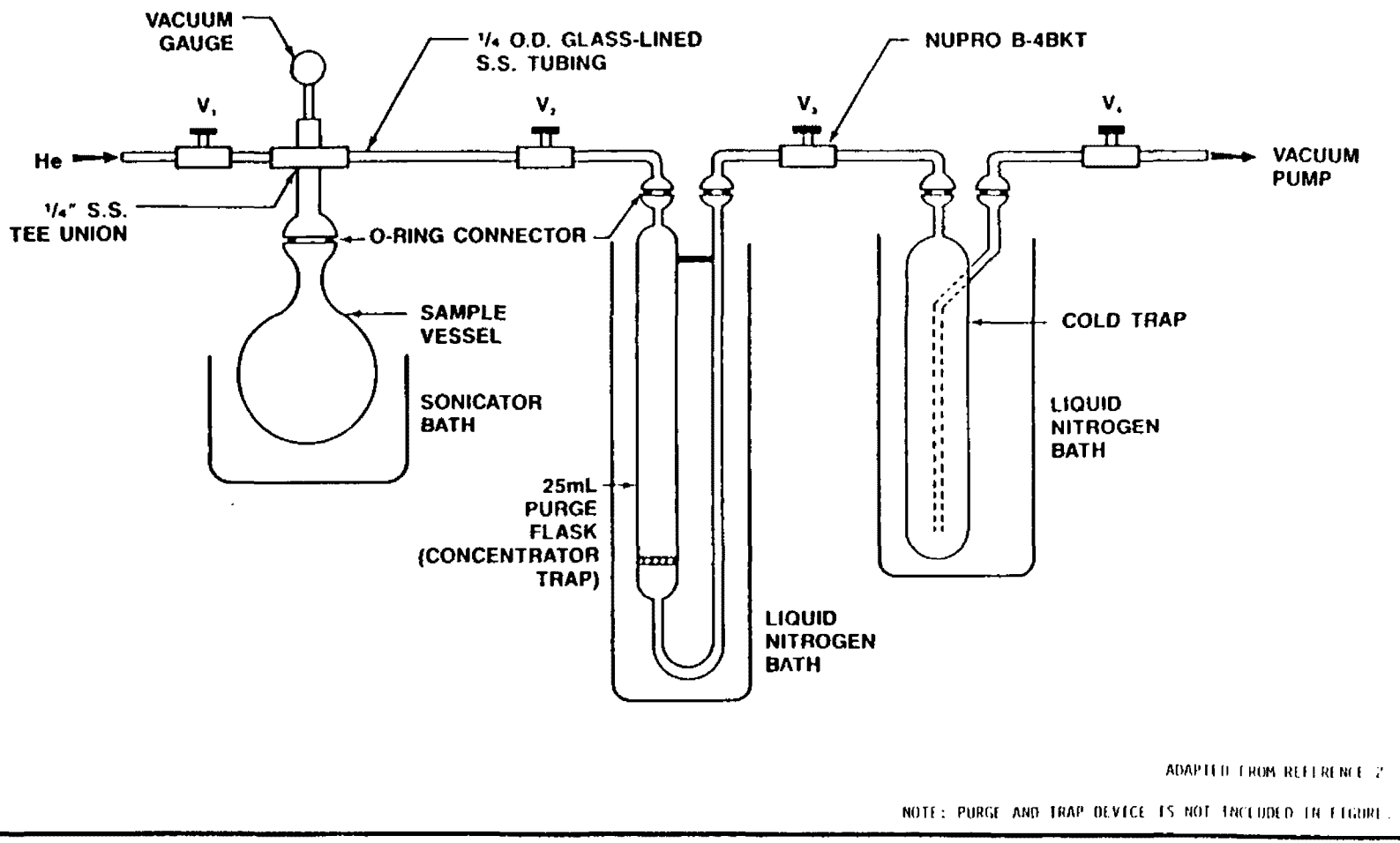
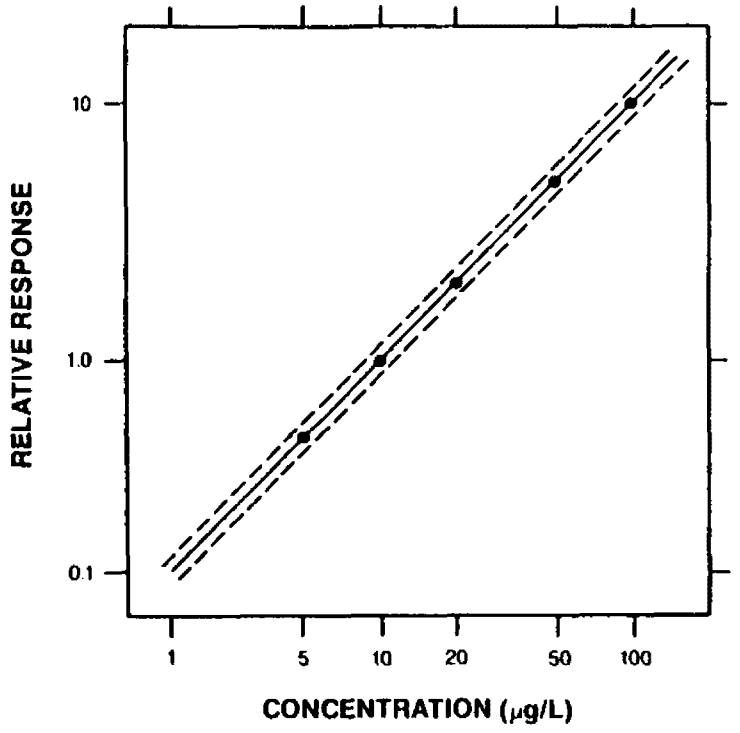
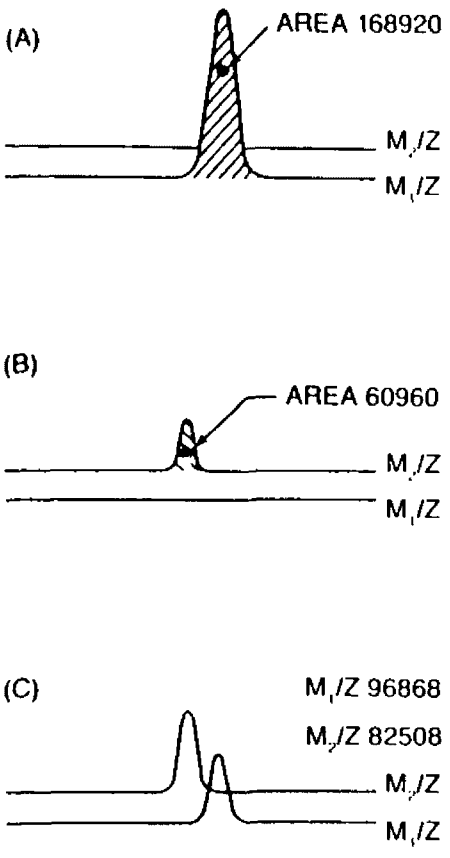


Figure II-1. Apparatus for vacuum distillation and cryogenic concentration.



ADAPTED FROM REFERENCE 3

2.



ADAPTED FROM REFERENCE 3

3.

Figure II-2. Relative response calibration curve.
Figure II-3. Extracted ion current profiles for (A) the unlabeled pollutant, (B) the labeled analog, and (C) a mixture of the labeled and the unlabeled compounds.

TABLE II-1. VOLATILE ORGANIC ANALYTES

Analyte	CASRN	ODES	Quantitation Ion (m/z)	Secondary Ion(s)
Acrolein	107-02-8	ACROLEIN	56	55
Acrylonitrile	107-13-1	ACRYLNTRLE	53	51, 52
Benzene	71-43-2	BENZENE	78	--
Bromodichloromethane	75-27-4	2CLBRMETHA	83	85, 129
Bromoform	75-25-2	BROMOFORM	173	171, 175
Bromomethane	74-83-9	METHYLBR	94	96
Carbon tetrachloride	56-23-5	CARBON TET	117	119, 121
Chlorobenzene	108-90-7	CLBNZ	112	114
Chloroethane	75-00-3	ETHYL CL	64	66
2-chloroethylvinyl ether	110-75-8	2-CLEVE	63	65, 106
Chloroform	67-66-1	CHLOROFORM	83	85
Chloromethane	74-87-3	METHYL CL	50	52
Dibromochloromethane	124-48-1	2BRCLMETH	129	206, 208, 127
1,1-dichloroethane	75-34-3	11-2CLETH	63	65, 83
1,2-dichloroethane	107-06-2	12-2CLETH	62	64, 98
1,1-dichloroethene	75-35-4	11-2CLETHE	96	61, 98
trans-1,2-dichloroethene	156-60-5	12-2CLETHE	96	61, 98
1,2-dichloropropane	78-87-5	12-2CLPRP	63	65, 114
cis-1,3-dichloropropene	10061-01-5	C13-2CLPRE	75	77
trans-1,3-dichloropropene	10061-02-6	T13-2CLPRP	75	77
Ethylbenzene	100-41-4	ETHYLBENZ	106	91
Methylene chloride	75-09-2	METHYLE CL	84	86
1,1,2,2-tetrachloroethane	79-34-5	4CLETHAN	83	85, 168
Tetrachloroethene	127-18-4	4CLETHE	164	129, 131, 166
Toluene	108-88-3	TOLUENE	92	91
1,1,1-trichloroethane	71-55-6	111-3CLETH	97	99, 117, 119
1,1,2-trichloroethane	79-00-5	112-3CLETH	97	83, 85, 99
Trichloroethene	79-01-6	3CLETHE	130	95, 97, 132
Vinyl chloride	75-01-4	VINYL CL	62	64

TABLE II-2. BFB MASS-INTENSITY SPECIFICATION

Mass	Intensity Required
50	15-40% of mass 95
75	30-60% of mass 95
95	Base peak, 100% relative abundance
96	5-9% of mass 95
173	<2% of mass 174
174	>50% of mass 95
175	5-9% of mass 174
176	>95% but <101% of mass 174
177	5-9% of mass 176.

TABLE II-3. PERCENT SPIKE RECOVERIES FOR VOLATILE PRIORITY POLLUTANTS USING VACUUM DISTILLATION[a]

Spiking Compound	Average Percent Recovery (Water)[b]	Average Percent Recovery (Tissue)[c]
Chloromethane	105 ± 22	85 ± 22
Bromomethane	110 ± 23	126 ± 75
Vinyl chloride	83 ± 12	64 ± 11
Chloroethane	103 ± 16	69 ± 22
Methylene chloride	126 ± 22	LC[d]
1,1-dichloroethene	98 ± 5	74 ± 8
1,1-dichloroethane	96 ± 5	90 ± 6
trans-1,2-dichloroethene	98 ± 5	86 ± 9
Chloroform	93 ± 8	107 ± 31
1,2-dichloroethane	98 ± 10	92 ± 5
1,1,1-trichloroethane	104 ± 9	92 ± 8
Carbon tetrachloride	102 ± 10	91 ± 9
Acrylonitrile	85 ± 13	NA[e]
Bromodichloromethane	108 ± 10	64 ± 11
1,2-dichloropropane	104 ± 7	54 ± 7
trans-1,3-dichloropropene	109 ± 9	52 ± 9
Trichloroethene	105 ± 9	65 ± 11
Benzene	106 ± 7	57 ± 10
Dibromochloromethane	102 ± 11	56 ± 9
1,1,2-trichloroethane	95 ± 8	66 ± 7
cis-1,3-dichloropropene	109 ± 9	54 9
Bromoform	104 ± 14	ND[f]
Tetrachloroetnene	105 ± 9	ND
1,1,2,2-tetrachloroethane	90 ± 9	61 ± 10
Toluene	106 ± 7	ND
Chlorobenzene	101 ± 7	64 ± 15
Ethylbenzene	103 ± 5	ND
2-chloroethyl vinyl ether	94 ± 50	--
Acrolein	113 ± 76	NA
Average compound recovery	102 ± 8	76 ± 20

[a] From references 1 and 2.
[b] Reagent water was spiked with 25 ug/L of each compound except acrolein and acrylonitrile, which were added at 100 ug/L. The recoveries are averaged from 9 analyses and were calculated by comparing vacuum extracted determinations to determinations for which spikes were added directly to a purge-and-trap device.
[c] Ten-gram fish samples were spiked at 25 ppb. The recoveries were averaged from 12 analyses.
[d] Laboratory contamination of fish prevented the generation of valid data.
[e] Compound was not added to this matrix.
[f] Not determined.

SECTION III

ANALYSIS OF METALS AND METALLOIDS IN ESTUARINE AND MARINE TISSUES

CONTENTS

ANALYSIS OF METALS AND METALLOIDS IN ESTUARINE AND MARINE TISSUES

1.0 SCOPE AND APPLICATION

1.1 This method is designed to determine antimony, arsenic, beryllium, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, thallium, and zinc concentrations in biological tissue samples. The method may be used for the analysis of varying tissue types such as edible muscle and livers of estuarine and marine organisms.

1.2 A universal wet oxidation (acid digestion) procedure is recommended that is capable of providing a clean extract suitable for analysis by atomic absorption spectrophotometry (AAS). This digestion has proven effective when determining the priority pollutant metals listed above (with the possible exception of beryllium and thallium) (e.g., Table III-2). Because of a lack of reference materials certified for beryllium and thallium, little is known regarding method suitability for these elements.

1.3 Limits of detection (LOD) are listed in Table III-1. These may vary depending on the element being measured, method of detection, and instrumental sensitivity.

2.0 SUMMARY OF METHOD

2.1 A macerated 5-g sample of tissue is homogenized wet, subsampled and digested using a wet oxidation method. The resulting extract is analyzed for the metals of interest using various atomic absorption (AA) techniques such as:

- direct aspiration (DFAA) = for higher concentration metals
- graphite furnace (GFAA) = for lower concentration metals
- hydride generation (HYDAA) = for hydride forming elements (antimony, arsenic, selenium)
- cold vapor (CVAA) = for mercury.

2.2 Alternative methods of detection (e.g., inductively coupled plasma emission spectrometry) may be used providing their performance and limitations have been established.

3.0 DEFINITIONS

Certified Reference Materials (CRM): A homogeneous sample that has been analyzed a sufficient number of times by numerous qualified laboratories. The data are compiled and certified values are determined through statistical analysis. A number of CRM are commercially available in a wide range of matrices for metals analyses (e.g., NBS Oyster Tissue, SRM 1566) (Reference 1).

Control Standard: A solution, independent of the calibration standards whose analyte concentration is known. These are often analyzed as an external check after calibration.

Limit of Detection (LOD): The LOD is the lowest concentration level that can be determined to be statistically different from a blank. The recommended value for LOD is 3σ, where σ is the standard deviation of the blank in replicate analyses (reference 2).

Matrix Modifier: A reagent added to a sample that alters some aspect of its composition (references 3-5).

4.0 INTERFERENCES

4.1 Interferences should be considered to be any chemical or physical phenomenon that can influence the accuracy of the data during an analytical

operation. These can have eitner a positive or a negative effect on the result depending on their nature.

4.2 Contamination of the sample can occur during any stage of collection, handling, storage, or analysis. Potential contaminant sources must be known and steps should be taken to minimize or eliminate them. Some of the most common sources of contamination include prolonged exposure of the tissue to metal-containing fumes and dust; insufficiently clean sample containers, storage facilities and testing apparatus; and the use of contaminated reagents during analysis (reference 6).

In general, clean laboratory procedures are extremely important when performing trace metal analysis.

4.3 Most instrumental methods are prone to matrix interferences, which can either suppress or enhance the analyte signal. If a matrix interference is suspected, its effect should be determined and corrective action taken. Some common matrix interferences are listed below along with suggested corrective measures (references 7, 8).

4.3.1 High sample viscosity - usually due to dissolved solids and high acid content. Match the matrix of the calibration standards with the samples where possible.

4.3.2 Non-specific absorption (light scatter) - usually due to dissolved solids or suspended particulates, which absorb analyte radiation. Background correction (see instrument manufacturer's instructions) should be used whenever this occurs.

4.4 Many chemical interferences, some of which are poorly understood, can occur during instrumental analysis of the sample extracts. A great many of these interferences have been addressed in the literature and in most cases a sample pretreatment or instrumental modification has been proposed as a remedy (e.g., reference 9).

5.0 SAFETY

Laboratory personnel should be well versed in standard laboratory safety practices. It is the responsibility of all staff and management to ensure that safety training is mandatory. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of data handling sheets should also be made available to all personnel involved in these analyses. Additional information on laboratory safety can be found in references 10-12.

5.1 Chemicals and reagents should be properly labelled and stored in an area appropriate to their properties. Any reagents whose composition or properties may change with time must be dated and properly disposed of on or before the expiration date.

5.2 Areas where strong oxidizing agents and flammable or explosive materials are used should be well labeled and the necessary restrictions imposed.

5.3 Where laboratory apparatus and instrumentation are used, the manufacturer's safety precautions should be strictly followed.

6.0 APPARATUS AND EQUIPMENT

6.1 Sample Containers - wide-mouth screw cap jars made of either glass or non-contaminating plastic (linear or high density polyethylene or equivalent). All containers should be pre-rinsed with dilute acid and distilled deionized water (DDW) as described in Sect. 10.6.

6.2 Dissection Tools - scalpels should be made of high-quality, corrosion-resistant stainless steel, while tweezers and cutting surfaces should be plastic or teflon. All tools should be thoroughly rinsed with DDW prior to use and between samples.

6.3 Tissue Grinder/Homogenizer - (e.g., Tekmar Tissuemizer, Tekmar Co., Cincinnati, OH) a standard tissue homogenizer can be used with minor modifications. If the apparatus contains stainless steel parts, they should be replaced with tantalum or titanium. Stainless steel blades used during homogenization have been found to be a source of nickel and chromium contamination.

6.4 Digestion Vessels - 125 mL borosilicate glass Erlenmeyer flasks equipped with all glass reflux caps (Tuttle covers). Tuttle covers or equivalent reflux caps are essential for preventing evaporative loss of volatile compounds or elements during high temperature digestion. They are commercially available (Fisher Scientific) or are easily produced from borosilicate test tubes.

6.5 Hot Plate - a thermostatically controlled plate with a range of 75 to 400° C.

6.6 Fume Hood - a properly constructed hood capable of withstanding acid fumes. It must be equipped with an exhaust fan having sufficient capacity to remove all fumes.

6.7 Atomic Absorption Spectrophotometer (AAS).

6.7.1 The AAS must have sufficient sensitivity and stability to perform within the specifications required by the method (Sect. 11). The instrument should have automatic background correction, direct aspiration flame, as well as flameless capabilities. The instrument must have a routine maintenance program to ensure proper performance and trouble-free operation. All source lamps should be handled with care and the exit windows kept free of dust and fingerprints. Periodic intensity and stability checks of the lamps should be made. Replace any lamps showing signs of deterioration (reference 13).

6.7.2 A graphite furnace (also called carbon rod) attachment for the AAS is recommended when determining most elements in the low concentration ranges. Most, if not all, AAS manufacturers offer this equipment as an

accessory. The stability and sensitivity afforded by the furnace is typically one to two orders of magnitude better than direct aspiration (reference 14).

6.7.3 In addition to the graphite furnace, another flameless attachment can be used in conjunction with the AAS to determine the hydride-forming elements (arsenic, antimony, and selenium). Most such attachments may also be used to analyze for mercury using the cold vapor technique. These methods are preferable to the graphite furnace since they vaporize the analyte from the sample matrix prior to detection.

7.0 REAGENTS AND CONSUMABLE MATERIALS

The purity of all reagents used for trace metal determinations is extremely important. Reagents should be checked for purity prior to use to confirm the absence of contamination (reference 6). Low level analyses will require Ultrex grade acids (J.T. Baker) or equivalent. Instra-Analyzed grade acids (J.T. Baker) or equivalent may be suitable for less sensitive analyses. Copper contamination may be particularly troublesome when Instra-Analyzed acids are used.

7.1 Distilled Deionized Water (DDW) - a water purified by distillation (or equivalent) followed by conditioning with a mixed bed ion exchanger. Such units are commercially available and yield a water with a typical resistivity of 18 megohms/cm.

7.2 Hydrochloric Acid - concentrated (35 percent).

7.3 Hydroxylamine Hydrochloride [20 percent (w/v)]: - dissolve 20 g of American Chemical Society (ACS) grade $NH_2OH \cdot HCl$ in 100 mL of DDW. Store in a precleaned glass or plastic bottle - prepare weekly.

7.4 Nitric Acid - concentrated (70 percent).

7.5 Perchloric Acid - concentrated (70 percent).

7.6 Sodium Borohydride, ACS Grade Granular or Powder.

7.7 Sodium Hydroxide, ACS Grade - pellets or flakes.

7.8 Stannous Chloride [20 percent (w/v)] - dissolve 20 g of ACS grade $SnCl_2$ in 20 mL of concentrated hydrochloric acid. Warm gently until solution clears, cool and add DDW until the solution reaches a 100 mL volume. Store in a precleaned glass or plastic bottle - prepare fresh daily.

7.9 Stock Standard Solutions - These standards (typically 1,000 ppm) can be purchased as certified solutions or prepared from ACS grade metal salts and pure compounds. Suitable procedures for preparing stock solutions are well documented (e.g., reference 15) and include the steps below.

7.9.1 Accurately weigh 1,000 mg of pure metal or metal equivalent of the salt and dissolve in a minimum amount (usually about 20 mL) of an appropriate acid. Once the reagent is dissolved, dilute the solution to 1,000 mL with DDW and store in a precleaned plastic bottle. The solution is usually stable for at least 1 year but must be checked periodically against an in-house control standard (Sect. 10).

8.0 SAMPLE COLLECTION, PREPARATION, AND STORAGE

8.1 The major difficulty in trace metal analyses of tissue samples is controlling contamination of the sample. In the field, sources of contamination include sampling gear, winches or steel cables, engine exhaust, dust, or ice used for cooling (reference 16). Care must be taken during handling to avoid these and any other possible sources of contamination. For example, stainless steel collection and handling devices (e.g., grab samplers or sieves used for infaunal collection from sediments) are suitable. The ship should be positioned such that the engine exhausts do not fall on deck during sampling. To avoid contamination from melting ice, the samples should be wrapped in aluminum foil and placed in watertight plastic bags. The outer skin of the fish or shell of the shellfish is protection against metals contamination from the aluminum foil.

8.2 Sample resection (i.e., surgical removal of tissue) and any subsampling of the organisms should be carried out in a controlled environment (e.g., a dust-free room). In most cases, this requires that the organisms be transported on ice to a laboratory, rather than being resected on board the sampling vessel. It is recommended that whole organisms not be frozen prior to resection if analyses will be conducted only on selected tissues, because freezing may cause internal organs to rupture and contaminate other tissue. If organisms are eviscerated on board the survey vessel, the remaining tissue (e.g., muscle) may be wrapped as described above and frozen.

8.3 Resection is best performed under "clean room" conditions. The "clean room" should have positive pressure and filtered air. The "clean room" should also be entirely metal-free and isolated from all samples high in contaminants (e.g., hazardous waste). At a minimum, care should be taken to avoid contamination from dust, instruments, and all materials that may contact the samples. The best equipment to use for trace metal analyses is made of quartz, TFE, polypropylene, or polyethylene. Quartz utensils are ideal but expensive. To control contamination when resecting tissue, separate sets of utensils should be used for removing outer tissue and for removing tissue for analysis. For bench liners and bottles, borosilicate glass would be preferred over plastic if trace organic analyses are to be performed on the same sample.

8.4 Resection should be conducted by or under the supervision of a competent biologist. For fish samples, special care must be taken to avoid contaminating target tissues (especially muscle) with slime and/or adhering sediment from the fish exterior (skin) during resection. The incision "troughs" are subject to such contamination; thus, they should not be included in the sample. In the case of muscle, a "core" of tissue is taken from within the area boarded by the incision troughs, without contacting them. Unless specifically sought as a sample, the dark muscle tissue that may exist in the vicinity of the lateral line should not be mixed with the light muscle tissue that constitutes the rest of the muscle tissue mass.

8.5 After the appropriate tissues are resected, samples should be stored in suitable containers (Sect. 6.1) and frozen at -20° C until analysis. Although specific holding times have not been recommended by U.S. EPA or other federal agencies (e.g., National Bureau of Standards), a holding time of 6 mo (except for mercury samples, which should be held no longer than 28 days) would be consistent with that recommended for water samples.

9.0 CALIBRATION AND STANDARDIZATION

9.1 Calibration standards are prepared by serial dilutions of the stock solutions. The acid matrix of the standrds should be as closely matched to the samples as possible (i.e., approximately 1 percent (v/v) HNO_3 and 4 percent (v/v) $HClO_4$).

Mixed standards of more than one element may be prepared only after their compatibility has been determined. Some common mixed standards are as follows:

- Cd, Cu, Pb, Ni, and Zn
- As, Se, and Sb

9.1.1 Do not add an incompatible anion to a mixed or single element standard. For example, adding chloride to a silver standard could form a precipitate of silver chloride (AgCl).

9.1.2 Do not mix metals that are incompatible in solution. For example, lead and chromium may form a precipitate of lead chromate ($PbCrO_4$).

9.2 Concentration ranges of the standards should bracket those for the samples to be analyzed. At least four analyses (one blank and three standards of increasing concentration) should be used to calibrate the instrument at the begining of each shift.

9.3 Stability of a calibration standard varies with element, acid matrix, concentration, and presence of other elements. As a general rule, standards

should be continuously monitored and replaced when necessary. As a matter of protocol, the following can be used as a guideline:

less than 0.1 ppm	-	prepare daily
0.1 to 1 ppm	-	prepare weekly
1.0 to 10 ppm	-	prepare monthly
10 to 100 ppm	-	prepare quarterly
100^{+} ppm	-	prepare yearly (at a minimum)

9.4 Initial Standardization - follow manufacturer's suggestions for standardizing the instrument and check sensitivity performance with specifications. If performance is acceptable, proceed with analysis; if not, refer to manufacturer's troubleshooting guide.

9.5 After standardizing the instrument, analyze an independent control standard as a check. If the result is acceptable, proceed; otherwise, troubleshoot calibration standards, control standard, or instrument.

9.6 Ongoing Calibration (reference 17) - the instrument should be tested with a single point calibration every 2 h during an analysis run or at a frequency of 10 percent of the analyses, whichever is more frequent. A calibration check must also be run after the last sample in a laboratory shift. A standard concentration in the middle of the initial calibration range should be used.

If the difference between the ongoing calibration result and the known standard concentration is greater than $\pm$10 percent (or $\pm$20 percent for mercury analysis), the instrument must be recalibrated and the preceding 10 samples reanalyzed for the analytes affected.

9.7 In the event that a sample is outside of the linear response of the instrument, it must be diluted to within range or reanalyzed using a less sensitive setup. This is commonly accomplished by calibrating the instrument with higher concentration standards using a secondary or tertiary wavelength having less sensitivity.

10.0 QUALITY CONTROL [see reference 17 and Quality Assurance/Quality Control (QA/QC) for 301(h) Monitoring Programs: Guidance on Field and Laboratory Methods (Tetra Tech 1986).]

A quality control program enables the assessment of the precision and accuracy of data. Precision is estimated by analysis of replicates. Accuracy is estimated by the analysis of blanks, spiked samples, and/or laboratory control samples (reference 18).

10.1 Replicates can be chosen to reflect the precision of most stages of the overall analytical method. Replicates can consist of different subsamples of a tissue homogenate or replicate instrumental analyses of the same digestion extract.

10.1.1 Replicate analyses of tissue subsamples are important because "the greatest potential for sample deterioration and/or contamination occurs during preanalysis steps of sample collection, handling, preservation, and storage" (reference 19).

10.1.2 Replicate analyses of a digestate focus only on the bench chemistry and/or instrumental variability of the method. Together with replicate analysis of tissue subsamples, they can be used to assess the impact of each stage on the overall precision of the analytical result.

10.1.3 At least one replicate (a subsample of a tissue homogenate) must be analyzed from each group of samples of a similar matrix type and concentration for each batch of samples or for each 20 samples, whichever is more frequent. If two analytical methods are used for the same element in a batch of samples, duplicates must be run by each method used.

The relative percent differences (RPD) for each component are calculated as follows:

$$RPD = \frac{D_1 - D_2}{(D_1 + D_2)/2} \times 100$$

where

D_1 = first sample value.

D_2 = second sample value.

10.2 As in the case of replicates, blanks can be chosen to address most stages of the overall analytical method. They include transportation, dissection, reagent, and calibration blanks.

10.2.1 Transportation blanks are derived from empty containers that have been stored with samples in the field and carried with them to the laboratory. A small amount of 5 percent (v/v) HNO_3 is used to rinse the inside of the container. The acid rinse is then retained for analysis. Transportation blanks serve as estimates of contamination during preanalysis steps (Sect. 10.1.1).

10.2.2 Resection blanks are used to estimate concentration from resection utensils that may carry over from one sample to the next. They are prepared by collecting a final rinse after cleaning utensils that have been used for resection. The final rinse should be performed with a known volume of 5 percent (v/v) HNO_3. One resection blank should be analyzed for each batch of samples.

10.2.3 Reagent (preparation) blanks are aliquots of 5 percent (v/v) HNO_3 that are processed through each sample preparation step (e.g., reagent addition, digestion, dilution). At least one reagent blank must be prepared for each batch of samples or for every 20 samples, whichever is more frequent. Reagent blanks serve as estimators of contamination resulting from the chemical analysis steps.

All samples with at least one analyte concentration that is less than 10 times the corresponding concentration in the associated reagent blank must be redigested and reanalyzed.

10.2.4 Calibration blanks consist of 5 percent (v/v) HNO_3 and are analyzed each time the instrument is calibrated, at the beginning of each

analysis run, and at a frequency of 10 percent during a run. Calibration blanks are used to ensure that the analytical instrument is not introducing false positive results during analysis. (Ongoing calibration quality assurance is discussed in Sect. 9.6.)

10.3 The results obtained from the reagent blanks can be used to calculate the LOD (Sect. 3) for the method. This is the assigned minimum value above which reliable data can be reported. Results for at least the reagent blank should be reported with the final data set.

10.4 Spiked samples are samples to which small volumes of standard solutions of the elements of interest have been added. Spiked samples provide a means of assessing losses during digestion, distillation, or other pretreatment steps. The spike is added before the pretreatment steps and should be 0.5 to 2.0 times the concentrations of the elements in the sample. At least one spiked sample must be analyzed for each batch of samples of a similar matrix type and concentration or for each 20 samples, whichever is more frequent.

10.4.1 The percent recovery for each element is calculated as follows:

$$\% \text{ Recovery} = \frac{(\text{spike + sample result}) - (\text{sample result})}{(\text{spike added})} \times 100$$

Spike percent recoveries should not be used to determine a correction factor to compensate for losses.

10.4.2 If graphite furnace AA is used, a single analytical spike is required <u>after</u> any digestion steps to determine if the method of standard additions (MSA) is required (reference 17 was used to develop this section).

The spike should be added at a concentration (in the sample) that is twice the LOD. The unspiked sample aliquot must be compensated for any volume change in the spiked samples by addition of DDW to the unspiked

sample aliquot. The percent recovery of the spike should be calculated as in Sect. 10.4.1

10.4.2.1 If the sample absorbance or concentration is >50 percent of the spike[1] and the spike recovery is between 85 percent and 115 percent, the sample should be quantified directly from the calibration curve.

10.4.2.2 If the spike recovery is less than 40 percent, the sample must be diluted and rerun with another spike. Dilute the sample by a factor of 5 to 10 and rerun. This step must only be performed once. If after dilution the spike recovery is still <40 percent, there are interferences associated with the instrumental technique that prevent GFAA analysis of the sample.

10.4.2.3 If the spike recovery is >40 percent and the sample absorbance or concentration is <50 percent of the spike[1], report the analyte as less than the LOD or less than the LOD times the dilution factor if the sample was diluted.

10.4.2.4 If the sample absorbance or concentration is >50 percent of the spike[1] and the spike recovery is <85 percent or >115 percent, the sample must be quantified by MSA.

10.4.2.5 The following procedures should be incorporated into MSA analyses.

a) Data from MSA calculations must be within the linear range as determined by the calibration curve generated at the beginning of the analytical run.

---

[1][Note that spike[1] is defined throughout Sect. 10.4.2 as (absorbance or concentration of spike sample) minus (absorbance or concentration of the sample.]

b) The sample and three spikes must be analyzed consecutively for MSA quantitation (the "initial" spike run data is specifically excluded from use in the MSA quantitation).

c) Spikes (post-digestion, as for the "initial" spike in Sect. 10.4.2) should be prepared such that:

- Spike 1 is approximately 50 percent of the sample absorbance.

- Spike 2 is approximately 100 percent of the sample absorbance.

- Spike 3 is approximately 150 percent of the sample absorbance.

d) The data for each MSA analysis should be clearly identified in the raw data documentation along with the slope, intercept and correlation coefficient (r) for the least squares fit of the data.

10.5 Laboratory control samples are certified reference materials (CRM) submitted blind to the laboratory. CRM provide an estimate of the accuracy of the overall method. A CRM must be chosen that has a similar matrix to samples and contains all the analytes. CRM can be purchased from a number of agencies and are available in a range of matrices (e.g., U.S. EPA Trace Metals in Fish Tissue or NBS Oyster Tissue).

10.5.1 Unlike an analyte spike (Sect. 10.4), a CRM tests the dissolution technique as well as instrument calibration and matrix interferences.

10.5.2 A minimum of one CRM should be analyzed for each survey or 2 percent of the total number of samples (i.e., 1 per 50 samples), whichever is more frequent).

10.5.3 The percent recovery for each element for the overall method is calculated as follows:

$$\% \text{ Recovery} = \frac{x}{REF} \times 100$$

where

x = the analytical result for the element
REF = the certified result for the element.

The data obtained for each reference material should be used to troubleshoot the method if results fall outside the acceptable range (i.e., the 95 percent confidence interval). Percent recovery values should not be used to determine a correction factor to compensate for apparent procedural losses.

10.6 Maintenance of Records - the data obtained from any QC work should be recorded in an organized manner to allow for easy retrieval and reviewing. If sufficient data have been collected, it is recommended that these be plotted on a control chart for a quick visual assessment. A typical control chart for CRM results is presented in Figure III-1.

10.6.1 The quality control chart can be used to determine if the following recommended guidelines are met:

10.6.1.1 Not more than 5 percent of the results lie outside two standard deviations (warning limit). A result outside three standard deviations requires action.

10.6.1.2 There are no regular periodic variations.

10.7 Cleaning and preparation of labware is an integral part of a quality assurance/quality control (QA/QC) program. Many cleaning procedures have been proposed in the literature that are suitable for decontaminating equipment. The main concerns with cleaning are removing elements of interest

from labware while maintaining an inactive surface. Some cleaning procedures tend to be too harsh, producing an surface with an ion exchange capacity. In this case a solution could partially or completely "lose" an analyte to the container walls (references 20, 21).

10.7.1 If at all possible, labware should not be used for work where analyte concentrations vary by more than ten times. For example, never use glassware for tissue analysis that has also been used for sediments. If one can use dedicated glassware, the cleaning requirements are greatly simplified.

10.7.2 A good universal cleaning procedure for glass and plasticware is outlined below.

10.7.2.1 Wash labware with a metal-free detergent and warm water.

10.7.2.2 Rinse at least three times with tap water followed by distilled deionized water (DDW).

10.7.2.3 Soak equipment or labware in a dilute acid (25 percent HNO_3) bath for 24 h. If possible, the bath should be maintained at an elevated temperature (70° C).

10.7.2.4 Rinse labware with large volumes of DDW and use immediately. If a time lapse must exist, the apparatus should be stored under dust-free conditions and rinsed further with DDW prior to use.

NOTES:
- Change the acid batch periodically such that no significant buildup of metals occurs.
- At no time should a metal containing reagent such as chromic acid be used.

10.8 Round Robin or Interlaboratory Check Programs - In addition to the quality control measures discussed above, all laboratories should participate in interlaboratory check programs.

11.0 PROCEDURE

11.1 Homogenize samples prior to analysis to ensure that a representative aliquot is taken. Any grinder or homogenizer that has been found to be free of contamination may be used (Sect. 6.3). Samples should be ground wet to avoid losses of volatile elements (e.g., Hg, Se) during drying. The liquid associated with a sample after thawing should be retained as part of the sample.

11.2 Transfer the sample paste to a container suitable for storage. If not immediately analyzed, the samples should be frozen (-20° C) until required. Containers should be tightly sealed to prevent moisture loss or gain during storage.

11.3 Dry Weight Determination - if sample size permits and dry-weight concentrations are required, dry weight determinations may be performed as follows: transfer an aliquot of approximately 3 g (weighed to the nearest 0.1 g) to a preweighed dish. Allow the sample to dry in an oven at 105° C overnight, and determine the solid residue weight to the nearest 0.1 g. The percent total solids is calculated as:

$$T_S = [\text{dry residue wt (g)}]/[\text{wet sample wt (g)}]$$

Dry weight determinations should not be made at the cost of having insufficient sample for metals analysis. Significant decreases in the size of samples used for extraction will decrease attainable detection limits.

11.4 Accurately weigh representative aliquots of homogenized tissue to the nearest 0.1 mg. If sample size permits, approximately 5 g is required to maintain optimum detection limits. Transfer the weighed tissue to a precleaned 125-mL Erlenmeyer flask equipped with an all-glass reflux cap. Analyze a sufficient number of reagent blanks, sample duplicates, analyte spikes, and certified reference materials concurrently (Sect. 10).

11.5 Add 10.0 mL of concentrated nitric acid (ACS grade or better), replace cap and swirl. Allow flask to stand at room temperature for about 15 hours in a dust-free ventilated environment. Periodically swirl the contents to help solubilize the tissue.

11.6 After 15 hours, gently heat the flask to approximately 100° C - hold at this temperature for 1 hour. Gradually increase the temperature in 50° C increments to a maximum of 250° C. Continue digesting until all tissue has been solubilized. This usually takes about 4 hours. Do not rush the initial digestion as losses of volatile elements will likely occur. Once digestion is complete, cool flasks to room temperature and add 4.0 mL of perchloric acid.

CAUTION: Perchloric acid is a strong oxidizing agent. The analyst must be fully aware of the precautions associated with its use. This procedure (i.e., use of perchloric acid at sub-fuming temperatures) has been safely performed without a perchloric hood, but a perchloric hood is strongly recommended nonetheless. Laboratories that do not carefully monitor perchloric acid digestions will be endangered without perchloric hoods. Safety precautions and background information pertaining to perchloric acid can be found in reference 22.

11.7 Return flasks to the hotplate which has been cooled to about 200° C. Continue heating for 1 hour, then increase plate temperature to 300° C. Hold at this temperature until all traces of nitric acid fumes have disappeared and the solutions have become clear. Do not overheat flasks or allow perchloric fumes (dense white) to appear. If perchloric fumes appear, reduce heat immediately. Remove the extracts when clear and cool them to room temperature.

11.8 When the digestion is complete, rinse the caps into the flasks and transfer the extract to a precleaned 100-mL volumetric flask. Rinse the Erlenmeyer flask three times with DDW and combine with the extract previously

added to the volumetric flask. Adjust the volume with DDW and transfer to a precleaned plastic bottle.

NOTE: Some elements are not as stable as others in solution and therefore should be analyzed first. Stability can be determined by daily analysis of the extracts, however, the following can be used as a guideline:

Sb, Pb, Hg, Se and Ag - analyze within one day
As and Cd - analyze within two days
Cr, Cu, Ni and Zn - analyze within one week
Be and Tl - to be determined.

11.9 Instrumental Analysis - The extracts will be analyzed using various techniques of atomic absorption spectrophotometry (AAS). The method of choice (i.e., GFAA vs HYDAA) depends on instrument availability, analyte concentration and sample matrix. In some instances it may be useful to use more than one AAS method to confirm a result.

11.9.1 Follow the manufacturer's instructions for initial setup and calibrate as outlined in Sect. 9 of this method. As every instrument responds uniquely to a given set of conditions, it is the analyst's responsibility to develop the optimum set of parameters. Use calibration standards and CRM to ensure that optimum conditions exist.

11.9.2 Table III-1 lists some general information for each of the priority pollutant metals.

11.9.3 It is possible to use alternate methods of detection providing they have been validated using a sufficient number of previously analyzed samples or CRM.

11.10 All data generated must be clearly recorded on a strip chart, printer or manually logged in prepared tables. The order in which the extracts are analyzed should be the same as it appears in the records. The data, when assembled, should be reported in consistent units (i.e., mg/L) to

avoid errors when calculating the final results (ug/g). The final report should contain all necessary methodology, results, quality control data (e.g., reagent blank values) and limits of detection for each element. The report must clearly state if any data were blank-corrected.

12.0 CALCULATIONS

12.1 All results are reported as micrograms of element per wet gram of tissue:

$$\text{ug/g ELEMENT (wet weight basis)} = \frac{C \times V}{W}$$

where:

C = concentration (may be blank corrected) of element in final extract (ug/mL)
V = volume of final extract (mL)
W = weight of wet tissue (g)

Reagent blank corrections may be made and blank values must always be reported.

13.0 PRECISION AND ACCURACY

In order to estimate precision and accuracy (single lab, multi-operator), a number of CRM and analyte spikes were analyzed using this method. Table III-2 summarizes typical data obtained. No data are currently available for beryllium or thallium.

14.0 REFERENCES

1. Taylor, J.K. 1985. Standard reference materials: handbook for SRM users. National Bureau of Standards Special Publicaton 260-100. National Bureau of Standards, Washington, DC.

2. Keith, L.J., W. Crummet, J. Deegan, Jr., R.A. Libby, J.K. Taylor, and G. Wentler. 1983. Principles of environmental analysis. Anal. Chem. 55:2210-2218.

3. Manning, D.C., and W. Slavin. 1983. The determinatin of trace elements in natural waters using the stabilized temperature platform furnace. Applied Spectroscopy 37:1-11.

4. Hinderberger, E.J., M.L. Kaiser, and S.R. Koirtyohann. 1981. Furnace atomic absorption analysis of biological samples using the L'vov platform and matrix modification. Atomic Spectroscopy 2:1-7.

5. Sturgeon, R.E., S.N. Willie, and S.S. Berman. 1985. Preconcentration of selenium and antimony from seawater for determination by graphite furance atomic absorption spectrometry. Anal. Chem. 57:6-9.

6. Murphy, T.J. 1976. The role of the analytical blank in accurate trace analysis. pp. 509-539. In: Accuracy in Trace Analysis: Sampling, Sample Handling, and Analysis. National Bureau of Standards Special Publication 422. National Bureau of Standards, Washington, DC.

7. Skoog, D.A. 1985. Principles of Instrumental Analysis. Saunders, Philadelphia, PA. pp. 270-279.

8. Veillon, C. 1976. Optical atomic spectroscopic methods. pp. 123-181. In: Trace Analysis: Spectroscopic Methods for Elements. D. Winefordner (ed). Wiley, New York.

9. Slavin, W., G.R. Carnrick, and D.C. Manning. 1984. Chloride interferences in graphite furnace atomic absorption spectrometry. Anal. Chem 56:163-8.

10. Carcinogens - working with carcinogens. DHEW, PHS, CDC, NIOSH. Publication 77-206 (Aug. 1977).

11. OSHA safety and health standards, general industry. OSHA 2206, 29 CFR 1910 (revised Jan. 1976).

12. Safety in academic chemistry laboratories. ACS Publications, Committee on Chemical Safety, 3rd Edition (1979).

13. Cantle, J.E. (ed). 1982. Atomic absorption spectrometry. Elsevier, New York.

14. Fuller, C.W. 1978. Electrothermal atomization for atomic absorption spectroscopy. The Chemical Society, London.

15. U.S. Environmental Protection Agency. 1979. Methods for chemical analysis of water and wastes. pp. 202.1-289.2. U.S. Environmental Protection Agency Environmental Monitoring and Support Laboratory. Cincinnati, OH.

16. Mart, L. 1979. Prevention of contamination and other accuracy risks in voltammetric trace metal analysis of natural waters. Part II: Collection of surface water samples. Fresenius Z. Anal. Chem. 299:97-102.

17. U.S. Environmental Protection Agency. 1985. U.S. EPA Contract Laboratory Program - statement of work for inorganic analyses, multi-media multi-concentration.

18. U.S. Environmental Protection Agency. 1983. Guidance for preparation of combined work/quality assurance project plans for water monitoring. Office of Water Regulations and Standards, U.S. EPA, Washington, DC. 33 pp.

19. Plumb, R.H., Jr. 1981. Procedures for handling and chemical analysis of sediment and water samples. Technical Report EPA/CE-81-1. Environmental Protection Agency/Corps of Engineers Technical Committee on Criteria for Dredged and Fill Material, U.S. Army Waterways Experiment Station, Vicksburg, MS. 471 pp.

20. Batley, G.E., and D. Gardner. 1977. Sampling and storage of natural waters for trace metal analysis. Water Res. 44:745-756.

21. Laxen, D.P.H., and R.M. Harrison. 1981. Cleaning methods for polythene containers prior to the determination of trace metals in freshwater samples. Anal. Chem. 53:345-350.

22. Schilt, A.A. 1979. Perchloric acid and perchlorates. G. Frederick Smith Chemical Company, Columbus, OH.