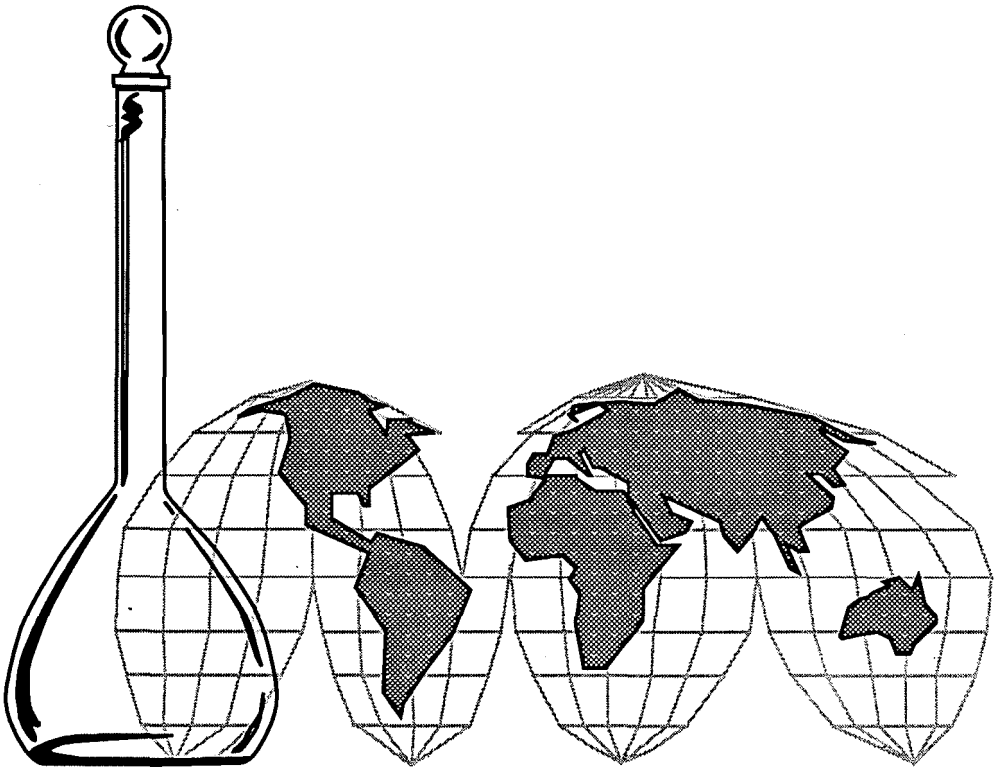
NOAA Technical Memorandum NOS ORCA 77

National Status and Trends Program
for Marine Environmental Quality

USE OF STANDARDS AND REFERENCE MATERIALS IN THE MEASUREMENT OF CHLORINATED HYDROCARBON RESIDUES

CHEMISTRY WORKBOOK



Silver Spring, Maryland
March, 1994

noaa NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION

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

NOAA Technical Memorandum NOS ORCA 77

USE OF STANDARDS AND REFERENCE MATERIALS IN THE MEASUREMENT OF CHLORINATED HYDROCARBON RESIDUES

CHEMISTRY WORKBOOK

T. L. Wade and A. Y. Cantillo

Silver Spring, Maryland
March, 1994

United States Department of Commerce — Ronald H. Brown, Secretary

National Oceanic and Atmospheric Administration — D. James Baker, Under Secretary

National Ocean Service — W. Stanley Wilson, Assistant Administrator

TABLE OF CONTENTS

PREFACE i
LIST OF TABLES ii
LIST OF FIGURES iii

ABSTRACT 1
1. INTRODUCTION 1
    1.1. Purpose 2
    1.2. Facilities and Personnel 2
    1.3. Approach 2
    1.4. Warning 3
2. SAMPLE TRACKING 3
    2.1. Collection 3
    2.2. Archival 3
    2.3. Preparation 4
3. ANALYTE ISOLATION 4
    3.1. Introduction 4
    3.2. Summary of Extraction Methods 5
4. INSTRUMENTAL ANALYSES 7
    4.1. Introduction 7
    4.2. Initialization 7
    4.3. Optimize Operating Conditions 7
    4.4. Calibration 9
    4.5. Reference Materials 12
    4.6. Analytical Sequence 13
    4.7. Quantification 13
    4.8. Method Detection Limit 15
    4.9. QA/QC Validation 15
    4.10. Control Charts 16
    4.11. Data Reporting and Archiving 17
5. EXAMPLE CALCULATIONS 17
    5.1. Introduction 17
    5.2. Examples 17
        5.2.1. Example 1. Preparation of Stock Solutions 17
        5.2.2. Example 2. Preparation of Calibration Solutions 18
        5.2.3. Example 3. Determination of Percent Moisture 19
        5.2.4. Example 4. Determination of Percent Solvent Extractable Material Weight 20
        5.2.5. Example 5. Required Sample Weight 21
        5.2.6. Example 6. Calibration Curve for the Analysis of Chlorinated Compounds Using 4,4'-DDE Calculation as an Example. 22
            5.2.6.1. Calibration Curve 22
            5.2.6.2. Concentration Calculations 23
            5.2.6.3. Percent Recovery 24
        5.2.7. Example 7. Determination of Method Detection Limit 24
            5.2.7.1. MDL Calculation 25
            5.2.7.2. Upper and Lower 95% Confidence Limits 25
        5.2.8. Example 8. Spiked Blank Calculations 26
        5.2.9. Example 9. Spiked Matrix Calculations 27
        5.2.10. Example 10. Reference Material Calculations 28
6. ANSWERS 28
7. REFERENCES 31

8. APPENDICES 32
8.1. Example Forms 32
8.1.1. Sample receiving and integrity form 33
8.1.2. Sample processing form 34
8.1.3. Sample transfer form 35
8.1.4. Sample information form 36
8.1.5. Sample information sheet 37
8.1.6. Laboratory sample logbook 38
8.1.7. Laboratory sample logbook 39
8.2. Selected Pesticide/PCB Structures and CAS Numbers 40
8.3. Standard Operating Procedures of TAMU/GERG 42
8.3.1. SOP-9016 - Extraction of Biological Tissues for Chlorinated Hydrocarbon Analysis 42
8.3.2. SOP-9017 - Quantitative Determination of Chlorinated Hydrocarbons 47
8.4. Information on NIST SRM 1974, Organics in Mussel Tissue (*Mytilus edulis*) 55
8.5. List of Contributors and Reviewers 58

PREFACE

This Workbook has a straight forward objective: to provide clear examples of the benefits of using standards and reference materials in a chemical measurement process. To put the role of standards and reference materials in context, the authors have prescribed other activities such as extraction and analytical methodology. The reader must realize that use of standards and reference materials represents just one part of a quality management program. Many other critical parts (e.g., program design, training, data analysis) are not described here. Nevertheless, the Group of Experts on Standards and Reference Materials believes that this Workbook fills a need. We hope that users of this Workbook will communicate their suggestions for improvements so that future editions will be more valuable.

John A. Calder
Chairman
Group of Experts on Standards and Reference Materials
NOAA
Silver Spring, MD

LIST OF TABLES

1. Quality assurance requirements....2

2. Analytes measured by GERG for the International Mussel Watch Program....8

3. GC/ECD operating conditions for pesticides and PCB analyses....9

4. Calibration standard concentrations, amount of internal standard and recovery standard added to samples and peak areas....21

5. Concentrations determined for selected analytes in spiked oyster tissue, mean and standard deviation....25

6. Spike blank results....26

7. Spiked matrix concentration results....27

8. Intercomparison exercise results....28

9. Chlorinated hydrocarbons of interest....48

10. Minimum GC requirements and operating conditions....50

11. Certified concentrations available for SRM 1974....56

12. Noncertified concentrations available for SRM 1974....57

LIST OF FIGURES

1. Water extraction procedure.....4

2. Sediment extraction procedure.....5

3. Tissue extraction procedure.....5

4. Typical GC/ECD chromatogram of pesticides and PCB analysis.....10

5. Typical analytical sequence printout from GC microprocessor.....14

6. 1991 TAMU results of analysis of SRM 1566a, Oyster Tissue, as a control material for fluoranthrene, 4,4'-DDE and PCB 8.....16

7. Sample calibration curve.....22

USE OF STANDARDS AND REFERENCE MATERIALS IN THE MEASUREMENT OF CHLORINATED HYDROCARBON RESIDUES
CHEMISTRY WORKBOOK

T. L. Wade
Geochemical and Environmental Research Group
College of Geosciences and Maritime Studies
Texas A&M University
833 Graham Road
College Station, TX 77845

A. Y. Cantillo
NOAA/NOS/ORCA
1305 East West Hwy., 10614
Silver Spring, MD 20852

ABSTRACT

This document is a workbook on the use of standards and reference materials for a QA/QC program for marine pollution studies of chlorinated hydrocarbons. As part of their mission, the Group of Experts on Standards and Reference Materials (GESREM) developed this workbook for laboratories that are initiating organic contaminant analyses of environmental samples. The first sections of this workbook give details of sample collection, archival, extraction, instrumental analyses, and data reduction under proper QA/QC procedures. The last sections give examples of how the calculations and procedures are actually applied in a laboratory. This workbook was prepared under sponsorship of the Intergovernmental Oceanographic Commission (IOC) of the United Nations Educational, Scientific, and Cultural Organization (UNESCO), and is printed simultaneously by IOC and the National Oceanic and atmospheric Administration (NOAA) to increase its distribution.

1. INTRODUCTION

One of the frustrations that has plagued environmental chemists is the inability to determine if valid comparison of data among laboratories can be made. Validity can be tested by comparing results of analyses of common standards and reference materials (RM). The value of RMs is evidenced by the increase in their availability and use (Cantillo, 1992). If the same concentration of an analyte in an RM is obtained by two independent methods, and the analyte concentrations agree within analytical uncertainty, then that material can be assigned certified concentrations and is termed a certified reference material (CRM) for that analyte. As part of their mission, the Group of Experts on Standards and Reference Materials (GESREM) developed this workbook on the use of RMs and CRMs for laboratories that are initiating the organic contaminant analyses of environmental samples.

The first sections of this workbook give details of sample collection, archival, extraction, instrumental analyses, and data reduction under proper Quality Assurance/Quality Control (QA/QC) procedures, since the quality of any analysis is also dependent on all these steps. The last sections give examples of how the calculations and procedures are actually applied in a laboratory. If the readers are familiar with collection and extraction procedures, they may go to Section 5 and the example calculations.

The development and implementation of a quality assurance protocol is a combination of good field collection and laboratory practice, and common sense. The QA/QC issues detailed in this manual are summarized in Table 1.

Table 1. Quality assurance requirements

1.	Collecting representative and unaltered samples.
2.	Preserving sample integrity until analysis.
3.	Use of standards and reagents of demonstrated purity.
4.	Accurate preparation and dilution of standards.
5.	Ongoing assessment of standards for degradation.
6.	Calibration of working standards against reference standards.
7.	Ongoing assessment of the method using blanks, spike blanks, duplicates, spiked matrix, and reference materials.
8.	Validation of long term method stability using laboratory reference material.
9.	Validation by intercomparison with other laboratories.
10.	Confirmation of analytical results by a second method (e.g., GC/MS).
11.	Validation of all electronic data manipulations.
12.	Examination of chromatograms and final data by pesticide experts.
13.	Final independent QA/QC approval.

1.1. Purpose

The purpose of this workbook is to provide a guide for using standards and reference materials in an overall quality assurance/quality control program. It uses examples tailored to marine pollution programs such as the International Mussel Watch and other regional and national marine sentinel organism monitoring programs, but it should help in establishing the QA/QC component of any environmental monitoring program.

1.2. Facilities and Personnel

It is assumed that adequately trained personnel and facilities are available for pesticide and polychlorinated biphenyl (PCB) analyses in environmental samples. In particular, it is assumed that the analyst is familiar with extraction techniques and gas chromatography (GC) using an electron capture detector (ECD), and that facilities are available for the preservation, extraction, purification, and analyses of samples. This includes a GC system with capillary column capabilities, an integrator, reagents, and pure standards of the individual analytes to be determined.

1.3. Approach

This workbook is not an exhaustive description of analytical techniques used in environmental analyses. However, because of their importance to an overall QA/QC program, a summary of sample collection, storage, tracking, analyte isolation, detection, quantitation, data validation and reporting are provided. This will be done by describing the process required to establish and validate methods at an analytical facility that has never before done pesticide/PCB analyses. Examples of all calculations are provided so that the user of this workbook can have actual examples to see how all equations are applied.

1.4. Warning

This workbook is not a "cookbook." Procedures described should not be followed thoughtlessly. Analysis of trace organic contaminants in environmental media is an area of active research and development. The perfect or universal method has not been developed. Thus, all techniques described in this workbook are given as examples to show how and when various standards and reference materials should be used. Although the techniques described have proven to be useful to the authors and represent the current "routine" practice of the National Oceanic and Atmospheric Administration's (NOAA) National Status and Trends (NS&T) Program, neither NOAA, GESREM, nor its sponsors, recommend or endorse these techniques for other applications.

2. SAMPLE TRACKING

The QA/QC of the project starts with sample collection. Collection protocols used must result in a representative sample, unaltered during collection, preservation, and shipping. Examples of sample tracking forms can be found in Section 8.

2.1. Collection

Sample containers free of contaminants and made of inert materials, such as glass or Teflon, must be used. To determine if field contamination is a problem, field blanks are often collected and analyzed to assess the possibility that the samples were contaminated in the field with the analyte or any material that might interfere with analyses. It is usually impossible to replicate all the steps of sample collection without actually collecting a sample. Materials suspected as potential contaminants during field operations can be collected and analyzed by the same procedures as the samples. It is also critical that samples are properly labeled with adequate information to provide unambiguous identification.

Typically for organic analyses, water samples are stored in precleaned amber bottles and preserved by adding acid (HCl) to reduce the pH to less than 2. Alternatively, samples may be preserved by adding dichloromethane at a 1:40 volume ratio of dichloromethane:sample water. Sediments are stored in precleaned glass jars and frozen (-20°C) in the field or as soon as possible. Tissue samples are wrapped in solvent-cleaned aluminum foil or stored in glass jars and frozen (-20°C) in the field or as soon as possible.

2.2. Archival

Careful procedures for custody of samples must be established. Unique file numbers are assigned to all samples, and batch numbers to all sample sets in order to assure traceability through the various analytical steps. All field information is verified before sample analyses begin. Samples are checked by a Sample Custodian against the collection identification (ID) numbers. If all the samples and collection identification numbers match, then file numbers are assigned and analyses of the samples may begin. A sample integrity form is then prepared. If the collection identification numbers and sample identification numbers do not match or if other problems exist (i.e., broken jars or thawed samples), these problems must be resolved and/or detailed on the sample integrity form before samples are assigned file numbers and sample analyses are initiated. The sample integrity form should be dated and signed by the Sample Custodian and a copy kept on file.

2.3. Preparation

Samples may require further processing before storage. For example, the tissue of interest may be dissected or the whole organism homogenized. If more than one tissue from the same organism is analyzed, each must be assigned a unique file number. Samples are homogenized so that duplicate sample analyses are within a specified precision (i.e., relative percent difference of 10% or less).

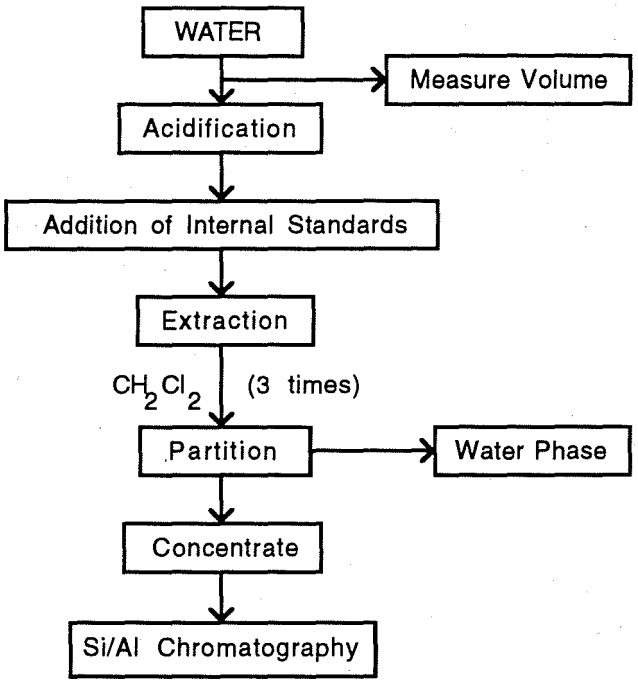


Figure 1. Water extraction procedure.

3. ANALYTE ISOLATION

3.1. Introduction

This section describes analytical procedures for the determination of trace levels of organic compounds in water, sediments, and tissues. The extraction procedures described for tissues are the ones used by the Geochemical and Environmental Research Group (GERG), Texas A&M University (TAMU), for analyses of International Mussel Watch and the NOAA NS&T Program samples. The procedures provide accurate, precise, and reproducible methods for sample collection, component isolation, and trace organic compound identification and quantification. The techniques have been extensively tested and successfully proven through multi-laboratory intercalibrations. Major steps of the analytical methods are summarized in Figures 1, 2, and 3, and are detailed below.

All glassware is cleaned before use by washing in soap solution, rinsing with distilled water, and heating at 400°C for 4 hr. Solvents must not contain detectable concentrations of analytes. Solvent purity is checked by evaporating the normal volume of solvent used in the analytical procedure to the normal final volume. The concentrated solvent is then analyzed by the GC/ECD to insure that no analytes are detected. Solvents are purchased in identifiable batches and approved on a per batch basis.

Each set of samples (10 to 20 samples) should be accompanied by a procedural blank and, when available, reference material of the same matrix. The procedural blank and reference material are processed through the entire analytical scheme in a manner identical to samples. Procedural blanks include all reagents, solvents, and internal standards. Procedural blanks are used to determine the concentration of an analyte that is inadvertently introduced in the sample as the result of the analytical process. Procedural blanks should be below the environmental concentration of interest. Duplicates, spiked blanks and matrix spike are sometimes used when suitable reference materials are not available. The frequency at which these control samples are analyzed depends on the objectives of the analytical program.

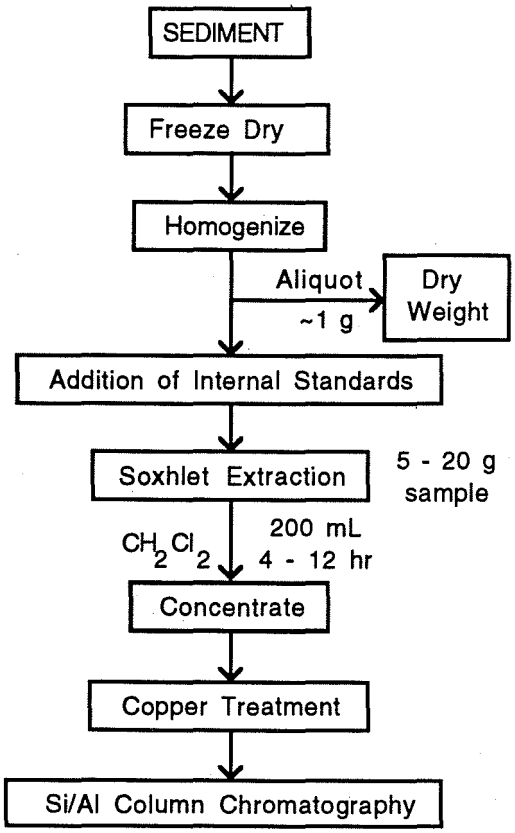


Figure 2. Sediment extraction procedure.

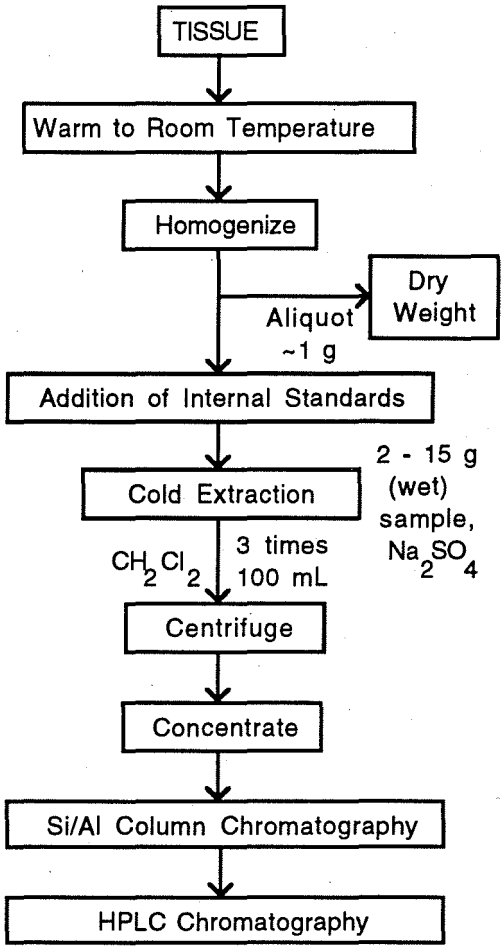


Figure 3. Tissue extraction procedure.

3.2. Summary of Extraction Methods

Internal standards (ISs) are added to all samples immediately before extraction. The IS mixture for chlorinated hydrocarbons contains 4,4'-dibromooctafluorobiphenyl (DBOFB), PCB 103, and PCB 198 (PCB numbering system is that of Ballschmiter and Zell, 1980). These ISs normally are resolved from substances found in most samples. The amount of each IS added to the 10-g wet weight International Mussel Watch samples by GERG was 100 ng. Other ISs can be used, such as 4,4'-DDT-d_8, gamma-chlordene, Ronnel, PCB 209, PCB 112, and octachloronaphthalene.

A flow-chart of the water extraction procedure is provided in Figure 1. Acidify water samples to a pH of 2 or less with concentrated HCl. Add internal standards and dichloromethane at 12% of the water volume. For example, for a 1 L sample, 120 mL of dichloromethane would be added. Extract the water in a separatory funnel. Shake the water and dichloromethane in the separatory funnel and allow the phases to separate. Remove the lower denser dichloromethane layer. Repeat the extraction two more times with the appropriate volume of dichloromethane. Discard the water phase, dry the organic phase with sodium sulfate, and concentrate the combined organic phases to 20 mL, containing the pesticide/PCB analytes, in a water bath using a three-ball Snyder column. Then transfer the extract to a 25-mL Kuderna-Danish concentrator

tube for evaporation and exchange into 2 mL of hexane. The sample extract is now ready for purification by silica gel/alumina column chromatography.

The sediment extraction procedure is shown as a flow-chart in Figure 2. Freeze-dry, remove detritus such as stones and shells, grind and Soxhlet-extract 5 - 20 g of sediment (dry weight) for at least 4 hr with 200 mL of dichloromethane. Add internal standards after freeze-drying and grinding and prior to extraction. Concentrate the organic phase to approximately 10-15 mL in a flat bottom flask equipped with a three-ball Snyder condenser. Further concentrate and exchange the extract into 2 mL of hexane in a 25-mL Kuderna-Danish concentrator tube in a water bath. Extracts are now ready for purification by silica gel/alumina column chromatography.

The tissue extraction flow-chart is detailed in Figure 3. If available, use 2 - 15 g of tissue (wet weight) for analysis, though smaller samples can be accommodated. Dry tissue samples by grinding with Na_2SO_4, add internal standards, and macerate them in 100 mL of dichloromethane with a Tissuemizer for 3 min. Centrifuge the mixture and decant the extract. Repeat this process two additional times on the settled solids. Concentrate extracts to 20 mL as described above and exchange to 2 mL of hexane in a 25-mL Kuderna-Danish concentrator tube in a water bath. The extracts are now ready for purification by silica gel/alumina column chromatography.

Separation of chlorinated hydrocarbons from aliphatic hydrocarbons and some polar lipids is accomplished with silica gel/alumina column chromatography. The silica gel is activated prior to use by heating at 170°C for 12 hr and then deactivated using 5% water by weight. The alumina is activated by heating at 400°C for 4 hr, and deactivated using 1% water by weight. The column is then slurry-packed using dichloromethane first with 10 g of alumina and then with 20 g of silica gel. For sediment samples, add copper, activated with concentrated HCl, to the top of the column to remove elemental sulfur. Some sediment samples may require additional treatment. Copper is added to the concentrated extract until the bright copper color persists. However, some researchers report that 4,4'-DDT may react with copper and therefore extracts should be treated with a minimum amount of copper. Add 50 mL of pentane and drain to the top of the column. Transfer the extract in 2 mL of hexane to the column. The column elution volumes are determined in the laboratory using standards containing aliphatic and aromatic hydrocarbons, pesticides, and PCBs. Elution volumes listed are only estimates. Then elute the column with pentane and collect 50 mL containing the aliphatic hydrocarbons (f_1). Continue to elute the column with 200 mL of 1:1 dichloromethane:pentane to isolate the chlorinated hydrocarbon fraction (f_2). Reserve f_1 for later analysis if required, and concentrate f_2 using the procedure described above.

Tissue sample extracts are subjected to a further cleanup/fractionation procedure (Krahn *et al.*, 1988) using an automated high performance liquid chromatography (HPLC) method that isolates polycyclic aromatic hydrocarbons (PAHs), pesticides, and PCBs from sample materials (lipids) that may interfere with the analyses. Sephadex columns can also could be used if an HPLC is not available (Ramos and Prohaska, 1981).

The HPLC method involves injecting a 0.5-mL sample extract in an HPLC system with two size exclusion columns connected in series (22.5 x 250 mm Phenogel 100 Angstrom columns) and collecting the desired fractions in collector vials at the predesignated time. For example, at Texas A&M, a Spectro Physics Model 8100 HPLC with a Gilson 231/401 auto sampler is used. The HPLC unit is equipped with an ultraviolet detector and provides a constant isocratic flow of approximately 7 mL/min. A precolumn (8 x 50 mm Phenogel 100 Angstrom) is connected to the two main columns, and it can be backflushed periodically (this is done daily when samples are being processed) to remove sample matrix contamination from the system. The sample

extracts are injected onto the columns with an autosampler that is capable of introducing 0.5-mL volumes of sample extracts, with minimum sample loss in the transfer lines and sample loop. Use a 0.9-mL sample loop to prevent loss of sample during sample loading. Program the fraction collector to synchronize processing with the autosampler and HPLC pump. Dichloromethane filtered through a 0.45-mm filter is used as the mobile phase. Approximately 40 mL fractions are collected for each sample.

The time interval during which the desired fraction is collected is based on the retention times of two selected compounds that encompass the analytes of interest, DBOFB and perylene. DBOFB elutes at about the same time as the first analyte and perylene is the last to elute. Collection of the sample fraction begins approximately 1.5 min before the elution of the first marker (DBOFB) and ends 2.0 min after the elution of the last marker (perylene).

After a new column is installed or any other major maintenance activity is conducted on the HPLC, run a standard containing the major analytes and representative interferences (e.g., corn oil) and calculate the recoveries of the analytes from a GC analysis. The methods described above are used to isolate pesticides and PCBs from other possible interfering components of the samples. There are many other approaches that may work equally as well.

4. INSTRUMENTAL ANALYSES

4.1. Introduction

The most widely used instrumental technique for the separation of chlorinated hydrocarbons is gas chromatography (GC). The most widely used detector for chlorinated pesticide analyses is the electron capture detector (ECD), because of its sensitivity to halogenated compounds. However, its inherent sensitivity renders it susceptible to electronic noise and other problems.

4.2. Initialization

To achieve maximum performance from an ECD, operating conditions must be precisely controlled. The instrument must be set up to the manufacturer's specifications, which require an adequate power supply, high purity gases, clean solvents, pure standards, an adequate temperature and moisture-controlled environment, and a capillary column. Initial testing is accomplished by installing a test column supplied by the manufacturer and injecting a standard under specified operating conditions. For example, a standard containing 1-5 picograms of *gamma*-HCH and aldrin in 1 µL of isooctane may be used to test the ECD detector.

The GC is held at the maximum oven temperature overnight to condition the system, and the ECD noise and drift are checked. If the instrument does not pass the noise and drift test as defined by the manufacturer, appropriate actions must be taken. The instrument may be conditioned longer, and checks made for air leaks, electrical connections checked, and other steps taken. If the instrument passes the noise and drift test, 1 µL of a test solution is injected. The compounds in the test solution, when analyzed under the specific test conditions on the test column, have to meet requirements for peak separation, peak height, and retention time. Meeting these conditions ensures that the instrument is operating up to the manufacturers specifications. At this time, the test column can be removed and the analytical column to be used for sample analyses can be installed and the instrument operating parameters set.

4.3. Optimize Operating Conditions

The specific GC/ECD analytical conditions developed are dependent on the analytes of concern and the precision, accuracy, and required detection limits. As an example, the workbook will

Table 2. Analytes measured by GERG for the International Mussel Watch Program.

Analytes	CAS Numbers◊	Analytes	CAS Numbers◊
2,4'- DDD	53-19-0	Heptachlor	76-44-8
4,4'- DDD	72-54-8	Heptachlor epoxide	1024-57-3
2,4'- DDE	3424-82-6	Hexachlorobenzene	118-74-1
4,4'- DDE	72-55-9	*alpha*-HCH	319-84-6
2,4'- DDT	789-02-6	*beta*-HCH	319-85-7
4,4'- DDT	50-29-3	*delta*-HCH	319-86-8
Aldrin	309-00-2	*gamma*-HCH	58-89-9
alpha-Chlordane	5103-71-9	Mirex	2385-85-5
gamma-Chlordane	5105-74-2	*cis*-Nonachlor	5103-73-1
Dieldrin	60-57-1	*trans*-Nonachlor	39765-80-5
Endrin	72-20-8	Oxychlordane	26880-48-8

Individual PCB congeners	IUPAC Numbers	CAS registry numbers
2,4'-Dichlorobiphenyl	8	34883-43-7
2,2',5-Trichlorobiphenyl	18	37680-65-2
2,4,4'-Trichlorobiphenyl	28	7012-37-5
2,2',3,5'-Tetrachlorobiphenyl	44	41464-39-5
2,2',5,5'-Tetrachlorobiphenyl	52	35693-99-3
2,3',4,4'-Tetrachlorobiphenyl	66	32598-10-0
3,3',4,4'-Tetrachlorobiphenyl	77* (110)	32598-13-3 (38380-03-9)
2,2',4,5,5'-Pentachlorobiphenyl	101	37680-73-2
2,3,3',4,4'-Pentachlorobiphenyl	105	32598-14-4
2,3',4,4',5-Pentachlorobiphenyl	118	31508-00-6
3,3',4,4',5-Pentachlorobiphenyl	126*	57465-28-8
2,2',3,3',4,4'-Hexachlorobiphenyl	128	38380-07-3
2,2',3,4,4',5'-Hexachlorobiphenyl	138	35065-28-2
2,2',4,4',5,5'-Hexachlorobiphenyl	153	35065-27-1
2,2',3,3',4,4',5-Heptachlorobiphenyl	170	35065-30-6
2,2',3,4,4',5,5'-Heptachlorobiphenyl	180	36065-29-3
2,2',3,4',5,5',6-Heptachlorobiphenyl	187 (182,159)	52663-68-0 (60145-23-5, 39635-35-3)
2,2',3,3',4,4',5,6-Octachorobiphenyl	195	52663-78-2
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	206	40186-72-9
2,2',3,3',4,4',5,5',6,6'-Decachlorobiphenyl	209	2051-24-3

◊ Chemical Abstracts Service registry numbers.

* PCB 77 and 126 are planar PCBs contained in the calibration mixture but analyses requires further separation (i.e., carbon columns.

PCB 110 coelutes with PCB 77, and PCB 187 with 182 and 159 under the procedures described in the workbook.

Table 3. GC/ECD operating conditions for pesticides and PCB analyses.

Injector (splitless):			
Injector Temp.:	280°C		
Detector Temp.:	325°C		
Range:	10		
Autosampler Injector:			
Vol.:	2 µL		
Rate:	5 µL/sec		
Column:	30 m, 0.25 µm film, 0.25 mm internal diameter, DB-5 fused silica		
Run Time:	94.33 min		
	Level 1	Level 2	Level 3
Temp 1	100°C	140°C	250°C
Hold Time	1 min	1 min	1 min
Rate	5°/min	1.5°/min	10°/min
Temp 2	140°C	250°C	300°C
Final Hold	-	-	5 min
Carrier Gas:	He at 15.5 psi, 30.3 cm/sec at 100°C		
Make-up Gas:	N_2 at 20 mL/min		

use NOAA NS&T Program and International Mussel Watch Program requirements. The pesticides/PCB determined as part of these programs are listed in Table 2, and selected structures are shown in Section 8.2. Pesticides and PCBs are not separated before GC analyses. Therefore, GC/ECD conditions must allow for the separation of all the analytes in a single GC run. The GC/ECD conditions that were developed for the NOAA NS&T program and were used for the International Mussel Watch are provided in Table 3. Refinement of these techniques may be required for some applications. A typical chromatogram of a pesticide/PCB standard is shown in Figure 4. Development of these GC conditions and determination of analyte retention times requires the use of pure standards of all the analytes. It may be necessary to have several mixtures so that analytes that have similar retention times are not in the same standard solution, enabling their retention order to be determined. When analyzing commercial PCB formulations (i.e., Aroclors, Clophen and others), it is not possible to resolve all congeners under analytical conditions described here. Total resolution may require additional chromatographic separation before GC/ECD analyses and/or use of a different polarity GC/ECD column. Whether or not such steps are taken depends on the purpose of the study and is usually dictated by cost.

4.4. Calibration

The calibration of the GC/ECD requires the use of calibration standards made with compounds and solvents of known purity. When available, standard solutions of the required purity can be purchased. Alternatively, pure compounds can be weighed and diluted with appropriate solvent(s) using volumetric glassware. It is necessary to weigh a minimum of at least 1 mg to

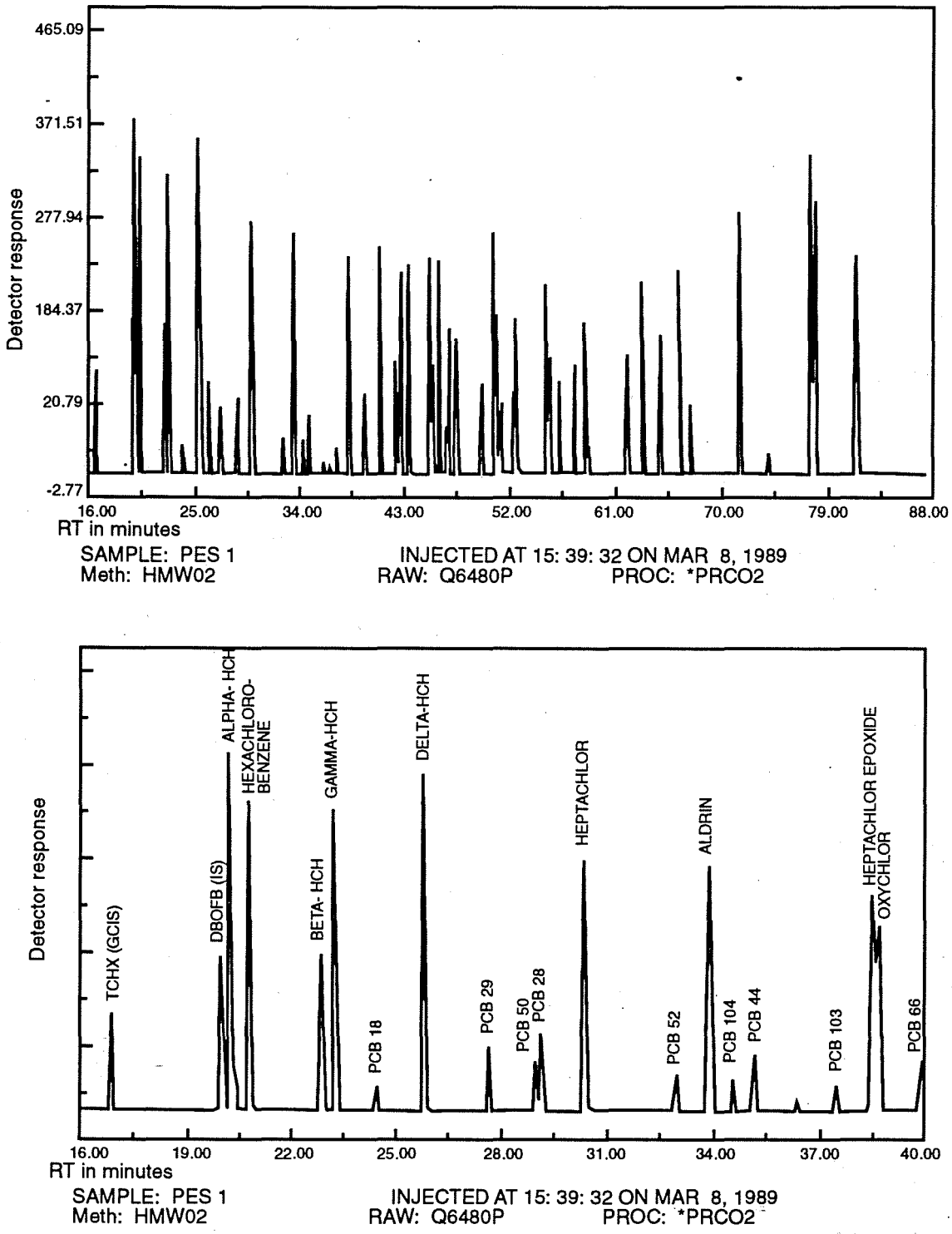


Figure 4. Typical GC/ECD chromatogram of pesticides and PCB analysis.

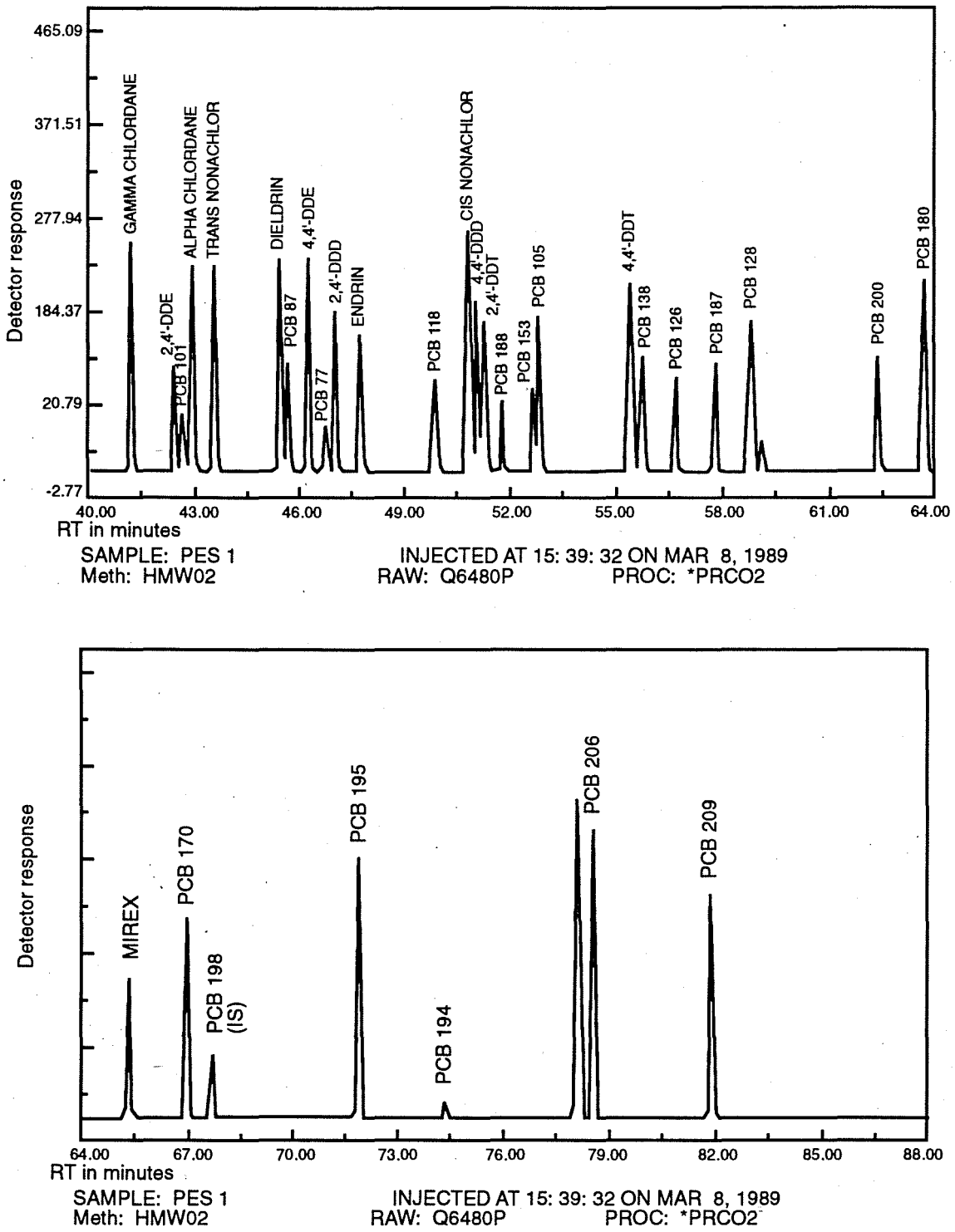


Figure 4. Typical GC/ECD chromatogram of pesticides and PCB analysis (cont.).

provide a weight with an uncertainty of less than one percent. Concentrations of stock solutions are normally made in the µg/mL range. In order to produce calibration solutions, dilution and combination of various stock solutions is required. The GC/ECD must be calibrated at a minimum of three concentrations other than zero that include the concentration range of interest expected for actual samples.

The calibration solution should be comprised of, at a minimum, the chlorinated hydrocarbons listed in Table 2, as well as all internal standards and recovery standards. Calibration standards are prepared in the concentration range of 5 to 200 ng/mL. Internal standard and recovery standards are added at a constant concentration of 100 ng/mL to all calibration standards. The internal standard compounds for all sample types are DBOFB, PCB 103, and PCB 198. The recovery standard is tetrachlorometaxylene (TCMX).

A typical calibration procedure is described in detail in Section 5.2.6. This method requires a calculator or a computer to define relationships between concentration and response using non-linear equations. An alternative calibration approach is to determine the linear range of the ECD and then dilute all samples until they are in the linear range of the detector.

4.5. Reference Materials

Ideally, CRMs should be used by laboratories to validate their analytical methods and assess accuracy. However, if CRMs are not available, RMs with information values can be substituted (see Section 8.4). The term reference material (RM) will be used to indicate either CRMs or other RMs. Even after validation, these methods must be monitored to verify that they continue to produce acceptable data and are in a state of statistical control. The RM matrix and analyte concentrations used should be a reasonable match to that of the samples to be analyzed (Taylor, 1983a). It is virtually impossible to find a RM that meets all of the matrix and concentration requirements and, therefore, some compromises are inevitable. However, the availability of RMs has increased in the past few years and their use is the best available approach to determination of the precision and accuracy of measured data.

The chemical measurement process should be evaluated and must be in a state of statistical control before the use of an RM begins. This means that the measurement process must be stable and capable of producing results with a reasonable standard deviation. The standard deviation must be small enough to allow the purpose of the measurements to be achieved. For example, the bias of the method will be a minimum of two times the uncertainty of the standard deviation (Taylor, 1983b and 1983c; Greenberg *et al.*, 1992).

The use of RMs that have undergone extensive testing ensures a homogeneous sample for replicate analyses to determine the precision and accuracy of a method. To provide a standard deviation when validating a method, the minimum number of analyses is three. The resulting mean and standard deviation can then be compared to the certified or information values provided with the RM.

The RM of choice should be analyzed as if it were a sample using the standard analytical protocol, including calculations. It is desirable to analyze the RM as a blind test to ensure that it receives no special handling or treatment. Once the data are available, the results are compared to the certified or information values for the RM. Initially, if the results determined for an analyte are within the reported value plus or minus one standard deviation, the method for that analyte is acceptable. Depending on the purpose of the analyses, some analytes may be acceptable even if they only agree within plus or minus three standard deviations. The measurements may have an adequate precision (an acceptable standard deviation), but the result may not agree with the reported concentration. This is caused by a bias in the method. If

the bias is consistent over a wide concentration range, then it theoretically could be corrected by simple multiplication of the data by a factor. However, many times bias is not a linear function. The method should be improved to eliminate the bias if possible or an alternate method chosen. Total statistical treatment of the data is beyond the scope of this workbook but can be found in Taylor (1985b) and references therein.

If the analyses of the RM shows the method to have acceptable accuracy for the purpose of the study, then analyses of actual samples should proceed. The RM should be reanalyzed at a frequency of once for every 20 samples to confirm that the method remains in a state of statistical control. The RM should be analyzed, preferably blind, as part of a normal sample set. Whenever the RM analysis shows that desired method accuracy has not been met, the affected sample sets should be reanalyzed. It is also necessary to determine the cause of the failure and correct it. If the frequent use of RMs makes them cost prohibitive, a secondary laboratory reference material, produced within a laboratory, can be used after standardization against the RM, using the same analytical procedure. Like RMs, this secondary material should be homogeneous in order to provide a consistent standard.

4.6. Analytical Sequence

The analytical sequence for the GC/ECD is designed to ensure that the instrument is in calibration throughout the analytical run. The initial injection should consist of a standard containing the internal standards, aldrin, and 4,4'-DDT. This initial injection determines if active sites exist in the GC injection port. Active sites cause breakdown of aldrin and 4,4'-DDT to dieldrin and 4,4'-DDE, respectively. If a peak for either dieldrin or 4,4'-DDE with an area greater than 5% of the area of aldrin or 4,4'-DDT is found, the injection liner must be cleaned or replaced. The GC/ECD is calibrated either prior to or during the analytical run. When the instrument is calibrated prior to analyses, samples are not analyzed until the calibration is acceptable (i.e., calibration curve fit r^2 of at least 0.995). During an analytical run, a mid-level standard is analyzed after every six samples to insure that the instrument is still in calibration. An alternative method is to analyze four concentration levels of calibration standards as part of the analytical string (every 6 samples). These standards are used to determine the calibration curve. However, if the r^2 is not at least 0.995, all samples must be rerun. A typical sequence for the second method is shown in Figure 5, where PES 1, 2, 4, and 5 are calibration mixtures.

4.7. Quantification

Concentrations of analytes in a sample are determined from a calibration curve. The ratio of the area of the internal standard to the analyte in the sample is used to determine the amount, usually in ng, of the analyte present in the sample. Once the amount of analyte is determined, it is divided by the volume or weight (wet or dry) of sample extracted in order to provide the concentration. The normal units of concentration used are ng/L, µg/L, ng/g, µg/g. For sediment or tissue samples, it is always important to determine if the results are based on the wet or dry weight of the sample. For example, most human health tissue advisories for pesticides are based on wet weight, while monitoring data typically is reported on a dry weight basis.

Due to the potential for artifacts created by the high degree of automation in today's analytical systems, visual and manual calculation checks are essential. Visually inspect every GC pattern to verify proper selection of peaks for quantitative calculations based on retention times. Analyte retention times must be verified versus known standards analyzed after every six samples. After calibration curves are established, all standards are recalculated to verify the calibration file in the GC microprocessor. Tabulations and control charts of daily detector

FEB 15, 1993 11:08
SEQUENCE: SEQ13 ON CRN 08
CHANNEL 13

SUBSEQUENCE 1

METHOD
HMW13

DIAL G-PRG PARAM-FILE
/ N

#WSHS #PMPS STOP
0, 15, 1

ISO POST-BTL# POST-#WSHS
NO, 35, 5

SAMPLES

	SAMPLE-NAME	BTL#	PROC-FILE	RAW-FILE	%DIL-F	STD-AMT	SMP-AMT
1	SOL CHK	1,	*PRC13	S0013P	100.00,	1.0000,	1.0000
2	PES 5	2,	*PRC13	S4828P	100.00,	1.0000,	1.0000
3	EP146	3,	*PRC13	C5661P	100.00,	1.0000,	1.0000
4	EP146	4,	*PRC13	C5662P	100.00,	1.0000,	1.0000
5	EP146	5,	*PRC13	C5663P	100.00,	1.0000,	1.0000
6	EP146	6,	*PRC13	C5664P	100.00,	1.0000,	1.0000
7	EP146	7,	*PRC13	C5665P	100.00,	1.0000,	1.0000
8	PES 2	8,	*PRC13	S4829P	100.00,	1.0000,	1.0000
9	EP146	9,	*PRC13	C5666P	100.00,	1.0000,	1.0000
10	EP146	10,	*PRC13	C5667P	100.00,	1.0000,	1.0000
11	EP146	11,	*PRC13	C5668P	100.00,	1.0000,	1.0000
12	EP146	12,	*PRC13	C5669P	100.00,	1.0000,	1.0000
13	EP146	13,	*PRC13	C5670P	100.00,	1.0000,	1.0000
14	EP146	14,	*PRC13	C5671P	100.00,	1.0000,	1.0000
15	PES 4	15,	*PRC13	S4830P	100.00,	1.0000,	1.0000
16	EP146	16,	*PRC13	Q4600P	100.00,	1.0000,	1.0000
17	EP146	17,	*PRC13	Q4601P	100.00,	1.0000,	1.0000
18	EP146	18,	*PRC13	Q4602P	100.00,	1.0000,	1.0000
19	EP146	19,	*PRC13	Q4603P	100.00,	1.0000,	1.0000
20	EP146	20,	*PRC13	Q4604P	100.00,	1.0000,	1.0000
21	PES 1	21,	*PRC13	S4831P	100.00,	1.0000,	1.0000
22	PCB MIX	22,	*PRC13	S4832P	100.00,	1.0000,	1.0000

23 /E

Figure 5. Typical analytical sequence printout from GC microprocessor. PES are pesticide/PCB calibration mixtures of differing concentrations with PES 1 and PES 5 having concentrations of 200 and 5 ng/mL respectively. EP 146 notations indicate the extraction page/batch of the samples. Q samples (RAW-FILE column) are QA/QC samples, including procedural blanks, reference materials, matrix spikes, and duplicates.

response for internal standards are maintained in order to assess detector stability with time, and to highlight any unusual shifts in sensitivity. Raw data files are archived so reintegration of individual peaks is possible.

4.8. Method Detection Limit

Details of the definition and procedure for determining the method detection limit have been described (Fed. Reg., Vol 89, Oct 26, 1984 p. 198 to 199), and only an example of their application will be provided here. "The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined in a given matrix containing the analyte."

The actual process of determining the MDL requires the analyses of at least seven replicates of a sample matrix that contains the analyte of interest at a level of one to five times the expected MDL. It is apparent that MDL determination is an iterative process. The minimum MDL must be in the range of 2.5 to 5 times the instrumental signal/noise ratio. A real sample is used to determine MDLs. If it does not contain any particular analyte at concentrations above the expected MDL, then those analytes should be added at concentrations not to exceed five times the expected MDL. All steps of the normal analytical procedure should then be performed on these samples. If sample concentrations are routinely corrected for the analytes found in procedural blanks, then seven procedural blanks must also be analyzed and each one used to correct one of the seven matrix analyses.

After successful analyses of the samples used for MDL determination, calculate the MDL from the standard deviation of the seven or more replicates and the student t value at the 99% confidence level with an "n" of seven. This corresponds to 3.143 times the standard deviation. The lower 95% confidence limit (LCL) and upper confidence limit (UCL) can also be determined using the appropriate student t values. The MDL is method specific and, therefore, changes in the method can affect the MDL. The MDL is reported in the same units as a normal sample analyses. Care must be taken in applying MDLs. For example, the MDL is dependent on the volume or weight of sample extracted. If the MDL was determined using a 10-gram sample and a 1-gram sample is extracted, the MDL will probably be 10 times higher for the 1-gram sample. The most rigorous approach, however, would be to determine the MDL for 1-gram sample extractions. The accuracy and precision of the method decreases as you approach the MDL. Care must be taken in interpretation of concentrations near the MDL. The required MDL is normally dictated by the project objectives. The MDL can be lowered by extracting a larger sample, concentrating the final extract to a smaller volume and injecting more of the sample extract. However, if these options are used, the amount of internal standard added to the extraction must also be adjusted.

4.9. QA/QC Validation

Before data are reported, its quality must be validated. This is a check to see that all of the accuracy and precision criteria for the analyses have been met. These acceptance criteria should be established before the analyses begin and the fact that they were or were not met should be documented. If the criteria were not met, the corrective action taken should be documented. For example, if a calibration curve had a r^2 of less than 0.995, the GC column might be replaced and all samples in that sample batch may have to be reanalyzed. The QA/QC validation should be approved by an individual not directly involved with the analyses.

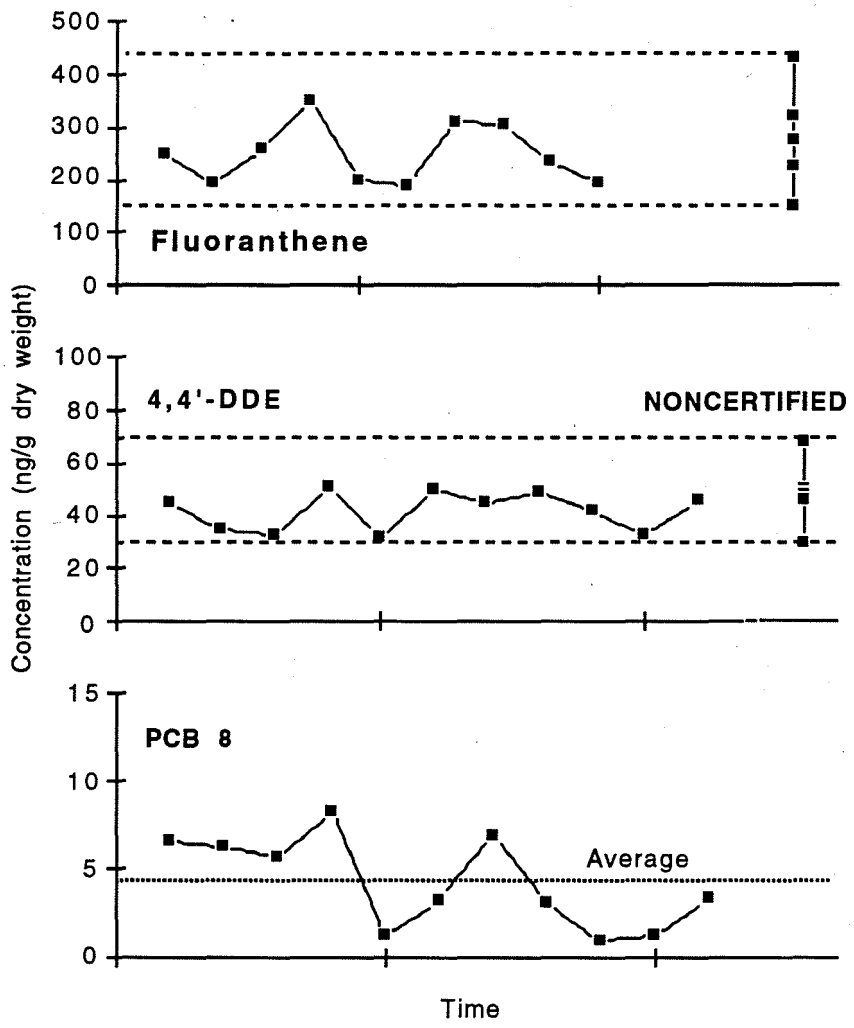


Figure 6. 1991 Results of analysis by TAMU of NIST SRM 1566a, Oyster Tissue, as control material for fluoranthene, 4,4'-DDE and PCB 8 (ng/g dry weight). No certified values for pesticides are available for SRM 1566a. [Symbols to the right denote the certified value, if available, the 95% uncertainty range, and ±35% of the uncertainty range. The average of all the determinations of PCB 8 is shown by the dotted line.]

4.10. Control Charts

Control charts are a graphical presentation of test results with respect to time or measurement sequence together with the limits within which the measurements are expected to lie when the analytical system is in a state of statistical control (Taylor, 1985a and 1985b). Control charts are one of the most common ways of monitoring the performance of a measurements process. The NOAA NS&T Program requires that a certain percentage of the analytical sample string be QA/QC materials, including materials with known analyte concentrations. The results of repeated analysis of RMs and CRMs during the 1991 analysis year for GERG are shown in Figure 6.

The certified values and uncertainties found in the US National Institute of Standards and Technology (NIST) Certificate of Analysis for SRMs describe, statistically, the range in which there is a 95% probability the true value is found. Ideally, an analyst should be able to achieve the same statistical distribution upon repeated analysis of a CRM. In practice, the effort to do this is usually not warranted and a more generous and realistic variability is acceptable. For example, the NS&T Program allowed a range of 35% above and below the uncertainties listed in the NIST Certificate of Analysis. For a certified value, x, and uncertainty, y, the ±35% range for NS&T analysis is

$$(x + y) + [0.35(x + y)]$$

to

$$(x - y) - [0.35(x - y)].$$

These uncertainty ranges are noted in the example control charts.

4.11. Data Reporting and Archiving

After the data have passed the QA/QC validation process, they are ready to be reported. Along with the data, other parameters need to be specified to enable others to assess its quality so informed interpretations of the data can be made. Information on the method detection limits and confidence intervals is necessary to insure proper interpretation of the data and comparison to existing data sets. Ancillary data are often required for complete interpretation of the data. Ancillary data include such parameters as percent moisture, percent lipids, grain size, total organic carbon, and others, and these parameters are commonly used during data interpretation. Finally, the data must be archived. All raw and final data should be saved so if questions about the data arise, the needed information can be readily retrieved in order to address these questions.

5. EXAMPLE CALCULATIONS

5.1. Introduction

This section provides some actual examples of calculations, and data on actual GC/ECD calibrations and sample analyses. Some of the calculations are simplified by using DDE as an example analyte, but the calculations apply to all of the pesticides/PCBs analyzed. Examples of forms used to track samples (from collection through analyses) are provided in Section 8.1. Different forms and calculation procedures can be adapted to the specific needs of researchers for their research program.

5.2. Examples

Examples of typical calculations and problems are provided on the following sections. Answers to the problems are found in Section 6.

5.2.1. Example 1. Preparation of Stock Solutions

Problem:

Prepare stock solutions of 4,4'-DDE and PCB 103 at a concentration approximately 10 µg/mL.

Solution:

The 4,4'-DDE stock solution is prepared by dissolving approximately 1 mg of 4,4'-DDE in hexane in a 100 mL volumetric flask and bringing the volume to exactly 100 mL. If the actual weight of 4,4'-DDE was 1.10 mg, then

$$\left[\frac{1.10 \text{ mg DDE}}{100 \text{ mL solvent}}\right]\left[\frac{1000 \text{ µg}}{\text{mg}}\right]=\left[\frac{11.0 \text{ µg DDE}}{\text{mL}}\right]$$

Similarly, the PCB 103 stock solution is prepared by dissolving approximately 1 mg of PCB 103 in hexane in a 100 mL volumetric flask and bring the volume to exactly 100 mL. If the actual weight of PCB 103 was 1.09 mg, then

$$\left[\frac{1.09 \text{ mg PCB 103}}{100 \text{ mL solvent}}\right]\left[\frac{1000 \text{ µg}}{\text{mg}}\right]=\left[\frac{10.9 \text{ µg PCB 103}}{\text{mL}}\right]$$

The concentrations of the stock solutions will be 11.0 µg 4,4'-DDE/mL and 10.9 µg PCB 103/mL.

5.2.2. Example 2. Preparation of Calibration Solutions

Problem:

Use the stock solutions to prepare the calibration solutions. The concentration of the 4,4'-DDE calibration solutions should be approximately 5, 40, 80, and 200 ng/mL, while the concentration of PCB 103 remains constant at approximately 100 ng/mL.

Solution:

To prepare the 200 ng/mL calibration solution, mix 0.2 mL of the 4,4'-DDE solution and 0.1 mL of the PCB 103 solution and dilute with solvent to 10 mL in a volumetric flask.

$$\left[\frac{0.2 \text{ mL DDE soln.}}{10 \text{ mL final volume}}\right]\left[\frac{11 \text{ µg DDE}}{\text{mL}}\right]\left[\frac{1000 \text{ ng}}{\text{µg}}\right]=\left[\frac{220 \text{ ng DDE}}{\text{mL mixed soln.}}\right]$$

$$\left[\frac{0.1 \text{ mL PCB 103 soln.}}{10 \text{ mL final volume}}\right]\left[\frac{10.9 \text{ µg PCB 103}}{\text{mL}}\right]\left[\frac{1000 \text{ ng}}{\text{µg}}\right]=\left[\frac{109 \text{ ng PCB 103}}{\text{mL mixed soln.}}\right]$$

Similarly, use the volumes listed below to prepare the appropriate calibration solutions. Note differences in volumes. The table below summarizes the dilutions. Repeat the calculations above for each mixture to ensure that they are correct.

4,4'-DDE soln. (mL)	PCB 103 soln. (mL)	Final vol. (mL)	Final 4,4'-DDE (ng/mL)	Final PCB 103 (ng/mL)
0.05	1.0	100	5.5	109
0.4	1.0	100	44	109
0.8	1.0	100	88	109
0.2	0.1	10	220	109

Answer the following:

Why is the concentration of PCB 103, the internal standard, kept constant?

5.2.3. Example 3. Determination of Percent Moisture

Problem:

Many environmental measurements require the results to be reported on a dry weight of sample basis. The percent (%) moisture or water content in the sample is determined by weighing an aliquot of the sample before and after drying. The drying can be done by heating in an oven or freeze drying.

Solution:

Weigh an empty container (i.e., Pyrex beaker) that will be used to hold the sample while it is dried.

$$\text{Beaker wt.} = 8.37 \text{ g}$$

Add the wet sample to the beaker and reweigh. Calculate the wet weight of the sample.

$$\text{Beaker wt. + wet sample wt.} = 10.06 \text{ g}$$
$$\text{Wet sample wt.} = 10.06 \text{ g} - 8.37 \text{ g} = 1.69 \text{ g}$$

Dry the sample until the sample attains a constant weight (all the water has evaporated). To do this, the sample is cooled to room temperature and weighed. The sample is then returned to the oven or freeze dryer, dried again, cooled, and reweighed. When the weight no longer decreases (i.e., within measurement error), it is assumed to be at a constant weight.

$$\text{Beaker wt. + dry sample wt.} = 9.66 \text{ g}$$
$$\text{Dry sample wt.} = 9.66 \text{ g} - \text{beaker wt.} = 9.66 \text{ g} - 8.37 \text{ g} = 1.29 \text{ g}$$

Calculate percent dry sample weight.

$$\text{Percent dry sample weight} = \left[\frac{\text{Sample dry wt.}}{\text{Sample wet wt.}}\right] (100\%)$$

$$= \left[\frac{1.29}{1.69}\right] (100\%) = 76.3\%$$

Calculate percent moisture

$$\text{Percent moisture} = \left[\frac{\text{Sample water wt.}}{\text{Sample wet wt.}}\right] 100\%$$

$$= \left[\frac{0.40}{1.69}\right] 100\% = 23.7\%$$

or

Percent moisture + percent dry sample = 100%

Calculate the percent dry weight and percent moisture for these samples to see if they are correct.

Beaker wt. (g)	Beaker plus wet sample wt. (g)	Beaker plus dry sample wt. (g)	Percent dry sample	Percent moisture
8.22	9.92	9.38	68.2	31.8
8.16	9.73	9.12	61.1	38.9

5.2.4. Example 4. Determination of Percent Solvent Extractable Material Weight

Problem

The percent solvent extractable material weight of a sample is operationally defined as the weight of material extracted with the solvent employed. An aliquot of the sample extract is taken, the solvent is removed, and the residue is weighed to determine the percent of the total sample weight that was extracted by the solvent used. The results are normally reported in percent. For tissue samples, the extractable weight is called percent lipids; for water and sediments, it is called oil and grease.

Procedure

A 20 mL aliquot is removed from a 270 mL sample extract for determination of percent extractable weight. The 20 mL aliquot is evaporated to a volume of 1 mL. From the 1 mL concentrate, 100 µL is removed and placed on a tared filter paper The solvent is allowed to evaporate and the residue on the filter paper is weighed.

Measurements

Sample dry weight extracted = 8.82 g
Total volume of extract = 270 mL
Sample aliquot removed = 20 mL
Final concentration aliquot volume = 1 mL = 1000 µL
Amount of final aliquot volume weighed = 100 µL
Weight of 100 µL of final concentrated aliquot after solvent evaporated = 0.719 mg

Solution

$$100\ \mu L \left[\frac{20\ mL\ aliquot \left[\frac{8.82\ g\ sample}{270\ mL\ solvent} \right]}{1000\ \mu L} \right] = 0.065\ g\ sample\ in\ the\ 100\ \mu L\ aliquot$$

The 100 µL aliquot weighed 0.719 mg, therefore

$$Extractable\ weight = \frac{0.719\ mg}{0.065\ g} = \frac{11.06\ mg}{g}$$

Percent extractable weight = 1.106 %

5.2.5. Example 5. Required Sample Weight

Problem

The method detection limit for 4,4'-DDE is 0.25 ng/g for a normal tissue extraction of 2 grams dry weight. A detection limit of 0.05 ng/g is required for a toxicity study. How much tissue must be extracted in order to achieve the required detection limit?

Solution

The weight of detectable 4,4'-DDE is the detection limit times the normal sample size. Therefore,

$$\text{Weight of 4,4'-DDE detectable} = (0.25 \text{ ng/g})\ (2 \text{ g}) = 0.50 \text{ ng}$$

$$\text{Weight required} = \frac{0.50 \text{ ng}}{0.05 \text{ ng/g}} = 10 \text{ g dry wt.}$$

Table 4. Calibration standard concentrations (ng/mL), amount of internal standard (IS) and recovery standard (RS) added to samples (ng), and resulting peak areas.

	RS (TCMX)	IS (PCB 103)	Analyte (4,4'-DDE)	Ratio (Analyte/IS)
Concentrations (ng/mL)				
Level 1	100.6	106.3	198.1	1.8636
Level 2	100.6	106.3	79.2	0.7451
Level 3	100.6	106.3	39.6	0.3725
Level 4	100.6	106.3	19.8	0.1863
Amount added (ng)				
Sample A	100.6	106.3		
Sample B	100.6	106.3		
Sample C	100.6	106.3		
Peak areas:				
Level 1	80363	32865	189284	5.7594
Level 2	92167	38067	95585	2.5110
Level 3	88246	37641	50547	1.3429
Level 4	98669	40371	29208	0.7235
Sample A	86759	29800	81500	2.7349
Sample B	88724	31752	67852	2.1369
Sample C	89796	30257	378674	12.5153

Discuss the following:

A. Will your method be reliable for that much sample?

B. How else can you increase your MDL without extracting more sample?

5.2.6. Example 6. Calibration Curve for the Analysis of Chlorinated Compounds Using 4,4'-DDE Calculation as an Example.

5.2.6.1. Calibration Curve

To calibrate the GC-ECD, four calibration solutions were used. The concentration range for each analyte is from 20 to 200 ng/mL. Each calibration solution has at least one internal standard (IS) (PCB 103 in this case), and one recovery standard (RS). The concentrations and areas for the internal standard, recovery standard, and 4,4'-DDE are given in Table 4.

The calibration curve is constructed by plotting the concentrations of 4,4'-DDE divided by the concentration of the IS, PCB 103, for each standard solution, versus the areas of 4,4'-DDE divided by the area of the IS in each standard solution. A plot of the data in Table 4 is shown in Figure 7.

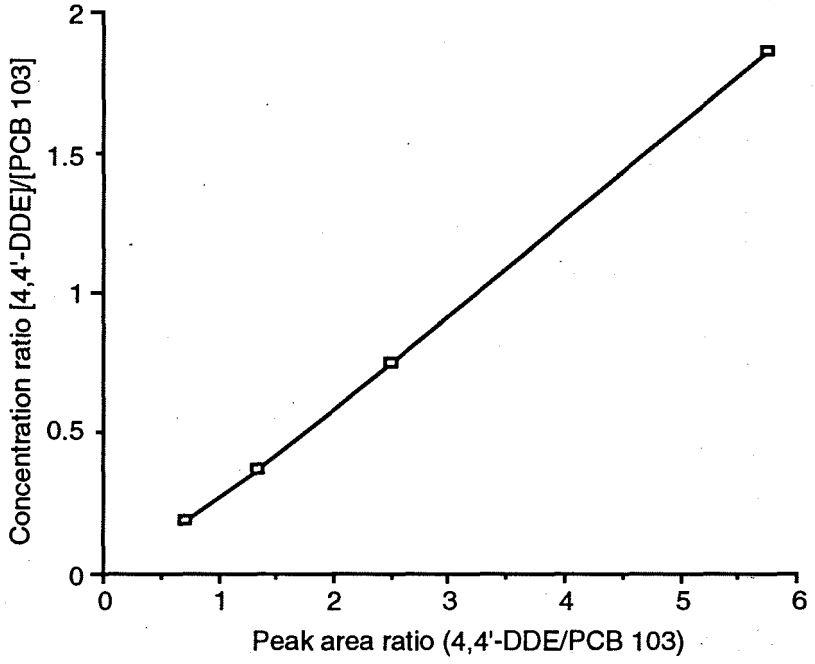


Figure 7. Sample calibration curve

The best line fit for GC/ECD data is often an exponential equation of the form:

$$y = A\ (x)^B$$

where y is the ratio of the concentration of 4,4'-DDE divided by the concentration of the IS, x is the ratio of the area of the 4,4'-DDE chromatogram peak divided by the peak area of the IS and A and B are constants. From a line fit program, it was determined that A is equal to 0.2677 and B is equal to 1.1095. Therefore,

$$y = 0.2677\ (x)^{1.1095}$$

A calibration curve must be determined for every analyte of interest, 4,4'-DDE is only one example. The exponential coefficient, B, is 1.1095 indicating that the curve is nearly linear. However, more accurate results are obtained using an exponential equation rather than a linear one.

5.2.6.2. Concentration Calculations

Once the calibration curve has been found to be acceptable (i.e., $r^2 > 0.995$), the equation is used to calculate the concentration of the corresponding analyte, in this case 4,4'-DDE. For example, consider sample A with the peak areas of IS (PCB 103) and 4,4'-DDE as given in Table 4. The ratio of the area of 4,4'-DDE to the IS (Table 4) is substituted into Equation 2.

$$y = 0.2677\ (2.7349)^{1.1095} = 0.8174$$

Where y is the ratio of the concentrations of 4,4'-DDE and the IS. The amount of IS added to the sample before extraction is known. Thus,

$$y = \frac{[DDE]}{[IS]} = 0.8174$$

and

$$[4,4'\text{-DDE}] = 0.8174\ [IS]$$

Since both 4,4'-DDE and IS are in the same sample extract, then

$$\text{ng 4,4'-DDE in extract} = 0.8174\ (\text{total ng IS in extract})$$

If it is assumed that both 4,4'-DDE and IS have the same recovery, the amount of 4,4'-DDE in the original sample can be calculated when the amount of IS added to the sample is substituted into the equation above.

$$\text{ng of 4,4'-DDE} = (0.8174)\ (106.3\ \text{ng}) = 86.88\ \text{ng}$$

To express the total amount of 4,4'-DDE in the sample as a concentration, it is necessary to divide the amount of 4,4'-DDE in ng by the wet or dry weight of sample extracted.

$$\text{Amount of 4,4'-DDE in the sample} = \frac{86.88\ \text{ng DDE}}{10\ \text{g sample extracted}} = \frac{8.688\ \text{ng DDE}}{\text{g}}$$

Calculate the concentrations for samples B and C from the data in Table 4.

5.2.6.3. Percent Recovery

The overall recovery of the internal standard can be calculated based on the IS and RS peak areas and concentrations in a calibration standard compared to their peak areas and concentrations in the sample using the equation

$$\% \text{ IS Recovery} = (R_1)\ (R_2)\ R_3)\ (R_4)\ (100)$$

where

R_1 = Area of IS/Area of RS in the sample

R_2 = Amount IS/Amount of RS in calibration solution

R_3 = Area of RS/Area of IS in calibration solution

R_4 = Amount of RS/Amount of IS in sample.

The percent IS recovery for Sample A from Table 4 and calibration mixture level 1 is calculated as follows,

$$R_1 = \frac{29800}{86759} = 0.343$$

$$R_2 = \frac{0.1063}{0.1006} = 1.056$$

$$R_3 = \frac{80363}{32865} = 2.445$$

$$R_4 = \frac{100.6}{106.3} = 0.946$$

Percent IS recovery Sample A = (0.343) (1.056) (2.445) (0.946) (100) = 83.8%

Calculate the percent recovery for samples B and C from the data provided in Table 4.

5.2.7. Example 7. Determination of Method Detection Limit

Oysters expected to contain negligible concentrations of analytes were obtained from a clean source (such as a local supermarket) and homogenized. Initial analyses indicated the absence of most of the analytes of interest with some exceptions (i.e., 4,4'-DDE and 4,4'-DDD). The oyster sample was then spiked with the analytes of interest. In Table 4, the concentration of selected analytes for 7 replicate analyses of the spiked oyster tissue are reported as well as the mean and standard deviation. The Method Detection Limit (MDL) can be calculated using the following formula:

$$MDL = t_{(n-1)}\ S$$

where n is the number of replicates, S is the standard deviation, and t is the standard t-value for 99% confidence level and n-1 degrees of freedom. For 7 replicates and 6 degrees of freedom, t is 3.143.

MDL (for 7 replicates) = 3.143 S

5.2.7.1. MDL Calculation

Calculate the MDL for dieldrin using the data in Table 5.

MDL = (3.143) (0.91) = 2.86 ng/g

5.2.7.2. Upper and Lower 95% Confidence Limits

The lower and upper 95% confidence limit (LCL and UCL) for 7 replicates can be calculated using the student t values for 95% confidence limit.

LCL = 0.64 MDL

UCL = 2.20 MDL

For example, the MDL for dieldrin is 2.86 ng/g. Therefore, LCL and UCL for dieldrin are:

LCL = (0.64) (2.86 ng/g) = 1.83 ng/g

UCL = (2.20) (2.86 ng/g) = 6.29 ng/g

Table 5. Concentrations (ng/g) determined for selected analytes in spiked oyster tissue, mean and standard deviation (S).

Sample	Dry Weight	Dieldrin	*trans*-Nonachlor	4,4'-DDE
1	1.31	9.27	10.19	22.87
2	1.38	9.03	9.84	22.19
3	1.27	9.06	9.21	22.65
4	1.38	7.62	8.58	20.64
5	1.21	8.84	9.04	23.98
6	1.24	7.95	9.11	20.10
7	1.14	6.86	9.45	19.11
Mean	1.28	8.38	9.35	21.65
S		0.91	0.54	1.74
MDL		2.86		
LCL		1.83		
UCL		6.29		

Table 6. Spike blank results.

	Dieldrin	*trans*-Nonachlor	4,4'-DDE
Spike Added (ng)	36.6	30.8	34.6
Spike Blank (ng)	31.2	27.9	35.2
Percent Recovery	85.5		

Do or answer the following:

A. Calculate the MDL, LCL, and UCL for *trans*-nonachlor and 4,4'-DDE from the information available in Table 5.

B. Why is the MDL for 4,4'-DDE so high?

C. Estimate the MDL for dieldrin if 12.7 grams of dry weight of sample were extracted. Do you think this is a legitimate MDL?

5.2.8. Example 8. Spiked Blank Calculations

Procedure:

Approximately 40 ng of each analyte of interest was "spiked" into a procedural blank. The spike blank thus produced was processed through the entire analytical procedure. The spike blank does not contain any sample and therefore does not have a weight. When the concentration is calculated, a weight of the average sample weight, or 1 g can be used. If no weight is used, the results are the weight of the analyte in ng. This can be compared directly to the amount in ng of spike added to the sample (Table 6).

The percent recovery of dieldrin is calculated as follows. The amount of dieldrin added to the spike blank was 36.6 ng. After processing the spike through the entire analytical procedure, the amount of dieldrin calculated to be present was 31.32 ng. What is the percent recovery of dieldrin?

Solution:

$$\text{Percent Recovery of Dieldrin} = \frac{\text{Amount found}}{\text{Amount added}} (100\%) = \frac{31.3 \text{ ng}}{36.6 \text{ ng}} (100\%) = 85.6\%$$

Do or answer the following:

A. Calculate recoveries for *trans*-nonachlor and 4,4'-DDE.

B. Is it possible to have a percent recovery greater than 100%? Explain.

Table 7. Spiked matrix concentration results (ng/g unless noted)

Description	Dry weight (g)	Dieldrin	*trans*-Nonachlor	4,4'-DDE
Spike Amount (ng)		36.6	30.8	34.6
Original Sample Concentration (ng/g)	10.33	1.15	0.34	66.54
Spiked Sample Concentration (ng/g)	9.92	4.54	2.93	73.78
Calculated Spike Concentration (ng/g)		3.68		
Spike Recovered (ng/g)		3.39		
% Recovery		92.1%		

5.2.9. Example 9. Spiked Matrix Calculations

Procedure:

Spiked matrix samples are often analyzed as part of QA/QC protocols. Approximately 40 ng of the analytes of interest are spiked into a sample. Another aliquot of the same sample is analyzed without spiking. Typical results are shown in Table 7.

Calculate the percent recovery of dieldrin as follows. The amount of dieldrin spiked into the sample was 36.6 ng, but the sample had some dieldrin already present (1.15 ng/g). The final concentration of the dieldrin in the spike sample, if no dieldrin was present, is calculated as follows:

$$\text{Spiked concentration} = \frac{\text{Spike amount (ng)}}{\text{Sample (g)}} = \frac{36.6 \text{ ng}}{9.92 \text{ g}} = 3.68 \text{ ng/g}$$

However, the total concentration in the sample is the sum of the amount of dieldrin spiked into the sample plus the amount present originally. Therefore,

$$\text{Spiked concentration} = \text{Total conc. measured} - \text{Conc. in unspiked sample.}$$

$$= 4.54 \text{ ng/g} - 1.15 \text{ ng/g} = 3.39 \text{ ng/g}$$

The percent recovery for dieldrin is

$$\text{Percent Recovery} = \frac{\text{Spike recovered}}{\text{Spike added}} (100\%) = \frac{3.39 \text{ ng/g}}{3.68 \text{ ng/g}} (100\%) = 92.1\%$$

Do or answer the following:

A. Calculate the percent recovery for *trans*-nonachlor and 4,4'-DDE.

B. Explain the 4,4'-DDE recovery.

Table 8. Intercomparison exercise results (ng/g)

Aliquot 1			Aliquot 2	Aliquot 3	Aliquot 4
Injection 1	Injection 2	Injection 3			
gamma-HCH					
0.47	0.52	0.53	0.51	0.47	0.41
4,4'-DDE					
50.19	50.31	50.43	48.12	49.74	47.85

5.2.10. Example 10. Reference Material Calculations

An intercomparison exercise using tissue homogenate was conducted to provide a consensus value for selected analytes as listed below. Each laboratory extracted four aliquots of the sample. Aliquot 1 was analyzed three times and the other three aliquots were analyzed once. Analysis was done using GC/ECD. The results for *gamma*-HCH and 4,4'-DDE are shown in Table 8.

Do or answer the following:

A. Calculate the mean, standard deviation, and coefficient of variation, CV, for the three injections of Aliquot 1, and for Aliquots 2, 3, and 4.

B. Where do you find the lowest coefficients of variation and why?

C. Calculate the coefficient of variation for the extraction.

D. Can you judge the accuracy and precision of the laboratory from the above data?

6. ANSWERS

Section 5.2.2.

The concentration of PCB 103 is kept constant because it is constant in the final sample extract. However, analyte concentrations in different samples are not constant. Analytes are added to the calibrtion standards at concentrations that are expected to bracket the analyte concentrations in the samples.

Section 5.2.5.

A. It may not be. There may be too much lipid material for column techniques. The excess lipids could interfere with the GC/ECD analysis. The method would need to be tested with the higher sample weight.

B. The MDL may be decreased by concentration to a smaller final volume or injection of more of the original sample volume if less internal standard is added before extraction.

Section 5.2.6.2.

The concentration of samples B and C are 6.61 and 46.97 ng/g dry weight respectively, assuming a 10 g sample. Note that the ratio for sample C is above the ratios for the highest calibration (Level 1) solution. Since the ECD is a non-linear detector, this concentration is not reliable. The sample must be diluted and more internal standard added. A better solution is to re-extract 5 to 10 times less sample weight.

Section 5.2.6.3.

The percent recovery for samples B and C are 87.5% and 82.3% respectively.

Section 5.2.7.2.

A.

	trans-Nonachlor	4,4'-DDE
MDL	1.70	5.47
LCL	1.09	3.50
UCL	3.74	12.03

B. The oyster sample before spiking was found to contain 4,4'-DDE. The sample concentration after spiking may be greater than 5 times the actual MDL, which violates one of the criteria for determining MDLs.

C. If 10 times more sample is extracted (12.7 vs. 1.27 g dry weight) and the final extract volume is the same, then the MDL should go down by a factor of 10 (i.e. 2.86 to 0.286) However, extracting 12.7 grams dry weight of oyster increases the amount of lipids co-extracted by a factor of 10. The increased sample size may cause a large mass of material to overload the chromatography columns. This causes problems with peak separation of the analytes from lipids, peak tailing and non-reproduceable results.

Section 5.2.8.

A. The percent recovery of *trans*-nonaclor is 90.6%, and that of 4,4'-DDE is 101.6%.

B. The recovery of 4,4'-DDE is greater than 100. This can result from uncertainty of the determination of the analyte. It can also be found if the internal standard has a lower recovery through the procedure than the analyte. QA/QC criteria are set up to ensure that the accuracy and precision of the data within an acceptable set limits of the percent recovery of spike blanks.

Section 5.2.9.

A.

Description	Dry weight (g)	Dieldrin	*trans*-Nonachlor	4,4'-DDE
Spike Amount (ng)		36.6	30.8	34.6
Original Sample Concentration (ng/g)	10.33	1.15	0.34	66.54
Spiked Sample Concentration (ng/g)	9.92	4.54	2.93	73.78
Calculated Spike Concentration (ng/g)		3.68	3.10	3.48
Spike Recovered (ng/g)		3.39	2.59	7.24
% Recovery		92.9%	83.5%	208.0%

B. The sample that was spiked had a high concentration of 4,4'-DDE. Therefore, two large numbers (3556, 3327) are subtracted to produce a small number (229). The uncertainty of determining the 4,4'-DDE concentration is about ±10%. Therefore, the uncertainty of the sample concentration is ±350 ng/g. It is obvious from this example that spiking a sample that already has substantial amounts of an analyte provides dubious estimates.

Section 5.2.10.

A.

gamma-HCH (ng/g)

	Aliquot 1				
Injection 1	Injection 2	Injection 3	Mean	SD	CV
0.47	0.52	0.53	0.51	0.03	6.34
Aliquot 2	Aliquot 3	Aliquot 4	Mean	SD	CV
0.51	0.47	0.41	0.46	0.05	10.86

4,4'-DDE (ng/g)

	Aliquot 1				
Injection 1	Injection 2	Injection 3	Mean	SD	CV
50.19	50.31	50.43	50.31	0.12	0.24
Aliquot 2	Aliquot 3	Aliquot 4	Mean	SD	CV
48.12	49.74	47.85	49.39	1.14	2.31

B. The lowest coefficient of variation is found for the triple injection of Aliquot 1. This indicates that the coefficient of variation for replicate injections of the same extract at a concentration of approximately 50 ng/g was about 0.24%, and at a concentrations of approximately 0.51 ng/g, 6.34%. In both cases, the replicate extraction had higher coefficients of variation. The total coefficient of variation, CV_T , should be equal to the extraction coefficient of variation, CV_E, and the GC/ECD analysis coefficient of variation, CV_A.

C. $$CV_T = CV_A + CV_E$$

For *gamma*-HCH,

$$10.98 = 6.38 + CV_E$$

$$CV_E = 4.52.$$

For 4,4'-DDE, CV_E is 2.07.

D. The precision, yes. Accuracy, no.

7. REFERENCES

Ballschmiter, K., and M. Zell (1980) Analysis of Polychlorinated Biphenyls (PCB) by Glass Capillary Gas Chromatography-Composition of Technical Aroclor- and Clophen-PCB Mixtures. Fresenius Z. Anal. Chem., 302:20-31.

Cantillo, A. Y. (1992) Standards and Reference Materials for Marine Science. Third Edition, NOAA Tech. Memo. NOS ORCA 68. NOAA, Rockville, MD, USA. 577 pp.

Federal Register (1984) (Oct. 26), Vol. 89, 198-9.

Greenberg, A. E., L. S. Clesceri and A. D. Eaton (eds.) (1992) Standard Methods for the Examination of Water and Wastewater. 18th Edition. Prepared and published jointly by the American Public Health Assoc., the American Water Works Assoc., and the Water Environment Federation. American Public Health Assoc., Washington, DC. I-1 - I-41.

Krahn, M. M., L. K. Moore, R. G. Bogar, C. A. Wigren. S. - L. Chan, and D. W. Brown (1988) High-performance liquid chromatography method for isolating organic contaminants from tissue and sediment extracts. J. Chromatogr., 437:161-75.

Ramos, L., and P. G. Prohaska (1981) Sephadex LH-20 chromatography of extracts of marine sediments and biological samples for the isolation of polynuclear aromatic hydrocarbons. J. Chromatogr., 211:284-9.

Taylor, J. K. (1983a) Reference Materials - What they are and how they should be used. J. Testing and Evaluation, 11:355.

Taylor, J. K. (1983b) Principles of Quality Assurance of Chemical Measurements. NBSIR 83-3105. National Bureau of Standards, Gaithersburg, MD, USA.

Taylor, J. K. (1983c) Validation of analytical methods. Anal. Chem., 55:600A-4A.

Taylor, J. K. (1985a) The Quest for Quality Assurance. American Lab., October, 67-75.

Taylor, J. K. (1985b) Standard Reference Materials: Handbook for SRM Users. NBS Special Publication 260-100, National Bureau of Standards, Gaithersburg, MD, USA. 85 pp.

8. APPENDICES

8.1. Example Forms

8.1.1. Sample receiving and integrity form

SAMPLE RECEIVING / INTEGRITY REPORT

Catalog: _____ Date Received: _____

From: _____

1. Number of shipping containers _____ Condition of shipping containers

Comments	Damaged	Undamaged

2. Airbill present? No Yes

Airbill number

3. Custody seals on containers? No Yes Intact Not intact

Comments

4. Chain of custody records? No Yes List of numbers:

List of numbers:		

5. General sample condition/temperature: Dry Ice Frozen Cool Unrefrigerated

Comments

6. List of damaged or broken containers (if any):

7. Number of samples expected: _____ Number of samples received: _____

8. Problems/discrepancies:

9. Resolutions

10. Checked in by: _____ Date: _____

8.1.2. Sample processing form

SAMPLE PROCESSING FORM

Catalog number: _____

1. Received by: _____ on _____ S T
2. Number of samples expected: _____ B W
3. Number of samples received: _____ O _____
4. Discrepancies/resolutions:

5. Aliquots? yes no by _____ on _____
   - Trace metals yes no by _____ on _____
   - AVS yes no by _____ on _____
   - TOC yes no by _____ on _____
   - Grain size yes no by _____ on _____
   - Other labs yes no by _____ on _____
6. Tissue labeled by _____ on _____
   Sediments labeled by _____ on _____
7. Tissumized by _____ on _____
   Composited/aliquoted by _____ on _____
8. Taken to freeze dryer by _____ on _____
9. Checked out by Returned:
   - Yellow lab _____ on _____ / / _____
   - Trace metal lab _____ on _____ / / _____
   - EV Lab _____ on _____ / / _____
   - Grain size lab _____ on _____ / / _____
10. Archived by _____ on _____

Dry weights:

Sediments by _____ on _____

_____ _____

8.1.3. Sample transfer form

SAMPLE TRANSFER FORM

Catalog number/name: _____

List of samples signed out:

Total: _____ Date: _____ Time: _____

Signature of recipient: _____

Signature of custodian: _____

Samples returned: Date: _____ Time: _____

Comments:

Signature of recipient: _____

Signature of custodian: _____

IMW SAMPLES 1991 - 1992

Sample ID	Sample ID	Country	Location	Bivalve	Number of individuals	Total wet weight (g)
IMW 1004	ARHU-2B	Argentina	Hudson	*Corbicula fluminea*	40	190
IMW 1008	ARAT-1B	Argentina	Atalaya	*Corbicula fluminea*	40	170
IMW 1020	URSL-1B	Uruguay	Santa Lucia	*Corbicula fluminea*	100	130
IMW 1024	ARMP-2B	Argentina	Mar del Plata	*Mytilus platensis*	15	140
IMW 1027	ARPC-1A	Argentina	Pehuen-co	*Bracchidontes rodrigezii*	100	160
IMW 1029	ARAP-1A	Argentina	Arroyo Parejas	*Bracchidontes rodrigezii*	100	170
IMW 1033	ARRA-2A	Argentina	Rawson	*Mytilus platensis*	50	170
IMW 1037	ARCA-1A	Argentina	Bahia Camarones	*Aulacomya ater*	30	130
IMW 1043	ARCA-1C	Argentina	Bahia Camarones	*Mytilus platensis*	30	130
IMW 1040	ARCA-2B	Argentina	Bahia Camarones	*Aulacomya ater*	30	130
IMW 1052	ARPL-1B	Argentina	Punta Loyola	*Mytilus platensis*	25	170
IMW 1057	PAPB-1A	Panama	Portobelo	*Isognomon alatus*	100	120
IMW 1064	PAPC-1B	Panama	Punta Chame	*Anadara tuberculosa*	10	180
IMW 1066	NIIA-1B	Nicaragua	Isla Aserradores	*Anadara tuberculosa*	16	150
IMW 1072	CREJ-1B	Costa Rica	Estero Jicabal	*Anadara tuberculosa*	10	130
IMW 1075	CRIP-1A	Costa Rica	Isla Paloma	*Anadara grandis*	7	170
IMW 1077	CREC-1A	Costa Rica	Estero Cocoroca	*Anadara tuberculosa*	20	200
IMW 1079	CREC-1C	Costa Rica	Estero Cocoroca	*Anadara similis*	20	230
IMW 1082	CRGO-1B	Costa Rica	Golfito	*Anadara tuberculosa*	20	130
IMW 1088	CRGO-1C	Costa Rica	Golfito	*Anadara similis*	20	160
IMW 1085	CRGO-1E	Costa Rica	Golfito	*Prototaca sp.*	15	110
IMW 1092C	PAPA-1B	Panama	Puerto Almirante	To be identified	12	180
IMW 1086C	COBC-1B	Colombia	Bahia Cartagena	*Crassostrea rizhophorae*	50	120
IMW 1998C	COBC-2B	Colombia	Bahia Cartagena	*Crassostrea rizhophorae*	50	160
IMW 1100	COCG-1B	Colombia	Cienaga Grande	*Crassostrea sp.*	15	150
IMW 1105C	COBT-1A	Colombia	Bahua Tumaco	*Anadara tuberculosa*	15	130
IMW 1107C	COBT-1C	Colombia	Bahua Tumaco	*Anadara similis*	15	110
IMW 1111C	COBT-3A	Colombia	Bahua Tumaco	*Anadara tuberculosa*	15	200
IMW 1113C	COBT-3C	Colombia	Bahua Tumaco	*Anadara similis*	15	130

IMW SAMPLE INVENTORY 1991 - 1992

Laboratory file number	Sample ID	Date collected	Date received	Sample type	No. of containers	Comments/description	Analyses			
C3921	ARHU-1A			T		Argentina Hudson Corbicula fluminea				
C3922	ARHU-1B			T		Argentina Hudson Corbicula fluminea				
C3923	IARHU-2A			T		Argentina Hudson Corbicula fluminea				
C3924	IARHU-2B			T		Argentina Hudson Corbicula fluminea				
C3925	IARHU-3A			T		Argentina Hudson Corbicula fluminea				
C3926	ARHU-3B			T		Argentina Hudson Corbicula fluminea				
C3927	ARAT-1A			T		Argentina Atalaya Corbicula fluminea				
C3928	IARAT-1B			T		Argentina Atalaya Corbicula fluminea				
C3929	ARAT-2A			T		Argentina Atalaya Corbicula fluminea				
C3930	ARAT-2B			T		Argentina Atalaya Corbicula fluminea				
C3931	ARAT-3A			T		Argentina Atalaya Corbicula fluminea				
C3932	ARAT-3B			T		Argentina Atalaya Corbicula fluminea				
C3933	URPE-1A			T		Uruguay Punta del Este Mytilus platensis				
C3934	URPE-1B			T		Uruguay Punta del Este Mytilus platensis				
C3935	URPE-2A			T		Uruguay Punta del Este Mytilus platensis				
C3936	URPE-2B			T		Uruguay Punta del Este Mytilus platensis				
C3937	URPE-3A			T		Uruguay Punta del Este Mytilus platensis				
C3938	URPE-3B			T		Uruguay Punta del Este Mytilus platensis				
C3939	URSL-1A			T		Uruguay Santa Lucia Corbicula fluminea				
C3940	URSL-1B			T		Uruguay Santa Lucia Corbicula fluminea				
C3941	ARMP-1A			T		Argentina Mar del Plata Mytilus platensis				
C3942	ARMP-1B			T		Argentina Mar del Plata Mytilus platensis				
C3943	ARMP-2A			T		Argentina Mar del Plata Mytilus platensis				
C3944	ARMP-2B			T		Argentina Mar del Plata Mytilus platensis				
C3945	ARMP-3A			T		Argentina Mar del Plata Mytilus platensis				
C3946	ARMP-3B			T		Argentina Mar del Plata Mytilus platensis				
C3947	ARPC-1A			T		Argentina Pehuen-co Bracchidontes rodrigezii				
C3948	ARPC-1B			T		Argentina Pehuen-co Bracchidontes rodrigezii				

Sample types: S = sediments. T = tissues. W = water. O = slick.

Laboratory Sample Logbook

Tissues	Comments: International Mussel Watch 1991-1992	Surrogate #: / Spike #:

	File no.	Sample description	Wet wt. (g)	Dry wt. (%)	Dry wt. (g)	Comments
1	C3986	IMW1066	15.640	11.80	1.85	
2	C10474	IMW1272	15.880	15.80	2.51	
3	C3924	IMW1004	15.430	14.00	2.16	
4	C4125	IMW1205	15.420	15.82	2.44	
5	C4031	IMW111C	15.475	10.91	1.69	
6	C3957	IMW1037	15.471	18.17	2.81	
7	C10472	IMW1263	15.185	13.83	2.10	
8	C3953	IMW1033	15.394	18.37	2.83	
9	C4161	IMW1241C	15.547	20.17	3.14	
10	C3947	IMW1027	15.789	13.33	2.11	
11	Q9769	QA/QC C3947	15.294	12.71	1.94	
12	Q9770	NIST	5.00	11.18	0.56	
13	Q9771	Blank				
14	Q9772	Spike blank	15.128	13.12	1.98	

QA

- Sample prep. Initial/date
- Exraction Initial/date
- Concentration Initial/date
- Si/Al Column Initial/date
- Concentration Initial/date
- HPLC Initial/date
- Concentration Initial/date
- GC/ECD prep. Initial/date
- GC/MS prep. Initial/date
- GC/FID prep. Initial/date

Lab Manager Initial/date

QA/QC Officer Initial/date

8.1.7. Laboratory sample logbook

PESTICIDE/ ORGANICS ANALYSIS DATA SHEET

EPA Sample No:

Lab name: _____ Contract: _____

Lab Code: _____ Case No.: _____ SAS No.: _____ SDG No.: _____

Matrix (sediment/water/tissue): TISSUE Lab sample ID: _____

Sample (weight or volume): _____ Lab file ID: _____

% Moisture: _____ Decanted? (yes/no): _____ Date received: _____

Extraction (sepF/Cont/Sonc): _____ Date extracted: _____

Concentrated extract vol. (µL): 1000 Date analyzed: _____

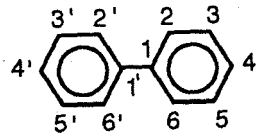
Injection vol. (µL): 1 Dilution factor: 1

GCP cleanup? (yes, how): HPLC pH: NA Sulfur cleanup? (no): _____

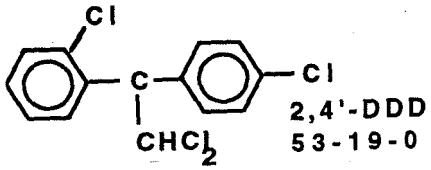
CAS No.	Compound	Concentration (µg/L or µg/kg)
319-84-6	*alpha*-HCH	
118-74-1	Hexachlorobenzene	
319-85-7	*beta*-HCH	
58-89-9	*gamma*-HCH	
319-86-8	*delta*-HCH	
76-44-8	Heptachlor	
1024-57-3	Heptachlor epoxide	
26880-48-8	Oxychlordane	
5105-74-2	*gamma*-Chlordane	
5103-71-9	*alpha*-Chlordane	
39765-80-5	*trans*-Nonachlor	
5103-73-1	*cis*-Nonachlor	
309-00-2	Aldrin	
60-57-1	Dieldrin	
72-20-8	Endrin	
2385-85-5	Mirex	
3424-82-6	2,4'- DDE	
72-55-9	4,4'- DDE	
53-19-0	2,4'- DDD	
72-54-8	4,4'- DDD	
789-02-6	2,4'- DDT	
50-29-3	4,4'- DDT	

8.2. Selected Pesticide/PCB Structures and CAS Numbers. (Some hydrogen atoms omitted for clarity.)

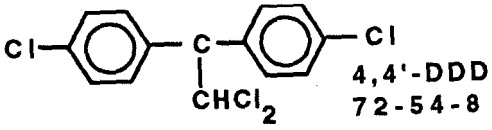
PCB parent structure



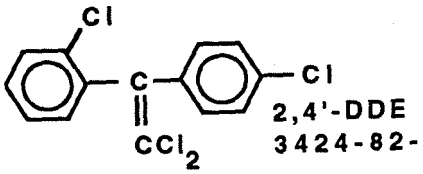
DDTs and metabolites



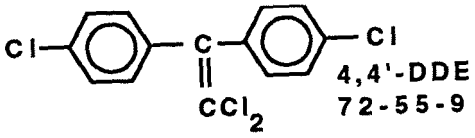
2,4'-DDD
53-19-0



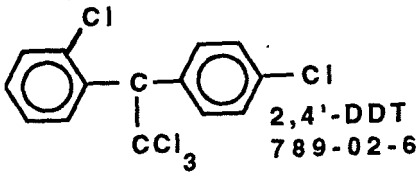
4,4'-DDD
72-54-8



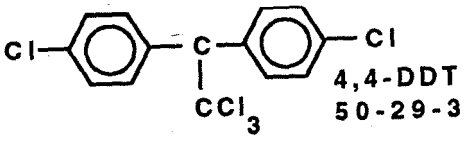
2,4'-DDE
3424-82-6



4,4'-DDE
72-55-9

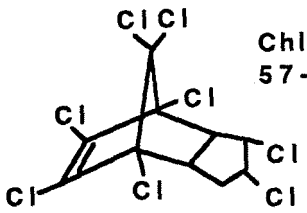


2,4'-DDT
789-02-6

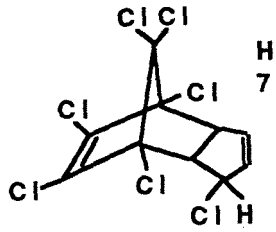


4,4-DDT
50-29-3

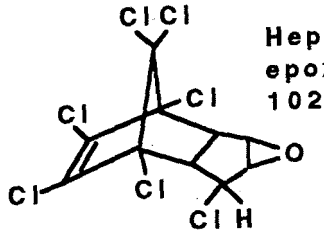
Cyclopentadiene family pesticides



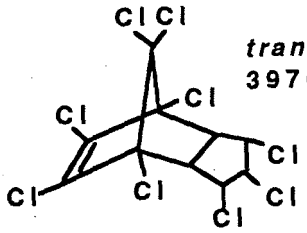
Chlordane
57-74-9



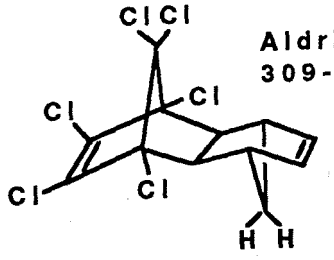
Heptachlor
76-44-8



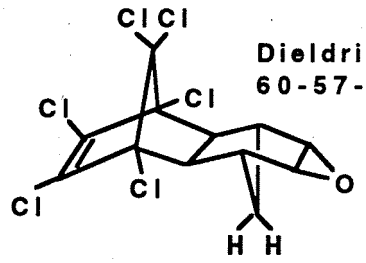
Heptachlor epoxide
1024-57-3



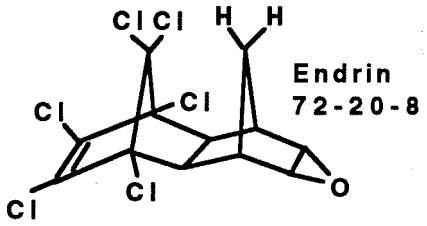
trans-Nonachlor
39765-80-5



Aldrin
309-00-2

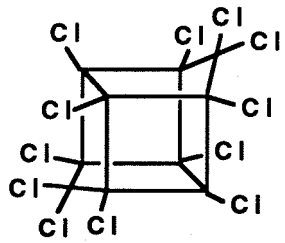


Dieldrin
60-57-1



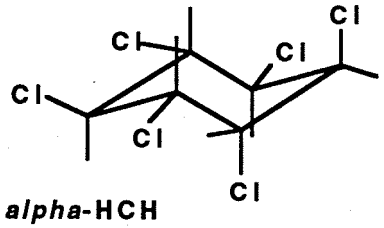
Endrin
72-20-8

Others

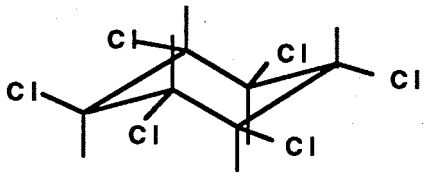


Mirex
2385-85-5

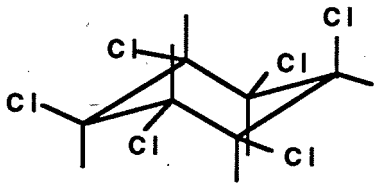
Hexachlorohexanes



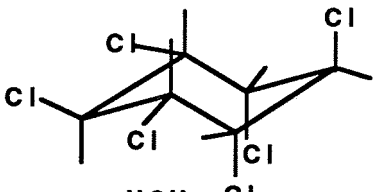
alpha-HCH
319-84-6



beta-HCH
319-85-7



delta-HCH
319-86-8



gamma-HCH
58-89-9

8.3. Standard Operating Procedures (SOPs) of TAMU/GERG

8.3.1. SOP-9016 - Extraction of Biological Tissues for Chlorinated Hydrocarbon Analysis

Geochemical and Environmental Research Group

STANDARD OPERATING PROCEDURES

EXTRACTION OF BIOLOGICAL TISSUES FOR CHLORINATED HYDROCARBON ANALYSIS

---

1. INTRODUCTION

Assessment of the environmental impact of man-made chlorinated pesticides and polychlorinated biphenyls (PCBs) requires their measurement in tissues at trace levels (parts per billion to parts per trillion).

Approximately 2 to 15 g of tissue is homogenized in a 200 mL centrifuge tube. The tissue is macerated in 100 mL of CH_2Cl_2 and 50 g Na_2SO_4. The eluate is dried with sodium sulfate, concentrated, and purified using a silica gel/alumina column to remove matrix interferences. Further purification by HPLC may be required to reduce matrix interferences. The chlorinated hydrocarbon extract is then concentrated for analysis by GERG SOP-9017.

2. SAMPLE COLLECTION, PRESERVATION AND STORAGE

2.1. Sample Collection

Tissue samples should be collected in precleaned glass jars and frozen (-20°C) in the field.

2.2. Sample Preservation and Storage

Store tissue samples at -20°C in the dark. Tissue samples should be shipped frozen to the laboratory and stored at -20°C in the dark until analysis. After subsampling, excess sample is stored at -20°C in the dark. Sample extracts are stored in the dark at/or below 4°C.

3. INTERFERENCES

Method interferences may be caused by lipid materials or other contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to false positives in GC/ECD detection. All materials used in this method are routinely demonstrated to be free from interferences by processing procedural blanks (one blank per 20 samples or each batch, whichever is more frequent).

Take care in dissecting tissue from animals with exoskeletons or shells. If possible, rinse the shells or exoskeleton free of extraneous materials prior to thawing and removal of tissue for analysis.

Matrix interferences may be caused by compounds that are coextracted from the sample. Biogenic materials that cause interferences in the analysis of tissue extracts are removed prior to GC/ECD analysis by silica/alumina and HPLC purification.

4. APPARATUS AND MATERIALS

4.1. Labware and Apparatus

Clean glassware by washing with detergent (micro cleaning solution) and rinsing with tap water. Then combust the glassware in a muffle furnace by heating at 400°C for at least 4 hr. Solvent rinses of acetone to dry the glassware followed by dichloromethane rinses may be substituted for the muffle furnace heating when determined to be appropriate by the analyst. After drying and cooling, seal and store glassware in a clean environment to prevent the accumulation of dust or other contaminants. Maintain stored glassware capped with combusted aluminum foil.

The following labware is needed to perform the tissue digestion and purification procedure:

Stainless Steel Knife or Shears: For dissecting animals.
Glass Centrifuge Tubes: 200 mL capacity.
Disposable Glass Pasteur Pipets: 1 mL.
Boiling Chips: Solvent extracted, Teflon.
Water Bath: Heated to 60-70°C.
Vials and Teflon Lined Caps: 1 mL to 7 mL capacity.
Nitrogen Gas Evaporation Unit
Tissumizers: Tekmar; Polytron homogenizer, or equivalent.
Flat Bottom Flasks: 100 mL and 500 mL capacity.
Concentrator Tubes: Kuderna-Danish 25 mL, graduated with ground glass stoppers.
Snyder Column: Kuderna-Danish - 3-ball column.
Erlenmeyer Flask: Various sizes.
Microliter Syringes: 1000, 500, 100, 50, and 10 µL capacity.
Balance: Top loading with an accuracy of 0.1 g.
Electrobalance: Cahn or equivalent, with an accuracy of 0.0001 mg.
Pyrex Glass Wool: Combusted at 400°C for 4 h.
Junior Orbital Shaker: or equivalent.
Chromatographic Column: 300 mm x 10 mm, with Pyrex glass wool plug at bottom and Teflon stopcock.
Microreaction Vessels: 2.0 mL or 1.0 mL autosampler vials with crimp cap septa.

Volumetric glassware used for sample measurement or introduction of internal standards must be calibrated.

4.2. Solvents and Reagents

The procedure requires the following:

Reagent Water: Reagent water contains no analytes above the method detection limit.
Solvents: Methylene chloride, hexane, pentane (pesticide quality or equivalent).
Sodium Sulfate: (ACS) Granular, anhydrous (purified by heating at 400°C for 4 hr in a shallow tray or other suitable method).
Alumina Oxide: Basic Brockmann 1, standard grade 150 mesh Aldrich 19,744-3 or equivalent. Combusted at 400°C for 4 hr and stored at 120°C prior to use.
Silica Gel: Grade 923, 100-200 mesh Aldrich 21,447-7 or equivalent, stored at 170°C before use.
Internal Standard Spiking Solutions: Refer to GERG SOP-9017 for preparation of appropriate surrogate spiking solution for chlorinated hydrocarbon analysis.
Matrix Spike Standard: Refer to GERG SOP-9017 for preparation of appropriate matrix spiking solution for chlorinated hydrocarbon analysis.

GC Internal Standard Solution: Refer to GERG SOP-9017 for preparation of appropriate internal standard spiking solution for chlorinated hydrocarbon analysis.

5. PROCEDURES

Mechanically macerate all tissue samples prior to extraction. Add internal standard after maceration and subsampling, but prior to digestion and extraction.

5.1. Preparation of Samples

5.1.1. Fish

While still partially frozen, rinse fish with reagent water if necessary to remove extraneous material. The edible portions of the fish or other target organs are dissected under contaminant free conditions. Pool sufficient tissue in a combusted mason jar and macerate using a Tissumizer or Polytron blender. Weigh approximately 2-15 g wet weight of the macerated tissue into a centrifuge tube.

5.1.2. Crabs, Sea Urchins, Brittle Stars

Rinse the animal(s) with reagent water if necessary to remove extraneous material. Dissect the tissue of interest under contaminant free conditions. Pool sufficient tissue in a combusted mason jar and macerate using a Tissumizer or Polytron blender. Weigh approximately 2-15 g wet weight of the macerated tissue into a centrifuge tube.

5.1.3. Clams, Mussels, Oysters

If necessary, rinse bivalves with reagent water to remove extraneous material. Shuck bivalves with a stainless steel knife and remove animal from shell. Pool sufficient tissue in a combusted mason jar and macerate using a Tissumizer or Polytron blender. Weigh approximately 2-15 g wet weight of the macerated tissue into a centrifuge tube.

5.1.4. Percent Moisture Determination

Remove a separate 5-g aliquot of macerated tissue, place in tared weighing pan, and weigh. Dry the tissue at 50°C, allow to cool and then reweigh. Calculate the percent moisture in the original sample from the weight loss.

5.2. Digestion and Extraction

Place the tissue in a 200 mL centrifuge tube. To each sample add 100 mL CH_2Cl_2, 50 g Na_2SO_4, and the appropriate amount of internal standard as described in GERG SOP-9017. Macerate the tissue for 3 min with the Tissumizer. Decant the CH_2Cl_2 into a 500 mL flat bottom flask. Centrifuge at approximately 2000 rpm for 5 min, if necessary.

Repeat the extraction two more times with 100 mL aliquots of CH_2Cl_2. Combine the CH_2Cl_2 aliquots in the 500- mL flat bottom flask. Remove a 20-mL aliquot for lipid determination.

Attach a 3-ball Snyder column to the 500-mL flat bottom flask. Add one clean boiling chip. Place the apparatus in a hot water bath (60-70°C) and concentrate to 10-20 mL.

Transfer the sample to a 25-mL concentrator tube. Rinse the 500-mL flat bottom flask with 5-10 mL of hexane and add to concentrator tube. Concentrate to 1 mL in a water bath held at 60-70°C.

5.3. Silica/Alumina Column Cleanup

Place a plug of glass wool and 2 cm of combusted sand in the glass chromatographic column. Fill the column with CH_2Cl_2.

Slurry pack 10 g of alumina (deactivated 1% with water) in CH_2Cl_2 into the column. Allow the alumina to settle.

Slurry pack 20 g of silica gel (deactivated 5% with water) in CH_2Cl_2 into the column. Allow to settle. Add 1 cm of combusted sand on top of the packed column. Allow CH_2Cl_2 to drain to the top of the sand.

Add 50 mL of pentane to the column and drain to the top of the sand.

Place a 500 mL flat bottom flask under the column. Transfer the sample extract in hexane to the column. Drain column to the top of the sand. Elute the column with 50-mL of pentane. Retain this fraction (f_1) for aliphatic hydrocarbon analysis. Rinse concentrator tube twice with 1 mL of 50:50 pentane:dichloromethane, add it to the column and drain the column to the sand layer. Add 200 mL of 50:50/pentane:CH_2Cl_2 to the column, elute at 1 mL/min and collect the effluent. This fraction contains the chlorinated hydrocarbons.

Concentrate the extracts as described in the last two steps of Section 5.2.

5.4. HPLC Cleanup

If necessary, further purify the fraction from Section 5.3 in 0.8 mL of CH_2Cl_2 by high performance liquid chromatography using an Spectra-Physics SP800 ternary HPLC pump and two size exclusion columns connected in series (22.5 x 250 mm Phenogel 100 Å columns), and a precolumn (8 x 50 mm Phenogel 100 A). Filtered (0.45 µm) dichloromethane is used as the mobile phase. Inject the samples onto the columns with an autosampler (Gilson Model 321) that is capable of introducing a 1.0 mL sample size. The HPLC unit is equipped with a UV absorbance detector (Model Water 440-Millipore). Collect the fractions containing the compounds of interest into 50-mL vials using the LKB Bromma 2211 fraction collector.

The time interval in which the desired fraction is collected is based on the retention times of 4,4'-dibromooctafluorobiphenyl (DBOFB) and perylene. Typically, these retention time markers are analyzed three times. Collection of the sample fraction begins 1.5 min before the elution of DBOFB and ends 2 min after the elution of perylene. Assuming a constant isocratic flow of the mobile phase of 7 mL/min, the total time needed to collect the fraction is approximately 7 min. At the end of every batch of ten samples, the marker standard mixture is run again to check retention time. After running a batch of samples (20), the columns are flushed and the precolumn is backflushed to remove sample matrix contamination from the system. On average, purification time is approximately 35 min.

5.5. Preparation for Instrumental Analysis

Concentrate the extract to 1 mL. The chlorinated hydrocarbon fraction is then analyzed by GERG SOP-9017.

6. QUALITY CONTROL

Quality control samples are processed in a manner identical to actual samples.

Analyze a method blank with every 20 samples, or with every sample set, whichever is more frequent. Blank levels should be no more than 3 times method detection limit (MDL). If blank levels for any component are above 3 times MDL, samples analyzed in that sample set should be re-extracted and reanalyzed. If insufficient sample is available for extraction, the data will be reported but flagged to indicate that it was out of control.

Analyze matrix spike/matrix spike duplicate (MS/MSD) samples with every 20 samples, or with every sample set, whichever is more frequent. The appropriate spiking level is 10 times the MDL.

Spike internal standard into every sample and QC sample. The appropriate spiking level is 10 times the MDL.

Internal standard and matrix spike recovery acceptance criteria are described in detail in GERG SOP-9017.

Reference Materials: Tissue reference material, when available, is analyzed for chlorinated hydrocarbons with each analytical sample string of approximately 20 samples. Control charts for these analyses are then established and maintained.

7. REPORTING AND PERFORMANCE CRITERIA

Reporting units are ng/g.

The minimum performance standard for the method is detection of 1.0 ng/g for individual compounds.

The effective minimum performance standard can be adjusted by decreasing final sample volume, increasing sample amount and/or increasing volume injected on the GC-ECD. However, the amount of internal standard added must also be lowered.

8.3.2. SOP-9017 - Quantitative Determination of Chlorinated Hydrocarbons

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURES SOP-9017

QUANTITATIVE DETERMINATION OF CHLORINATED HYDROCARBONS

---

1. INTRODUCTION

The quantitative method described in this document determines chlorinated hydrocarbons (e.g. chlorinated pesticides and PCBs) in sample extracts. The method is based on high resolution, capillary gas chromatography using electron capture detection (GC/ECD).

Extracts should be prepared as described in the appropriate GERG SOP's.

Sample collection, preservation, storage, and holding times are discussed under the analytical procedures for sample extraction and purification.

2. APPARATUS AND MATERIALS

A gas chromatograph with a split/splitless injection system, capillary column capability and a electron capture detector (ECD) is utilized.

2.1. GC Column

A 30-m long x 0.25-mm I.D. fused silica capillary column with DB-5 bonded phase (J&W Scientific or equivalent) should be used. The column should provide good resolution of chlorinated hydrocarbons, surrogates, and internal standards.

2.2. Autosampler

The autosampler is capable of making 1-4 µL injections.

3. REAGENTS

3.1. Calibration Solution

The calibration solution is comprised of, at a minimum, the chlorinated hydrocarbons indicated with an asterisk in Table 9.

Table 9. Chlorinated hydrocarbons of interest.

Chlorinated Pesticides

2,4'-DDD*	Aldrin*	*gamma*-HCH *
2,4'-DDE*	*alpha*-Chlordane*	Heptachlor epoxide*
2,4'-DDT*	*alpha*-HCH *	Heptachlor*
4,4'-DDD*	*beta*-HCH *	Hexachlorobenzene*
4,4'-DDE*	*delta*-HCH *	Lindane*
4,4'-DDT*	Dieldrin*	Mirex*
	Endrin*	*trans*-Nonachlor*

Polychlorinated Biphenyls

Dichlorobiphenyls	Pentachlorobiphenyls	Heptachlorobiphenyls
7	100	178
8 *	88	187/182/159*
15	92	183
	84	185
Trichlorobiphenyls	101*	174
18*	99	177
24	83	171
16/32	97	172
26	87	180*
25	85	191
31	82	170*
28*	107/108	189
33	118/108/149*	
22	114	Octachlorobiphenyls
37	105*	202
	126*	200
Tetrachlorobiphenyls		201
45	Hexachlorobiphenyls	196
46	136	195*
52*	151	194
49	144	205
47/48	149	
44*	146	Nonachlorobiphenyls
42	153*	208
41/64	141	206*
40	137	
74	138*	Decachlorobiphenyls
70	158	209*
66*	129	
60/56	159	
77/110*	128*	
	167	

PCB numbers from Ballschmiter and Zell (1980)
* Compounds in the calibration mixture.
Slash indicates coelution of PCB congeners, some of which coelute at a different chlorination level. For example, 77/110 indicates that PCB 77 and PCB 110 coelute. PCB 110 is a pentachlorobiphenyl while PCB 77 is a tetrachlorobiphenyl.

Calibration standards should be prepared in the concentration range of 5 to 200 ng/mL (at four concentrations) at a minimum. Internal standard and GC internal standard compounds should be added at 100 ng/mL to all calibration standards.

3.2. Internal Standard Spiking Solution

The internal standards for all sample types are 4,4'-dibromooctafluorobiphenyl (DBOFB), PCB 103, and PCB 198. An internal standard solution is made by weighing an appropriate aliquot of pure material into a volumetric flask and diluting to volume with hexane. Internal standards are added to each sample at a concentration of approximately 10 times the MDL. For higher concentrations of chlorinated hydrocarbons, the internal standard concentrations can be appropriately increased.

3.3. GC Internal Standard Solution

The GC internal standard for this analysis is tetrachloro-m-xylene (TCMX). The GC internal standard solution is made by weighing an appropriate aliquot of pure material into a volumetric flask and diluting to volume with hexane. GC internal standard should be added to each sample extract to obtain a final concentration of approximately 100 ng/mL. For higher concentrations, the GC internal standard concentration is appropriately increased.

3.4. Matrix Recovery Spiking Solution

The matrix spiking solution consists of chlorinated pesticides and PCBs, indicated with an asterisk in Table C.1.

The matrix spike is added to samples at a concentration approximately 10x the MDL. If higher concentrations are expected, the matrix spike is appropriately increased.

3.5. Retention Index Solution

The calibration mixture is also used as a retention index solution as well as Aroclor mixtures.

4. PROCEDURE

4.1. Sample Extraction and Purification

Water samples are extracted and purified (optional) following GERG SOP-9014. Sediment samples are extracted and purified following GERG SOP-9015. Tissue samples are extracted and purified following GERG SOP-9016.

4.2. High Resolution GC-ECD Analysis

4.2.1. GC Conditions

For the analysis of chlorinated hydrocarbons, the analytical system, or its equivalent, should include, at a minimum, the conditions listed in Table 10.

Table 10. Minimum GC requirements and operating conditions.

Instrument:	Hewlett-Packard 5880A or Varian 3500 Series
Features:	Split/splitless capillary inlet system, HP-1000 LAS 3357 data acquisition system
Inlet:	Splitless
Detector:	Electron Capture
Column:	0.25-mm I.D. x 30-m DB-5 fused silica capillary column (J&W Scientific)
Gases:	
Carrier:	Helium 1 mL/min
Make-Up:	Argon/methane (95/5) or Nitrogen, 20 ml/min.
Temperatures:	
Injection port:	275°C
Detector:	325°C
Oven Program:	100°C for 1 min., then 5°C/min. to 140°C, hold 1 min.; 1.5°C/min to 250°C, hold 1 min.; 10°C/min to 300°C, hold 5 min.

The GC oven temperature program may be modified to improve resolution.

Calibration:	Four-point calibration (5 or 20, 40, 80, and 200 ng/mL)
Quantification:	Surrogate standard/calibration

4.2.2. Calibration

Pesticide/PCB calibration is done as part of the analytical run. The four calibration mixtures are interspersed with actual samples during the GC/ECD analyses. The calibration curve is then based on these four standards. If the calibration curve has an r^2 of 0.995 or higher for all analytes present in the samples, it is accepted, if not, the calibration standards as well as all the samples must be reanalyzed by GC/ECD. This procedure is superior to the procedure where the instrument is initially calibrated at four points and then mid-level standards are run during the analytical run. This latter calibration only insures that mid-level samples remain in calibration. Since the ECD detector is nonlinear, a one point check on its calibration is not as rigorous as calibration during the GC/ECD run.

4.2.3. Sample Analysis

As discussed in Section 4.2.2 calibration mixture, actual samples, and QA samples (blanks, matrix spikes, SRM, etc.) are run as one analytical sequence.

Sample injections of 1 to 4 µL are made with an autosampling device.

If the response for any peak exceeds the highest calibration solution, the extract is diluted, more surrogate solution added, and the sample reanalyzed for those analytes that exceed the calibration range.

4.2.4. Calculations

Concentrations in samples are based on surrogate standards added. All analyte concentrations are normally calculated from PCB 103 surrogate. The internal standard (TCMX) is used to calculate surrogate recoveries. In selected cases, DBOFB and/or PCB 198 may be used to calculate selected analytes concentrations, if it can be demonstrated that they produce more reliable data (i.e., if matrix interference occurs with PCB 103) based on % recoveries in spiked blanks, matrix spikes, or reference materials.

5. QUALITY ASSURANCE/QUALITY CONTROL (QA/QC) REQUIREMENTS

5.1. Calibration Checks

A four-point calibration curve establishes the response of the detector. The calibration curve is prepared using a non-linear calibration equation of the form:

$$Y = A\,(x)^B$$

$$Y = \frac{C_A}{C_{IS}} = A\left(\frac{A_A}{A_{IS}}\right)^B$$

where A is the slope of the line fit, B is the polynomial coefficient for the line fit, C_A is the concentration (ng/mL) of the analyte measured, C_S is the concentration of the IS (ng/mL) (PCB 103), A_A is the area for the analyte measured, and A_S is the area for the IS standard (PCB 103).

The calibration solutions that are analyzed as part of the analytical GC/ECD run are preceded by no more than six samples and no more than six samples are run between calibration mixtures. Acceptance criteria for the calibration curve is outlined in Section 4.2.2.

5.2. Method Blank Analysis

An acceptable method blank analysis does not contain any target compound at concentration 3 times greater than the MDL. If the method blank does not meet these criteria, the analytical system is out of control and the source of the contamination must be investigated, corrected, and documented before further sample analysis proceeds.

5.3. Surrogate Standards Analysis

All samples and quality control samples are spiked with DBOFB, PCB 103, and PCB 198. The surrogate standard solution will be spiked into the sample prior to extraction in an attempt to minimize individual sample matrix effects associated with sample preparation and analysis.

The laboratory will take corrective action whenever the recovery of the surrogate used to quantitate is outside of the 40 to 130% range.

The following corrective action will be taken when an out of control event occurs:

a. Calculations are checked to assure that no errors have been made.

b. The surrogate standard solutions are checked for degradation, contamination, etc., and instrument performance is checked.

c. If the surrogate could not be measured because the sample required dilution or only a portion of the sample was analyzed, or matrix interference occurs with only one surrogate, no corrective action is required. The surrogate recovery is properly annotated.

d. If the steps above fail to reveal a problem, the sample or extract is reanalyzed. If reanalysis of the extract yields surrogate recoveries within the stated limits, then the reanalysis data is reported. If upon reinjection QA criteria are still violated, the sample will be submitted for re-extraction if sufficient sample is available. If the sample was completely consumed, the data will be reported but designated as outside the QA criteria.

5.4. Matrix Spike Analysis

Matrix spikes when required are analyzed with each sample set. A sample is randomly chosen, split into subsample(s), and subsample(s) are fortified with the matrix spike. The acceptable matrix spike recovery criteria are:

> The average recoveries for all compounds, except hexachlorobenzene (HCB) and *beta*-HCH, must fall between 40 and 120%. Recoveries of HCB and *beta*-HCH are acceptable if they are greater than 10%.

If the matrix spike criteria are not met, the matrix spike will be reinjected on the GC. If the reinjected matrix spike analysis meets the criteria, then the reanalysis data is reported. If none of the analytes that are in violation are present in the sample the violation is noted but no action is required. If analytes that are present in the sample are in violation, the entire batch of samples are submitted for re-extraction if sufficient sample is available. If the sample was completely consumed, the data will be reported but designated as outside the QA criteria.

5.5. Method Detection Limit

The method detection limit is determined following the procedures outlined in the Federal Register (1984), Vol. 49, No. 209: 198-199.

5.6. GC Resolution

The target compounds, surrogates, and internal standard should be resolved from one another and from interfering compounds. When they are not, coelutions are documented.

5.7. Reference Material Analysis

When available, standard reference materials (SRM) or laboratory reference materials will be analyzed for chlorinated hydrocarbons. One sample will be analyzed per batch of samples. The result should agree within ±3 standard deviations of the mean of the previously reported data for laboratory reference material. For SRM, the results should agree within 50 to 125% of certified values or ± 35% of reference values. The data produced are used to construct control charts.

6. CALCULATIONS

6.1. Chlorinated Hydrocarbon Calculations

All calculations are based on the internal standards added before extraction and purification. The actual sample concentration (C, see section 7.1 for reporting units) for each compound is calculated by the following formula:

$$Y = A\left(\frac{A_a}{A_{SU}}\right)^B\left(\frac{I_{SU}}{S_{DW}}\right)$$

where A is the slope of the curve fit, B is the polynomial coefficient for the line fit, A_a is the area for the analyte measured, A_{SU} is the area for the IS (PCB 103), I_{SU} is the amount of IS added to the sample, and S_{DW} is the sample dry weight.

6.2. Calculation Notes

To each sample, a specific amount of surrogate standard is added. The recovery of these compounds is monitored in each sample using the response of TCMX, the internal standard (I_{gc}), added to the final extract just prior to GC/ECD analyses.

$$\text{Percent IS recovery} = (R_1\ R_2\ R_3\ R_4)(100\%)$$

where R_1 is the ratio of the surrogate peak area to that of the internal standard in the sample, R_2 is the ratio of the surrogate concentration to that of the internal standard in one of the calibration mixtures, R_3 is the ratio of the internal standard peak areato that of the surrogate in one of the calibration mixtures, and R_4 is ratio of the amount of internal standard (I_{gc}) added to sample just prior to GC analysis to that of the surrogate standard added to sample just prior to the sample extraction.

7. REPORTING

7.1. Reporting Units

Data is reported in ng/g dry weight. If the data is required on a wet weight basis, the sample wet weight (S_{WW}) can be substituted in the equation in Section 6.1 for S_{DW}.

The effective minimum performance standard can be adjusted by decreasing final sample volume, increasing sample amount, and/or increasing volume injected on the GC-ECD.

7.2. Minimum Method Performance Criteria

The minimum method performance standard for tissues is 0.5 ng/g for individual compounds.

7.3. Significant Figures

Results are reported to three (3) significant figures.

7.4. Surrogate Recovery

Surrogate recoveries are reported for each sample analyzed.

7.5. Matrix Spike

Matrix spike recoveries, when required, are reported for each batch of samples analyzed.

7.6. Reference Materials

When available, the results of the analysis of reference materials is reported for each batch of samples analyzed.

8.4. Information on NIST SRM 1974, Organics in Mussel Tissue (*Mytilus edulis*).

NIST SRM 1974 is intended primarily for use in validating analytical methods for the determination of selected PAHs in marine bivalve tissue or materials of similar matrix. Development of this SRM was partially funded by the NOAA National Status and Trends Program. Complete preparation and analysis information can be found in the Certificate of Analysis (NIST, 1991) and in Wise et al. (1991).

The mussels (*Mytilus edulis*) used for the preparation of this SRM were collected on December 1, 1987 from Dorchester Bar within Boston Harbor, MA (42° 18.25' N, 71° 02.31' W). Approximately 2400 individual mussels were collected by hand at low tide. The samples were transported to the Battelle New England Laboratory (Duxbury, MA) where the mussels were rinsed in a tank supplied with pumped sea water. Rocks and other debris were removed. The samples were placed in insulated, Teflon-lined wooden containers, frozen and transported to NIST on dry ice. The samples were transferred to Teflon bags and stored in a liquid nitrogen vapor freezer (-120°C) until shucked. The mussel tissue was removed from the shell using the following procedure. The mussels were allowed to warm up to about 0°C. The tissue was removed from the shell using a titanium knife, placed in Teflon bags, and immediately returned to a liquid nitrogen freezer. The frozen mussel tissue was pulverized using a cryogenic procedure. After mixing, the mussel tissue homogenate were aliquoted into pre-cooled glass bottles. The bottles of SRM 1974 have been stored at -80°C since preparation.

The certified concentrations for polycyclic aromatic hydrocarbons (PAHs) are listed in Table 11, and noncertified concentrations for other PAHs, PCBs, chlorinated pesticides, and elements in Table 12.

SRM 1974 can be purchased for US$334 (50 g). Price subject to change without notice. Please contact the National Institute of Standards and Technology, Office of Standard Reference Materials, Gaithersburg, MD 20899, USA.

Table 11. Certified concentrations available for SRM 1974.

PAHs (ng/g dry weight):

Compound	Value	Uncertainty (±)	Compound	Value	Uncertainty (±)
Anthracene	6.1	1.7	Fluoranthene	272	47
Benzo[*b*]fluoranthene	52.3	9.4	Pyrene	276	30
Benzo[*ghi*]perylene	20.0	2.3	Perylene	8.5	2.4
Benzo[*a*]pyrene	18.6	3.8	Phenanthrene	45	11
Indeno[1,2,3-*cd*]=pyrene	14.6	2.7			

Table 12. Noncertified concentrations available for SRM 1974.

PAHs (ng/g dry weight):

Compound	Value	Uncertainty (±)
1-Methylnaphthalene	9	2
1-Methylphenanthrene	19	5
1,3-, 2,10-, 3,9-, and 3,10-Dimethylphenanthrenes	91	17
1,6- and 2,9-Dimethylphenanthrene	47	11
1,7-Dimethylphenanthrene	42	9
2,6-Dimethylphenanthrene	37	7
2,7-Dimethylphenanthrene	35	9
2- and 9-Ethylphenanthrenes and 3,6-dimethylphenanthrene	34	8
2-Methylnaphthalene	17	4
9-Methyl- and 4-methylphenanthrene	22	5
Benzo[*a*]fluoranthene	4.1	1.2
Benzo[*e*]pyrene	81	6
Benzo[*j*]fluoranthene and benzo[k]fluoranthene	35	6
Benzo[*k*]fluoranthene	24	1
Benz[*a*]anthracene	37	3
Chrysene/Triphenylene	124	11
Dibenz[*a,h*]anthracene	2.8	0.1
Fluorene	12	2
Indeno[1,2,3-*cd*]fluoranthene	3.9	0.6

PCBs (ng/g dry weight):

Compound	Value	Uncertainty (±)	Compound	Value	Uncertainty (±)
PCB 18	24	9	PCB 128	15	2
PCB 28	62	3	PCB 138, PCB 163,		
PCB 44	65	23	PCB 164	110	11
PCB 52	98	39	PCB 153	145	8
PCB 66	110	5	PCB 180	13	1
PCB 101, PCB 90	105	11	PCB 187, PCB 182	30	1
PCB 105	45	3			
PCB 118	110	5			

PCBs are listed according to Ballschmitter and Zell (1980). The major PCB congener is listed first, the others listed are minor components.

Chlorinated Pesticides (ng/g dry weight)

Compound	Value	Uncertainty (±)	Compound	Value	Uncertainty (±)
2,4'-DDD	20	7	4,4'-DDT	3	2
2,4'-DDE	5.8	0.6	*cis*-Chlordane	26	1
2,4'-DDT	4	1	Dieldrin	8	4
4,4'-DDD	68	3	*trans*-Nonachlor	21	5
4,4'-DDE	48	2			

Table 12. Noncertified concentrations available for SRM 1974 (cont.).

Element (μg/g dry weight except where noted)

Compound	Value	Uncertainty	Compound	Value	Uncertainty
Na (%)	3.29	0.09	Rb	5.67	0.16
Mg (%)	0.48	0.03	Sr	60	14
Al	503	46	Mo	2.0	0.5
Cl (%)	6.04	0.17	Ag	0.854	0.021
K (%)	1.10	0.33	Cd	1.4	0.4
Sc	0.085	0.009	Sb	0.0262	0.0002
V	1.55	0.29	Cs	0.040	0.003
Cr	2.61	0.21	La	0.35	0.08
Mn	10.2	1.2	Ce	0.53	0.13
Co	0.38	0.01	Sm	0.064	0.014
Fe	500	27	Eu	0.012	0.002
Ni	1.00	0.08	Hf	0.05	0.03
Cu	9.2	1.9	Ta	0.018	0.003
Zn	91.6	3.8	Au	0.0476	0.0010
As	9.72	0.35	Hg	0.194	0.014
Se	2.00	0.06	Pb	9.7	0.6
Br	373	18	Th	0.07	0.02

References:

National Institute of Standards and Technology (1991) Certificate of analysis, SRM 1974, Organics in mussel tissue (*Mytilus edulis*). National Institute of Standards and Technology, Gaithersburg, MD.

Wise, S. A., B. A. Benner, R. G. Christensen, B. J. Koster, J. Kurz, M. M. Schantz, and R. Zeisler (1991) Preparation and analysis of a frozen mussel tissue reference material for the determination of trace organic constituents. Env. Sci. Technol., 25(10):1695-1704.

8.5. List of Contributors and Reviewers

Dr. Neil Andersen
Chairman, GIPME
National Science Foundation
Washington, DC 20550 USA
202 357 7910
FAX: 202 357 7621

Dr. Shier Berman
National Research Council of Canada
Institute for Environmental Chemistry
Ottawa, K1A OR6 Canada
613 993 2359
FAX: 613 993 2451

Dr. J. M. Bewers
Bedford Institute of Oceanography
PO Box 1006 Dartmouth
Nova Scotia, B2Y 4A2 Canada
902 426 2371
FAX: 902 426 6695

Dr. Robert Boyd
National Research Council of Canada
Institute for Marine Biosciences
Halifax, Nova Scotia B3H 321 Canada
902 426 6503
FAX: 902 426 9413

Dr. John Calder
NOAA/OAR
1335 East West Hwy, rm. 4134
Silver Spring, MD 20910 USA
301 713 2465
FAX: 301 713 0666

Dr. Rodger Dawson
Chesapeake Biological Laboratory
University of Maryland
Box 38
Solomons, MD 20688 USA
410 326 7284
FAX: 410 326 6342

Dr. Yu Guohui
Marine Standard Materials Centre
Second Institute of Oceanography
State of Oceanic Administration
Hongzhou 310012 China
0571 876 924
FAX: 0571 871 539

Dr. Chidi Ibe
Senior Assistant Secretary
Intergovernmental Oceanographic
Commission
UNESCO
1 rue Miollis
75015 Paris, France
4568 3992
FAX: 33 1 4056 9316

Dr. Anthony Knap
Bermuda Biological Station
Ferry Road
St. Georges, Bermuda

Mr. Gunnar Lauenstein
NOAA/NOS/ORCA
N/ORCA21
Rockville, MD 20852 USA
301 713 3028
FAX: 301 713 4388

Dr. Laurence Mee
IAEA - MEL
10, avenue des Castellans
MC 9800 Monaco
33 9205 2222
FAX: 33 9205 7744

Dr. Thomas O'Connor
NOAA/NOS/ORCA
N/ORCA21
Rockville, MD 20852 USA
301 713 3028
FAX: 301 713 4388

Ms. Reenie Parris
National Institute of Standards and Technology
Center for Analytical Chemistry
Gaithersburg, MD 20899 USA
301 975 3103
FAX: 301 926 8671

Dr. Jose Sericano
Geochemical and Environmental Research Group
Texas A&M University
833 Graham Road
College Station, TX 77845 USA
409 690 0095
FAX: 409 690 0059

Dr. Graham Topping
QUASIMEME Project Manager
Marine Laboratory
PO Box 101, Victoria Rd.
Aberdeen, AB9 8DB
Scotland

Dr. Alan Walton
Centre Oceanographique de Rimouski
310, Allée des Ursulines
Rimouski, Quebec, G5L 3A1
Canada

Dr. David Wells
QUASIMEME Project Manager
Marine Laboratory
PO Box 101, Victoria Rd.
Aberdeen, AB9 8DB
Scotland