U.S. Army Corps of Engineers

New England District
Concord, Massachusetts

QUALITY ASSURANCE PROJECT PLAN

Volume III
Appendices B, C, D

8 January 1999 (DCN: GEP2-123098-AAET)

Revised May 2003 (DCN: GE-022803-ABLZ)

Environmental Remediation Contract
General Electric (GE)/Housatonic River Project
Pittsfield, Massachusetts

Contract No. DACW33-00-D-0006

WESTON SOLUTIONS

QUALITY ASSURANCE PROJECT PLAN, FINAL (REVISED 2003)

ENVIRONMENTAL REMEDIATION CONTRACT GENERAL ELECTRIC (GE) HOUSATONIC RIVER PROJECT PITTSFIELD, MASSACHUSETTS

Volume III—Appendices B, C, and D

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Contract No. DACW33-00-D-0006

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TABLE OF CONTENTS

VOLUME III

APPENDIX B—DATA EVALUATION DELIVERABLES
APPENDIX C—QAPP ADDENDUM FOR TISSUE ANALYSIS
APPENDIX D—STANDARD OPERATING PROCEDURES—TISSUE ANALYSES

APPENDIX D

STANDARD OPERATING PROCEDURES—TISSUE ANALYSES

TABLE OF CONTENTS—APPENDIX D

SOP-9214 Confirmation of Analytes by Full Scan Gas Chromatography/Mass Spectrometry

SOP-9415 Determination of Percent Dry Weight for Biological Tissues

SOP-9701 Procedure for the Preparation or Modification of Standard Operating Procedures

SOP-9702 Personnel Selection and Training Requirements

SOP-9703 Procedure for the Preparation or Modification of Quality Assurance Manuals, Safety Manuals, and Quality Assurance Project Plans

SOP-9705 Procedure for Initial Preparation of Samples

SOP-9706 Procedure for Receiving Samples

SOP-9707 Procedure for Storage of Samples

SOP-9708 Procedure for Sample Tracking

SOP-9709 Procedure for Archival and Final Disposition of Samples

SOP-9711 Preparation of Fish Composites

SOP-9713 Procedure for Documentation, Quality Control, Verification, and Preparation of Analytical Standards for the Analysis of Chlorinated Pesticides and PCBs

SOP-9714 Procedure for Documentation, Quality Control, Verification, and Preparation of Analytical Standards for the Analysis of Tetra- through Octa-Chlorinated Dibenzo-p-Dioxins (PCDDs) and Dibenzofurans (PCDFs) by Isotope Dilution High Resolution Gas Chromatography/High Resolution Mass Spectrometry (HRGC/HRMS)

SOP-9719 Procedures for the Extraction of Tissues and Purification of Extracts for Analysis of Polychlorinated Dibenzo-p-Dioxins (PCDD) and Polychlorinated Dibenzofurans (PCDF)

SOP-9720 Silica/Alumina Column Chromatography for the Purification of Extracts from Tissue, Sediments, and Soil Samples Prior to the Analysis of Aliphatics and Polynuclear Aromatic Hydrocarbons, Polychlorinated Biphenyls, and Organochlorine Pesticides

TABLE OF CONTENTS—APPENDIX D
(Continued)

SOP-9722 Quantitative Determination of Tetra- through Octa-Polychlorinated Dibenzo-p-Dioxins (PCDDs) and Dibenzofurans (PCDFs) by Isotope Dilution High Resolution Gas Chromatography/High Resolution Mass Spectrometry (HRGC/HRMS)

SOP-9724 Purification of Extracts by Gel Permeation Chromatography Prior to the Analysis of Polynuclear Aromatic Hydrocarbons (PAH) and Organochlorine Pesticides (OC)/Polychlorinated Biphenyls (PCB) Analysis

SOP-9727 Determination of Percent Lipid in Biological Tissue

SOP-9807 Extraction of Biological Tissues for the Analysis of Polynuclear Aromatic Hydrocarbons, Polychlorinated Biphenyls, and Organochlorine Pesticides

SOP-9808 Separation of Polychlorinated Biphenyls from Organochlorine Pesticides by Silica Gel Column Chromatography

SOP-9810 Quantitative Determination of Chlorinated Hydrocarbons by Gas Chromatography/Electron Capture Detection

SOP-9811 Charcoal:Silica Gel Column Chromatography for the Separation of Planar Polychlorinated Biphenyls in Sample Extracts Prior to Gas Chromatography-Electron Capture Detector Analysis

SOP-9728 Quantitative Determination of Planar Polychlorinated Biphenyls (PL-PCBs) by Isotope Dilution High Resolution Gas Chromatography/High Resolution Mass Spectrometry

SOP-9733 Quantitative Determination of Polynuclear Aromatic Hydrocarbons by Gas Chromatography/Mass Spectrometry Using the Selected Ion Monitoring Mode

SOP-9804 Extracting Sediment Samples Using Accelerated Solvent Extractor for Surface Prospecting Aliphatic and Aromatic Hydrocarbon Analyses

SOP-9901 Confirmation of Analytes Using Gas Chromatography/Mass Spectrometry with the Selected Ion Monitoring Technique

SOP-0008 Quantitative Determination of N-Alkanes, Pristane, Phytane, Total Resolved and Unresolved Complex Mixtures, and Total Petroleum Hydrocarbons

SOP-0101 Extraction and Analysis of Plant Pigment Samples using High Performance Liquid Chromatography (HPLC)

SOP-9702
PERSONNEL SELECTION AND TRAINING REQUIREMENTS

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURE SOP-9702

PERSONNEL SELECTION AND TRAINING REQUIREMENTS

This document presents the procedures used in the performance of the above administrative activities.

Director 6/5/98 Date

Quality Assurance Manager 6/4/98 Date

SOP Author/Revision By: G. Ekman

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Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURE SOP-9702

PERSONNEL SELECTION AND TRAINING REQUIREMENTS

1.0 PURPOSE

1.1 Summary

The purpose of this procedure is to establish the selection and training requirements for personnel involved in the operation, maintenance, and technical support activities of the Geochemical and Environmental Research Group (GERG), a center for applied research within the College of Geosciences at Texas A&M University.

1.2 Application

The provisions of this SOP apply to GERG operations, staff, and management.

2.0 SAFETY

2.1 The hazards, toxicity or carcinogenicity of each compound or reagent used in GERG's standard operating procedures have not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory maintains Material Safety Data Sheets (MSDS) which contain information regarding the safe handling of chemicals used at GERG's facilities. A reference file of MSDS is available to all personnel involved with these materials. All laboratory personnel should direct any questions regarding safety issues to their supervisors or the Safety Officer.

3.0 RESPONSIBILITIES AND AUTHORITIES

3.1 The Director, Deputy Directors, and Financial Manager of GERG are responsible for ensuring the formulation and implementation of all policies associated with personnel selection and training.

3.2 GERG's Managers and Supervisors are responsible for ensuring compliance with all policies associated with personnel selection and training.

3.3 The Quality Assurance (QA) Manager is responsible for developing the QC documentation forms involved in personnel training.

3.4 GERG's Managers and Supervisors are responsible for initial training, on-going training, and documentation of up-to-date training records for all persons they supervise.

3.5 GERG's Safety Officer is responsible for all general safety related training, on-going general training, and documentation of up-to-date records associated with safety for personnel involved in the operation, maintenance and technical support of GERG's research and service organization.

3.6 The QA Manager is responsible for maintaining training documentation and other certifications for personnel associated with the operation, maintenance and technical support of GERG activities.

4.0 PERSONNEL SELECTION

4.1 GERG selects and assigns personnel on the basis of experience and education. Personnel are selected for a position after consideration of their ability to meet written job performance requirements and prior documented performance in relevant activities.

4.2 In addition to information required on formal job applications, GERG may require an applicant's official academic transcripts, interviews with GERG managers and the right to contact an applicant's references. GERG personnel selection procedures comply with all TAMU Human Resources policies.

5.0 TRAINING

5.1 Training for each employee is based on an evaluation of the requirements needed to perform a task or to meet QC objectives.

5.2 The primary training occurs on-the-job, but if deemed appropriate by the Manager or Supervisor, it is supplemented with classroom type training and/or seminars.

5.3 To supplement GERG in-house training, a variety of personnel training programs are available through the Texas A&M University System. This type of training is encouraged by GERG management.

5.4 Initial and continuing training is provided as necessary to ensure that GERG personnel are qualified to perform their job. The adequacy of training is evaluated during each employee's performance evaluation.

5.5 The primary training instruments are the Proficiency Orientation Checklists (POC) and the Standard Operating Procedures (SOPs). Personnel are considered proficient for their position based upon job performance, completion of the appropriate POC(s) for their position as well as documentation of special training (i.e., licenses, certifications, degrees).

5.6 **Initial Training**

5.6.1 The QA Manager will provide the employee with a copy of the appropriate POC for their position and will provide the file copy of the same POC to their Manager or Supervisor. When applicable, the Manager or Supervisor will supply the untrained employee with a copy of the appropriate SOPs. Copies of SOPs are maintained within each work area.

5.6.1.1 The employee may keep their copy of the POC. When training is completed for a section of the POC, the Manager or Supervisor and the employee will sign-off on the file copy of the POC. The Manager or Supervisor then gives the signed file copy to the QA Manager.

5.6.1.2 Each POC specifies that training for Section A (Safety) and Section B (General), and when applicable, Section C (Standards and Sample Handling) must be completed and signed. Those repetitive portions of the various

POCs have to be documented only once for each employee.

5.6.1.3 When training for other sections of the POC is completed, the Manager or Supervisor obtains the file copy from the QA Manager so that these sections may be signed. The Manager or Supervisor must return the signed file copy to the QA Manager.

5.6.1.4 An employee's training may be documented on more then one POC, as long as the Manager or Supervisor responsible for the training on a specific POC certifies the employee's skills.

5.6.2 When an SOP is used as the basis for training, the untrained employee will be provided with sufficient time to read and to formulate any questions.

5.6.2.1 The Manager or Supervisor will discuss the SOP and evaluate the employee's understanding of terms and concepts basic to the SOP.

5.6.2.2 After the Supervisor is satisfied the employee understands the procedures as outlined in the SOP, a staff member will be assigned to the untrained employee for on-the-job training. The untrained employee will only work under the direct supervision of the experienced staff member.

5.6.2.3 The Manager or Supervisor may assign a test set of samples or other specific activities to the newly-trained employee. The newly-trained employee will be allowed to operate independently only after he/she demonstrates that any applicable test set of samples or other job specific activities meet the necessary QC or job performance objectives.

5.6.2.4 The newly-trained employee's performance and applicable QC acceptance criteria will be monitored by the Manager or Supervisor.

5.7 Continuing training includes periodic review of applicable performance goals, POCs, and SOP's by the Manager or Supervisor.

5.8 The Manager or Supervisor must provide their employees the following work area specific training:

1. Information on hazardous chemicals known to be present in the employee's work area and to which the employee may be exposed including:

   A. location by work area,
   B. specific hazards, including acute and chronic effects,
   C. safe handling procedures.

2. Work area location of MSDSs or procedures for obtaining MSDSs.

3. How to obtain and use appropriate personal protective equipment and/or first aid treatment to be used with respect to the hazardous chemicals in the work area.

4. Instructions on spill cleanup procedures and proper disposal of hazardous chemicals specific to that work area.

5.9 As needed, the Manager or Supervisor may initiate retraining for any employee using applicable SOPs.

5.10 Supervisory and Management Training

5.10.1 Training seminars for enhancing Supervisory skills will be provided as appropriate to management and senior staff. Training in Supervisory skills will include:

1. Leadership skills;
2. Interpersonal communication;
3. Command responsibilities and limits;
4. Motivation of personnel;
5. Problem analysis and decision making;
6. Performance evaluation techniques;

7. Discipline procedure;
8. Interviewing techniques;
9. Workmen's compensation, and
10. Administrative policies and procedures.

5.10.2 Management training programs will be provided as appropriate to senior staff on an annual basis. Management training includes:

1. Supervisory skills training;
2. Leadership skills;
3. Strategic planning;
4. Effective communication skills;
5. Team building;
6. Quality assurance and quality control;
7. Facility security and emergency plans;
8. Purchasing;
9. Material storage;
10. Facility modifications;
11. Environmental issues, and
12. Budgeting.

6.0 RECORD REQUIREMENTS

6.1 The QA Manager is responsible for maintaining training documentation for each employee. Training files must be kept current and at a minimum should include:

1. With the exception of student workers, temporary employees, and graduate students, maintain original and updated resumes usually including education, experience, and employment history;

2. Appropriate POC(s); and,

3. Documentation of general safety training and other training programs completed along with copies of any Continuing Education Units (CEUs), qualifications or certifications achieved.

6.2 The Supervisor or Manager is responsible for documenting work area specific safety training and other job-related training activities for all employees under their supervision and for providing any appropriate documentation of this training to the QA Manager.

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SOP-9703
PROCEDURE FOR THE PREPARATION OR MODIFICATION OF QUALITY ASSURANCE MANUALS, SAFETY MANUALS, AND QUALITY ASSURANCE PROJECT PLANS

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURES

SOP-9703

PROCEDURE FOR THE PREPARATION OR MODIFICATION OF QUALITY ASSURANCE MANUALS, SAFETY MANUALS, AND QUALITY ASSURANCE PROJECT PLANS

This document presents the procedures used in the performance of the above administrative activities.

Director — Date: 4/30/97

Quality Assurance Manager — Date: 4/30/97

SOP Author/Revision By: Grace Ekman

PROCEDURE FOR THE PREPARATION OR MODIFICATION OF QUALITY ASSURANCE MANUALS, SAFETY MANUALS, AND QUALITY ASSURANCE PROJECT PLANS

1.0 PURPOSE

This document provides procedures for the staff at the Geochemical and Environmental Research Group (GERG) of the College of Geosciences and Maritime Studies at Texas A&M University to follow when developing new documents or modifying existing documents such as quality assurance manuals, safety manuals and quality assurance project plans (QAPPs).

1.1 Summary

Quality assurance manuals, safety manuals, and QAPPs must be prepared based upon sound technical and management principles, including the use of standardized formats, an internal review process, and management sign-off. A need to modify documents may occur as a result of project revision or the introduction of new field or analytical techniques.

1.2 Application

The provisions of this SOP apply to GERG operations, staff, and management activities.

2.0 SAFETY

The hazards, toxicity or carcinogenicity of each compound or reagent used in GERG's standard operating procedures have not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory maintains Material Safety Data Sheets (MSDS) which contain information regarding the safe handling of chemicals used at GERG's facilities. A reference file of MSDS is available to all personnel involved with these materials. All laboratory personnel should direct any questions regarding safety issues to their supervisors or the Safety Officer.

3.0 DOCUMENT PREPARATION AND MAINTENANCE

3.1 Quality Assurance/Quality Control Manuals

3.1.1 The responsibility for the preparation of GERG's comprehensive Quality Assurance/Quality Control (QA/QC) Manuals (e.g., Quality Assurance Management Plan, Generic Quality Assurance Manual, etc.) is delegated to the Quality Assurance (QA) Manager.

3.1.2 Comprehensive QA/QC manuals will be reviewed by designated management and laboratory staff. These documents require a cover sheet (see Figure 1) displaying, as a minimum, the signatures of the Director of GERG and the Quality Assurance Manager. Depending upon the type of document, other signatures may be required.

3.1.3 The original copy of any new or revised QA/QC document will be maintained by the QA Manager and distributed as appropriate to the management staff. Requests for additional copies of such documents should be directed to the QA Manager.

3.2 Safety Manuals and Related Documents

3.2.1 The responsibility for the preparation of GERG's manuals and protocols related to safety is delegated to the TAMU Office of Safety and Health and to GERG's Safety Officer.

3.2.2 The format used for GERG's safety manuals and protocols must meet TAMU guidelines.

3.2.3 GERG's safety manuals and protocols must be reviewed by selected laboratory and management staff. These documents require a cover sheet (see Figure 1) displaying, as a minimum, the signatures of the Director of GERG and the Safety Officer. Depending upon the type of document, other signatures may be required.

3.2.4 The original copy of any new or revised safety document will be maintained by the Safety Officer and distributed as appropriate to

GERG staff. Requests for additional copies of such documents should be directed to the Safety Officer.

3.3 Quality Assurance Project Plans

3.3.1 Each Principal Investigator or Project Manager has responsibility for the preparation of a Quality Assurance Project Plan (QAPP) when it is required for a project.

3.3.2 At a minimum, the QAPP shall consist of the following form and content unless another specific format is required by the client.

3.3.2.1 Title page, with provision for approval signatures (see Figure 1);

3.3.2.2 Table of contents;

3.3.2.3 Brief project description;

3.3.2.4 Project organization and responsibilities;

3.3.2.5 QA objectives for measurement data in terms of precision, accuracy, completeness, representativeness and comparability;

3.3.2.6 Sampling procedures when applicable, or a statement of "other's" responsibility;

3.3.2.7 Sample custody;

3.3.2.8 Calibration procedures, criteria and frequency;

3.3.2.9 Analytical procedures;

3.3.2.10 Data reduction, validation and reporting;

3.3.2.11 Internal quality control checks and frequency;

3.3.2.12 QA performance audits, systems audits and frequency;

3.3.2.13 QA reports to management;

3.3.2.14 Preventative maintenance procedures and schedules;

3.3.2.15 Specific procedures routinely used to assess data precision and accuracy, representativeness, comparability, and completeness for the parameter(s) involved; and

3.3.2.16 Corrective action.

3.3.3 Prior to submittal to the client, all QAPPs must be reviewed and approved by the QA Manager. The QAPP must have a cover sheet (see Figure 1) displaying, as a minimum, the signatures of the Project Manager, the Quality Assurance Manager, and the Director of GERG (or the appropriate designee). Depending upon the project, other signatures may also be required.

3.3.4 The original copy of any new or revised QAPP will be maintained by the QA Manager and distributed as appropriate to management staff. Requests for additional copies of such documents should be directed to the QA Manager.

4.0 DOCUMENT REVISION RESPONSIBILITY, FORMAT, AND REVIEW PROTOCOL

4.1 Revision Origination

4.1.1 All employees are encouraged to present their suggestions and their observations regarding quality improvement. Continuous improvement is a guiding principle in all aspects of GERG's operations.

4.1.2 The originator of the suggestion should describe the observation/problem and the proposed resolution to his or her supervisor or through the appropriate chain of command to the QA Manager.

4.2 Revision Evaluation

4.2.1 The Quality Assurance (QA) Manager shall evaluate the suggested revisions and will make a decision on the validity and need for new or revised documents.

4.2.1.1 After review of a problem area and QA implications, the suggestion will either be adopted, thereby resulting in a new or revised manual, or the concern will be addressed by a memorandum from the QA Manager.

4.3 Revision Format, Review, and Approval Protocol

4.3.1 All proposed changes to manuals and QAPPs must follow the procedures specified in this SOP. The revised or new document must, after thorough review, be approved or rejected by the QA Manager or the Safety Officer and the Director.

4.3.2 The original copy of any new or revised manual or QAPP will be maintained and copies distributed to appropriate management staff. Requests for additional copies of such documents should be directed to the QA Manager or Safety Officer.

4.4 Client Notification, Review and Approval

4.4.1 If a new or revised QAPP affects an on-going project, the client must be notified. The client must approve the revision prior to its use on the project. At the client's request, the project may be continued with the original version of the QAPP.

Figure 1-- Cover sheet

(FOR Manuals or QAPPs with appropriate font and spacing:)

DOCUMENT TITLE

for

DOCUMENT SUBJECT

prepared by

GEOCHEMICAL AND ENVIRONMENTAL RESEARCH GROUP
Texas A&M University
833 Graham Road
College Station, TX 77845
409-862-2323

prepared for

CLIENT/PROJECT NAME
ADDRESS

(SIGNATURE LINES/DATES -e.g.-)

Project Manager	Date
Quality Assurance Manager	Date
Director	Date

GERG MANUAL XXXX

SOP-9705
PROCEDURE FOR INITIAL PREPARATION OF SAMPLES

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURES SOP-9705

PROCEDURE FOR INITIAL PREPARATION OF SAMPLES

This document presents the procedures used in the performance of the above laboratory activities.

Susan E Ekmon
Quality Assurance Manager

5/2/97
Date

Author/Revision By: Jennifer Pintkowski

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURES SOP-9705

PROCEDURE FOR INITIAL PREPARATION OF SAMPLES

1.0 PURPOSE

This procedure is used by staff of the Geochemical and Environmental Research Group (GERG) of the College of Geosciences and Maritime Studies at Texas A&M University for the initial preparation of samples.

1.1 APPLICATION

1.1.1 Preliminary sample preparation is performed in accordance with the client's guidelines described in their Quality Assurance Project Plan (QAPP) or GERG's SOPs. The following processes are to be used as generic guidelines unless specified otherwise in project documentation.

2.0 SAFETY

The hazards, toxicity or carcinogenicity of each compound or reagent used in GERG's standard operating procedures have not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory maintains Material Safety Data Sheets (MSDS) which contain information regarding the safe handling of chemicals used at GERG's facilities. A reference file of MSDS is available to all personnel involved with these materials. All laboratory personnel should direct any questions regarding safety issues to their supervisors or the Safety Officer.

3.0 REAGENTS AND MATERIALS

3.1 REAGENTS

Methylene Chloride: Cat. 300-4*DK; pesticide grade or equivalent, lot tested

Methanol: Cat. 230-4*DK; pesticide grade or equivalent, lot tested

3.2 MATERIALS

Balance: Top loading with an accuracy of 0.01 g, calibrated daily (data maintained in logbook)

Band saw: Stainless steel blade, Biro Model 11 or equivalent

Blender/Mixer: Stainless steel blades, Bamix de luxe, Daily blender mixer, or equivalent

Grinder/Chopper: Stainless steel blades, Hobart or equivalent

Tissumizer: Pro Scientific, Tekmar homogenizer or equivalent

Aluminum foil: Heavy duty, combusted or rinsed with methylene chloride

Dissection board: Teflon, washed, dried and rinsed with methylene chloride

Forceps: Stainless steel, washed, dried and rinsed with methylene chloride

Scissors: Stainless steel, washed, dried and rinsed with methylene chloride

Gloves: Latex, powder-free

Jars: Glass, 250 mL or 500 mL, with Teflon lined lids, precleaned to meet U.S. EPA "Specifications and Guidance for Contaminant-Free Sample Containers"

Cutting tools (knives, electric knives, machetes, meat cleavers, scissors, scalpels, etc.): Stainless steel, washed, dried and rinsed with methylene chloride

Plastic bags: 1 or 2 gallon capacity, polyethylene, zipper-locked, Ziplock or equivalent

Plastic snap cap vial: 20 dram polycarbonate or equivalent

Plastic tubs: 3 or 10 gallon capacity, opaque, with lids, Rubbermaid or equivalent

Spatulas: Stainless steel, washed, dried and rinsed with methylene chloride

4.0 ANIMAL TISSUE SAMPLES

4.1 Dissection is accomplished with Methanol/Methylene Chloride rinsed tools (i.e. stainless steel forceps, scissors, knives, etc.) and care is taken not to contaminate the sample. To the extent possible, the outer surface of the sample is washed with HPLC water to remove extraneous material prior to dissection. The dissected sample is placed in a pre-cleaned jar with the appropriate identification label attached. The samples are stored in the walk-in freezer at -20 ± 10°C until further processing.

4.2 Homogenization of large samples (i.e., whole fish, rats, ducks, etc.) is accomplished with a Biro Model 11 (or equivalent), stainless steel Band saw and/or a stainless steel food grinder/chopper. To the extent possible, the outer surface of the sample is washed with HPLC water to remove extraneous material prior to homogenization. The samples are then stored in the walk-in freezer at approximately -20 ± 10°C until further processing is required.

4.3 Homogenization of smaller samples is done with a Tekmar™ Tissumizer, household blender or hand blender. Between samples all pieces of equipment are cleaned with S/P™ Brand Micro-All Purpose Liquid Cleaner, rinsed with deionized water, and then rinsed with methanol followed by methylene chloride. To the extent possible, the outer surface of the sample is washed with HPLC water to remove extraneous materials. The samples are stored in the walk-in freezer at -20 ± 10°C until further processing is required.

5.0 SEDIMENTS

5.1 Unless otherwise directed by the client, extraneous material (i.e., leaves, rocks, twigs) are removed prior to homogenization and aliquot preparation.

5.2 Sediment samples are homogenized by stirring vigorously with a clean stainless steel or Teflon utensil and an aliquot is placed in a pre-cleaned labeled glass jar for organic analysis, a whirlpak for grain size and/or polycarbonate vial for metals.

5.3 Samples requiring ambient storage are maintained in designated cabinets or shelving areas. The sediment samples requiring frozen storage are stored in the walk-in freezer at approximately -20 ± 10°C until further processing. Grain size samples are stored in the walk-in cooler (4° ± 2°C).

5.4 Wet weights of separate aliquots are taken at this time for later sediment percent moisture determination (See appropriate GERG SOP).

6.0 PLANTS

6.1 Care should be taken to not touch the plants with gloves or hands.

6.2 Plants are cut into small pieces with methanol/methylene chloride rinsed scissors and homogenized with a solvent rinsed stainless steel or Teflon utensil.

6.3 The samples are stored in the walk-in freezer at approximately -20 ± 10°C until laboratory processing.

7.0 WATER

7.1 Water sample bottles only require the attachment of GERG's labels.

7.2 The samples for organic analysis are stored in the walk-in cooler at 4° ± 2°C until laboratory processing.

7.3 Inorganic aqueous samples for metals analysis should be shipped and stored at ambient temperatures unless specified otherwise by the client. However, aqueous samples requiring hexavalent chromium or mercury determination are shipped and stored at 4° ± 2°C unless specified otherwise by the client.

8.0 BILE

8.1 Bile samples are held in the freezer at approximately -20 ± 10°C with no further processing required.

9.0 MISCELLANEOUS MATRICES

9.1 Samples requiring ambient storage are maintained in designated cabinets or shelving areas.

9.2 Samples having "miscellaneous" matrices (i.e., creosote/oil) are prepared as required according to written procedures provided by the Program Manager or Project Administrator.

SOP-9706
PROCEDURE FOR RECEIVING SAMPLES

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURES SOP-9706

PROCEDURE FOR RECEIVING SAMPLES

This document presents the procedures used in the performance of the above laboratory activities.

Lissa E Ekman
Quality Assurance Manager

5/2/97
Date

Author/Revision By: Jennifer Pintkowski

PROCEDURES FOR RECEIVING SAMPLES

1.0 PURPOSE

This procedure is used by the staff of the Geochemical and Environmental Research Group (GERG) of the College of Geosciences and Maritimes Studies at Texas A&M University for receiving samples.

1.1 APPLICATION

1.1.1 All samples processed through GERG's Sample Custodial Facilities are checked in according to routine chain-of-custody requirements.

2.0 SAFETY

The hazards, toxicity or carcinogenicity of each compound or reagent used in GERG's standard operating procedures have not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory maintains Material Safety Data Sheets (MSDS) which contain information regarding the safe handling of chemicals used at GERG's facilities. A reference file of MSDS is available to all personnel involved with these materials. All laboratory personnel should direct any questions regarding safety issues to their supervisors or the Safety Officer.

3.0 REQUIRED SAMPLE RECEIPT DOCUMENTATION

3.1 The Sample Receipt Log (Figure 1) and Sample Receiving/Integrity Report (SR/IR; Figure 2) are completed for all samples during the check-in process.

3.2 Shipping containers, airbills and custody seals are inspected and documented.

3.2.1 Shipping containers are opened and inspected (inside the biological safety cabinet) for damage, leakage and the presence of airbills. Airbill numbers are noted on the SR/IR and the airbill is included in the client binder.

3.2.2 Custody seals, if present, are inspected for integrity and the originals are included in the client binder.

4.0 INSPECTION OF CONTENTS

4.1 Check for chain of custody (COC) records or traffic reports and any other paperwork (i.e., letters, shipping labels, etc.).

4.2 Check the temperature of samples using an NIST certified thermometer. The thermometer is inserted as close to the samples as possible and the shipping container is re-closed for approximately 5 minutes.

4.3 The temperature of unrefrigerated samples will be recorded upon receipt. Acceptable temperature ranges for frozen or cooled samples are:

4.3.1 Samples shipped on dry ice: ≤ 0°C.

4.3.2 Ice/blue cooled samples: between 4 ± 2°C.

4.3.3 Samples received outside these limits are flagged and the client is notified.

4.4 Inorganic aqueous samples for metals analysis should be shipped and stored at ambient temperature (not refrigerated except for samples requiring hexavalent chromium or mercury) unless otherwise specified by the client.

4.5 Note the presence or absence of sample tags or custody seals on sample containers on the SR/IR.

4.6 Note the presence or absence of dry ice, regular ice, or blue ice and record the temperature of the samples (see Section 4.2) on the SR/IR.

5.0 CHECK-IN OF SAMPLE CONTAINERS

5.1 Check sample container ID numbers and contents for absolute agreement with shipping documents. Use a photocopy of shipping documents for marking during check-in procedure.

5.1.1 Document any discrepancies on the COC and SR/IR.

5.2 Inspect the samples individually for damage (i.e., cracked lid, cracked or broken container, leakage, etc.) and record any comments on the COC and SR/IR.

5.3 After inspection of samples and notation of the conditions on the COC and SR/IR, transfer the samples to an opaque storage container labeled with an unique number and shelf location and place in an appropriate secure storage facility (i.e., freezer, cooler, or cabinet).

5.4 The Project Administrator is notified of any discrepancies between the shipping documents and the samples received. The client is notified and any discrepancies resolved.

5.5 The Sample Custodian provides the client with a "Notification of receipt" letter; a fax documenting a copy of the signed COC; or confirmation by phone on a client-by-client basis. A log of phone confirmation is maintained by the Sample Custodian.

5.6 The original shipping list or COC document is signed and dated on receipt and copies are made of all pertinent documents. Copies are given to the Project Administrator. The Sample Control group keeps the original and places in a client binder which is stored in a secure location.

5.7 Shipping containers are returned to the client if so directed in the project plan.

6.0 FILE NUMBERS AND ANALYTICAL REQUESTS

6.1 Samples are logged into the Paradox database (GERG Information Network (GIN)) and sequential laboratory ID file numbers are computer generated for each sample. The database also includes the field identification number, date collected, date received by the laboratory, analyses requested, and any appropriate comments.

6.2 Sample Control personnel generate labels for each sample which includes the laboratory identification number, the field identification

number, the type of sample, and the sample delivery group identification. These labels are securely attached to the original sample containers with transparent tape.

7.0 DOCUMENTATION

7.1 The Project Administrator (PA) provides Project Initiation Forms and Environmental Analytical Request Forms to the Sample Control group. These include the Purchase Order Number and costs for each analysis. This information is entered into GIN. Copies of Project Initiation/Analytical Forms are made, with the originals going to the PA Assistant for the PA file and distribution.

8.0 EXAMPLE FORMS

8.1 Sample Receipt Log.
8.2 Sample Receiving/Integrity Report (SR/IR).
8.3 Notification of receipt letter.

GERG

Texas A&M University

Sample Receipt Log

Sample Delivery Group	Date	Client	Client ID	Gerg ID	Carrier	# of Containers	Client PI	GERG PI	Initials

8.1 Example Sample Receipt Log

GERG SAMPLE RECEIVING/ INTEGRITY REPORT

Catalog: _____ Date Received: _____ SDG#: _____

Sender: _____

1. Number of Shipping Containers:

Comments:

2. Airbill Present? No Yes

Shipping Company: Fedex UPS Airborne Express Other

Airbill Number | Comments:

3. Custody Seals on Container? No Yes Intact Not Intact

Comments:

4. Chain of Custody Records? No Yes

Comments:

5. General Sample Condition: Frozen Dry Ice Cool Blue Ice Unrefrigerated Ice

Temperature/Comments:

6. List of Broken Containers:

7. Number of Samples Expected: _____ Number of Samples Received: _____

8. Problems/ Discrepancies:

9. Resolutions:

10. Checked in by: _____ Date: _____

8.2 Example Sample Receiving/Integrity Report (SR/IR)

GERG
Geochemical and Environmental Research Group
833 Graham Road
College Station, Texas 77845
TEXAS A&M UNIVERSITY
Telephone: (409) 690-0095
FAX: (409) 690-0059
TELEX: 910-380-8722

August 7, 1995

Mr.

Dear Mr.

Enclosed is a copy of the Chain of Custody form for three (3) water samples received by the Geochemical and Environmental Research Group (GERG) on August 4, 1995. The samples were shipped on August 3, 1995, transported to College Station via Federal Express (Airbill# 0136939530) and received at GERG by Jennifer Pintkowski. The three samples were received cool and packed on ice. No discrepancies were noted. If you need any additional information, please contact me at (409)690-0095.

Sincerely yours,

Jennifer Pintkowski
Sample Receiving

JLP:jlp
cc: Jim Brooks
Jennifer Wong
Guy Denoux
Hank Chambers
Enclosure: Chain of Custody

8.3 Example Notification of receipt letter.

SOP-9707
PROCEDURE FOR STORAGE OF SAMPLES

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURES

SOP-9707

PROCEDURE FOR STORAGE OF SAMPLES

This document presents the procedures used in the performance of the above laboratory activities.

Quality Assurance Manager

5/2/97
Date

Author/Revision By: Jennifer Pintkowski

PROCEDURE FOR STORAGE OF SAMPLES

1.0 PURPOSE

1.1 This procedure is used by staff of the Geochemical and Environmental Research Group (GERG) of the College of Geosciences and Maritime Studies at Texas A&M University for the storage of samples.

2.0 SAFETY

The hazards, toxicity or carcinogenicity of each compound or reagent used in GERG's standard operating procedures have not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory maintains Material Safety Data Sheets (MSDS) which contain information regarding the safe handling of chemicals used at GERG's facilities. A reference file of MSDS is available to all personnel involved with these materials. All laboratory personnel should direct any questions regarding safety issues to their supervisors or the Safety Officer.

3.0 STORAGE FACILITIES

3.1 Immediately after check-in procedures are completed, samples are stored in secured freezers, refrigerators, or in ambient temperature cabinets or shelves as described in the client Quality Assurance Project Plan (QAPP), project documentation, or GERG SOPs.

3.2 Individual walk-in freezers and coolers are monitored continuously by an automatic system with an alarm to indicate failure. In addition, manual temperature determination using an NIST traceable thermometer is performed every normal working day for verifying temperature records. The manual temperature is recorded in the Temperature Log (Figure 1).

3.3 Any deviation from acceptable limits (-20°C ± 10°C for frozen samples or between 2 and 6°C for samples in walk-in cooler) is noted in the "Comments" section of temperature log book. Any corrective action taken is also recorded in the comments section.

4.0 INTERMEDIATE STORAGE

4.1 Soils, sediments, tissues, and bile are stored in the walk-in freezer (-20°C ± 10°C) until homogenization or analysis, unless otherwise specified in the QAPP or applicable GERG SOP's.

4.2 Water samples for organic analyses are stored in the walk-in cooler (4°C ± 2°C) until analysis, unless otherwise specified in the QAPP or applicable GERG SOP's.

4.3 Aqueous samples for metals analysis are stored at ambient temperature in cabinet or shelving areas unless otherwise specified by the client. However aqueous samples for determination of mercury or hexavalent chromium are shipped and stored in the walk-in cooler at 4° ± 2°C.

4.4 Other sample material (i.e., oils, creosote, wipes, etc.) are stored according to the QAPP, project documentation, or applicable GERG SOP's.

5.0 LOCATION OF STORED SAMPLES

5.1 The sample custodian maintains records of the location of stored samples based upon the designated cabinet, cooler or freezer and shelf number and is responsible for locating them when required by the laboratory staff.

6.0 EXAMPLE FORMS

6.1 Temperature Log

FREEZER & REFRIGERATOR TEMPERATURE LOG

Date/Time	Initials	Freezer Temp (°C)			Cooler Temp (°C)	Comments
		A	B	C		

38-16 Freezer Temp Log

6.1 Example Temperature Log Form

SOP-9708
PROCEDURE FOR SAMPLE TRACKING

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURES SOP-9708

PROCEDURE FOR SAMPLE TRACKING

This document presents the procedures used in the performance of the above laboratory activities.

Quality Assurance Manager

5/2/97
Date

Author/Revision By: Jennifer Pintkowski

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURES SOP-9708

PROCEDURE FOR SAMPLE TRACKING

1.0 PURPOSE

This procedure is used by the staff of the Geochemical and Environmental Research Group of the College of Geosciences and Maritime Studies at Texas A&M University for maintaining records of the location of original samples and any aliquots prepared from samples for analysis at this facility.

2.0 SAFETY

The hazards, toxicity or carcinogenicity of each compound or reagent used in GERG's standard operating procedures have not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory maintains Material Safety Data Sheets (MSDS) which contain information regarding the safe handling of chemicals used at GERG's facilities. A reference file of MSDS is available to all personnel involved with these materials. All laboratory personnel should direct any questions regarding safety issues to their supervisors or the Safety Officer.

3.0 SAMPLE TRACKING PROCEDURE

3.1 The sample custodian is responsible for sample storage and sample transfer to the appropriate internal or external laboratory.

3.2 All samples are handled under routine chain-of-custody procedures and are accompanied by a Sample Transfer Form (Figure 1). When samples require shipment outside of the city, a sample information sheet will accompany the shipment. A fax will be sent to the recipient to inform them that the samples will be arriving. The fax will include the following: total number of samples, analysis required, and airbill number.

4.0 SAMPLE TRANSFER FORM/SIGN OUT

4.1 The Sample Transfer Form specifies the sample delivery group (SDG) number, a list of the samples or sample aliquots included and the time and date of transfer.

4.2 The Sample Transfer Form is signed by both the recipient and a member of the sample control group.

4.2.1 If the samples or sample aliquots are not to be returned to the Sample Custodian, they are referred to as "keepers" and this is noted on the Sample Transfer Form.

4.3 The recipient of the samples is responsible for their safe handling, analysis and storage until the unused samples or sample aliquots are returned to the Sample Custodian.

5.0 SAMPLE TRANSFER FORM/SIGN IN

5.1 The samples must be returned in a timely fashion to the sample custodian when the recipient is finished with them.

5.2 The returned samples or sample aliquots are checked against the list of those signed out, the time and date noted, and the Sample Transfer Form is signed by both the recipient and a member of the sample control group.

5.2.1 If any sample or sample aliquot has been completely consumed, this is noted on the Sample Transfer Form.

5.3 The Sample Transfer Form becomes a permanent part of the appropriate client binder for the specific SDG involved.

5.4 Samples are then returned to the appropriate storage area or secured facility.

6.0 EXAMPLE FORMS

6.1 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: _____

Figure 1. Example Sample Transfer Form

SOP-9709
PROCEDURE FOR ARCHIVAL AND FINAL DISPOSITION OF SAMPLES

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURES SOP-9709

PROCEDURE FOR ARCHIVAL AND FINAL DISPOSITION OF SAMPLES

This document presents the procedures used in the performance of the above laboratory activities.

Grace E Ekman
Quality Assurance Manager

5/2/97
Date

Author/Revision By: Jennifer Pintkowski

PROCEDURE FOR ARCHIVAL AND FINAL DISPOSITION OF SAMPLES

1.0 PURPOSE

This procedure is used by staff of the Geochemical and Environmental Research Group (GERG) of the College of Geosciences and Maritime Studies at Texas A&M University for sample archival and final disposition of samples.

2.0 SAFETY

The hazards, toxicity or carcinogenicity of each compound or reagent used in GERG's standard operating procedures have not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory maintains Material Safety Data Sheets (MSDS) which contain information regarding the safe handling of chemicals used at GERG's facilities. A reference file of MSDS is available to all personnel involved with these materials. All laboratory personnel should direct any questions regarding safety issues to their supervisors or the Safety Officer.

3.0 ARCHIVAL IN FREEZER, COOLER, OR CABINET

3.1 All samples or remaining materials are retained by the sample control group after analysis and stored in the appropriate sample storage area (i.e., freezer, walk-in cooler, cabinet, etc.) to minimize deterioration until final disposition is authorized by the Project Administrator (PA) or Project Manager (PM).

3.1.1 Original samples containers are disposed of after all of the sample is consumed. unless specified otherwise by the client. The ID and all label information is verified before disposal, and its disposal is documented on Sample Transfer Form and/or Catalog Tracking Form.

3.2 All storage facilities are secured and access is limited to authorized personnel.

3.3 Samples are stored in opaque containers indexed with an identification number/descriptor and freezer shelf location. The shelf location is

recorded in GIN and on the Tracking Form (Figure 1) in sample control binders.

3.4 Archival space for high security samples, accessed only by the sample custodian, is available.

3.5 Samples are held in archive for 90 days following completion of the analytical work and acceptance by client. Project Administrators are responsible for notification of client at the end of the 90 day period and determine the ultimate sample disposal procedures. Long term archive requires written authorization from the laboratory manager if not specified in project documentation or QAPP.

4.0 FINAL DISPOSITION OF SAMPLES

4.1 Samples are retained in archive until notification by the project originator is given in writing for the final disposition of the samples.

4.2 Samples are disposed of according to all applicable TAMU Policies.

CLIENT/SDG: _____

Storage Location: _____

Receiving Information:
Received by:_____

Date Received:_____

Number of Samples Received:_____
Number Expected:_____

S_____**T**_____**W**_____**O**_____

Grain Size required? YES NO
Grain Size shipped by:_____
Date shipped:_____

Metals Required? YES NO
Date Metals delivered to Presley:_____

Archived by:_____
Date Archived:_____

Disposed by:_____
Date Disposed:_____

Comments:_____
_____
_____

Figure 1. Example Tracking Form

SOP-9711
PREPARATION OF FISH COMPOSITES

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURES SOP-9711

PREPARATION OF FISH COMPOSITES

This document presents the procedures used in the performance of the above laboratory activities.

Quality Assurance Manager

5/2/97
Date

Author/Revision By: Jennifer Pintkowski

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURES SOP-9711

PREPARATION OF FISH COMPOSITES

1.0 PURPOSE

This procedure is used by the staff of the Geochemical and Environmental Research Group (GERG) of the College of Geosciences and Maritime Studies at Texas A&M University for the preparation and homogenization of fish samples prior to analysis

1.1 SUMMARY

A single composite fish sample provides an "average" fish which can be analyzed to give a single average measurement of an analyte. A composite can be useful (1) if there is not enough of a single fish or fish sample to analyze, (2) if multiple analyses are to be performed, or (3) if the analysis cost is high in relation to the cost of collecting individual samples.

Individual fish (whole, filleted with skins, or filleted without skins) are homogenized separately according to the relevant Quality Assurance Project Plan (QAPP) or applicable GERG SOP. An equal-sized subsample of each homogenate is combined to make up the composite sample. The standard homogenate composite weight is 100 to 200 g, but a smaller amount may be used if fewer analyses are being performed or if tissue availability is limited. This is in accordance with U.S. Department of the Interior, National Biological Survey, Research Information Bulletin No. 23, 1994; Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Vol. 1, EPA 823-R-93-002, Aug. 1993; and EMAP-Near Coastal Program.

2.0 SAFETY

The hazards, toxicity or carcinogenicity of each compound or reagent used in GERG's standard operating procedures have not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory maintains Material Safety Data Sheets (MSDS) which contain information regarding the safe handling of chemicals used at GERG's facilities. A reference file of MSDS is available to all personnel involved with these materials. All laboratory personnel should direct any questions regarding safety issues to their supervisors or the Safety Officer.

3.0 QUALITY CONTROL

3.1 Make a note in the appropriate section of the Sample Receiving/Integrity Report if anything out of the ordinary happens to the sample.

3.2 Use Standard Laboratory Practice when filling out all paperwork. Use black or blue waterproof ink. When correcting an entry, use one single line through the bad entry, date and initial change, and state the reason for the change. Write a large letter Z through all the empty sample lines, date and initial.

4.0 APPARATUS AND MATERIALS

4.1 Apparatus

Balance: top loading with an accuracy of ± 0.01 g, calibrated prior to use (data in logbook)

Band saw: stainless steel blade, Biro Model 11 or equivalent

Blender/mixer: stainless steel blades, Bamix de luxe, Daily blender mixer, or equivalent

Grinder/chopper: stainless steel blades, Hobart or equivalent

Tissumizer: Pro Scientific, Tekmar homogenizer or equivalent

4.2 Labware

Aluminum foil: heavy duty, combusted or rinsed with methylene chloride

Dissection board: Teflon, washed, dried and rinsed with methylene chloride

Forceps: stainless steel, washed, dried and rinsed with methylene chloride

Gloves: latex, powder-free

Jars: glass, 250 mL or 500 mL, with Teflon lined lids, precleaned to meet U.S. EPA "Specifications and Guidance for Contaminant-Free Sample Containers"

Cutting tools (knives, electric knives, machetes, meat cleavers, scissors, scalpels, etc.): stainless steel, washed, dried and rinsed with methylene chloride

Plastic bags: 1 or 2 gallon capacity, polyethylene, zipper-locked, Ziplock or equivalent

Plastic snap cap vial: 20 dram polycarbonate or equivalent

Plastic tubs: 3 or 10 gallon capacity, opaque, with lids, Rubbermaid or equivalent

Spatulas: stainless steel, washed, dried and rinsed with methylene chloride

5.0 SOLVENTS AND REAGENTS

Cleaning liquid: MICRO, or equivalent

Methanol: Baxter; Cat. 230-4DK; pesticide grade or equivalent

Methylene Chloride: Baxter; Cat. 300-4DK; pesticide grade or equivalent; lot tested

Water: HPLC grade; Baxter; Cat. 365-4DK or equivalent

6.0 PROCEDURE

6.1 Obtain the appropriate instructions from the client listed on the Environmental Analysis Request Form(s) for the sample set from the Project Administrator/Manager. Be certain that the type of fish sample is given (e.g. whole, filleted with skin, filleted without skin).

6.1.1 It is preferable to have at least 100 - 200 g of composite.

6.1.2 Label the pre-cleaned jars or plastic containers with preprinted labels (Project, Catalog ID or SDG, Sample ID, and Client Sample Descriptor) for the original samples and the composites.

6.2 Retrieve the frozen fish to be composited from the freezer. Choose the appropriate number of fish for compositing. If possible, choose fish that have the same approximate length: the length of the smallest

individual should be no less than 75% of the length of the largest individual.

6.3 Remove aluminum foil wrapping, if present, from each one. It may be necessary to let the outside of the fish thaw slightly and/or rinse the fish with distilled water to facilitate removal of the foil.

6.3.1 Wash the outside of each fish with HPLC grade water to remove any contaminants.

6.3.2 Note that scaling, skinning, filleting and homogenization is easier when the fish tissue is still partially frozen. Fish should only be thawed to the point where it becomes possible to make an incision into the flesh.

7.0 PREPARATION OF INDIVIDUAL WHOLE FISH:

7.1 Cut each fish into small pieces (approximately 2" x 2"). For large fish (> 1 ft), use a band saw, which has been cleaned with dilute MICRO solution, rinsed with HPLC Grade water and allowed to dry. For smaller fish (<1 ft), use a clean electric knife.

7.2 Grind the fish chunks using a meat grinder, which has been cleaned with dilute MICRO solution, and rinsed successively with DI water, methanol and methylene chloride. Very small samples can be homogenized using a tissumizer or blender/mixer.

7.2.1 The sample must be homogenized to the point that no lumps or chunks are visible and the mixture appears homogeneous.

7.2.2 For organic analyses, fill a prelabeled 500 mL jar, 1" from the top with the homogenized sample (use a 250 mL jar for smaller samples).

7.2.3 For metals analyses, fill a clean, pre-weighed plastic jar or polyethylene bag for the homogenized sample.

7.2.4 The remaining tissue is placed in a 1-2 gallon zipper-locked plastic bag, marked Excess Tissue and hand labeled with the Catalog number and the sample ID.

7.3 Store all individual samples, homogenates and aliquots in the appropriate freezer at -20°C ± 10°C in an opaque plastic tub labeled with the Client Designator and the Sample Delivery Group and storage location.

8.0 PREPARATION OF INDIVIDUAL FILLETED FISH:

8.1 Lay the fish down flat on a clean cutting board and remove the scales and adhering slime by scraping from the tail to the head using the blade-edge of a clean stainless steel knife. To control cross-contamination, rinse the cutting board and knife with HPLC grade water between fish.

8.2 Wash the outside of each fish using HPLC grade water and place the fish on a clean cutting board. Allow each fish to thaw only to the point where it becomes possible to make an incision into the flesh. The fish should not be allowed to thaw completely. Ideally, the removal of the fillet should occur while ice crystals are still present in the muscle tissue.

8.3 Make a shallow cut through the skin on either side of the dorsal fin from the top of the head to the tail. Make a cut behind the entire length of the gill cover, cutting through the skin and flesh to the bone. Make a cut along the belly from the base of the pectoral fin to the tail. This cut is made on both sides of the anus and the fin directly behind it. Do not cut into the gut cavity. If the skin is to be removed (e.g. for catfish and other fish without scales or at client's request), loosen the skin just behind the gills and pull it off between knife blade and thumb toward the tail. Pliers may be necessary.

8.4 Remove the fillet. Note that the fillet should include the belly flap. The dark muscle tissue that may exist in the vicinity of the lateral line should not be separated from the light muscle tissue that constitutes the rest of the muscle tissue mass. Bones which may still be present in the flesh after filleting should be carefully removed.

8.5 Cut the fillet into smaller pieces using an appropriate cutting tool, if necessary, and homogenize the tissue using a meat grinder, grinder/mixer or tissumizer, which has been cleaned with dilute

MICRO solution, and rinsed successively with HPLC Grade water, methanol and methylene chloride.

8.5.1 The sample must be homogenized to the point that no lumps or chunks are visible and the mixture appears homogeneous.

8.5.2 Fill a prelabeled 500 mL jar, 1" from the top with the homogenized sample. Use a prelabeled 250 mL jar for smaller samples.

8.5.3 The remaining tissue is placed in a 1-2 gallon zipper-locked plastic bag, marked Excess Tissue and hand labeled with the Catalog number and the sample ID.

8.6 Calibrate the toploading balance according to the manufacturer's instructions. Note the date, calibration weight and the calibrator's initials in the calibration logbook for that particular balance, located near the balance.

8.7 Weigh equal amounts (± 1%) of each homogenized fish sample into a clean stainless steel or glass container so that the total weight of the composite is appropriate for all analyses according to the Environmental Analysis Request Form. Accounting for % dry weight and lipid determination, QC samples and possible re-analyses, at least 100 - 200 g of composite tissue is ideal.

8.7.1 Note that the amount of fish to be extracted also depends upon the detection limits required of the project. Consult with the Project Administrator (PA) or the Principal Investigator (PI) to ensure that adequate sample is prepared for multi-analytical techniques.

8.8 Thoroughly homogenize the composite. Transfer the composite to a labeled precleaned glass jar for organic analyses. For metals analyses, use a clean, pre-weighed plastic jar or polyethylene bag.

8.9 Store all individual samples, homogenated composites, and aliquots in the appropriate freezer at -20°C ± 10°C in an opaque plastic tub labeled with the Client Designator and the Sample Delivery Group and storage location.

SOP-9713
PROCEDURE FOR DOCUMENTATION, QUALITY CONTROL, VERIFICATION, AND PREPARATION OF ANALYTICAL STANDARDS FOR THE ANALYSIS OF CHLORINATED PESTICIDES AND PCBs

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURES

SOP-9713

PROCEDURE FOR DOCUMENTATION, QUALITY CONTROL, VERIFICATION, AND PREPARATION OF ANALYTICAL STANDARDS FOR THE ANALYSIS OF CHLORINATED PESTICIDES AND PCBS.

This document presents the procedures, materials, and quality control used in the performance of the above preparation activities.

Quality Assurance Manager

5/14/97
Date

Author/Revision By: Jose Sericano

PROCEDURE FOR DOCUMENTATION, QUALITY CONTROL VERIFICATION, AND PREPARATION OF ANALYTICAL STANDARDS FOR THE ANALYSIS OF CHLORINATED PESTICIDES AND PCBS.

1.0 PURPOSE

This provides the procedures for the documentation of purchased analytical standard materials, their quality control (QC) and verification criteria, and their preparation for use in the analysis of Chlorinated Pesticides and PCBs which are used by staff of the Geochemical and Environmental Research Group (GERG) of the College of Geosciences and Maritime Studies at Texas A&M University.

1.1 Summary of Method

The use of properly prepared standards and reagents is essential for any analytical procedure. All materials purchased for use in calibration, spiking activities, precision and accuracy evaluation, or other analytical instrumental evaluations must be documented in a Standards Logbook as described in this standard operating procedure (SOP). Prior to use, and after certain preparatory steps, purchased standards must be evaluated for both purity and appropriate concentration by GERG staff, and must be shown to meet the appropriate QC criteria. Procedures ensuring traceability of these standard materials from receipt, QC verification, preparation, aliquot identification, analytical use, and consumption or disposal are provided in this SOP.

1.2 Applicability

The procedures included in this SOP apply to the preparation of analytical standards used in the analysis of all matrices for Chlorinated Pesticides and PCBs performed by gas chromatography with electron capture detector by the GERG staff.

1.2.1 Interferences

High purity reagents and solvents must be used, and all equipment and glassware must be scrupulously cleaned. Gloves, certain plastic components, and certain greases must not be used if these result in sample contamination.

1.2.2 Special Precautions

Chlorinated Pesticides and Polychlorinated Biphenyls (PCBs) should be considered hazardous and direct physical contact should be avoided. Some of these compounds are known carcinogens or suspected carcinogenic agents. Exposure to some of these chemicals has been shown to produce liver injury in animals. Therefore, exposure by all routes should be minimized. Wear chemical goggles, face shield, gloves, and chemical resistant clothing such as a laboratory coat and/or a rubber apron to prevent contact with eyes, skin, and clothing. These chemicals should only be used by persons trained in the safe handling of hazardous chemicals. For more detailed information regarding the handling of a particular chlorinated compound, please refer to the appropriate Material Safety Data Sheet.

2.0 SAFETY

The hazards, toxicity or carcinogenicity of each compound or reagent used in GERG's standard operating procedures have not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory maintains Material Safety Data Sheets (MSDS) which contain information regarding the safe handling of chemicals used at GERG's facilities. A reference file of MSDS is available to all personnel involved with these materials. All laboratory personnel should direct any questions regarding safety issues to their supervisors or the Safety Officer.

3.0 QUALITY CONTROL REQUIREMENTS

The following information must be obtained and applicable analytical activities must be performed, evaluated and approved prior to the use of any standard material in the laboratory.

3.1 Certificate of Analysis (COA) and Purity

3.1.1 All purchased organic materials for use as standards in analytical activities at GERG shall have a certificate of analysis specifying the content, purity and concentration of the material and its traceability to NIST, NRCC or USEPA standard materials.

3.1.1.1 Certified Standard Reference Materials (SRMs) available from NIST, NRCC and others do not require analysis prior to use. However, when their use requires mixing with other standards or dilution activities (i.e., when used for calibration standards), the criteria described in Section 3.2 apply to any subsequent use.

3.1.1.2 Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or an independent source. Addition of a commercial stock solution to small amounts of a solvent like acetone to enhance its dispersion in a sample does not require the verification procedures described in Section 3.2.

3.1.1.3 Standards which are commercially prepared or those prepared in the laboratory should be replaced when the comparison to check standards indicates deterioration in the quality of the standard.

3.1.1.4 When compound purity is assayed to be 96% or greater, the weight may be used without correction to calculate the concentration in the diluted standard solution. Specific gravity (density) may be used to determine the correct volume to use to provide the required amount of a neat solution.

3.2 Verification of Purity and Concentration of Purchased Materials

3.2.1 Certified Standard Reference Materials (SRMs) available from NIST, NRCC and other vendors do not require analysis prior to use.

3.2.2 After appropriate dilution and mixing of purchased standard materials, the resulting standard solution is analyzed under normal conditions with an NIST certified standard or the former standard solution, using the former calibration curve.

3.2.2.1 The chromatogram of the new standard is compared to the NIST certified standard or to the former standard for the proper target analyte peaks and retention times; for the appropriate area and response factors; and for the appropriate concentration in the diluted standard solution.

3.2.3 The prepared standard must meet the following QC acceptance criteria:

3.2.3.1 No unidentified or interfering peaks are present;

3.2.3.2 No degradation of the new solution is evidenced by reduced or missing peaks;

3.2.3.3 Target analytes are within the appropriate retention time windows;

3.2.3.4 Areas or concentrations are within ±10% of the areas or concentrations of the former solution.

3.2.4 If, after evaluation, the new standard does not meet these QC acceptance criteria, its failure must be documented in the appropriate standard logbook and it must be disposed of in accordance with appropriate waste disposal procedures.

3.2.5 If the new standard, after evaluation, meets these QC acceptance criteria, its acceptability must be documented in the appropriate standard logbook prior to use (see Section 6).

4.0 APPARATUS AND MATERIALS

4.1 Glassware and Hardware

4.1.1 Hamilton Syringes with non-beveled needle;

500 µL
1000 µL

4.1.2 Volumetric Flasks, Class "A";

25 mL
50 mL
100 mL
1000 mL

4.1.3 Volumetric pipettes, Class "A";

1 mL
2 mL
5 mL
10 mL
25 mL

4.1.4 Standard storage containers;

15 mL glass vials with Teflon-lined caps
40 mL glass vials with Teflon-lined caps.

4.1.5 Pipette bulbs for specified volumetric pipettes.

4.2 Instrumentation

4.2.1 Cahn Electrobalance capable of weighing to 0.001 mg; or equivalent

4.2.2 Gas chromatograph equipped with an ECD: instrumentation specified in the appropriate analytical SOP is used for QC verification of prepared standards

5.0 REAGENTS AND CONSUMABLE MATERIALS

5.1 Reagents and Solvents

5.1.1 Hexane: Baxter; Cat. 300-4; Pesticide grade or equivalent

5.2 Analytical Standards

The following is a table of the target analytes, internal standards and surrogates used in making the standards used for the analysis of chlorinated pesticides and PCBs, along with the manufacturer and catalog number. A vendor/company supplying the equivalent quality of standards can be used.

5.2.1 Chlorinated Pesticide and PCB mix. Each analyte at a concentration of 20 µg/mL.

1,2,4,5-tetrachlorobenzene	endrin
1,2,3,4-tetrachlorobenzene	mirex
pentachlorobenzene	PCB 8
hexachlorobenzene	PCB 18
pentachloroanisole	PCB 28
alpha-HCH	PCB 29
beta-HCH	PCB 44
delta-HCH	PCB 52
gamma-HCH (lindane)	PCB 66
heptachlor	PCB 87
heptachlor epoxide	PCB 101
alpha-chlordane	PCB 105
gamma-chlordane	PCB 110
oxychlordane	PCB 118
cis-nonachlor	PCB 128
trans-nonachlor	PCB 138
2,4'-DDE	PCB 153
4,4'-DDE	PCB 170
2,4'-DDD	PCB 180
4,4'-DDD	PCB 187
2,4'-DDT	PCB 195
4,4'-DDT	PCB 200
aldrin	PCB 206
dieldrin	PCB 209
endosulfan II	

5.2.2 Dicofol; >95%; ULTRA Scientific; PST-391

5.2.3 Chlorpyrifos; >95%; ULTRA Scientific; PST-480

5.2.4 4,4' dibromooctafluorobiphenyl; 99%; Aldrich; 10199-0

5.2.5 2,4,5,6 tetrachloro-m-xylene; 99%; Aldrich; 11375-1

5.2.6 PCB 103; >97%; ULTRA Scientific; RCP-040

5.2.7 PCB 198; >97%; ULTRA Scientific; RCP-075

5.3 Consumable Materials

5.3.1 Aluminum foil: combusted for 4 hours at 440°C and rinsed with methylene chloride prior use.

6.0 REQUIRED DOCUMENTATION OF STANDARD MATERIALS

6.1 General Information and Definitions

When available, standard materials are generally obtained as either a high purity solid or a high purity solution of specific density, purity, and/or concentration. These solutions may be purchased commercially as "neat" single component solutions or as multi-component solutions to be diluted to desired concentration. Standards may also be prepared in the laboratory by weighing solids and preparing a solution; measuring liquid volumes (corrected for density) and diluting as needed; or by actual synthesis or derivatization of required materials.

6.1.1 Stock Standards

Purchased stock standard solutions, at the appropriate concentration may be used without further dilution for certain analyses if they are certified by the manufacturers or another independent source. Stock standards may also be prepared from purchased standards, which are diluted and/or mixed in Class A volumetric glassware with the appropriate solvent. The resulting concentration is expressed in the appropriate concentration units {milligrams per liter (mg/L), nanograms per liter (ng/L), picograms per microliter (pg/μL)}. After preparation and before use, prepared stock standards must be checked using the procedures described in Section 3.2 to verify that all required components are present at the appropriate concentrations.

6.1.2 Intermediate Standards

Intermediate standards are dilutions of the specific stock standard materials which are prepared as solutions in Class A volumetric glassware using the appropriate solvent to achieve desired concentrations. After preparation and before use, intermediate standards must be checked using the procedures provided in Section 3.2 to verify that all required components are present at the appropriate concentrations.

6.1.3 Working Standards

Working standards are dilutions and/or combinations of two or more of the specified intermediate standards prepared in Class A volumetric glassware using the appropriate solvent to achieve the desired concentrations. After preparation and before use, working standards must be checked using the procedures described in Section 3.2 to verify that all required components are present at the appropriate concentrations.

6.2 Traceability and Documentation of Purchased Standard Materials

Traceability and documentation of the purity, concentration, preparation, use and ultimate consumption or disposal of standard materials is essential to the quality control of an analytical method. Purchased standard materials must have a Certificate of Analysis (COA), have a purity of 96% or greater, or must be ACS reagent grade or better. All handling, storage, dissolution, and treatment must be performed in strict accordance with this SOP.

6.2.1 All standard material, including SRMs, must be checked on receipt for reasonable limits of the expiration date and to verify a correct match (purity, content and concentration) of the COA or other purity criteria with both the standard container label and the associated purchase order.

6.2.1.1 If any of the above are not correct, do not use or log-in the standard. Contact the laboratory supervisor for the appropriate corrective action.

6.2.2 The verified standard material must then be initialed, the receipt date and logbook page must be written upon the label using waterproof ink (e.g., Rec'd 9/11/95-ABE -MSNP9-99).

6.2.3 The standard material must then be logged into the Neat/Purchased (Figure 1) or Intermediate (Figure 2) Logbook upon completion of these first two steps and prior to any use in the laboratory.

6.2.3.1 Using the information on the COA or standard label, completely fill in the information requested on the logbook page.

6.2.3.2 If the standard contains multiple analytes or if the concentration of the analytes in the solution vary, reference the information designated for these areas to the COA.

6.2.3.3 The Assay Date may be marked NA for Not Applicable if a COA is received. However, it must be completed and the assay attached or a storage location referenced if: there is no COA received; if there is a question of degradation; or if there is a need to prove non-deterioration of a properly stored but technically expired container of standard materials or solutions.

6.2.3.4 The correct storage location and conditions for standard materials must be indicated and must be updated if storage facilities are modified.

NOTE: Standard materials must not be stored with samples and volatile standards should not be stored with other non-volatile standard materials.

6.2.3.5 If multiple aliquots are received or if the original standard material is divided into separate storage containers, an unique identification must be placed on the label of the container, and each separate aliquot must be identified in the logbook.

6.2.3.6 The COA should be attached to the back of the logbook page, using both staples and transparent tape,

after the appropriate information has been entered on the front of the logbook page.

6.2.3.61 The original COA for SRMs should be given to the QA Manager and a copy maintained on file in the laboratory area where the SRM is used. The COA for an SRM is usually too large to be attached to a logbook page.

6.2.3.7 As the standard material or its aliquots are used, the forward reference section of the logbook page must be completed, indicating the subsequent logbook location documenting use of the material for preparation of an Intermediate (Figure 2) or Working Standard (Figure 3) Logbook.

6.2.3.8 All information requested on subsequent logbook pages must be completed prior to actual use of the standard material for an analytical activity. "Back Ref" refers to the prior reference page for the standard material being prepared on the current page.

6.3 Requirements for Storage, Aliquot Preparation, and Expiration Dates

6.3.1 All neat or purchased solutions and solids, and all prepared solutions described in this SOP are maintained in a freezer (or at the temperature specified by the manufacturer) while not in use.

6.3.2 Neat or purchased solutions maintained in their original container are labelled to indicate the date of receipt, logbook page of entry, and the initials of the person performing the login activity.

6.3.3 Stock solutions are labelled with the stock ID, date and logbook page for preparation, and the initials of the person performing the preparation activity. The volumetric flask containing the stock solution is capped, wrapped with Teflon tape and maintained in a freezer while not in use.

6.3.4 After preparation according to this SOP, all other solutions are split into approximately equivalent volumes in 15 mL or 40 mL containers with screw top Teflon lids, and labelled with solution ID, aliquot ID, the date and logbook page for preparation, and the initials of the person performing the preparation activity. These aliquots are maintained in a freezer at while not in use.

6.3.5 A specific single solution aliquot is used in laboratory activities until it is either 90% consumed or routine analysis indicates that degradation or excessive concentration has occurred. At that point, the balance of the solution is disposed of and the aliquot is logged out of the Standard Logbook.

6.3.6 When not specified by the manufacturer, all standards included in this SOP are given an expiration date of one year from the date of receipt or of preparation. However, with proper storage, some analytes may exhibit sufficient stability to be used beyond this expiration date, as long as the analytical results do not indicate degradation and/or excessive concentration.

7.0 PREPARATION OF STOCK, WORKING, AND CALIBRATION STANDARD SOLUTIONS

7.1 Preparation of Stock Solution A

7.1.1 Using a Class A volumetric pipette and a pipette bulb, two mL of the purchased Chlorinated Pesticide and PCB Mix are added to a 50 mL volumetric flask containing approximately 25 mL of hexane.

7.1.2 The volumetric flask is filled to the mark with hexane, for a total volume of 50 mL in the standard solution.

7.1.3 The initial concentration of each analyte in the Chlorinated Pesticide and PCB Mix is 20 µg/mL.

7.1.4 After dilution to 50 mL, the concentration of each analyte in Stock Solution A is 0.80 µg/mL.

7.1.5 The volumetric flask is labelled "Stock Solution A" and the concentration, standard logbook page, initials of the preparer, and the date of preparation are also written on the label.

7.2 Preparation of the Dicofol Stock Solution

7.2.1 A 25 mL volumetric flask is to be filled with approximately 20 mL of hexane prior to adding the dicofol.

7.2.2 Approximately 0.500 mg of the dicofol neat standard (solid compound) is weighed on an aluminum foil "boat" that has been cleaned with methylene chloride.

7.2.3 The solid dicofol is added to the volumetric flask after it has been weighed and the volumetric flask is filled to the 25 mL mark using hexane.

7.2.4 The concentration of dicofol in this dicofol stock solution is approximately 20 µg/mL. The actual weight of dicofol is used to calculate the actual final concentration of this stock solution.

7.2.5 The volumetric flask is labelled "Dicofol Stock Solution" and the concentration, standard logbook page, initials of the preparer, and the date of preparation are written on the label.

7.3 Preparation of the Dicofol Working Solution

7.3.1 Using a Class A volumetric pipette and a pipette bulb, two mL of the Dicofol Stock Solution are added to a 50 mL volumetric flask containing about 25 mL of hexane.

7.3.2 The volumetric flask is then filled to the mark with hexane for a total volume of 50 mL of the dicofol working solution.

7.3.3 After dilution, the concentration of dicofol is approximately 0.80 µg/mL.

7.3.4 The volumetric flask is labelled "Dicofol Working Solution" and the concentration, standard logbook page, initials of the preparer, and the date of preparation are written on the label.

7.4 Preparation of the Chlorpyrifos Stock Solution

7.4.1 A 25 mL volumetric flask is filled with approximately 20 mL of hexane prior to adding the chlorpyrifos.

7.4.2 Approximately 0.500 mg of the chlorpyrifos neat standard (solid compound) is weighed on an aluminum foil "boat" that has been cleaned with methylene chloride.

7.4.3 The solid chlorpyrifos is added to the volumetric flask after it has been weighed and the volumetric flask is then filled to the 25 mL mark using hexane.

7.4.4 The concentration of chlorpyrifos in this chlorpyrifos stock solution is approximately 20 µg/mL. The actual weight of chlorpyrifos is used to calculate the actual final concentration of this stock solution.

7.4.5 The volumetric flask is labelled "Chlorpyrifos Stock Solution" and the concentration, standard logbook page, initials of the preparer, and the date of preparation are written on the label.

7.5 Preparation of the Chlorpyrifos Working Solution

7.5.1 Using a Class A volumetric pipette and a pipette bulb, two mL of the Chlorpyrifos Stock Solution are added to a 50 mL volumetric flask containing about 25 mL of hexane.

7.5.2 The volumetric flask is filled to the mark with hexane for a total volume of 50 mL of chlorpyrifos working solution.

7.5.3 After dilution, the concentration of chlorpyrifos is approximately 0.80 µg/mL.

7.5.4 The volumetric flask is labelled "Chlorpyrifos Working Solution" and the concentration, standard logbook page,

initials of the preparer, and the date of preparation are written on the label.

7.6 Preparation of Chlorinated Pesticide and PCB Calibration Standards

The following standard solutions are combined in five separate 100 mL volumetric flasks and are called OC CAL 1, OC CAL 2, OC CAL 3, OC CAL 4 and OC CAL 5.

After preparation according to this SOP, the solutions are split into approximately equivalent volumes in 15 mL or 40 mL containers with screw top Teflon lids.

The container is labelled with the solution ID, the aliquot ID, the date and logbook page for preparation, and the initials of the person performing the preparation activity.

These aliquots are sealed with Teflon tape and maintained in a freezer while not in use.

7.6.1 OC CAL 1

Using separate Class A pipettes and pipettes bulbs, the following solutions are added to a 100 mL volumetric flask:

25.0 mL of Stock Solution A (see section 7.1),
25.0 mL of Dicofol Working Solution (see section 7.3),
25.0 mL of Chlorpyrifos Working Solution (see section 7.5),
10 mL of STOCIS (see section 9.0),
10 mL of STTCMX Solution (see section 10.0).

Add hexane to the mark for a final volume of 100 mL.

The concentration of each chlorinated pesticide and PCB in OC CAL 1 is approximately 0.200 µg/mL.

The concentrations of the surrogate and internal standards in OC CAL 1 are approximately 0.100 µg/mL each.

7.6.2 OC CAL 2

Using separate Class A pipettes and pipettes bulbs, the following solutions are added to a 100 mL volumetric flask containing about 30 mL of hexane:

10.0 mL of Stock Solution A (see section 7.1),
10.0 mL of Dicofol Working Solution (see section 7.3),
10.0 mL of Chlorpyrifos Working Solution (see section 7.5),
10 mL of STOCIS (see section 9.0),
10 mL of STTCMX Solution (see section 10.0).

Add hexane to the mark for a total volume of 100 mL.

The concentration of each chlorinated pesticide and PCB in OC CAL 2 is approximately 0.080 µg/mL.

The concentrations of the Surrogate and Internal Standards in OC CAL 2 are approximately 0.100 µg/mL each.

7.6.3 OC CAL 3

Using separate Class A pipettes and pipettes bulbs, the following solutions are added to a 100 mL volumetric flask containing 50 mL of hexane:

5.0 mL of Stock Solution A (see section 7.1),
5.0 mL of Dicofol Working Solution (see section 7.3),
5.0 mL of Chlorpyrifos Working Solution (see section 7.5),
10 mL of STOCIS (see section 9.0),
10 mL of STTCMX Solution (see section 10.0).

Add hexane to the mark for a total volume of 100 mL.

The concentration of each chlorinated pesticide and PCB in OC CAL 3 is approximately 0.040 µg/mL.

The concentrations of the Surrogate and Internal Standards OC CAL 3 are approximately 0.100 µg/mL each.

7.6.4 OC CAL 4

Using separate Class A pipettes and pipettes bulbs, add the following solutions to a 100 mL volumetric flask containing about 50 mL of hexane:

2.5 mL of Stock Solution A (see section 7.1),
2.5 mL of Dicofol Working Solution (see section 7.3),
2.5 mL of Chlorpyrifos Working Solution (see section 7.5),
10 mL of STOCIS (see section 9.0),
10 mL of STTCMX Solution (see section 10.0).

Add hexane to the mark for a total volume of 100 mL.

The concentration of each Chlorinated Pesticide and PCB in OC CAL 4 is approximately 0.020 µg/mL.

The concentrations of the Surrogate and Internal Standards in OC CAL 4 are approximately 0.100 µg/mL each.

7.6.5 OC CAL 5

Using separate Class A pipettes and pipettes bulbs and syringes, the following solutions are added to a 100 mL volumetric flask containing about 50 mL of hexane:

0.63 mL of Stock Solution A (see section 7.1),
0.63 mL of Dicofol Working Solution (see section 7.3),
0.63 mL of Chlorpyrifos Working Solution (see section 7.5),
10 mL of STOCIS (see section 9.0),
10 mL of STTCMX Solution (see section 10.0).

Add hexane to the mark for a total volume of 100 mL.

The concentration of each Chlorinated Pesticide and PCB in OC CAL 5 is approximately 0.005 µg/mL.

The concentrations of the Surrogate and Internal Standards in OC CAL 5 are approximately 0.100 µg/mL each.

8.0 PREPARATION OF MATRIX SPIKE SOLUTIONS

8.1 Using separate Class A pipettes and pipettes bulbs, the following solutions are added to a 50 mL volumetric flask containing about 25 mL of hexane:

1.0 mL of the purchased Chlorinated Pesticide and PCB Mix,

1.0 mL of Dicofol Stock Solution (see section 7.3),
1.0 mL of Chlorpyrifos Stock Solution (see section 7.5)

Add hexane to the mark for a total volume of 50 mL.

The Matrix Spike Solution shall be named STOCSP-XX-YY; where XX is the log reference page number and YY is the bottle number for that batch.

After preparation according to this SOP, the matrix spike solutions are split into approximately equivalent volumes in 15 mL or 40 mL containers with screw top Teflon lids.

The container is labelled with solution ID, aliquot ID, the date and logbook page for preparation, and the initials of the person performing the preparation activity.

These aliquots are sealed with Teflon tape and maintained in a freezer while not in use.

The concentration of each chlorinated pesticide and PCB is approximately 0.400 µg/mL.

9.0 PREPARATION OF SURROGATE STANDARD SOLUTIONS

A 1000 mL volumetric flask is filled with approximately 800 mL of hexane prior to adding the solid surrogate compounds.

Approximately 1.000 mg of the following neat surrogate standards (solid compounds) is weighed on aluminum foil "boats" that have been cleaned with methylene chloride.

4,4' dibromooctafluorobiphenyl (DBOFB)
PCB 103
PCB 198

Each surrogate compound is added to the volumetric flask after it has been weighed, and hexane is added to the volumetric flask to the 1000 mL mark.

After preparation according to this SOP, the surrogate solutions are split into approximately equivalent volumes in 15 mL or 40 mL containers with screw top Teflon lids.

The container is labelled with solution ID, aliquot ID, the date and logbook page for preparation, and the initials of the person performing the preparation activity. These aliquots are sealed with Teflon tape and stored in a freezer while not in use.

The Surrogate Standard Solution shall be named STOCIS-XX-YY; where XX is the log reference page number and YY is the bottle number for that batch.

The concentration of each surrogate compound is approximately 1.0 µg/mL.

10.0 PREPARATION OF INTERNAL STANDARD SOLUTIONS

A 1000 mL volumetric flask is to be filled with approximately 800 mL of hexane prior to adding the compound.

Approximately 1.000 mg of the neat standard (solid compound) is weighed on an aluminum foil "boat" that has been cleaned with methylene chloride.

The internal standard compound, tetrachloro-m-xylene (TCMX), is added to the volumetric flask after it has been weighed. Hexane is then added to the volumetric flask to the 1000 mL mark.

After preparation according to this SOP, the surrogate solutions are split into approximately equivalent volumes in 15 mL or 40 mL containers with screw top Teflon lids.

The container is labelled with solution ID, aliquot ID, the date and logbook page for preparation, and the initials of the person performing the preparation activity. These aliquots are sealed with Teflon tape and stored in a freezer while not in use.

The Internal Standard Solution is named STTCMX-XX-YY; where XX is the log reference page number and YY is the bottle number for that batch.

The concentration of the internal standard compound in this solution is approximately 1.0 µg/mL.

11.0 PREPARATION OF METHOD DETECTION LIMIT SOLUTIONS

OC CAL 5 is diluted as appropriate for the matrix being studied and then used to spike samples in the laboratory for the determination of the method detection limits. Refer to section 7 for the OC CAL 5 preparation steps.

12.0 PREPARATION OF INDEPENDENT CONTINUING CALIBRATION CHECK SOLUTIONS

OC CAL 3, which is not used to calibrate the GC, is used as the Independent Continuing Calibration Check solution. Refer to section 7 for the OC CAL 3 preparation steps.

13.0 DOCUMENTATION REQUIREMENTS

All documents pertaining to verification of standards shall be maintained by the GC/ECD Supervisor in folders.

14.0 LIST OF FORMS

14.1 Neat/Purchased Logbook Form
14.2 Intermediate Logbook Form
14.3 Working Standards Logbook Form

Neat / Purchased—PEST/PCB Ref. Page: _____

Analyte: _____ Method: _____

Source: _____ Cat #: _____ Lot #: _____

Amt Rcvd: _____ Conc.: _____ Date: _____

Evaluation

Purity: _____ Source COA Rcvd?: ☐ Yes ☐ No ☐ Attached

Known Impurities: _____

Assay Date: _____ Initials: _____ Results: _____

Storage Location: _____

Packaging: _____

Conditions: _____

Date Rcvd: _____ Initials: _____ Exp Date: _____

Method of Disposal: _____

ID of Aliquot Prepared: _____

Comments: _____

Forward References

Reference Pg	Date	Initials	Reference Pg	Date	Initials

1995-2oc Lab Side—Purchased/Pest

Figure 1. Neat/Purchased Logbook Form

Intermediate Standards Log—PEST/PCB

Ref Page: _____

Analyte: _____ Method: _____

Preparation

Back Ref	Amount	

Storage

Location: _____

Packaging: _____

Conditions: _____

Prep Date: _____ Initials: _____ Exp Date: _____ *after opened*

Method of Disposal: _____

ID of Aliquot Prepared: _____

Comments: _____

Forward References

Reference Pg	Date	Initials	By	Result

1995-21c.Lab Stds—Intermediate/Pest

Figure 2. Intermediate Logbook Form

Working Standards Log—PEST/PCB Ref. Page: _____

Analyte: _____ Method: _____

Matrix: _____ Conc.: _____

Preparation

Back Ref	Lot #	Amount	Description

Storage Location: _____

Packaging: _____

Conditions: _____

Prep Date: _____ Initials: _____ Exp Date: _____

Method of Disposal: _____

ID of Aliquot Prepared: _____

Comments: _____

1995-23c.Lab Stds—Log/Pest

(page 1/2)

Figure 3. Working Standards Logbook Form

Working Standards Log—PEST/PCB

Ref. Page: __________

Forward References

Std. ID	Ref. Page	Date	Initials	Exp/Cons.	Date	Initials

1995-23c Lab Side—Log/Pest

(page 2/2)

Figure 3. Cont.

SOP-9714
PROCEDURE FOR DOCUMENTATION, QUALITY CONTROL, VERIFICATION, AND PREPARATION OF ANALYTICAL STANDARDS FOR THE ANALYSIS OF TETRA- THROUGH OCTA-CHLORINATED DIBENZO-P-DIOXINS (PCDDs) AND DIBENZOFURANS (PCDFs) BY ISOTOPE DILUTION HIGH RESOLUTION GAS CHROMATOGRAPHY/HIGH RESOLUTION MASS SPECTROMETRY (HRGC/HRMS)

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURES

SOP-9714

PROCEDURE FOR DOCUMENTATION, QUALITY CONTROL, VERIFICATION, AND PREPARATION OF ANALYTICAL STANDARDS FOR THE ANALYSIS OF TETRA- THROUGH OCTA-CHLORINATED DIBENZO-*p*-DIOXINS (PCDDS) AND DIBENZOFURANS (PCDFS) BY ISOTOPE DILUTION HIGH RESOLUTION GAS CHROMATOGRAPHY/HIGH RESOLUTION MASS SPECTROMETRY (HRGC/HRMS)

This document presents the procedures, materials, and quality control used in the performance of the above preparation activities.

Laura E Ekman
Quality Assurance Manager

5/2/97
Date

Author/Revision By: Laura Chambers

PROCEDURE FOR DOCUMENTATION, QUALITY CONTROL, VERIFICATION, AND PREPARATION OF ANALYTICAL STANDARDS FOR THE ANALYSIS OF TETRA- THROUGH OCTA-CHLORINATED DIBENZO-*p*-DIOXINS (PCDDS) AND DIBENZOFURANS (PCDFS) BY ISOTOPE DILUTION HIGH RESOLUTION GAS CHROMATOGRAPHY/HIGH RESOLUTION MASS SPECTROMETRY (HRGC/HRMS)

1.0 PURPOSE

This document provides the procedures for the documentation of purchased analytical standard materials, their quality control (QC) and verification criteria, and their preparation for use in the analysis of tetra- through octa-chlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) by isotope dilution high resolution gas chromatography/high resolution mass spectrometry which is used by staff of the Geochemical and Environmental Research Group (GERG) of the College of Geosciences and Maritime Studies at Texas A&M University.

1.1 SUMMARY OF METHOD

The use of properly prepared standards and reagents is essential for any analytical procedure. All materials purchased for use in calibration, spiking activities, precision and accuracy evaluation, or other analytical instrumental evaluations must be documented in a Standards Logbook as described in this standard operating procedure (SOP). Prior to use and after certain preparatory steps, purchased standards must be evaluated for both purity and appropriate concentration by GERG staff, and must be shown to meet the appropriate QC criteria. Procedures ensuring traceability of these standard materials from receipt, QC verification, preparation, aliquot identification, analytical use, and consumption or disposal are provided in this SOP.

1.2 APPLICABILITY

The procedures included in this SOP apply to the preparation of analytical standards used in the analysis of all matrices for tetra- through octa-chlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) by isotope dilution high resolution gas chromatography/high resolution mass spectrometry by the GERG staff.

1.2.1 Interferences

High purity reagents and solvents must be used, and all equipment and glassware must be scrupulously cleaned. Gloves, certain plastic components, and certain greases must not be used if these result in sample contamination.

1.2.2 Special Precautions

Hazards associated with the dioxin/furan standards in the MSDS include the following information:

Hazards: While little toxicity data on these compounds is available, they are potentially highly toxic. Dioxins and dibenzofurans substituted in the 2,3,7 and 8 positions are especially likely to exhibit toxic effects including teratogenicity, mutagenicity, and carcinogenicity. Exposure to microgram quantities could result in toxic effects. Only experienced personnel should be allowed to work with these chemicals.

Toxic exposure routes are potentially high via oral, dermal, enterperitoneal routes.

Personnel protection including an impervious laboratory apron or coverall should be worn while handling this standard. Neoprene gloves are preferred. An acceptable substitute would be two dissimilar types of gloves such as PVC gloves worn over latex exam gloves. Open only in a fume hood or glove box. Respiratory protection (full-face air line or cartridge type respirator) is required.

If this standard is spilled, absorb as much as possible with activated charcoal and place in an appropriate container. Solvent-wash all contaminated surfaces with toluene and absorbent paper followed by washing with a strong soap and water solution. Do not re-enter the contaminated area until the Safety Officer (or other responsible person) has verified that the area has been properly cleaned. Dispose of all contaminated materials in sealed steel containers.

If skin contact occurs, remove all contaminated clothing and immediately flood skin with water. Wash the affected skin areas with soap and water for 15 minutes. If symptoms persist or develop, seek medical attention.

If any laboratory personnel should inhale this chemical, remove them at once to open air and arrange for immediate transportation to a medical facility.

If eye contact occurs, check for contact lenses and remove them at once if present. Immediately flush eyes with water for 15 minutes. Do not use oil or ointment in eyes. Arrange immediate transportation to a medical facility.

If ingestion occurs and the exposed person is convulsing or unconscious, do not attempt first aid. Transport immediately to a hospital emergency room or poison control center. If the victim is conscious, administer large volumes of liquid. Transport at once to a medical facility.

Symptoms of ingestion of microgram quantities of these compounds may cause liver and kidney damage, chloracne and death.

2.0 SAFETY

The hazards, toxicity or carcinogenicity of each compound or reagent used in GERG's standard operating procedures have not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory maintains Material Safety Data Sheets (MSDS) which contain information regarding the safe handling of chemicals used at GERG's facilities. A reference file of MSDS is available to all personnel involved with these materials. All laboratory personnel should direct any questions regarding safety issues to their supervisors or the Safety Officer.

3.0 QUALITY CONTROL REQUIREMENTS

The following information is obtained and applicable analytical activities are performed, evaluated and approved prior to the use of any standard material in the laboratory.

3.1 Certificate of Analysis (COA) and Purity

3.1.1 All purchased organic materials for use as standards in analytical activities at GERG shall have a certificate of analysis specifying the content, purity and concentration of the material and its traceability to NIST, NRCC or USEPA standard materials.

3.1.1.1 Certified Standard Reference Materials (SRMs) available from NIST, NRCC and others do not require analysis prior to use. However, when their use requires mixing with other standards or dilution activities (i.e., when used for calibration standards), the criteria provided in Section 3.2 apply to any subsequent use.

3.1.1.2 Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or an independent source. Addition of a commercial stock solution to small amounts of a solvent like acetone to enhance its dispersion in a sample does not require the verification procedures described in Section 3.2.

3.1.1.3 Standards which are commercially prepared or those prepared in the laboratory should be replaced when the comparison to check standards indicates deterioration in the quality of the standard.

3.1.1.4 When compound purity is assayed to be 96% or greater, the weight may be used without correction to calculate the concentration in the diluted standard solution.

3.2 Verification of Purity and Concentration of Purchased Materials

3.2.1 Certified Standard Reference Materials (SRMs) available from NIST, NRCC and other vendors do not require analysis prior to use.

3.2.2 The prepared standard must meet the following QC acceptance criteria:

3.2.2.1 No unidentified or interfering peaks are present;

3.2.2.2 No degradation of the new solution is evidenced by missing peaks;

3.2.2.3 Target analytes meet the QC acceptance criteria specified in the SOP for instrumental analysis of PCDDs and PCDFs.

3.2.3 If, after evaluation, the new standard does not meet these QC acceptance criteria, its failure is documented in the appropriate standard logbook and it is disposed of in accordance with appropriate waste disposal procedure.

4.0 APPARATUS AND MATERIALS

4.1 Glassware and Hardware

4.1.1 Pipettes-Disposable, Pasteur: 150 mm x 5 mm (i.d.); combusted

4.1.2 Vials with Teflon Lined Caps: 2 mL capacity; amber; washed, rinsed and combusted

4.1.3 Teardrop Vials With Teflon Lined Caps: clear glass; washed, rinsed and combusted

4.2 Instrumentation normal to analysis for QC verification is specified in the instrumental SOP for PCDD and PCDF.

5.0 ANALYTICAL STANDARDS

All analytical standards are purchased as solutions in nonane, have a chemical purity of ≥98% unless specifically noted in the COA, and are of isotopic purity of >99% unless specifically noted in the COA.

5.1 EPA-1613CVS, Calibration and Verification Solutions CS1-CS5 at concentrations specified in EPA Method 1613. Contents: 500 µL each CS1, CS2, CS4, and CS5, 1 mL CS3 (5 ampoules total). Purchased from CHEMSYN SCIENCE LABORATORIES/WELLINGTON LABORATORIES (CSL/WL) or other appropriate vendor.

5.2 EPA-1613CS3, Calibration Verification Solution CS3 purchased individually as needed at concentrations specified in EPA Method 1613. Contents: 1 mL. Purchased from CHEMSYN SCIENCE LABORATORIES/WELLINGTON LABORATORIES (CSL/WL) or other appropriate vendor.

5.3 EPA-1613CSS, Cleanup Standard Spiking Solution at concentrations specified in EPA Method 1613. Contents: 1 mL. Purchased from CHEMSYN SCIENCE LABORATORIES/WELLINGTON LABORATORIES (CSL/WL) or other appropriate vendor.

5.4 EPA-1613LCS, Labeled Compound Stock Solution at concentrations specified in EPA Method 1613. Contents: 1 mL. Purchased from CHEMSYN SCIENCE LABORATORIES/WELLINGTON LABORATORIES (CSL/WL) or other appropriate vendor.

5.5 EPA-1613PAR, PAR Stock Solution at concentrations specified in EPA Method 1613. Contents: 1 mL. Purchased from CHEMSYN SCIENCE LABORATORIES/WELLINGTON LABORATORIES (CSL/WL) or other appropriate vendor.

5.6 EPA-1613ISS, Internal Standard Spiking Solution at concentrations specified in EPA Method 1613. Contents: 1 mL. Purchased from CHEMSYN SCIENCE LABORATORIES/WELLINGTON LABORATORIES (CSL/WL) or other appropriate vendor.

5.7 EPADB-5CWDS, DB-5 Colum Window Defining Standard Mixture at concentrations specified in EPA Method 1613. Contents: 1.2 mL.

Purchased from CHEMSYN SCIENCE LABORATORIES/WELLINGTON LABORATORIES (CSL/WL) or other appropriate vendor.

5.8 EPADB-5TCDD, DB-5 Column TCDD Isomer Specificity Test Standard Mixture at concentrations specified in EPA Method 1613. Contents: 1.2 mL. Purchased from CHEMSYN SCIENCE LABORATORIES/WELLINGTON LABORATORIES (CSL/WL) or other appropriate vendor.

5.9 EPADB-225TCDF, DB-225 Column TCDF Isomer Specificity Test Standard Solution at concentrations specified in EPA Method 1613. Contents: 1.2 mL. Purchased from CHEMSYN SCIENCE LABORATORIES/WELLINGTON LABORATORIES (CSL/WL) or other appropriate vendor.

6.0 REQUIRED DOCUMENTATION OF STANDARD MATERIALS

6.1 General Information and Definitions

When available, standard materials are generally obtained as either a high purity solid or a high purity solution of specific density, purity and concentration. These solutions are purchased commercially as single component solutions or as multi-component solutions to be diluted to desired concentration. Standards may also be prepared in the laboratory by weighting solids and preparing a solution; measuring liquid volumes (corrected for density) and diluting as needed; or by actual synthesis or derivatization of required materials.

6.1.1 Stock Standards

Purchased stock standard solutions, at the appropriate concentration may be used without further dilution for certain analyses if they are certified by the manufacturers or another independent source. Stock standards may also be prepared from purchased standards, which are diluted and/or mixed in Class A volumetric glassware with the appropriate solvent. The resulting concentration is expressed in the appropriate concentration units {milligrams per liter (mg/L), nanograms per liter (ng/L), picograms per microliter (pg/µL)}. After preparation and before use, prepared stock standards are checked using the procedures described in Section 3.2 to verify that all required components are present at the appropriate concentration.

6.1.2 Intermediate Standards

Intermediate standards are dilutions of the specific stock standard materials which are prepared as solutions in Class A volumetric glassware using the appropriate solvent to achieve desired concentrations. After preparation and before use, intermediate standards are checked using the procedures described in Section 3.2 to verify that all required components are present at the appropriate concentration.

6.1.3 Working Standards

Working standards are dilutions or combinations of two or more of the specified intermediate standards prepared in Class A volumetric glassware using the appropriate solvent to achieve the desired concentrations. After preparation and before use, working standards are checked using the procedures described in Section 3.2 to verify that all required components are present at the appropriate concentration.

6.2 Traceability and Documentation of Purchased Standard Materials

Traceability and documentation of the purity, concentration, preparation, use and ultimate consumption or disposal of a standard materials is essential to the quality control of an analytical method. Purchased standard materials must have a Certificate of Analysis (COA), have a purity of 96% or greater, or are ACS reagent grade or better. All handling, storage, dissolution, and treatment are performed in strict accordance with this SOP.

6.2.1 All standard material, including SRMs are checked on receipt for reasonable limits on the expiration date and a correct match (purity, content and concentration) of the COA or other purity criteria with both the standard container label and the associated purchase order.

6.2.1.1 If any of the above are not correct, do not use or long-in the standard. Contact the supervisor for the appropriate action.

6.2.2 The standard material is then be initialed, the receipt date and logbook page are written upon the label using waterproof ink (i.e., Rec'd 9/11/95-ABE -MSNP999).

6.2.3 The standard material is then be logged into the Stock/Purchased (Figure 1) Logbook upon completion of these first two steps and prior to any use in the laboratory.

6.2.3.1 Using the information on the COA or standard label, completely fill in the information requested on the logbook page.

6.2.3.2 If the standard contains multiple analytes or if the concentration of the analytes in the solution vary, reference the information designated for these areas to the COA.

6.2.3.3 The Assay Date may be marked NA for Not Applicable if a COA is received. However, it must be completed and the assay attached if there is no COA received; if there is a question of degradation; or if there is a need to prove non-degradation of a properly stored but technically expired container of standard materials.

6.2.3.4 The correct storage location and conditions for standard materials is be indicated on the form and updated if storage facilities are modified.

NOTE: Standard materials must not be stored with samples and volatile standards should be stored with other non-volatile materials.

6.2.3.5 If multiple aliquots are received or if the original standard material is divided into separate storag containers, a unique identification must be on th label of the container, and each separate aliquot i identified on the logbook page.

6.2.3.6 The COA is attached to the back of the logbook page after the appropriate information has been entered on the front of the logbook page.

6.2.3.61 The original COA for SRMs is given to the QA Manager and a copy maintained on file in the laboratory area where the SRM is used.

6.2.3.7 As the standard material or its aliquots are used, the forward reference section of the logbook page is completed, indicating the subsequent logbook location documenting use of the material for sample preparation.

7.0 PREPARATION OF CALIBRATION AND VERIFICATION STANDARDS

All preparation and transfer of these standards is performed in a fume hood using appropriate safety protective apparel and equipment.

7.1 Use EPA-1613CVS, Calibration and Verification Solutions CS1-CS5.

7.2 Break open the ampoule and transfer entire contents to a 2 mL amber glass vial using a disposable pasteure pipette.

7.3 Label vial with "Stock/Purchased" page number, date, and initials, record transfer on "Stock/Purchased" form, and store vial in freezer.

7.4 Transfer small (i.e., 20-30 µL) aliquots to teardrop vials as necessary for instrumental analysis. Label the vials as CS1, CS2, etc.

8.0 PREPARATION OF MATRIX SPIKE SOLUTIONS

8.1 Use EPA-1613PAR, PAR Stock Solution.

8.2 Break open the ampoule and transfer entire contents to a 2 mL amber glass vial using a disposable pasteure pipette.

8.3 Label vial, with "Stock/Purchased" page number, date, and initials, record transfer on "Stock/Purchased" form, and store vial in freezer.

9.0 PREPARATION OF SURROGATE SOLUTIONS

9.1 Use EPA-1613LCS, Labeled Compound Stock Solution.

9.2 Break open the ampoule and transfer entire contents to a 2 mL amber glass vial using a disposable pasteure pipette.

9.3 Label vial with "Stock/Purchased" page number, date, and initials, record transfer on "Stock/Purchased" form, and store vial in freezer.

10.0 PREPARATION OF INTERNAL STANDARD SOLUTIONS

10.1 Use EPA-1613ISS, Internal Standard Spiking Solution.

10.2 Break open the ampoule and transfer entire contents to a 2 mL amber glass vial using a disposable pasteure pipette.

10.3 Label vial with "Stock/Purchased" page number, date, and initials, record transfer on "Stock/Purchased" form, and store vial in freezer.

11.0 PREPARATION OF CLEANUP STANDARD SPIKING SOLUTION

11.1 Use EPA-1613CSS, Cleanup Standard Spiking Solution.

11.2 Break open the ampoule and transfer entire contents to a 2 mL amber glass vial using a disposable pasteure pipette.

11.3 Label vial with "Stock/Purchased" page number, date, and initials, record transfer on "Stock/Purchased" form, and store vial in freezer.

12.0 PREPARATION OF DB-5 COLUMN PERFORMANCE SOLUTIONS

12.1 Use EPADB-5CWDS, DB-5 Column Window Defining Standard Mixture and EPADB-5TCDD, DB-5 Column TCDD Isomer Specificity Test Standard Mixture.

12.2 Break open both ampoules and transfer approximately half of each ampoule to each of two 2 mL amber glass vials using a disposable pasteure pipette.

12.3 Label vials with "Stock/Purchased" page number, date, and initials, record transfer on "Stock/Purchased" form, and store vials in freezer.

12.4 Transfer small (i.e., 20-30 µL) aliquots to teardrop vials as necessary for instrumental analysis. Label the vials CPSM/WDM, DB5.

13.0 PREPARATION OF DB-225 COLUMN PERFORMANCE SOLUTION

13.1 Use EPADB-225TCDF, DB-225 Column TCDF Isomer Specificity Test Standard Solution.

13.2 Break open the ampoule and transfer entire contents to a 2 mL amber glass vial using a disposable pasteure pipette.

13.3 Label vial, record transfer on "Stock/Purchased" form, and store vial in freezer.

13.4 Transfer small (i.e. 20-30 µL) aliquots to teardrop vials as necessary for instrumental analysis. Label the vials CPSM DB225.

13.0 DOCUMENTATION REQUIREMENTS

The "Stock/Purchased" forms and Certificate of Analysis for all purchased stock standards are maintained by the Laboratory Supervisor in a chronological file in the laboratory.

14.0 LIST OF FIGURES

14.1 Example Stock/Purchased Standard Logbook Form

Stock / Purchased—DIOXINS

Ref. Page: _____

Analytes: _____ Method: _____

Source: _____ Cat #: _____ Lot #: _____

Amt Rcvd: _____ Conc.: _____ Date: _____

Evaluation

Purity: _____ Source COA Rcvd?: ☐ Yes ☐ No ☐ Attached

Known Impurities: _____

Assay Date: _____ Initials: _____ Results: _____

Storage Location: _____

Packaging: _____

Conditions: Store in freezer when not in use.

Date Rcvd: _____ Initials: _____

Method of Disposal: Contact GERG's Safety Officer for the proper disposal of standard containers and solutions.

ID of Aliquot Prepared: Use page number, date, and initials.

Comments: _____

Forward References

Page	Date	Initials	Page	Date	Initials

1995-20b Lab Stds—Stock/Dioxin

Figure 1. Example Stock/Purchased Standard Logbook Form

Contract No.: DACW33-94-D-0009
DCN: GEP2-123098-AAET
Revision No.: 01
Date: 01/99

SOP-9719
PROCEDURES FOR THE EXTRACTION OF TISSUES AND PURIFICATION OF EXTRACTS FOR ANALYSIS OF POLYCHLORINATED DIBENZO-P-DIOXINS (PCDD) AND POLYCHLORINATED DIBENZOFURANS (PCDF)

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURES SOP-9719

PROCEDURES FOR THE EXTRACTION OF TISSUES AND PURIFICATION OF EXTRACTS FOR ANALYSIS OF POLYCHLORINATED DIBENZO-*p* -DIOXINS (PCDD) AND POLYCHLORINATED DIBENZOFURANS (PCDF)

This document presents the procedures used in the performance of the above preparation activities.

Susan E Ekman
Quality Assurance Manager

5/2/97
Date

Author/Revision By: B. Wang, L. Chambers

PROCEDURES FOR THE EXTRACTION OF TISSUES AND PURIFICATION OF EXTRACTS FOR ANALYSIS OF POLYCHLORINATED DIBENZO-*p* -DIOXINS (PCDD) AND POLYCHLORINATED DIBENZOFURANS (PCDF)

1.0 PURPOSE

This document provides the procedures for the extraction of biological tissue samples and purification of the extracts for the measurement of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) which are used by staff of the Geochemical and Environmental Research Group (GERG) of the College of Geosciences and Maritime Studies at Texas A&M University.

1.1 SUMMARY OF THE METHOD

This procedure uses matrix-specific extraction and analyte-specific purification steps to allow the determination of the 2,3,7,8-substituted PCDD and PCDF isomers using High Resolution Gas Chromatography/High Resolution Mass Spectrometry (HRMS). The method provides selective cleanup procedures to aid in the elimination of interfering contaminants.

A tissue sample is spiked with the Labeled Compound Spiking solution (LCSS) containing specified amounts of isotopically ($^{13}C_{12}$) labeled 2,3,7,8-substituted PCDDs/PCDFs and homogenized in a 200 mL centrifuge tube. The tissue is dried with 50 grams of sodium sulfate (Na_2SO_4) and extracted by maceration three times in 100 mL aliquots of methylene chloride (CH_2Cl_2). After extraction, the samples are spiked with a Cleanup Recovery Standard (CRS), $^{37}Cl_4$ -2,3,7,8-TCDD, to monitor losses through the extract purification steps. An aliquot for % lipid determination is then removed. The extract is dried with sodium sulfate, concentrated and subjected to a bulk purification involving a Silica Gel/Sulfuric Acid slurry. The extract is then processed through three column chromatographic procedures to remove co-extracted matrix interferences: a mixed bed silica gel column, a basic alumina column, and an activated charcoal column. All concentration steps are performed using tetradecane as a keeper and the final concentration step reduces the extract to approximately 10 µL tetradecane. An Internal Standard (ISS) of selected $^{13}C_{12}$-labeled PCDD is added to all final extracts before bringing the final volume of the extract to 20 µL of tetradecane

The identification and determination of the 2,3,7,8-substituted PCDDs and PCDF isomers are then conducted according to the appropriate GERG SOP.

1.2 APPLICABILITY

1.2.1 Matrix

This method is applicable to biological matrices such as bird, fish and mammal tissue, blubber, eggs, and body fluids.

1.2.2 Interferences

Interferences in the matrix, solvents, reagents, glassware and other sample processing hardware may yield discrete artifacts and/or other elevated baselines that may cause misinterpretation of chromatographic data. All materials used during the cleanup procedure must be demonstrated to be free from interferences under the same conditions of analysis by analyzing laboratory method blanks, at a frequency of one blank per 20 samples or one with each batch if the number of samples is less than 20.

Laboratory background contamination is minimized by washing the glassware according to the GERG SOP followed by sequential solvent rinsing of all clean glassware with methanol, acetone, methylene chloride, toluene, and hexane prior to use.

1.2.3 Special Precautions

1.2.3.1 Before and after subsampling, all tissue samples must be stored in the dark at -20°C ± 10°C. Sample extracts are stored in the dark at room temperature.

1.2.3.2 Hazards associated with the dioxin/furan standards in the MSDS include the following information:

Hazards: While little toxicity data on these compounds is available, they are potentially highly toxic. PCDDs and PCDFs substituted in the 2,3,7 and 8 positions are especially likely to exhibit toxic effects including teratogenicity, mutagenicity, and carcinogenicity. Exposure to microgram quantities could result in toxic effects. Only experienced personnel should be allowed to work with these chemicals.

Toxic exposure routes are potentially high via oral, dermal, and enterperitoneal routes.

Personnel protection should include an impervious laboratory apron or coverall should be worn while handling these standards. Neoprene gloves are preferred. An acceptable substitute would be two dissimilar types of gloves such as PVC gloves worn over latex exam gloves. Open standards only in a fume hood or glove box. Respiratory protection (full-face air line or cartridge type respirator) is required.

If this standard is spilled, absorb as much as possible with activated charcoal and place in an appropriate container. Solvent-wash all contaminated surfaces with toluene and absorbent paper followed by washing with a strong soap and water solution. Do not re-enter the contaminated area until the Safety Officer (or other responsible person) has verified that the area has been properly cleaned. Dispose of all contaminated materials in sealed steel containers.

If skin contact occurs, remove all contaminated clothing and immediately flood skin with water. Wash the affected skin areas with soap and water for 15 minutes. If symptoms persist or develop, seek medical attention.

If any laboratory personnel should inhale this chemical, remove them at once to open air and arrange for immediate transportation to a medical facility.

If eye contact occurs, check for contact lenses and remove them at once if present. Immediately flush eyes with water from any source for 15 minutes. Do not use oil or ointment in eyes. Arrange immediate transportation to a medical facility.

If ingestion occurs and the exposed person is convulsing or unconscious, do not attempt first aid. Transport immediately to a hospital emergency room or poison control center. If the

victim is conscious, administer large volumes of liquid. Transport at once to a medical facility.

Symptoms of ingestion of microgram quantities of these compounds may cause liver and kidney damage, chloracne and death.

1.2.4 Reporting Units

The reporting units are picograms per gram (pg/g) on a wet or dry weight basis (as specified by the client) for the target analytes. Lipids content is reported as a percent based on the dry weight of the sample. The % moisture (or solids) is reported with the data.

1.2.5 Minimum Levels (MLs)

The minimum level (ML) for each analyte is defined as the level at which the entire system must give a recognizable signal and acceptable calibration point. It is equivalent to the concentration of the lowest calibration standard, assuming that all method specific sample weights, volumes, and procedures have been employed. The MLs vary with degree of chlorination. Based on 10 grams (dry weight) of tissue extracted, the MLs are listed below (refer to EPA method 1613) .

Tetras	1.0 pg/g
Pentas, Hexas, Heptas	5.0 pg/g
Octas	10.0 pg/g

Minimum Levels are reported for all analytes in all samples.

2.0 SAFETY

The hazards, toxicity or carcinogenicity of each compound or reagent used in GERG's standard operating procedures have not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory maintains Material Safety Data Sheets (MSDS) which contain information regarding the safe handling of chemicals used at GERG's facilities. A reference file of MSDS is available to all personnel involved with these materials. All laboratory personnel should direct any questions regarding safety issues to their supervisors or the Safety Officer.

3.0 QUALITY CONTROL REQUIREMENTS

3.1 Method Blank (BLANK)

A Method Blank is used to demonstrate that the analytical method is free of contaminating interference. The BLANK is prepared by executing all of the specified extraction and extract purification steps except for the introduction of a sample. The BLANK is spiked with the Labeled Compound Spiking Solution (LCSS), the Clean-up Recovery Standard (CRS) and the Internal Standard (ISS) at the appropriate stages of the preparation. A BLANK must be run with each set of 20 or fewer samples.

3.2 Laboratory Blank Spike (LBS)

A Laboratory Blank Spike is used to demonstrate analytical accuracy of the method. It is prepared by executing all of the specified extraction and extraction purification steps except for the introduction of a sample. The LBS is spiked with the Precision and Recovery spiking solution (PAR), the Labeled Compound Spiking Solution (LCSS), the Clean-up Recovery Standard (CRS) and the Internal Standard (ISS) at the appropriate stages of the preparation. A LBS may be run with each set of 20 or fewer sample.

3.3 Ongoing Precision and Recovery (OPR)

An Ongoing Precision and Recovery sample is used to demonstrate analytical accuracy in the presence of a clean matrix. A OPR is prepared by executing all of the specified extraction and extract purification steps, using 5 mL corn oil spiked with the PAR LCSS, CRS, and ISS at the appropriate stage of preparation to replace the sample. One OPR sample must be run with each set of 20 or fewer samples when EPA Method 1613 is specifically required by the client.

3.4 Matrix Spike (MS)

A Matrix Spike is used to demonstrate analytical accuracy in the presence of a representative matrix. A MS is prepared by executing all of the specified extraction and purification steps on a selected sample. The MS is spiked with the Precision and Recovery spiking solution (PAR), the Labeled Compound Spiking Solution (LCSS), the Clean-up Recovery Standard (CRS) and the Internal Standard (ISS) at the appropriate stages of the preparation. The MS must be run with each set of 20 or

fewer samples. A Matrix Spike Duplicate (MSD) may be run with each set of 20 or fewer samples if required by the client.

3.5 Duplicate (DUP)

A sample Duplicate is used to demonstrate matrix homogeneity and analytical precision in the presence of a representative matrix. A DUP is prepared by executing all of the specified extraction and purification steps on replicate portions of selected sample. The DUP is spiked with the Labeled Compound Spiking Solution (LCSS), the Clean-up Recovery Standard (CRS) and the Internal Standard (ISS) at the appropriate stages of the preparation. A DUP must be run with each set of 20 or fewer samples.

3.6 Standard Reference Material (SRM)

Standard Reference Materials are used to demonstrate analytical accuracy on a certified reference matrix from an independent source. All of the specified extraction and purification steps are performed on the Standard Reference Material. The SRM is spiked with the Labeled Compound Spiking Solution (LCSS), the Clean-up Recovery Standard (CRS) and the Internal Standard (ISS) at the appropriate stages of the preparation. An SRM should be analyzed with each set of 20 or fewer samples, depending on availability. The SRM should represent, as closely as possible, the matrix being analyzed.

3.7 Labeled Compound Recovery

The percent recovery of the LCSS is used to monitor method performance on the sample matrix. All samples are spiked with LCSS, extracted, purified and analyzed according to this method. The QC acceptance criteria for % recovery of labeled compounds are as described in GERG's SOP for quantitative analysis of the PCDDs and PCDFs.

4.0 APPARATUS AND MATERIALS

4.1 Glassware and Hardware

The following laboratory glassware and hardware is needed to perform the tissue extraction and purification procedure:

Stainless Steel Knife or Shears: For dissecting tissue samples.

Stainless Steel Forceps

Glass Centrifuge Tubes: 200 mL capacity.

Pipettes-Disposable, Pasteur: 150 mm x 5 mm (i.d.).

Pipettes-Disposable, Serological: 10 mL x 8 mm (i.d.) for preparation of the carbon column.

Water Bath: Heated to 60-70°C

Vials and Teflon Lined Caps: 40 mL capacity.

Tissumizers: Teckmar Polytron homogenizer or equivalent.

Flat Bottom Flasks: 50, 125, 250 and 500 mL capacity.

Balance: Top loading with an accuracy of 0.001 grams.

Erlenmeyer Flask: 500 mL, with Teflon lined PVC flexible cap.

Microliter Syringes, Micro-pettor pipettes and Disposable micro-capillary pipettes: 1000, 250, 100, 50, 25, 20, 10, 5, 4, 3, 2 and 1 mL capacity.

Microreaction Vessels: 1.0, 2.0 and 3.0 mL capacity vials with screw cap and Teflon lined septa.

Chromatography Columns: 30 cm x 13 mm (i.d.) with 250 mL reservoir and Teflon stopcock.

Graduated Cylinders: 10, 25, 50, 100 and 4000 mL capacity.

Magnetic Stirring Plate: Nine-Place, independent stirring speeds for all vessels.

Nitrogen blowdown apparatus: Dry heat source and filtered nitrogen stream.

Rotary-evaporator: Buchi, model R144 or equivalent, equipped for automatic distillation and a low temperature water recirculator to assure an adequate recovery of the solvents being used.

Rotary-evaporator trap: 100 mL.

Desiccator: Stainless steel construction, no plastic components.

NOTE: No grease, oil, or any other lubricants are used on the desiccator or the rotovap junctions to avoid contamination.

4.2 Instrumentation

Not applicable.

5.0 REAGENTS AND CONSUMABLE MATERIALS

5.1 Reagents

5.1.1 **Purified Water**: HPLC Grade or better.

5.1.2 **1N Sodium Hydroxide Solution**: ACS reagent grade sodium hydroxide from J.T. Baker, Cat. # 3728-01 or equivalent; prepare a 1 N solution in purified water.

5.1.3 **Concentrated Sulfuric Acid**: J.T. Baker; Cat. # JT9673-00 or equivalent; 95.0-98.0%; suitable for trace metal analysis.

5.1.4 **Solvents**: Equivalent solvents from other source may be used after lot testing.

Methylene Chloride: Burdick and Jackson; Cat. # 300-4; Grade: High Purity
Hexane: Burdick and Jackson; Cat. # GC60393-4; Grade: Capillary GC/GC-MS Solvent
Acetone: Burdick and Jackson; Cat. # 010-4; Grade: High Purity Solvent
Toluene: Burdick and Jackson; Cat. # 347-4; Grade: High Purity Solvent

Methanol: Burdick and Jackson; Cat. # 230-4; Grade: High Purity Solvent
Tetradecane: Fluca Chemical Co.; Cat. # 87140; Highest Grade Available
Cyclohexane: Burdick and Jackson; Cat. # 053-4; Grade: High Purity Solvent

5.1.5 **Prepurified Nitrogen Gas**: Nitrogen gas used in final evaporation is purified through an activated carbon trap. No rubber or plastic is used in the nitrogen delivery system.

5.1.6 **Drierite**: W.A. Hammond; Stock #23005 or equivalent; desiccating 8 mesh, indicating.

5.1.7 **Anhydrous Sodium Sulfate:** J.T. Baker; Cat. #3891-05 or equivalent; Reagent Grade: Combusted at 400°C for 4 hours and stored covered with aluminum foil at 130°C.

5.1.8 **Silica Gel for Column Chromatography**: E.M. Science; Cat. #7734-5 or equivalent; Silica Gel 60, 70-230 mesh. Activated for >16 hours at 170°C. Stored covered with aluminum foil at 170°C.

5.1.9 **Basic Alumina**: E.M. Science; Cat. #AX0612-3 or equivalent; Aluminum Oxide, basic, chromatographic grade, 80-200 mesh, Alcoa Type GC-20. Combusted at 600°C for >16 hours. Stored covered with aluminum foil at 130°C. Use within 3 days, then reactivate or discard after that.

NOTE: Recoveries of the targeted analytes are strongly influenced by the degree of activation of the alumina. Extreme caution must be taken to avoid usage of the adsorbent after 3 days from the original activation.

5.1.10 **Activated Carbon**: AX-21 Carbon (Anderson Development Co.): Wash 100 grams of AX-21 carbon powder (as received) by suspending in 300 mL methanol and subsequently vacuum filtering through a pre-cleaned glass fiber filter fitted in a 350 mL Buchner funnel Rinse two times with 100 mL

methanol and vacuum dry. Keep the washed AX-21 carbon at 130°C for a minimum of 72 hours. Then store in the same oven covered with aluminum foil at 130°C.

5.1.11 **AX-21 Carbon/Silica Gel**: Combine 5 grams of prepared AX-21 Carbon with 95 g of prepared Silica Gel in a 500 mL Erlenmeyer with a Teflon lined PVC flexible screw cap (do not put the cap into the oven). Blend by shaking until a uniform color is achieved. Activate the mixture at 130°C for a minimum of 24 hours and store covered with aluminum foil in the same oven at 130°C.

5.1.12 **H_2SO_4/Silica Gel**: Prepare by mixing 100 grams of concentrated H_2SO_4 with 150 grams of activated silica gel. Shake and roll on a roller table for a minimum 2 hours. Store in 500 mL Erlenmeyer flask with Teflon lined PVC flexible screw cap at room temperature.

5.1.13 **NaOH/Silica Gel**: Prepare by mixing 33 mL of 1 N NaOH solution and 67 grams of activated silica gel. Stir and shake by hand until free flowing. Store in 500 mL Erlenmeyer flask with Teflon lined PVC flexible screw cap at room temperature.

5.2 Analytical standards

Analytical Standards are purchased as solution with certification of their purity, concentration and authenticity . The Labeled Compound Spiked Solution (LCSS), the Clean-up Recovery Standard (CRS), the Precision and Recovery Standard (PAR) and the Internal Standard (ISS) are used as received from the manufacturer without further treatment. When not being used, standards are stored in the dark at 4° ± 2°C in amber glass screw-capped vials with PTFE-lined caps.

5.2.1 Labeled Compound Spiking Solution (LCSS)

This solution contains the fifteen $^{13}C_{12}$-labeled PCDD and PCDF quantitation standards in nonane at the nominal concentrations listed in Table 1. Twenty (20) µL of the LCSS are diluted in 1 mL of acetone and spiked into each tissue sample prior to extraction.

5.2.2 Cleanup Recovery Standard (CRS)

This solution contains the cleanup recovery standard, $^{37}Cl_4$-2,3,7,8-TCDD, in nonane at the nominal concentration listed in Table 2. Five (5) µL of this solution are spiked into each sample extract immediately after the extraction and before any cleanup procedures are started.

5.2.3 Precision and Recovery Standard (PAR)

This solution contains the seventeen 2,3,7,8-substituted native PCDD and PCDF isomers at the nominal concentrations listed on Table 3. Five (5) µL of the PAR solution are diluted in 1 mL of acetone and spiked into the selected Laboratory Blank Spike (LBS), Matrix Spike (MS) and Matrix Spike Duplicate (MSD), if required, prior to extraction.

5.2.4 Internal Standard (ISS)

This solution contains two $^{13}C_{12}$-labeled PCDD isomers at the nominal concentrations listed in Table 4. Ten (10) µL of the ISS are added to the final sample extract before HRGC/HRMS analyses to determine the percent recoveries for the $^{13}C_{12}$-labeled compounds.

5.3 Standard Reference Materials

5.3.1 CARP-1

CARP-1 is a ground whole carp reference material for organochlorine compounds which contains nine of the seventeen target analytes at certified concentrations. This SRM is available from the National Research Council of Canada.

5.3.2 EDF-2524

EDF 2524 is a clean, natural matrix reference material gathered in clean waters with a history of sustaining relatively untainted fish. One analyte, 2,3,7,8-TCDF is present at a certified concentration. This SRM is available from Cambridge Isotope Laboratories.

5.3.3 EDF-2525

EDF-2525 is a contaminated natural matrix reference material gathered from a polluted Great Lakes region which contains selected target analytes at certified concentrations within the calibration range for this method. This SRM is available from Cambridge Isotope Laboratories.

5.3.4 EDF-2526

EDF-2526 is a fortified natural matrix reference material which contains all seventeen target analytes at certified concentrations within the calibration range for this method. This SRM is available from Cambridge Isotope Laboratories.

5.4 Miscellaneous Materials.

Boiling Chips: Teflon, solvent extracted with methylene chloride prior to use.

Glass Wool: Combusted at 400°C for at least 4 hours.

Stirring Bars: Teflon coated.

Glass Fiber Filter Paper: Gelman Type A/E or equivalent, Whatmann GF/F or equivalent

6.0 EXTRACTION AND CLEANUP PROCEDURES

6.1 Sample Preparation

While still partially frozen, rinse the tissue with reagent water if necessary to remove extraneous material. If required, 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 an aliquot of the macerated tissue to be extracted into a centrifuge tube. The preferred tissue sample size is 10 grams (wet weight), although smaller amounts may be used depending on sample availability.

NOTES: 1. Depending on sample availability, the minimum sample weight to be extracted may be modified by the HRMS analyst or supervisor.

2. All glassware is rinsed with methanol, acetone, methylene chloride, toluene, and hexane prior to use in this extraction procedure.

6.2 Percent Dry Weight Determination

6.2.1 Remove a separate 0.5-1 gram aliquot of the homogenized tissue composite. Place it in a tared weighing container, and weigh. Dry the tissue for at least 24 hours at 65°C, allow it to cool in a desiccator, and then re-weigh. Record the weight.

6.2.2 Reheat the sample to 65°C for at least 2 hours, again cool in a desiccator for 30 minutes to room temperature, and weigh. Record the weight.

6.2.3 If the difference between the first and second weight of the dried sample is less than 0.02 grams, calculate the % dry weight according to GERG's SOP for % dry weight determination, using the second weight. If the difference is greater than 0.02 grams, continue the heating, cooling and weighing process until the difference in the last two weights is less than 0.02 grams, and calculate the % dry weight based on the last weight.

6.3 Extraction Procedure (Tissumizing)

6.3.1 The tissumizer probe is washed with Micro, rinsed with tap water, and then rinsed with methanol, acetone, methylene chloride, toluene, and hexane prior to use.

6.3.2 The appropriate amount of tissue is weighed out in a solvent rinsed, labeled 200 mL centrifuge bottle.

6.3.3 Add 20 µL of dioxin LCSS to all the samples. The LCSS is first added to 1 mL acetone in a test tube. This acetone solution is then quantitatively transferred to the appropriate sample or blank. The test tube is rinsed three times with acetone, and the rinse solutions are also transferred to the corresponding sample.

6.3.3.1 Add the PAR standard to the LCSS/acetone mixture which will be used for the LBS, MS, and MSD as required. This acetone solution is then quantitatively transferred to the appropriate sample or blank. The test tube is rinsed three times with acetone, and the rinse solutions are also transferred to the corresponding sample.

6.3.4 Add 100 mL of methylene chloride to each sample

6.3.5 Add 40 mL of combusted, room temperature sodium sulfate to each sample immediately before tissumizing.

6.3.6 Macerate the tissue with tissumizer for three minutes.

6.3.7 Place a large funnel on top of a labeled 500 mL flat bottom flask, plug the stem with glass wool and add about 2 inches of room temperature sodium sulfate. Wet the sodium sulfate with methylene chloride.

6.3.8 Decant methylene chloride extract into the funnel in the labeled 500 mL flat bottom flask.

6.3.9 Add another 100 mL methylene chloride solution to the centrifuge bottle and repeat steps 6.3.6-6.3.9 two more times (a total of three methylene chloride extractions).

6.4 Addition of Cleanup Recovery Standard (CRS)

6.4.1 Add 5 µL CRS to each extract before proceeding to next step.

6.5 Determination of % Lipids

6.5.1 After the sample has been completely extracted and filtered into the 500 mL flask, draw a line to mark the volume of the extract. This volume is later determined for use in the calculation of the % lipid content.

6.5.2 Measure out approximately 20 mL of extract into a 25 mL graduated cylinder. Record the actual volume removed for lipid determination in the Lipid Logbook.

6.5.3 Place a funnel with glass filter paper on top of a labeled 125 mL flat bottom flask.

6.5.4 Add combusted, room temperature sodium sulfate to the funnel and pour the 20 mL of extract into it.

6.5.5 Rinse the graduated cylinder three times with methylene chloride and pour the rinsate into the funnel.

6.5.6 Put a ground glass stopper on the 125 mL flask.

6.5.7 Refer to GERG's SOP to complete the % lipid determination procedure. Determine the RPD and verify that it meets QC acceptance criteria.

6.6 **Solvent Exchange to Hexane.**

6.6.1 Add 5-8 pieces of solvent cleaned Teflon boiling chips to the remaining sample extract in the 500 mL flask.

Note: Teflon boiling chips are cleaned by rinsing repeatedly with methanol and methylene chloride (6-7 times) and are stored into a closed container before use.

6.6.2 Place a three ball Snyder column on top of the 500 mL flask.

6.6.3 Place the flask on a hot water bath (60-70°C) and concentrate down to about 10 mL.

6.6.4 Take the flask off the water bath, cool, and rinse down the Snyder column with hexane.

6.6.5 Add 120 mL of hexane with a graduated cylinder and place the flask back on the bath.

6.6.6 Let the extract boil until the top of the Snyder column becomes opaque, indicating that the methylene chloride is gone.

6.6.7 After the methylene chloride is completely boiled off, take the flask off the water bath, cool, and rinse down the Snyder column with hexane.

6.6.8 Remove the Snyder column and cap the sample flask with a glass stopper.

6.7 Sulfuric Acid (H_2SO_4)/Silica Gel Slurry

6.7.1 Preparation of the H_2SO_4/Silica Gel Slurry (see Section 5.1.12)

NOTE: All Silica Gel used in this SOP is placed in a oven at 170°C for a minimum of 16 hours and cooled to room temperature before use.

6.7.1.1 Weigh out 150 grams of Silica Gel in a 500 mL Erlenmeyer flask.

6.7.1.2 Repeat 6.7.1.1 with a second 500 mL Erlenmeyer flask.

6.7.1.3 Add 100 grams of concentrated sulfuric acid to the silica gel in each flask.

6.7.1.4 Cap the flasks with Teflon lined PVC screwcaps.

6.7.1.5 Tape the two flasks together at the caps with Teflon tape and mix by rolling on a roller table for a minimum of two hours.

6.7.2 Place the labeled flasks containing the hexane extracts on a magnetic stirring plate.

6.7.3 Add a Teflon coated magnetic stirring bar to each flask.

6.7.3.1 The magnetic stirring bars are rinsed with these solvents in this order: methanol, acetone, methylene chloride, toluene, and hexane.

6.7.3.2 Rinse the metal pair of a forceps with hexane.

6.7.3.3 Use the forceps to place the stirring bar in the sample, rinsing the forceps with hexane between picking up each stirring bar.

6.7.4 Turn the magnetic stirring plate on and set the rpms at a medium setting, making sure the sample is not splashing against the flask.

6.7.5 Using a 50 mL beaker, add approximately 40 grams of the H_2SO_4/silica gel to each flask.

6.7.6 Stir the samples for a minimum of 2 hours.

6.7.7 Filter the samples.

6.7.7.1 A large funnel is plugged with combusted glass wool.

6.7.7.2 Combusted, room temperature sodium sulfate is added to the funnel so that the glass wool is covered by the sodium sulfate.

6.7.7.3 Place the funnel on a labeled 250 mL flat bottom flask.

6.7.7.4 Pour the entire contents of the 500 mL flask into the funnel and rinse repeatedly with hexane until there is no more material in the original 500 mL flask.

6.7.7.5 After the liquid completely drains through the funnel, rinse the sodium sulfate in the funnel three times with 5 mL of hexane.

6.7.7.6 Discard the funnel contents, retaining the Teflon coated stirring bar, and cap the 250 mL sample flask with a glass stopper.

6.8 Rotovapping

6.8.1 Add 50 µL of tetradecane (n-C_{14}) to each sample extract using a 50 µL micropipette

6.8.1.1 Before using the micropipette, it must be rinsed with hexane about 10-20 times.

6.8.2 Set the rotovap to 250 mbars for evaporating hexane.

6.8.3 Rinse the rotovap trap with methanol, acetone, methylene chloride, toluene, and hexane before attaching the sample flask to it. Start the rotovap after the sample flask has been attached.

6.8.4 The samples are rotovapped to 50 µL of tetradecane (n-C_{14}).

6.9 Mixed Bed Silica Columns

6.9.1 Place a 300 mm x 13 mm (i.d.) column with a 250 mL reservoir upside down and rinse with these solvents in this order: methanol, acetone, methylene chloride, toluene, and hexane. Make sure that the solvents completely coat and rinse the inside the columns.

6.9.2 Turn the column right-side-up and pack it with the following (from bottom to top):

6.9.2.1 Combusted glass wool plug

6.9.2.2 1 scoop of combusted sand (about 1 cm)

6.9.2.3 1 gram activated, room temperature silica gel

6.9.2.4 4 grams NaOH/Silica Gel prepared as described in the following subsections (see Section 5.1.13).

6.9.2.4.1 NaOH/Silica Gel: Weigh out 67 grams of activated, room temperature silica gel in a 500 mL Elynmeyer flask.

6.9.2.4.2 Measure out 33 mL of 1 N NaOH in a graduated cylinder.

6.9.2.4.3 Pour the NaOH into the flask and cap with a Teflon lined PVC cap.

6.9.2.4.4 Shake the mixture until there are no more lumps

6.9.2.5 1 gram activated, room temperature silica gel

6.9.2.6 8 grams H_2SO_4/Silica Gel acid slurry

6.9.2.7 2 grams activated room temperature silica gel

6.9.2.8 1 scoop combusted, room temperature sodium sulfate (about 1 cm)

Outline of Column Packing, Section 6.9

(TOP)	1 cm	Na_2SO_4
	2 grams	Silica
	8 grams	40% H_2SO_4/Silica Gel
	1 grams	Silica
	4 grams	33% 1 N NaOH/Silica Gel
	1 grams	Silica
	1 cm	Quartz sand
(BOTTOM)		Glass wool plug

6.9.3 Place a waste jar under the completed column and add 25 mL hexane as a pre-rinse. The stopcock should be open.

6.9.4 Close the stopcock when the level of hexane is 1 cm above the sodium sulfate.

6.9.5 Rinse the bottom tip of the column with hexane.

6.9.6 Replace the waste jar with a properly labeled 250 mL flat bottom flask.

6.9.7 With the stopcock open, add sample to the column using a clean, combusted disposable pipette plugged with glass wool.

6.9.8 Rinse the 250 mL flask three times with 2 mL hexane and add the rinsate to the column using the same pipette for sample transfer and rinsates.

6.9.9 Let the solvent drain to the sodium sulfate level.

6.9.10 Add 120 mL hexane to the column with the stopcock open so that 1-2 drops per second occur.

6.9.11 Cover the column with a piece of aluminum foil.

6.9.12 After the hexane has completely passed through the column, add 50 µL tetradecane (n-C_{14}) to the collection flask. Rotovap to 50 µL tetradecane (n-C_{14}).

6.10 Basic Alumina Column

6.10.1 Place a 250 mm x 13 mm (i.d.) column with a 200 mL reservoir upside down and rinse with the following solvents: methanol, acetone, methylene chloride, toluene, and hexane. Make sure the solvents completely coat and rinse the inside of the column.

6.10.2 Turn the column right-side-up and pack it with the following (from bottom to top):

6.10.2.1 Combusted glass wool

6.10.2.2 One (1) scoop combusted sand (about 1 cm)

6.10.2.3 Six (6) grams activated, room temperature alumina prepared as described in the following sub-sections.

6.10.2.3.1 Alumina is placed into a 400 mL beaker with a ceramic evaporating dish as a lid.

6.10.2.3.2 Combust at 600°C for 16 hours, store covered at 130°C.

6.10.2.3.3 Let the alumina cool to room temperature before use.

NOTE: Alumina can only be used for three days after combustion. After that, it must be discarded.

6.10.2.4 1 scoop combusted sodium sulfate (about 1 cm)

Outline of Basic Alumina Column, Section 6.10

(TOP)	1 cm	Na_2SO_4
	6.0 grams	Basic Alumina
	1 cm	Quartz sand
(BOTTOM)		Glass wool plug

6.10.3 Place a waste jar under the column and add 25 mL hexane to rinse. The column stopcock should be open.

6.10.4 Close the stopcock when the level of the solvent is 1 cm above the sodium sulfate.

6.10.5 Rinse the bottom tip of the column with hexane.

6.10.6 With the stopcock closed, add sample extract to the column using a combusted disposable pipette with a glass wool plug.

6.10.7 Rinse the flask three times with 2 mL hexane and add the rinsate to the column using the same disposable pipette for the sample and rinsate transfer.

6.10.8 Drain the solvent to 1 cm above the surface of the sodium sulfate. Add 60 mL of hexane to the reservoir, drain the sodium sulfate level and close the stopcock.

6.10.9 Replace the waste jar with a labeled 125 mL flat bottom flask.

6.10.10 Add 40 mL of hexane/methylene chloride (70:30) to the top of the column.

6.10.12.1 The 70:30 solvent is made by adding 300 mL methylene chloride to 700 mL hexane in a 1 liter graduated cylinder.

6.10.12.2 The 70:30 solution is transferred to a 1000 mL flat bottom flask and mixed thoroughly before each use.

NOTE: The solvent mixture is only good for one day and should be remade for each set of samples. To minimize waste, the quantity prepared should reflect the number of samples using the correct proportions noted.

6.10.13 Adjust the stopcock flow to one drip per second and cap the column with a piece of combusted, solvent rinsed aluminum foil.

6.10.14 After the 70:30 solvent completely drains to the sodium sulfate level, add 50 µL tetredcane (n-C_{14}) to the labeled 125 mL sample flask. Rotovap the solution to the 50 µL tetradecane (n-C_{14}).

6.11 Charcoal Column

6.11.1 Pre-clean the AX-21 carbon.

6.11.1.1 Place a glass fiber filter paper in a 350 mL Buchner funnel.

6.11.1.2 Pour 100 g of AX-21 carbon power on top of the filter paper in the Buchner funnel.

6.11.1.3 Add 300 mL methanol to the carbon and vacuum filter the carbon/methanol mix.

6.11.1.4 Repeat the process two more times with methanol, then use vacuum to dry the carbon for 10 seconds.

6.11.1.5 Put the washed carbon in a oven at 130°C for a minimum of 72 hours before use.

6.11.2 Make 5% AX-21 carbon/silica gel mixture for the columns

6.11.2.1 Weigh out 95 grams of activated, room temperature silica gel in a 500 mL Erlynmeyer flask

6.11.2.2 Add 5 grams of washed, cooled AX-21 carbon to the flask.

6.11.2.3 Cap the flask with a piece of aluminum foil.

6.11.2.4 Shake the mixture by hand until a uniform black color is achieved.

6.11.3 Cut and combust the columns for charcoal.

6.11.3.1 Charcoal columns are made by cutting off both ends of a clean disposable serological pipette.

6.11.3.2 The pipettes are placed in a 4 L beaker and combusted for 4 hours at 440°C.

6.11.4 Plug the column with combusted glass wool approximate 1 1/2 inches from the end.

6.11.5 Place the column on the rack with a binder clip holding it in place. The end with the glass wool should be facing down.

6.11.6 With a small funnel and a spatula, add 1 cm of activated, room temperature silica gel to the top of the glass wool plug.

6.11.7 Add 1 gram of 5% AX-21 carbon/silica gel, being careful not to shake or pack the column.

6.11.8 Plug the open end with combusted glass wool. The glass wool should be touching the charcoal, but should not be forced down and pack the column.

Outline of Charcoal Column Section 6.11

(TOP)		Glass wool plug
	1.0 grams	5% AX-21/silica gel
	1 cm	Activated silica gel
(BOTTOM)		Glass wool plug

6.11.9 Add 22 mL of 50:50 methylene chloride/cyclohexane to a labeled 25 mL concentrator tube.

6.11.9.1 The 50:50 solvent is made by adding equal volume of methylene chloride and cyclohexane e.g., 500 mL of methylene chloride to 500 mL cyclohexane.

NOTE: The 50:50 solvent should be only used for one day and the remaining solvent should be discarded. The solution is remade for each new set of samples.

6.11.10 Place a combusted, disposable pasteur pipette into each labeled concentrator tube.

NOTE: Each sample should have its own concentrator tube and pipette so no cross contamination will occur. The concentrator tube should be labeled with the sample number. To minimize waste, the quantity prepared should reflect the number of samples, using the correct proportions noted.

6.11.11 Place a waste jar under the column.

6.11.12 Pre-rinse the column with 5 mL of 50:50 solvent.

6.11.13 Let the solvent drain to the glass wool level, flip the column over and rinse with another 5 mL of 50:50 solvent, collecting the solvent in the waste jar.

6.11.14 Let the solvent drain to the glass wool level. Using the combusted, disposable pipette with a glass wool plug, add concentrated sample extract from the 125 mL flask (Section 6.10) to the top of the column.

6.11.15 Rinse the flask with 2 mL of 50:50 solvent three times, using the same pipette for the removal of the sample and its rinsate, and add the rinsate to the column.

6.11.16 After the solvent and sample drain to the glass wool level, add the remaining 50:50 solvent to the column.

6.11.17 Add 20 mL of 75:20:5 solvent mixture to the concentrator tubes.

6.11.17.1 75:20:5 is made by mixing 750 mL methylene chloride with 200 mL of methanol and 50 mL of toluene in a 1000 mL graduated cylinder.

NOTE: This mixture is only good for one day and the remaining should be discarded after use. To minimize waste, the quantity prepared should reflect the number of samples using the correct proportions noted.

6.11.18 Pipette the 20 mL of 75:20:5 to the charcoal column and drain into the waste jar.

6.11.19 When the solvent completely drains down to the glass wool level and stops dripping, flip the column. Place a labeled 125

mL flat bottom flask in place of the waste jar. The long side (with silica gel on bottom) of the charcoal column should face upward.

6.11.20 Fill the concentrator tubes with 25 mL of toluene and pipette it into the column.

6.11.21 When the 25 mL is has been removed from the concentrator tube, add another 25 mL of toluene to the concentrator tube and pipette it into the column.

6.11.22 After the toluene completely drains into the 125 mL flask, add 20 µL tetradecane (n-C14) to the sample extract and rotovap to the 20 µL tetradecane (n-C14). The rotovap should be set to 80 mbars for evaporating toluene.

6.12 Final Evaporation

6.12.1 Prepare 2 mL tear-drop vials by labeling them with the sample numbers.

6.12.2 Add 20 µL of tetradecane (n-C14) to each vial.

6.12 3 Transfer the samples from the 125 mL flask to the vials using a disposable, combusted pasteur pipette with a glass wool plug. Fill the vials to 3/4 full of sample.

6.12.4 Place new combusted disposable glass pasteur pipettes in the nitrogen blowdown apparatus. These pipettes should have no glass wool in them.

6.12.5 Insert the Teflon nitrogen hoses into the pipettes.

6.12.6 Place the 2 mL tear-drop vials containing the sample on the nitrogen blowdown apparatus and turn on the heat.

6.12.7 Insert the pipette into the vials, making sure the tip of the pipette does not touch the sample.

6.12.8 With the individual nitrogen stream controls in the back of the hood completely off, turn on the main nitrogen line (to the left of the hood) up to 20 psi.

6.12.9 Slowly adjust the individual controls up until a slight movement of the sample surface is seen.

NOTE: Each individual control valve controls six nitrogen hoses. These should be turned up slowly so that the stream does not force sample out of the vials. When turning on one controller, other two controller are affected and must be adjusted. The best way to avoid contamination is by having all hoses open and inserting them one-by-one in the pipette to maintain total control of the flow on the samples. Do not turn off the individual controllers until the main controller is off.

6.12.10 As the volume of the vial is reduced, add rinsate from the 125 mL flask to the tear-drop vial.

6.12.10.1 Rinse each 125 mL flask three times with about 1 mL of toluene and add the rinsate to the tear-drop vial.

6.12.11 Blow down the sample to 20 µL tetradecane. Take the vials off and place them on a vial tray, leaving the vials uncapped.

6.12.12 Add 10 µL of the Internal standards (ISS) to each sample using a separate disposable micropipette for each sample.

6.12.13 Place the vials back on the blowdown apparatus making sure they are in the same position as before.

6.12.14 Blow the sample down to the 20 µL tetradecane (n-C_{14}) and take them off the blowdown apparatus.

6.12.15 Cap the sample and make a final label for each with its sample number on it.

7.0 DOCUMENTATION REQUIREMENTS

Copies of following documents must accompany the sample set in a labeled folder before it can be delivered for HRCG/HRMS analysis:

Chain of Custody documents

Sample Information Sheet

Analysis Request Form

Laboratory Bench Sheet

Sample dry weight and lipid bench-sheets

Other miscellaneous information.

8.0 ANALYTICAL PROCEDURE

All sample extracts are then analyzed by using HRGC/HRMS according to the procedures described in the appropriate GERG SOP.

Table 1. Composition of the Labeled Compound Spiking Solution (LCSS).

Analyte	Concentration (pg/µL)
$^{13}C_{12}$ - 2,3,7,8- TCDD	100
$^{13}C_{12}$ -2,3,7,8- TCDF	100
$^{13}C_{12}$ -1,2,3,7,8- PeCDD	100
$^{13}C_{12}$ -1,2,3,7,8- PeCDF	100
$^{13}C_{12}$ -2,3,4,7,8- PeCDF	100
$^{13}C_{12}$ -1,2,3,4,7,8- HxCDD	100
$^{13}C_{12}$ -1,2,3,6,7,8- HxCDD	100
$^{13}C_{12}$ -1,2,3,4,7,8- HxCDF	100
$^{13}C_{12}$ -1,2,3,6,7,8- HxCDF	100
$^{13}C_{12}$ -1,2,3,7,8,9- HxCDF	100
$^{13}C_{12}$ -2,3,4,6,7,8- HxCDF	100
$^{13}C_{12}$ -1,2,3,4,6,7,8-HpCDD	100
$^{13}C_{12}$ -1,2,3,4,6,7,8-HpCDF	100
$^{13}C_{12}$ -1,2,3,4,7,8,9-HpCDF	100
$^{13}C_{12}$ -OCDD	200

Solvent: Nonane

Table 2. Composition of the Clean-up Recovery Standard (CRS).

Analyte	Concentration (pg/µL)
$^{37}Cl_4$ -2,3,7,8- TCDD	40

Solvent: nonane

Table 3. Composition of the Precision and Recovery Standard (PAR)

Analyte	Concentration (pg/µL)
2,3,7,8- TCDD	40
2,3,7,8- TCDF	40
1,2,3,7,8- PeCDD	200
1,2,3,7,8- PeCDF	200
2,3,4,7,8- PeCDF	200
1,2,3,4,7,8- HxCDD	200
1,2,3,6,7,8- HxCDD	200
1,2,3,7,8,9- HxCDD	200
1,2,3,4,7,8- HxCDF	200
1,2,3,6,7,8- HxCDF	200
1,2,3,7,8,9- HxCDF	200
2,3,4,6,7,8- HxCDF	200
1,2,3,4,6,7,8-HpCDD	200
1,2,3,4,6,7,8-HpCDF	200
1,2,3,4,7,8,9-HpCDF	200
OCDD	400
OCDF	400

Solvent: nonane.

Table 4. Composition of the Internal Standard (ISS)

Analyte	Concentration (pg/µL)
$^{13}C_{12}$ -1,2,3,4- TCDD	200
$^{13}C_{12}$ -1,2,3,7,8,9- HxCDD	200

Solvent: nonane

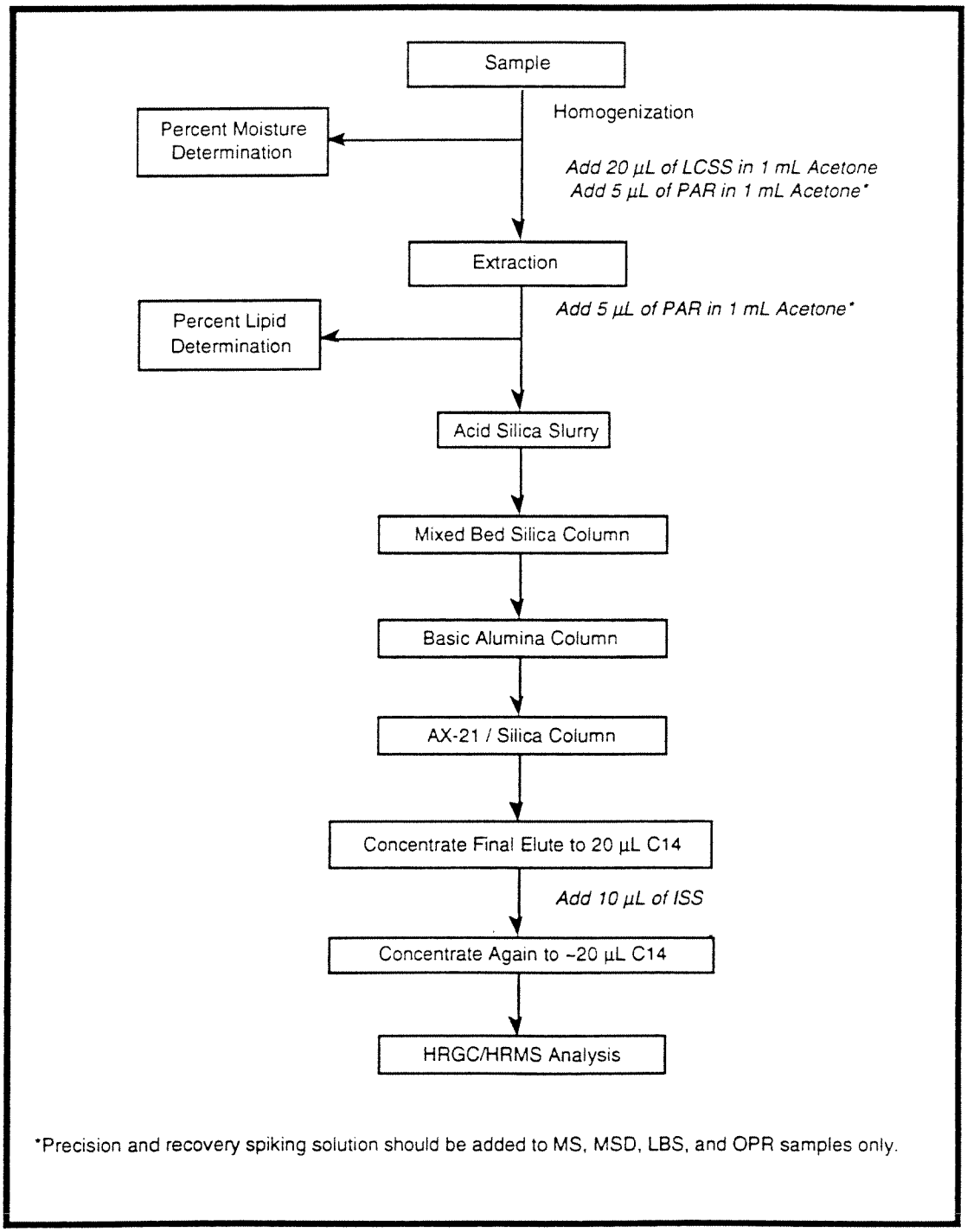

Figure 1. Flow Chart for Sample Processing

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SOP-9720
SILICA/ALUMINA COLUMN CHROMATOGRAPHY FOR THE PURIFICATION OF EXTRACTS FROM TISSUE, SEDIMENTS, AND SOIL SAMPLES PRIOR TO THE ANALYSIS OF ALIPHATICS AND POLYNUCLEAR AROMATIC HYDROCARBONS, POLYCHLORINATED BIPHENYLS, AND ORGANOCHLORINE PESTICIDES

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURE SOP-9720

SILICA/ALUMINA COLUMN CHROMATOGRAPHY FOR THE PURIFICATION OF EXTRACTS FROM TISSUE, SEDIMENTS, AND SOIL SAMPLES PRIOR TO THE ANALYSIS OF ALIPHATICS AND POLYNUCLEAR AROMATIC HYDROCARBONS, POLYCHLORINATED BIPHENYLS, AND ORGANOCHLORINE PESTICIDES

This document presents the procedures, materials, and quality control used in the performance of the above preparation activities.

Susan E Ekman
Quality Assurance Manager

5/2/97
Date

Author/Revision By: Y. Qian

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURE SOP-9720

SILICA/ALUMINA COLUMN CHROMATOGRAPHY FOR THE PURIFICATION OF EXTRACTS FROM TISSUE, SEDIMENTS, AND SOIL SAMPLES PRIOR TO THE ANALYSIS OF ALIPHATICS AND POLYNUCLEAR AROMATIC HYDROCARBONS, POLYCHLORINATED BIPHENYLS, AND ORGANOCHLORINE PESTICIDES

1.0 PURPOSE

This document provides the procedures for extract purification prior to analysis of aliphatic (AL) and aromatic (AR) hydrocarbons, polynuclear aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), and organochlorine pesticides (OC) which are used by staff of the Geochemical and Environmental Research Group (GERG) of the College of Geosciences and Marine Studies at Texas A&M University.

1.1 Summary

The extracts of biological tissue, sediments, and other type of samples obtained by organic solvent extraction may contain interferent materials. These non-desirable compounds in the extract may prevent accurate identification and quantification of the target compounds in a sample. Silica/alumina column chromatography is used to eliminate the interferent materials and allows quantitative recovery of the target compounds.

The extract purification steps using silica/alumina column chromatography described in this standard operating procedure (SOP) are used to isolate the aliphatic fraction (SA1) or aromatics and pesticides fraction (SA2) from the concentrated sample extract. The fraction containing the desired target compounds is collected and concentrated prior to analysis. Further purification by gel permeation chromatography (GPC) may be required, depending on the sample matrix type and analyte type.

1.2 Application

All tissue extracts require this silica/alumina column chromatography purification procedure prior to the analysis of PAHs and/or polychlorinated biphenyls (PCB). In addition, these procedures are required for biological tissue extracts when analysis of aliphatic compounds (AL) and aromatic compounds (AR) has been requested.

Sediment and soil extracts from samples specified for the analyses of OC and/or PCB also require purification using silica/alumina chromatography. Extracts from other samples matrices may use this purification procedure after appropriate method validation.

Note: Purification of extracts from sediment and soil specified for the analysis of only AL and/or AR usually follow the procedures outlined in the GERG SOP for use of the alumina column chromatography.

2.0 SAFETY

The hazards, toxicity or carcinogenicity of each compound or reagent used in GERG's standard operating procedures have not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory maintains Material Safety Data Sheets (MSDS) which contain information regarding the safe handling of chemicals used at GERG's facilities. A reference file of MSDS is available to all personnel involved with these materials. All laboratory personnel should direct any questions regarding safety issues to their supervisors or the Safety Officer.

3.0 QUALITY CONTROL

3.1 Process QC extracts (blank, Matrix Spike, Duplicate, etc.) in an identical manner as the other extracts.

3.2 Note in the Comments section of the Laboratory Sample Logbook if anything out of the ordinary occurred to the extract or if there are unusual extract characteristics (cloudy, precipitate present, etc.).

3.3 Use Standard Laboratory Practice when filling out all paperwork. Use black waterproof ink. When correcting an entry, use one single line through the incorrect entry, date and initial change. Write a large Z through all the empty sample lines, date and initial.

3.4 Observe good, clean, and safe laboratory practice.

4.0 APPARATUS AND MATERIALS

4.1 Glassware and Hardware

The following laboratory glassware and hardware are needed to perform the sample cleanup using silica/alumina column chromatography. All glassware is pre-cleaned according to the GERG SOP.

Balance: Top loading with an accuracy of 0.001 gram

Beakers: 40 mL and 300 mL capacity, borosilicate glass

Chromatographic Columns: 30 cm x 13 mm (i.d.) with 250 mL reservoir and Teflon stopcock.

Desiccator

Flat Bottom Flask: 250 mL, combusted at 440°C for 4 hours

Funnels: Borosilicate glass, assorted sizes

Glass rod: 40 cm x 0.7 cm

Pyrex Glass Wool: Combusted at 440°C for 4 hours.

Graduated Cylinders: 250 mL and 2000 mL capacity.

Muffle Furnace: Programmable temperature up to 900°C

Oven: Temperature programmable up to 200°C

Pasteur pipettes: 1 mL, disposable, combusted at 440°C for 4 hours

Rolling flask: 1000 mL capacity with interior studs to facilitate mixing

Roller-Table: Lortone, Inc., Model NF-1 or equivalent

Spatula: Stainless steel with a scoop at one end

Stoppers: Ground glass, 24/40 and 19/22, borosilicate glass

Forceps: Stainless steel

4.2 Reagents and Consumable Materials

Acid: Hydrochloric acid; EM science; Cat. #HX0603-3; reagent grade; concentrated 12N.

Anhydrous Sodium Sulfate: J.T. Baker; Cat. #3891-05; reagent grade or equivalent; granular powder. Combusted at 440°C for 4 hours and stored at 130°C

Alumina Oxide (alumina): Aldrich; Cat. #19944-3; Basic Brockmann I; chromatographic grade, ~150 mesh or equivalent; combusted at 440°C for 4 hours and stored at 130°C

Chromatographic Silica: Aldrich; Cat. # 21,447-7 or equivalent; Silica gel, grade 923, 100-200 mesh; activated at 170°C for at least 24 hours and stored at 170°C

Copper: 20-30 mesh; granular; J.T. Baker; Cat. # 1720-05.

Purified water: HPLC grade or equivalent; Mallinkrodt; Cat. #6795.

Sand: White quartz, -50 to +70 mesh; Sigma, Cat. #S-9887; combusted at 440°C for 4 hours.

Silica Gel: Desiccating, indicating 16-18 mesh; J.T. Baker; Cat. #3401-05.

Solvents:

Methylene chloride; Baxter; Cat. #300-4; pesticide grade or equivalent.
Methanol; Baxter; Cat. #230-4; pesticide grade or equivalent.
Pentane; Baxter; Cat. #312-4; pesticide grade or equivalent.
Hexane; Baxter; Cat. GC60393-4; GC/GC-MS grade or equivalent.

5.0 PROCEDURE FOR PREPARING COLUMNS

5.1 Documentation Required

Obtain a copy of the Organic Analysis Request Form and check the Laboratory Logbook Benchsheet for the extracts to be purified. Verify that the sample requires SA1 or SA2 column cleanup using the Organic Analysis Request Form and the Laboratory Sample Logbook. Determine if copper is required. Sediment samples

generally require copper for sulfur removal, whereas tissue samples, with the exception of eggs, usually do not require copper.

5.2 Preparation for Column Setup

5.2.1 Remove the sodium sulfate, alumina, and silica from the oven storage and place them in a desiccator to cool to room temperature.

5.2.2 Remove combusted sand from the 130°C oven and let it sit on the bench to cool to room temperature.

Note: When taking out materials from the oven, wear heat protective gloves or use large forceps capable of handling heavy objects. The beakers containing sand, silica, alumina, and sodium sulfate are heavy (up to 1000 grams), and are hot. Make sure all the beakers are properly covered with aluminum foil.

5.2.3 Preparation of Deactivated Silica and Alumina

5.2.5.1 Clean and calibrate the balance prior to use according to the instructions in the balance calibration logbook.

5.2.5.2 Place an empty rolling flask and funnel on the balance and tare the weight until the balance reading is 0.000 g.

5.2.5.3 Weigh the alumina into the flask. The total amount of alumina weighed should be the number of samples x 10 grams. Generally, extra alumina is weighed (at least 10 g) in case additional adsorbent is needed when building columns.

5.2.5.4 Deactivate the alumina by adding 1% (w/w) of HPLC water to the alumina flask while the flask is still sitting on the balance.

The amount of water added is calculated by multiplying the total weight of alumina x 0.01 (g). For example, if 171.2 g of alumina is in the flask, 1.7 g of water (171.2 x 0.01 ≈ 1.7) is added to the flask. Tare the weight of the alumina, then add the calculated amount of water into the flask.

5.2.5.5 Weigh the appropriate amount of silica into a separate rolling flask.

The total amount of silica should be the number of samples x 20 grams. Extra silica (at least 20 g) is usually weighed out in case additional silica is needed when building columns.

5.2.5.6 Deactivate the silica by adding 5% (w/w) of HPLC water to the silica flask.

The amount of water added is calculated by multiplying the total weight of silica x 0.05. For example, if 380 g of silica is weighed, the amount of water added to the silica is 19 g (380 x 0.05 = 19). Tare the weight, then add the calculated amount of water into the flask.

5.2.5.7 Clamp the two rolling flasks together at the joint and place them on the roller table. Turn on the roller table and let the chemicals mix for one hour to thoroughly mix and deactivate the silica and alumina.

5.2.6 Preparation of Activated Copper

5.2.6.1 Place approximately 50 g (for 24 samples) of granular copper into a 300 mL beaker.

5.2.6.2 Under a fume hood, carefully pour an appropriate amount of concentrated 12 N hydrochloric acid into the beaker. The copper should be submerged in the acid.

5.2.6.3 Stir the copper with a glass stirring bar gently for a few seconds and let the copper stand in the acid for 5 minutes.

5.2.6.4 Under a fume hood, place a funnel plugged with a piece of glass wool on top of another 300 mL beaker and pour the copper/acid mixture into the funnel.

5.2.6.5 Rinse the copper 3 times with HPLC water or until the rinsing water is neutral. To check that the acid was removed, place the funnel on another clean 250 mL beaker and rinse the copper again. Test the rinse water with a small amount of baking soda. If rinse reacts with the baking soda then additional rinsing with water is needed. If it does not react, place the funnel containing the copper on a clean beaker. The collected rinsing water is neutralized with baking soda and discarded.

5.2.6.6 Rinse the copper in the funnel with approximately 20 mL of methanol three to four times. Discard the methanol as waste.

5.2.6.7 Rinse the copper three or four times in the funnel with approximately 20 mL of methylene chloride. Discard the methylene chloride as waste.

5.2.6.8 Store the copper covered with hexane in a properly labeled beaker and cover with foil, noting date and identity of hexane as the solvent.

5.2.7 Preparation of Pentane/Methylene Chloride (50/50) Solution

5.2.7.1 The pentane/methylene chloride (50/50) solution is prepared by mixing 2 L pentane with 2 L methylene chloride. Use a 4 L labeled mixing bottle and a 2 L graduated cylinder to measure the solvents.

5.2.7.2 Measure 2 L pentane in the graduated cylinder and pour the pentane into the mixing bottle.

5.2.7.3 Using the graduated cylinder, measure 2 L methylene chloride and add it to the mixing bottle. Shake again immediately prior to use. Seal the mixing bottle and mix the solvents by shaking the bottle.

5.3 Column Setup

5.3.1 Clamp the glass chromatography columns (30 cm x 13 m m with a Teflon stopcock and a 250 mL reservoir) onto the column rack located in the hood. The number of columns should be the same as the number of sample extracts.

5.3.2 Put a 250 mL waste jar under each column to collect waste solvent.

5.3.3 Open the stopcock. Using labeled squeeze bottles, rinse the columns three times with approximately 5 mL of methanol each time followed by three 5 mL of rinses using methylene chloride. Make sure that the rinsing solvents completely coat and rinse the inside of the column.

5.3.4 Rinse the tips of a pair of forceps and scissors with methylene chloride. Cut a plug of glass wool with the scissors. Place the glass wool into the column using the forceps. Rinse a glass rod with methylene chloride and pack the glass wool into the bottom of the column with the glass rod.

5.3.5 Close the stopcock, fill the column with approximately 30 mL of methylene chloride and place a funnel on top of the column.

5.3.6 Add 2 cm of combusted sand (about 1-1/2 metal scoop) into each column through the funnel using a stainless steel scoop pre-rinsed with methylene chloride.

5.3.7 Place a 40 mL beaker on a clean calibrated balance. Tare the weight.

5.3.8 Weigh approximately 10 g (±0.1 g) of deactivated alumina.

5.3.9 Pour the alumina into the column through the funnel. Rinse the funnel and column reservoir with methylene chloride, making sure all of the alumina settles into the bottom of the column. Settle the alumina by opening stopcock to drain the solvent and gently tapping the column while draining the solvent to the surface of the alumina.

5.3.10 After adding alumina into every column, weigh approximately 20 g (±0.2 g) of deactivated silica for each column.

5.3.11 Add methylene chloride to the silica in the weighing beaker. Mix well with a glass rod to make a slurry, making sure no visible air bubbles are present.

5.3.12 Pour the silica/methylene chloride slurry into the column through the funnel. Rinse the beaker with methylene chloride and pour it into the column.

5.3.13 Rinse the funnel and the column reservoir with methylene chloride, making sure all the silica settles into the bottom of the column.

5.3.14 Open the stopcock and drain the solvent to a few centimeters above the silica surface.

5.3.15 Add approximately 2 cm of sodium sulfate into the column using a stainless steel scoop (about 1 and one-half scoops). If necessary, rinse the column reservoir with methylene chloride to make sure all the sodium sulfate settles onto the column.

5.3.16 If required, slowly add approximately 1 cm of activated granular copper evenly over the sodium sulfate, using a stainless steel scoop.

5.3.17 Open the stopcock and drain the solvent to the surface of sodium sulfate or copper.

5.3.18 Add 50 mL of pentane and drain the pentane to the surface of sodium sulfate or copper. Close the stopcock.

5.4 Extract Purification Using the Si/Al Column

5.4.1 Rinse the outside of the column tip (below the stopcock) into the waste jar using methylene chloride.

5.4.2 Replace the waste collecting jar with a 250 mL flat bottom flask. Discard solvent waste into appropriate waste container. Each flask should be appropriately labeled with extraction batch number, fraction type (SA1 or SA2), and extract ID number. This flask is used to collect the desired extract fraction.

5.4.3 Transfer the extracts from concentrating tubes to the column using disposable pipettes. The samples should already be concentrated and solvent exchanged into about 2 mL of hexane.

5.4.4 Open the stopcock and let the extract drain to the top surface of the column.

5.4.5 Close the stopcock.

5.4.6 Rinse the extract concentrating tube using with 1 mL of pentane/methylene chloride (50/50) solution and add the rinsing solution into the column with the same disposable pipettes.

5.4.7 Open the stopcock. Let the extract rinses drain to the surface of the column.

5.4.8 Close the stopcock. Repeat Steps 5.4.6 through 5.4.8 with two additional pentane/methylene chloride rinses of the concentrating tube.

5.4.9 For tissue extracts specified for the analysis of AL, AR, OC, and PCB, two fractions of the extract are generally prepared, either by preparing two extracts of the same sample or by quantitatively splitting the extract of one sample into two equal parts. One fraction of the extract (SA1) is used for AL analysis and the other fraction (SA2) is for AR, OC, or PCB analysis. Both fractions are purified by silica/alumina column chromatography.

5.4.9.1 For the SA1 fraction, measure 100 mL of pentane using a graduated cylinder. Add the solution to the column using a glass funnel. Pour the solvent slowly so that the surface of the column is not disturbed.

5.4.9.2 Open the stopcock and collect the extract in the 250 mL flat bottom flask at a flow rate of about 2 mL/min. The flow rate is controlled by adjusting the stopcock. Do not let the column run dry.

5.4.9.3 After all of the 100 mL of pentane has drained to the top of the column and is collected in the 250 mL flat bottom flask, add 40 mL of pentane/methylene chloride (50/50) solution into the column using a graduated cylinder. Collect the 40 mL of pentane/methylene chloride solution in the same flask. Do not let the column run dry.

5.4.9.4 Close the stopcock of the column.

5.4.9.5 This collected fraction is SA1; continue with step 5.4.11.

5.4.10 For extracts from tissues, sediment and soil samples requiring the analysis for AL, AR, OC, and/or PCBs, (SA2), after transferring the extracts and rinses to the top of the column (see Section 5.4.1-5.4.8), measure 200 mL of pentane/methylene chloride (50/50) solution using a graduated cylinder.

5.4.10.1 Place a 250 mL flat bottom flask labeled with the extraction batch, fraction type (SA2) and extract identifications under the column.

5.4.10.2 Add the solution to the column through a glass funnel. Pour the solution slowly so that the surface of the column is not disturbed.

5.4.10.3 Open the stopcock and elute the sample in the flat bottom flask at a flow rate of about 2 mL/min. The flow rate is controlled by adjusting the stopcock.

5.4.10.4 After all of the 200 mL of pentane/methylene chloride solution has passed through the column and is collected in the 250 mL flat bottom flask, close the stopcock of the column. This fraction is labeled SA2; continue with step 5.4.11.

5.4.11 Remove the collecting flask and cap it with a glass stopper. The collected samples are then transferred to the laboratory area where the extract concentration step can be performed.

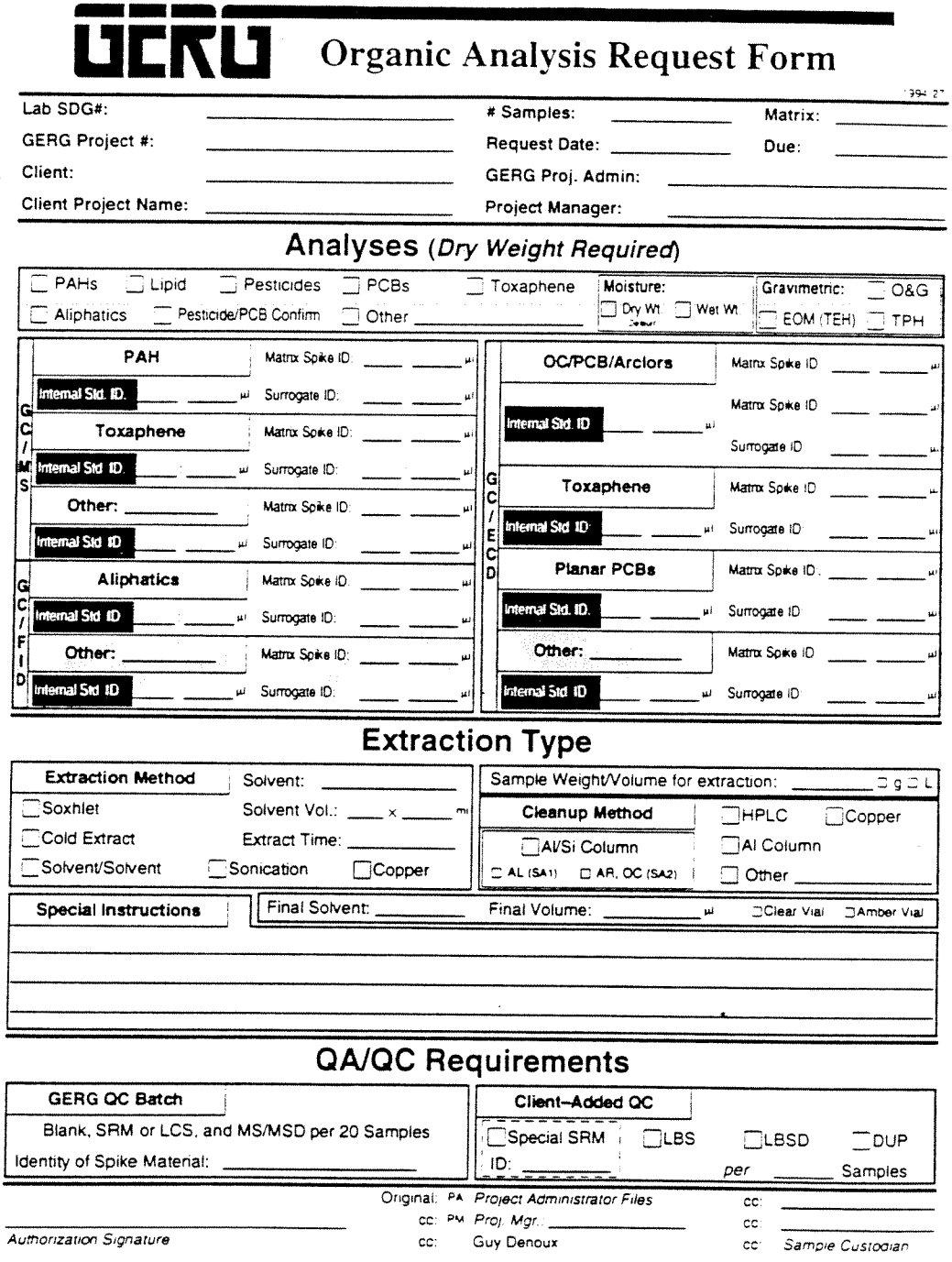
5.4.12 Sign the Laboratory Sample Logbook in the appropriate place with date and analyst's initial.

5.4.13 Dismantle the column by pouring out the alumina and silica from the column into a specified chemical waste container. Remove the glass wool plug from the column and discard the glass wool. Place the glass column in the cleaning area so that the column can be cleaned according to GERG SOP.

6.0 EXAMPLE FORMS

6.1 Example Organic Analysis Request Form
6.2 Example Laboratory Sample Logbook

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURE SOP-9720



GERG Organic Analysis Request Form

Lab SDG#: _____ # Samples: _____ Matrix: _____
GERG Project #: _____ Request Date: _____ Due: _____
Client: _____ GERG Proj. Admin: _____
Client Project Name: _____ Project Manager: _____

Analyses (*Dry Weight Required*)

☐ PAHs ☐ Lipid ☐ Pesticides ☐ PCBs ☐ Toxaphene | Moisture: ☐ Dry Wt ☐ Wet Wt | Gravimetric: ☐ O&G ☐ EOM (TEH) ☐ TPH
☐ Aliphatics ☐ Pesticide/PCB Confirm ☐ Other _____

GC/MS
PAH — Matrix Spike ID: ____ ____ µl — Internal Std. ID: ____ ____ µl — Surrogate ID: ____ ____ µl
Toxaphene — Matrix Spike ID: ____ ____ µl — Internal Std. ID: ____ ____ µl — Surrogate ID: ____ ____ µl
Other: _____ — Matrix Spike ID: ____ ____ µl — Internal Std. ID: ____ ____ µl — Surrogate ID: ____ ____ µl

GC/FID
Aliphatics — Matrix Spike ID: ____ ____ µl — Internal Std. ID: ____ ____ µl — Surrogate ID: ____ ____ µl
Other: _____ — Matrix Spike ID: ____ ____ µl — Internal Std. ID: ____ ____ µl — Surrogate ID: ____ ____ µl

GC/ECD
OC/PCB/Arclors — Matrix Spike ID: ____ ____ µl — Internal Std. ID: ____ ____ µl — Matrix Spike ID: ____ ____ µl — Surrogate ID: ____ ____ µl
Toxaphene — Matrix Spike ID: ____ ____ µl — Internal Std. ID: ____ ____ µl — Surrogate ID: ____ ____ µl
Planar PCBs — Matrix Spike ID: ____ ____ µl — Internal Std. ID: ____ ____ µl — Surrogate ID: ____ ____ µl
Other: _____ — Matrix Spike ID: ____ ____ µl — Internal Std. ID: ____ ____ µl — Surrogate ID: ____ ____ µl

Extraction Type

Extraction Method — Solvent: _____
☐ Soxhlet — Solvent Vol.: ____ x ____ ml
☐ Cold Extract — Extract Time: _____
☐ Solvent/Solvent ☐ Sonication ☐ Copper

Sample Weight/Volume for extraction: _____ ☐ g ☐ L

Cleanup Method — ☐ HPLC ☐ Copper
☐ Al/Si Column ☐ Al Column
☐ AL (SA1) ☐ AR, OC (SA2) ☐ Other _____

Special Instructions
Final Solvent: _____ Final Volume: _____ µl ☐ Clear Vial ☐ Amber Vial

QA/QC Requirements

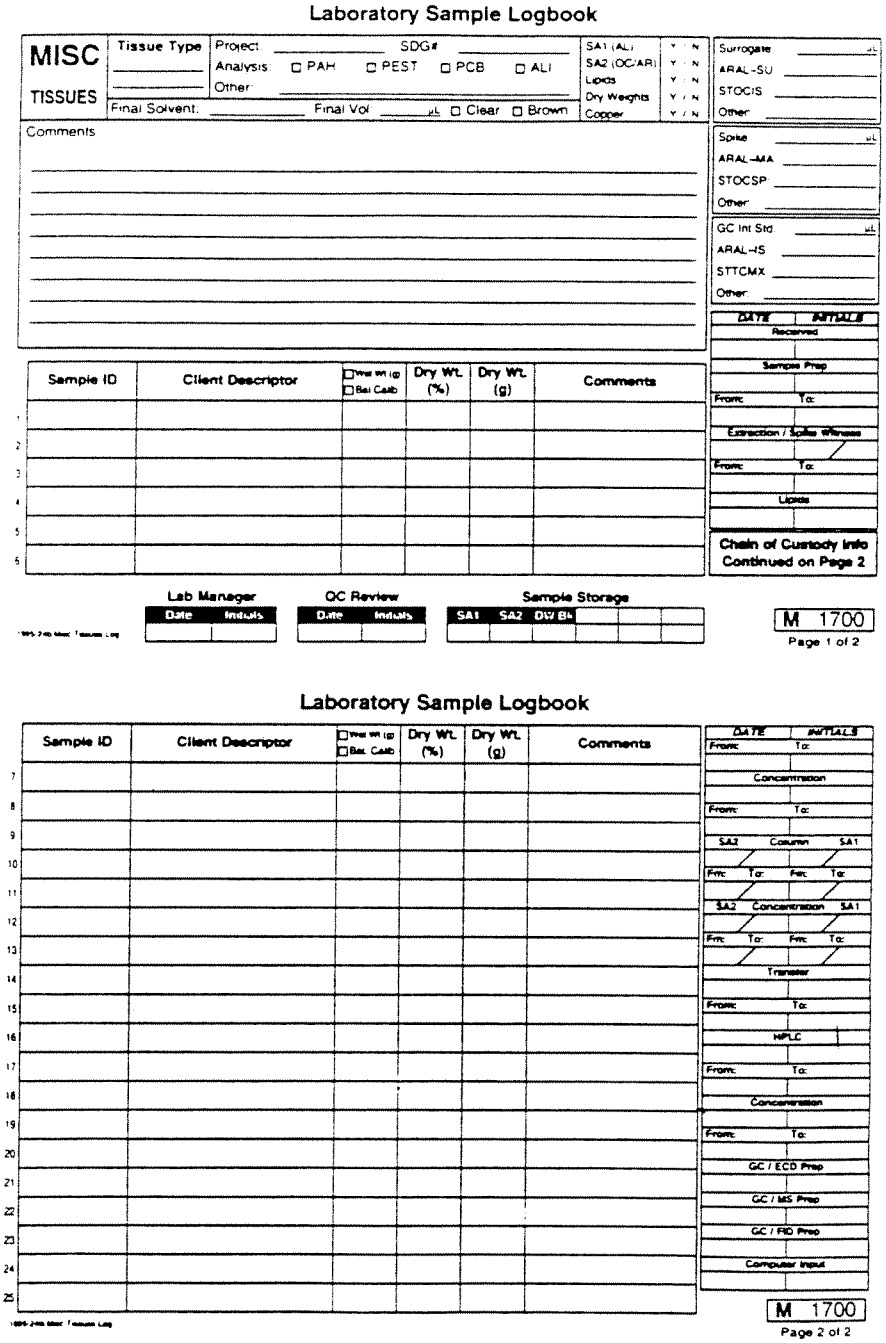
GERG QC Batch
Blank, SRM or LCS, and MS/MSD per 20 Samples
Identity of Spike Material: _____

Client-Added QC
☐ Special SRM ☐ LBS ☐ LBSD ☐ DUP
ID: _____ per _____ Samples

_____
Authorization Signature

Original: PA *Project Administrator Files* cc: _____
cc: PM *Proj. Mgr.* _____ cc: _____
cc: Guy Denoux cc: *Sample Custodian*

Figure 6.1. Example Organic Analysis Request Form



Laboratory Sample Logbook

MISC
TISSUES

Tissue Type
Project: SDG#
Analysis: ☐ PAH ☐ PEST ☐ PCB ☐ ALI
Other:
Final Solvent: Final Vol: µL ☐ Clear ☐ Brown

SA1 (ALI) Y / N
SA2 (OC/AR) Y / N
Lipids Y / N
Dry Weights Y / N
Copper Y / N

Surrogate µL
ARAL-SU
STOCIS
Other:

Spike µL
ARAL-MA
STOCSP
Other:

GC Int Std. µL
ARAL-IS
STTCMX
Other:

Comments

Sample ID	Client Descriptor	☐ Wet Wt (g) ☐ Bal. Calib.	Dry Wt. (%)	Dry Wt. (g)	Comments
1					
2					
3					
4					
5					
6					

DATE / INITIALS
Received
Sample Prep
From: To:
Extraction / Spike Aliquots
From: To:
Lipids
Chain of Custody Info
Continued on Page 2

Lab Manager: Date / Initials
QC Review: Date / Initials
Sample Storage: SA1 SA2 DW Bl.

M 1700
Page 1 of 2

Laboratory Sample Logbook

Sample ID	Client Descriptor	☐ Wet Wt (g) ☐ Bal. Calib.	Dry Wt. (%)	Dry Wt. (g)	Comments
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					

DATE / INITIALS
From: To:
Concentration
From: To:
SA2 Column SA1
Frm: To: Frm: To:
SA2 Concentration SA1
Frm: To: Frm: To:
Transfer
From: To:
HPLC
From: To:
Concentration
From: To:
GC / ECD Prep
GC / MS Prep
GC / FID Prep
Computer Input

M 1700
Page 2 of 2

Figure 6.2. Example Laboratory Sample Logbook

Contract No.: DACW33-94-D-0009
DCN: GEP2-123098-AAET
Revision No.: 01
Date: 01/99

SOP-9722
QUANTITATIVE DETERMINATION OF TETRA- THROUGH OCTA-POLYCHLORINATED DIBENZO-P-DIOXINS (PCDDs) AND DIBENZOFURANS (PCDFs) BY ISOTOPE DILUTION HIGH RESOLUTION GAS CHROMATOGRAPHY/HIGH RESOLUTION MASS SPECTROMETRY (HRGC/HRMS)

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURES

SOP-9722

QUANTITATIVE DETERMINATION OF TETRA- THROUGH OCTA-POLYCHLORINATED DIBENZO-*p*-DIOXINS (PCDDs) AND DIBENZOFURANS (PCDFs) BY ISOTOPE DILUTION HIGH RESOLUTION GAS CHROMATOGRAPHY/HIGH RESOLUTION MASS SPECTROMETRY (HRGC/HRMS)

This document presents the procedures, materials, and quality control used in the performance of the above instrumental activities.

Quality Assurance Manager

5/2/97
Date

Author/Revision by: L. Chambers

QUANTITATIVE DETERMINATION OF TETRA- THROUGH OCTA-POLYCHLORINATED DIBENZO-*p*-DIOXINS (PCDDs) AND DIBENZOFURANS (PCDFs) BY ISOTOPE DILUTION HIGH RESOLUTION GAS CHROMATOGRAPHY/HIGH RESOLUTION MASS SPECTROMETRY (HRGC/HRMS)

1.0 PURPOSE AND SUMMARY

This document provides the procedures used by the staff of the Geochemical and Environmental Research Group (GERG) of the College of Geosciences and Maritime Studies at Texas A&M University for the quantitative determination of tetra- through octa- polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) in sample extracts using isotope dilution high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS).

1.1 Applicability

The instrumental procedures described in this document are applicable to the quantitative analysis of extracts from aqueous, sediment, biological tissues and other matrices after appropriate extraction and purification.

1.2 Target Analyte List and CAS Registry Numbers

The chlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDDs and PCDFs) determined by this method and their CAS Registry numbers are listed in Table 1.

1.3 Retention Time References, Quantitation References, Relative Retention Times, and Minimum Levels

1.3.1 The retention time references, quantitation references, relative retention times and minimum levels for determination of PCDDs and PCDFs using this method are listed in Table 2.

1.3.2 The Minimum Level (ML) for each analyte is defined as the level at which the entire system must give a recognizable signal and an acceptable calibration point. It is equivalent to the concentration of the lowest calibration standard, assuming that all method specific sample weights, volumes, and

procedures have been employed. The ML varies with the degree of chlorination.

1.3.3 The sample specific Estimated Detection Limit (EDL) is the concentration of an analyte required to produce a signal with a peak height at least 3 times the background signal level. An EDL is calculated for each 2,3,7,8-substituted isomer that is not identified. The quantitation software (OPUSQuan) supplied by the instrument manufacturer calculates a sample specific-EDL using an algorithm based on the criteria outlined in EPA Method 8290, Section 7.9.5.1.1.

1.4 Applicable Concentration Range

The analyte concentrations of the calibration standards (CS1-CS5) used for instrument calibration are listed in Table 3.

1.5 Special Considerations

1.5.1 Hazards associated with the dioxin/furan standards in the MSDS include the following information:

Hazards: While little toxicity data on these compounds is available, they are potentially highly toxic. Dioxins and dibenzofurans substituted in the 2,3,7 and 8 positions are especially likely to exhibit toxic effects including teratogenicity, mutagenicity, and carcinogenicity. Exposure to microgram quantities could result in toxic effects. Only experienced personnel are allowed to handle these chemicals.

Toxic exposure routes are potentially high via oral, dermal, and enterperitoneal routes.

Personnel protection should include an impervious laboratory apron or coverall and should be worn while handling this standard. Neoprene gloves are preferred. An acceptable substitute would be two dissimilar types of gloves such as PVC gloves worn over latex exam gloves. Open only in a fume hood or glove box. Respiratory protection (full-face air line or cartridge type respirator) is required.

If the standard is spilled, absorb as much as possible with activated charcoal and place in an appropriate container. Solvent-wash all contaminated surfaces with toluene and absorbent paper followed by washing with a strong soap and water solution. Do not re-enter the contaminated area until the Safety Officer (or other responsible person) has verified that the area has been properly cleaned. Dispose of all contaminated materials in sealed steel containers.

If skin contact occurs, remove all contaminated clothing and immediately flood skin with water. Wash the affected skin areas with soap and water for 15 minutes. If symptoms develop, persist, or seek medical attention.

If any laboratory personnel should inhale this chemical, remove them at once to open air and arrange for immediate transportation to a medical facility.

If eye contact occurs, check for contact lenses and remove them at once if present. Immediately flush eyes with water from any source for 15 minutes. Do not use oil or ointment in eyes. Arrange immediate transportation to a medical facility.

If ingestion occurs and the exposed person is convulsing or unconscious, do not attempt first aid. Transport immediately to a hospital emergency room or poison control center. If the victim is conscious, administer large volumes of liquid. Transport at once to a medical facility.

Ingestion of microgram quantities of these compounds may cause liver and kidney damage, chloracne and death.

2.0 SAFETY

The hazards, toxicity or carcinogenicity of each compound or reagent used in GERG's standard operating procedures have not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory maintains Material Safety Data Sheets (MSDS) which contain

information regarding the safe handling of chemicals used at GERG's facilities. A reference file of MSDS is available to all personnel involved with these materials. All laboratory personnel should direct any questions regarding safety issues to their supervisors or the Safety Officer.

3.0 QUALITY CONTROL

3.1 Instrument Criteria

3.1.1 Mass Spectrometer Performance

3.1.1.1 The mass spectrometer must be operated in the electron ionization mode. A static resolving power of at least 10,000 (10 percent valley definition) must be demonstrated at appropriate masses before any analysis is performed. Static resolving power checks must be performed at the beginning of each 12 hour period of operation. Corrective action must be implemented whenever the resolving power does not meet the requirement.

3.1.1.2 Chromatography time for PCDDs and PCDFs exceed the long term mass stability of the mass spectrometer. Because the instrument is operated in the high-resolution mode, mass drifts of a few ppm can have serious adverse effects on instrument performance. Therefore, a mass drift correction is mandatory. To that effect, a lock-mass ion is selected from the reference compound (PFK) used for tuning the mass spectrometer. The selection of the lock-mass ion is dependent on the masses of the ions monitored within each descriptor (see Table 4). Any acceptable lock-mass ion at any mass between the lightest and heaviest ion in each descriptor can be used to monitor and correct mass drifts.

3.1.1.3 The level of the reference compound (PFK) metered into the ion chamber during HRGC/HRMS analyses is adjusted so that the amplitude of the most intense selected lock-mass ion signal does not exceed 10

percent of the full scale deflection for a given set of detector parameters. Suggested lock-mass ions are listed in Table 4.

3.1.1.4 Documentation of the instrument resolving power is accomplished by recording the peak profile of the reference compound peaks within each descriptor. The format of the peak profile representation must allow manual determination of the resolution, i.e., the horizontal axis must be a calibrated mass scale. The result of the peak width measurement performed at the 10 percent valley definition cannot exceed 100 ppm for all reference compound peaks recorded.

3.1.1.5 Total cycle time (dwell times plus switching times) for all ions within a single descriptor must be one second or less.

3.1.2 CG Column Performance

3.1.2.1 Using the GC column performance check solution (CPSM/WDM, see Table 5), the chromatographic separation between 2,3,7,8-TCDD and the peaks representing any other unlabeled TCDD isomers must be resolved with a valley of $\leq$ 25 percent, where:

Eq. 1 Valley percent = (x/y) (100)

x = height of the valley between 2,3,7,8-TCDD and the closest eluting isomer, and

y = the peak height of 2,3,7,8-TCDD

3.1.2.2 When confirmation analysis of the 2,3,7,8-TCDF isomer is required, chromatographic separation between 2,3,7,8-TCDF and the peaks representing any other unlabeled TCDF isomers must be

resolved with a valley of ≤ 25 percent using Eq. 1, replacing TCDD with TCDF.

3.1.2.3 The GC column performance check solution (CPSM/WDM, see Table 5) also contains the known first and last PCDD/PCDF eluters under the conditions specified in this method. Their retention times are used to determine the eight homologue retention time windows that are used for qualitative and quantitative purposes. The retention times for the switching of SIM ions characteristic of one homologous series to the next higher homologous series must be indicated in the Selected Ion Current Profile (SICP). Accurate switching at the appropriate times is absolutely necessary for accurate monitoring of these compounds.

3.1.2.4 The absolute retention time of 2,3,7,8-TCDD must be at least 25 minutes on the DB-5 column when EPA Method 1613 is required by the client.

3.1.2.5 The absolute retention time of TCDF must be at least 15 minutes on the DB-225 column when EPA Method 1613 is required by the client.

3.2 Analyte Identification Criteria

3.2.1 Retention Times

3.2.1.1 For 2,3,7,8-substituted congeners which have an isotopically labelled quantitation or internal standard present in the sample extract the retention time at maximum peak height of the sample components (i.e., the two ions used for quantitation purposes listed in Table 4) must be within -1 to +3 seconds of the isotopically labelled standard.

3.2.1.2 For 2,3,7,8-substituted compounds that do not have an isotopically labelled quantitation or internal

standard present in the sample extract, the retention time must fall within 0.005 retention time units of the relative retention times measured in the routine calibration. Identification of OCDF is based on its retention time relative to $^{13}C_{12}$-OCDD as determined from the daily routine calibration results.

3.2.1.3 For non-2,3,7,8-substituted compounds (tetra through hepta) the retention time must be within the corresponding homologous retention time window established by analyzing the column performance check solution.

3.2.1.4 The ion current responses for both ions used for quantitative purposes must silumltaneously reach their maxima (± 2 seconds).

3.2.1.5 The ion current responses for both ions used for the labelled standards must reach maximum simultaneously (± 2 seconds).

3.2.2 Ion Abundance Ratios

The integrated ion current profiles for the two ions used for quantitation purposes must have a ratio between the lower and upper limits established for the homologous series to which the peak is assigned. See Table 6 for the required theoretical ion ratios and their QC acceptance limits.

3.2.3 Signal-to-Noise Ratio

3.2.3.1 All ion current intensities must be ≥ 3 times noise level for positive identification of a PCDD or PCDF compound or a group of coeluting isomers.

3.2.3.2 The peaks representing the PCDDs and PCDFs and labelled compounds in the CS1 calibration solution must have signal-to-noise ratios greater than or equal to 10.

3.2.4 Polychlorinated Diphenyl Ether Interferences

In addition to the above criteria, the identification of a GC peak as a PCDF can only be made if no signal having a S/N > 3 is detected at the same retention time (±2 seconds) in the corresponding polychlorinated diphenyl ether (PCDPE, see Table 4) channel.

3.3 Calibration Criteria

3.3.1 Initial Calibration

3.3.1.1 All five high-resolution concentration calibration solutions listed in Table 3 (CS1 - CS5) must be used for the initial calibration.

3.3.1.2 The theoretical ion abundance ratios for all 17 native analytes and all 16 labelled compounds must be within the QC control limits specified in Table 6.

3.3.1.3 The signal-to-noise ratio for the GC signals present in each SICP (including the ones for the labelled standards) must be ≥ 10.

3.3.1.4 The percent relative standard deviations for the mean relative response factors from the 17 unlabeled standards must not exceed $\pm$ 20 percent, and those for the fifteen labelled reference compounds must not exceed $\pm$ 30 percent.

3.3.2 Continuing Calibration (VER for EPA Method 1613)

When EPA Method 1613 is required, the continuing calibration may be referred to as a verification of calibration (VER), and the criteria included in section 3.3.2.5 are not used.

3.3.2.1 Continuing calibration must be performed at the beginning of each 12 hour period after successful mass resolution and GC resolution performance checks have been completed. A routine calibration is also required at the end of a 12 hour shift.

3.3.2.2 The isotopic ratios for all 17 native analytes and all 16 labelled compounds must be within the control limits for the theoretical ion abdance ratios specified in Table 6.

3.3.2.3 The signal-to-noise ratio for the GC signals present in each SICP (including the ones for the labelled standards) must be ≥ 10.

3.3.2.4 If the client requires EPA 1613, the measured concentrations obtained during the routine calibration runs must be within the acceptance criteria specified in Table 7.

3.3.2.5 The QC acceptance criteria for the calculated relative response factors for the unlabeled standards must be within $\pm$ 20 percent of the mean values established during the initial calibration. QC acceptance criteria for the calculated relative response factors for the labelled standards must be within $\pm$ 30 percent of the mean values established during the initial calibration.

3.4 Criteria for QC Samples in an Analytical Batch

3.4.1 Method Blank (BLANK)

3.4.1.1 A Method Blank is used to demonstrate freedom from contamination in the analytical procedure, and is required for each set of 20 or fewer samples.

3.4.1.2 If any of the 2,3,7,8-substituted PCDDs or PCDFs are found in the blank at greater than the minimum level (Table 2), or if any potentially interfering compound is found in the blank at the minimum level for each level of chlorination given in Table 2, re-extraction of the analytical batch may be indicated. The analyst should refer to the relevant Quality

Assurance Project Plan for the necessary corrective action.

3.4.1.3 Analyst discretion is used when contamination is present which does not adversely effect the overall analytical effort.

3.4.1.4 If any of the 2,3,7,8-substituted PCDDs or PCDFs are found in the blank at greater than the minimum level, but are not detected in the analytical samples above this level, no action is required.

3.4.1.5 When target analytes are present in the method blank and in the analytical samples at concentrations above the Minimum Level, analytical data for those analytes in the samples must be flagged.

3.4.2 Laboratory Blank Spike (LBS)

3.4.2.1 A Laboratory Blank Spike (LBS) is used to demonstrate analytical accuracy of the method, and may be required with each set of 20 or fewer samples.

3.4.2.2 QC acceptance criteria for native analyte recoveries is 70 to 130% of the spiked amount.

3.4.2.3 If six or more of the seventeen target analytes are outside the acceptance criteria corrective action may be indicated. Corrective action may include recalculation and/or reanalysis of the LBS, instrument maintenance and/or recalibration, and re-extraction of the analytical batch.

3.4.3 Ongoing Precision and Recovery (OPR)

3.4.3.1 An Ongoing Precision and Recovery (OPR) sample is used to demonstrate analytical accuracy in the presence of a clean matrix when EPA method 1613 reporting requirements are required by the client.

3.4.3.2 The performance based QC acceptance criteria for the OPR are different for each target analyte. Those criteria are detailed in Table 7.

3.4.3.3 If any of the seventeen target analytes are outside the acceptance criteria corrective action is required. Corrective action may include recalculation and/or reanalysis of the OPR, instrument maintenance and/or recalibration, and re-extraction; or the analytical batch.

3.4.4 Matrix Spike (MS) and Matrix Spike Duplicate (MSD)

3.4.4.1 A Matrix Spike (MS) sample is used to demonstrate analytical accuracy in the presence of a representative matrix and is required for each set of 20 or fewer samples.

3.4.4.2 A Matrix Spike Duplicate (MSD) is used to demonstrate analytical accuracy and precision in the presence of a representative matrix and may be required for each set of 20 or fewer samples.

3.4.4.3 QC acceptance criteria for analyte recoveries in the MS and the MSD is 70 to 130% of the spiked amount.

3.4.4.4 If a Matrix Spike Duplicate (MSD) has been included with the analytical batch, the results obtained from the MS and MSD samples should agree within a Relative Standard Deviation (RSD) of 20%.

3.4.4.5 The MS and MSD acceptance criteria are advisory. However, if six or more of the seventeen target analytes are outside the QC acceptance criteria corrective action may be indicated. Corrective action may include recalculation and/or reanalysis of the MS and MSD; instrument maintenance and/or recalibration.

3.4.5 Duplicate (DUP)

3.4.5.1 A sample Duplicate (DUP) is used to demonstrate matrix homogeneity and analytical precision in the presence of a representative matrix, and is required with each set of 20 or fewer samples.

3.4.5.2 QC acceptance criteria for analyte concentrations greater than ten times the minimum level is a Relative Standard Deviation (RSD) of 20%.

3.4.5.3 The DUP acceptance criteria are advisory. However, if six or more of the seventeen target analytes are outside the QC acceptance criteria corrective action may be indicated. Corrective action may include: recalculation and/or reanalysis of the DUP and/or the original sample, instrument maintenance and/or recalibration.

3.4.6 Standard Reference Material (SRM)

3.4.6.1 A Standard Reference Material (SRM) is used to demonstrate analytical accuracy on a certified reference matrix from an independent source, and is required with each set of 20 or fewer samples.

3.4.6.2 When the SRM certificate provides a concentration range for the certified or concensus value of an analyte, measured analyte concentrations for the SRM should fall within that range, with no more than one-third of the analytes exceeding this criteria.

3.4.6.3 When the SRM certificate does not provide a concentration range, the acceptance criteria is 70 to 130% of the certified or concensus value of the analyte, with no more than one-third of the analytes exceeding this criteria.

3.4.6.4 The QC acceptance criteria is invalid when the analyte concentration falls below the minimum level listed in Table 2.

3.4.6.5 The SRM acceptance criteria are advisory. However, if more than 35% of the analytes fall outside the QC acceptance criteria, corrective action may be indicated. Corrective action may include recalculation and/or reanalysis of the SRM, instrument maintenance and/or recalibration.

3.4.7 Labelled Compound Recovery

3.4.7.1 All samples are spiked with the Labelled Compound Spiking Solution (LCSS) to monitor method performance on the sample matrix.

3.4.7.2 QC acceptance criteria for labelled compound recovery is 40 to 135%.

3.4.7.3 When EPA Method 1613 is required by the client, the method based acceptance criteria for labelled compound recoveries are different for each standard. See Table 8 for details.

3.4.7.4 If a labelled compound recovery falls below the acceptance criteria, but the sum of the areas of the affected peaks is 10% or greater than the area of the corresponding peaks in the continuing calibration standard, the analytical data are flagged accordingly.

3.4.7.5 If six or more of the fifteen labelled compounds are outside the acceptance criteria corrective action may be indicated. Corrective action may include: recalculation and/or reanalysis of the sample; instrument maintenance and/or recalibration; or re-extraction of the sample aliquot.

4.0 CHROMATOGRAPHIC CONDITIONS

4.1 Gas Chromatograph

4.1.1 The Gas Chromatograph (GC) used for this analytical method is an HP5890 Series II, or equivalent. The GC has an injection port designed for capillary columns and splitless injections, is capable of temperature ramp programming with an isothermal hold, and of meeting all of the performance specifications outlined in Section 3.1.2. A 2mL splitless injection is used for all extracts, blanks, calibration solutions and the performance check samples.

4.1.2 All injections are made with a CTC-2000S programmable autosampler, or equivalent.

4.1.3 The GC/MS interface components withstand 350∞C, and are designed so that the separation of 2,3,7,8-TCDD from the other TCDD isomers achieved in the gas chromatographic column is not appreciably degraded. The GC column is fitted directly into the mass spectrometer ion source without being exposed to the ionizing electron beam.

4.2 GC Columns

4.2.1 The GC column used for analysis of PCDDs and PCDFs and for isomer specificity of 2,3,7,8-TCDD is a 60 meter J&W DB5 or DB5MS column with a 0.25 mm ID and a 0.25 mm film thickness, or equivalent.

4.2.2 The GC column used for isomer specificity and confirmation of 2,3,7,8-TCDF is a 60 meter J&W DB225 column with 0.25 m m ID and 0.25 mm film thickness, or equivalent.

4.3 Operating Conditions

4.3.1 Operating conditions known to produce acceptable results with the recommended analytical column (DB5MS) are shown below:

Inject at 200°C, hold for 2 minutes
Ramp at 5°C per minute to 220°C, hold for 16 minutes
Ramp at 5°C per minute to 235°C, hold for 7 minutes
Ramp at 5°C per minute to 320°C, hold for 10 minutes

4.3.2 Operating conditions known to produce acceptable results with the recommended confirmation column (DB225) are shown below:

Inject at 200°C, hold for 1 minute
Ramp at 12°C per minute to 300°C, hold for 10 minutes

5.0 DETECTOR AND DATA SYSTEM CRITERIA

5.1 The detector used for these analyses is a VG AutoSpec Ultima, or equivalent, utilizing 28 to 40 eV electron impact ionization, capable of repetitively selectively monitoring a minimum of 12 exact m/z's at high resolution (≥10,000) during a period of approximately 1 second or less, and meeting all of the performance specifications outlined in Section 3.2.1.

5.2 A dedicated data system is employed to control the rapid multiple-ion monitoring process and to acquire the data. Quantitation data (peak areas and/or peak heights) and Selected Ion Monitoring (SIM) traces are acquired during the analyses and stored. The data system is capable of acquiring data at a minimum of 12 ions in a single scan. It is capable of switching to different sets of ions (descriptors) at specified times during an acquisition, and of providing hard copies of individual ion chromatograms for selected gas chromatographic time intervals. It is capable of acquiring mass spectral peak profiles and providing hard copies of peak profiles to demonstrate the required resolving power. The data system permits the measurement of noise on the base line.

6.0 INSTRUMENT CALIBRATION PROCEDURE

6.1 Initial Calibration

Initial calibration is required before any samples are analyzed for PCDDs and PCDFs. Initial calibration is also required if any continuing calibration verification does not meet the required criteria listed in Section 3.3.2. All five high resolution

concentration calibration solutions listed in Table 3 must be used for the initial calibration.

6.1.1 Tune the instrument with PFK as described in Section 3.1.1.

6.1.2 Inject 2 µL of the CPSM/WDM (Table 5) and acquire SIM mass spectral data as described in Section 3.1.1. Demonstrate and document that the criteria listed in Section 3.2.2 are met.

6.1.3 Using the same GC and MS conditions that produced acceptable results with the CPSM/WDM, analyze a 2 µL portion of each of the five concentration calibration solutions (CS1 - CS5) once, and demonstrate that the following conditions are met.

6.1.3.1 The ratio of integrated ion currents for the homologous series quantitation ions appearing in Table 6 must be within the indicated control limits set for each homologous series.

6.1.3.2 The ratio of integrated ion currents for the ions belonging to the $^{13}C_{12}$-labelled quantitation and internal standards must be within the control limits stipulated in Table 6.

NOTE: All ratios must be within the specified control limits simultaneously in one run. Otherwise, corrective action is necessary.

6.1.3.3 For each SICP and for each GC signal corresponding to the elution of a target analyte and of its $^{13}C_{12}$-labelled standard(s), the signal-to-noise (S/N) ratio must be greater than or equal to 10.

6.1.4 Refering to Table 2, calculate the 17 relative response factors (RRF) for unlabeled target analytes [RRF(n); n = 1 to 17] relative to their appropriate quantitation standards, and the RRFs for the $^{13}C_{12}$-labelled quantitation standards [RRF(m); m = 1 to 15] relative to the two internal standards according to the following equations.

$$Eq.\ 2\ \text{RRF(n)} = \frac{Ax * Cqs}{Aqs * Cx}$$

$$Eq.\ 3\ \text{RRF(m)} = \frac{Aqs * Cis}{Ais * Cqs}$$

where:

Ax = sum of the integrated ion abundances of the quantitation ions for unlabeled PCDDs and PCDFs,

Aqs = sum of the integrated ion abundances of the quantitation ions for the labelled quantitation standards,

Ais = sum of the integrated ion abundances of the quantitation ions for the labelled internal standards,

Cx = concentration of the unlabeled PCDD and/or PCDF analyte in the calibration solution (pg/mL),

Cqs = concentration of the $^{13}C_{12}$-labelled quantitation standard in the calibration solution (100 pg/mL), and

Cis = concentration of the $^{13}C_{12}$-labelled internal standard in calibration solution (100 pg/mL).

NOTE: The RRF(n) and RRF(m) are dimensionless quantities; the units used to express Cx, Cqs and Cis must be the same.

6.1.4.1 The native OCDF is quantitated against labelled OCDD. As a result the concentration of OCDF is corrected for the recovery of the labelled OCDD. In instances where OCDD and OCDF behave differently during sample extraction, concentration, and cleanup procedures, this may decrease the accuracy of the OCDF results. However, given the low toxicity of this compound relative to the other

dioxins and furans, the potential decrease in accuracy is not considered significant.

6.1.4.2 The 1,2,3,7,8,9-HxCDD isomer is quantitated using the averaged response of the labelled analogs of the other two 2,3,7,8-substituted HxCDDs. As a result, the concentration of 1,2,3,7,8,9-HxCDD is corrected for the average recovery of the other two HxCDDs.

6.1.4.3 Any peaks representing non-2,3,7,8-substituted PCDDs and PCDFs are quantitated using an average of the RRFs from all of the $^{13}C_{12}$-labelled 2,3,7,8-substituted isomers at the same level of chlorination.

6.1.5 Calculate the mean RRFs for the five calibrations solutions using the following equations.

Eq. 4 $$\overline{RRF(n)} = \left(\frac{1}{5}\right) \sum_{j=1}^{5} RRF_j(n)$$

Eq. 5 $$\overline{RRF(m)} = \left(\frac{1}{5}\right) \sum_{j=1}^{5} RRF_j(m)$$

where:

n = a particular 2,3,7,8-substituted PCDD/PCDF unlabeled target analyte (n = 1 to 17),

m = a particular $^{13}C_{12}$-labelled quantitation standard (m = 1 to 15), and

j = the injection number or calibration solution number (j = 1 to 5).

6.1.6 Determine and their respective percent relative standard deviations (%RSD). Verify and document that the initial calibration meets all of the acceptance criteria outlined in Section 3.3.1.

6.2 Continuing Calibration Verification

Routine calibration must be performed at the beginning of a 12 hour period after successful MS resolution and GC resolution performance checks. A routine calibration is also required at the end of a 12 hour shift.

6.2.1 Using the same GC and MS conditions that were used for the CPSM/WDM and the initial calibration, analyze a 2 µL portion of the midpoint calibration solution (CS3) with the following mass spectrometer operating parameters.

6.2.1.1 The ratio of integrated ion currents for the homologous series quantitation ions appearing in Table 6 must be within the indicated control limits set for each homologous series.

6.2.1.2 The ratio of integrated ion currents for the ions belonging to the $^{13}C_{12}$-labelled quantitation and internal standards must be within the control limits stipulated in Table 6.

NOTE: All ratios must be within the specified control limits simultaneously in one run. Otherwise, corrective action is necessary.

6.2.1.3 For each SICP and for each GC signal corresponding to the elution of a target analyte and of its $^{13}C_{12}$-labelled standard(s), the signal-to-noise (S/N) ratio must be greater than or equal to 10.

6.2.2 Refering to Table 2, calculate the concentrations of the 17 unlabeled target analytes, and the concentration and percent

recovery of the fifteen $^{13}C_{12}$-labelled quantitation standards in the CS3 standard using the following equations.

$$Eq.\ 6\ Cx = \frac{Ax * Cqs}{Aqs * \overline{RRF(n)}}$$

$$Eq.\ 7\ \%\ Recovery = \frac{Ax * Cis * 100}{Ais * Cqs * \overline{RRF(m)}}$$

where:

Ax = sum of the integrated ion abundances of the quantitation ions for unlabeled PCDDs and PCDFs,

Aqs = sum of the integrated ion abundances of the quantitation ions for the $^{13}C_{12}$-labelled quantitation standards,

Ais = sum of the integrated ion abundances of the quantitation ions for the $^{13}C_{12}$-labelled internal standards,

Cx = concentration of the unlabeled PCDDs and PCDFs isomers in pg/µL

Cqs = concentration of the $^{13}C_{12}$-labelled quantitation standard in the calibration solution (100 pg/µL),

Cis = concentration of the $^{13}C_{12}$-labelled internal standard in the calibration solution (100 pg/µL),

$\overline{RRF(n)}$ = mean relative response factor for the unlabeled target analyte relative to its $^{13}C_{12}$-labelled quantitation standard [RRF(n), with n = 1 to 17], and

$\overline{RRF(m)}$ = mean relative response factor for $^{13}C_{12}$-labelled quantitation standard relative to its $^{13}C_{12}$-labelled internal standard [RRF(m), with m = 1 to 15].

6.2.3 Verify and document that the continuing calibration verification meets the acceptance criteria outlined in Table 7 (Section 3.3.2.4).

NOTE: If EPA Method 1613 reporting has not been specifically requested by the client, the analyst may use the alternate calculation procedure and acceptance criteria described in Section 6.2.4.

6.2.4 Refering to Table 2, calculate the 17 relative response factors (RRF) for unlabeled target analytes [RRF(n); n = 1 to 17] relative to their appropriate quantitation standards (Eq. 2, Section 6.1.4), and the RRFs for the $^{13}C_{12}$-labelled quantitation standards [RRF(m); m = 1 to 15] relative to the two internal standards (Eq. 3, Section 6.1.4).

6.2.5 Verify and document that the continuing calibration verification meets the acceptance criteria outlined in Section 3.3.2.5.

7.0 ANALYTICAL STANDARDS

Analytical standards are purchased as solutions with certified purity, concentration and authenticity. The Labelled Compound Spiking Solution (LCSS), the Cleanup Recovery Standard (CRS), the Precision and Recovery standard (PAR), the Internal Standard (IS) and the instrument Calibration Solutions (CS1 - CS5) are used as received from the manufacturer without further treatment. When not being used, standards are stored in the dark at 40°C in screw-capped vials with PTFE-lined caps.

7.1 Labelled Compound Spiking Solution (LCSS)

This solution contains the fifteen $^{13}C_{12}$-labelled PCDD and PCDF quantitation standards in nonane at the nominal concentrations listed in Table 9. Twenty (20) µL of the LCSS is diluted in an appropriate solvent and spiked into each sample prior to extraction.

7.2 Cleanup Recovery Standard (CRS)

This solution contains the cleanup recovery standard $^{37}Cl_4$-2,3,7,8-TCDD in nonane at the nominal concentration listed in Table 9. Five (5) µL of this solution is diluted in an appropriate solvent and spiked into each sample extract immediately after the extraction and before any cleanup procedures are started.

7.3 Precision and Recovery Standard (PAR)

The solution contains the seventeen 2,3,7,8-substituted native PCDD and PCDF isomers at the nominal concentrations listed in Table 9. Five (5) µL of this solution is diluted in an appropriate solvent and spiked into the selected Matrix Spike (MS), Matrix Spike Duplicate (MSD), and/or Method Blank Spike (LBS) samples prior to extraction.

7.4 Internal Standard (IS)

This solution contains two $^{13}C_{12}$-labelled PCDD isomers at the nominal concentrations listed in Table 9. Ten (10) µL of the IS are added to the final sample extract before HRGC/HRMS analysis to determine the percent recoveries for the LCSS and CRS compounds.

7.5 Instrument Calibration Standards (CS1 - CS5)

These five solutions contain the seventeen 2,3,7,8-substituted native PCDDs and PCDFs, the $^{37}Cl_4$-2,3,7,8-TCDD cleanup standard, and the sixteen $^{13}C_{12}$-labelled quantitation and internal standards at the nominal concentrations listed in Table 3. The native analyte concentrations are homologue dependent, with the lowest concentrations for the tetrachlorinated dioxin and furan and the highest concentrations for the octachlorinated congeners. These solutions permit the relative response factors to be measured as a function of concentration. The CS3 standard is also used as the continuing calibration verification (VER).

7.6 Column Performance and Window Defining Mix (CPSM/WDM)

This solution contains the first and last eluting isomers (DB5MS column) for each homologous series from tetra- through heptachlorinated congeners. The solution also contains a series of other TCDD isomers for the purpose of documenting the chromatographic resolution of 2,3,7,8-TCDD on a DB5MS column.

The $^{13}C_{12}$-2378-TCDD is present for positive identification of the 2,3,7,8-TCDD isomer. See Table 5.

7.7 2,3,7,8-TCDF CPSM

This solution contains a series of three TCDF isomers for the purpose of documenting the chromatographic resolution of 2,3,7,8-TCDF on a DB225 analytical column. See Table 5.

8.0 REQUIRED SAMPLE DOCUMENTATION AND IDENTIFICATION

Copies of the following documents must accompany the sample set in a labelled folder when it is delivered to HRGC/HRMS analysis group:

Chain-of-custody documents
Sample Information Sheet(s)
Analysis Request Form(s)
Laboratory bench sheet
Dry weight bench sheet
Percent lipid bench sheet
Sample Action Request Form(s)

9.0 SAMPLE ANALYSIS

9.1 Tune the instrument with PFK as described in Section 3.1.1.

9.2 Inject 2 µL of the CPSM/WDM (Table 5) and acquire SIM mass spectral data as described in Section 3.1.1. Demonstrate and document that the criteria listed in Section 3.1.2 are met.

9.3 Inject 2 µL of the CS3 Calibration Verification solution (Table 3) and acquire SIM mass spectral data as described in Section 3.1.1. Demonstrate and document that the criteria listed in Section 3.3.2 are met.

NOTE: Alternately, samples may be analyzed immediately after an initial calibration if all the criteria listed in Section 3.3.1 have been met.

9.4 Inject 2 mL of toluene and acquire SIM mass spectral data as described in Section 3.1.1. Demonstrate and document that the analytical system is free of interfering contamination.

9.5 Inject 2 mL of the sample extract and acquire SIM mass spectral data under the same conditions that have been established to produce acceptable results, above.

9.6 Qualitative Identification

For a gas chromatographic peak to be identified as a PCDD or PCDF isomer, it must meet all of the criteria specified in Section 3.2.

9.7 Quantitative Identification

9.7.1 For gas chromatographic peaks that have met all the qualitative identification criteria, calculate the concentration of the PCDD or PCDF isomers using the following equation:

$$Eq.\ 8\ Cx = \frac{Ax * Qqs}{Aqs * W * \overline{RRF(n)}}$$

where:

Cx = concentration of the unlabeled PCDD and PCDF isomer in pg/g (ppt),

Ax = sum of the integrated ion abundances of the quantitation ions for unlabeled PCDDs and PCDFs,

Aqs = sum of the integrated ion abundances of the quantitation ions for the $^{13}C_{12}$-labelled quantitation standards,

Qqs = amount in pg of the $^{13}C_{12}$-labelled quantitation standard added to the sample immediately prior to extraction,

RRF(n) = mean relative response factor for the unlabeled target analyte relative to its $^{13}C_{12}$-labelled quantitation standard [RRF(n), with n = 1 to 17], and

W = weight, in grams, of the sample.

NOTE: If the analyte is identified as one of the 2,3,7,8-substituted PCDDs or PCDFs, the value for RRF(n) is used as calculated in Equation 4. If the analyte is a non-2,3,7,8-substituted congener the average of all RRFs for the homologous series is used in the calculation.

NOTE: If the sample is a water, the sample size is represented in liters (L), and the resulting concentration is in pg/L (ppq).

9.7.2 Calculate the percent recovery of the fifteen quantitation standards in the sample extract using the following equation:

$$Eq.\ 9\ \%\ \text{Recovery} = \frac{Aqs * Qis * 100}{Ais * Qqs * \overline{RRF(m)}}$$

where:

Aqs = sum of the integrated ion abundances of the quantitation ions for the $^{13}C_{12}$-labelled quantitation standards,

Ais = sum of the integrated ion abundances of the quantitation ions for the $^{13}C_{12}$-labelled internal standards,

Qqs = amount in pg of the $^{13}C_{12}$-labelled quantitation standard added to the sample immediately prior to extraction,

Qis = amount in pg of the $^{13}C_{12}$-labelled internal standard added to the sample extract in the last step of preparation,

$\overline{RRF(m)}$ = mean relative response factor for $^{13}C_{12}$-labelled quantitation standard relative to its $^{13}C_{12}$-labelled internal standard [RRF(m), with m = 1 to 15].

9.7.3 If the concentration in the final extract of any of the fifteen 2,3,7,8-substituted PCDD/PCDF compounds exceeds the upper method calibration limit listed in Table 3, the sample extract is diluted by an appropriate dilution factor and reanalyzed.

9.7.4 If requested, the total concentration for each homologous series of PCDD and PCDF is calculated by summing up the concentration of all positively identified isomers of each homologous series. The total should also include the 2,3,7,8-substituted congeners.

9.7.5 A sample specific Estimated Detection Limit (EDL) is calculated for each 2,3,7,8-substituted congener that is not identified, regardless of whether or not other non-2,3,7,8-substituted isomers are present. The EDL is the concentration of a given analyte required to produce a signal with a peak height of at least 3 times the background signal level. The quantitation software (OPUSQuan) supplied by the instrument manufacturer calculates a sample specific-EDL using an algorithm based on the criteria outlined in EPA Method 8290, Section 7.9.5.1.1.

9.7.6 Calculate and report the Relative Percent Difference (RPD) between duplicate sample results.

9.7.7 Calculate and report the % Recovery of native analytes in the Matrix Spike (MS) and Matrix Spike Duplicate (MSD) samples.

9.8 Confirmation Analysis

If the 2,3,7,8-TCDF isomer is identified at a concentration which is above the Minimum Level, the 2,3,7,8-TCDF concentration must be confirmed on a second analytical column (DB225) which is isomer specific for that analyte.

9.8.1 Using the steps outlined in 9.1 through 9.6, reanalyze the sample extract on a DB225 analytical column. Use the GC conditions described in Section 4.3.2, and the mass spectral

conditions described in section 3.1.1, monitoring only the ions listed in the first descriptor (Table 4).

9.8.2 Recalculate the concentration of 2,3,7,8-TCDF using Equation 8.

9.8.3 Report the lower of the two calculated concentrations for 2,3,7,8-TCDF (DB5 and DB225).

10.0 INSTRUMENT MAINTENANCE

10.1 Gas Chromatograph Maintenance

10.1.1 The syringe is cleaned by rinsing with appropriate solvent after each injection.

10.1.2 A new injection port liner and septum are installed at the beginning of each new run sequence, or more frequently if required.

10.1.3 A new injection port base plate is installed as needed when chromatographic resolution falls below the QC acceptance criteria.

10.1.4 One to two feet of the analytical column are removed as needed when chromatographic resolution falls below the QC acceptance criteria.

10.1.5 The tank of carrier gas (He) is replaced when the pressure falls below 500 psi.

10.1.6 All maintenance is recorded in the "HRGC/HRMS Maintenance Log" or noted in the instrument log book.

10.2 Mass Spectrometer Maintenance

10.2.1 The high emission filament is replaced as necessary.

10.2.2 The inner ion source assembly is cleaned and replaced as necessary.

10.2.3 The outer ion source assembly is cleaned and replaced as necessary.

10.2.4 The septum on the PFK reservoir is replaced as necessary.

10.2.5 The transfer line/re-entrant assembly is disassembled, cleaned, repaired and reassembled as necessary.

10.2.6 The rotary pump oil is changed yearly, or more frequently if indicated.

10.2.7 The diffusion pump oil is changed as necessary.

10.2.8 All maintenance is recorded in the "HRGC/HRMS Maintenance Log" or noted in the instrument log book.

11.0 DOCUMENTATION REQUIRED FOR ANALYTICAL RESULTS

11.1 All maintenance and repairs made to the gas chromatograph and/or the mass spectrometer are recorded in the "HRGC/HRMS Maintenance Log". Simple or routine operations (e.g.,. changing the filament) can be noted in the "HRGC/HRMS Instrument Run Log".

11.2 All injections and analytical run sequences are recorded in the "HRGC/HRMS Instrument Run Log".

11.3 For all Mass Spectral Calibrations document the instrument resolving power by recording the peak profile of the reference compound peaks within each descriptor. The format of the peak profile representation must allow manual determination of the resolution, i.e., the horizontal axis must be a calibrated mass scale. (Also see Section 3.2.1.3.)

11.4 For all Initial Calibrations the following documentation is printed and maintained for a period of not less than one year.

11.4.1 The Selected Ion Current Profile (SICP) for each ion in each calibration run (CS1 - CS5), including any manual integrations,

11.4.2 Listing of retention times and peak areas for all target analytes in each calibration run (CS1 - CS5),

11.4.3 Listing of the calculated Relative Response Factors (RRF(n) andRRF(m)) for all target analytes in each calibration run (CS1 - CS5), and

11.4.4 Listing of the calculated Average Relative Response Factors ($\overline{RRF(n)}$ and $\overline{RRF(m)}$) for all target analytes, the standard deviation and percent relative standard deviation for each $\overline{RRF(n)}$ and $\overline{RRF(m)}$.

11.5 For all Continuing Calibrations the following documentation is printed and maintained for a period of not less than one year.

11.5.1 The Selected Ion Current Profile (SICP) for each ion in the calibration run (CS3), including any manual integrations,

11.5.2 Listing of retention times and peak areas for all target analytes in the calibration run (CS3),

11.5.3 Listing of the analyte concentrations for all target analytes in the calibration run (CS3), and

11.5.4 Listing of the analyte concentrations compared to the QC acceptance criteria listed in Table 7. For EPA Method 1613 reporting this listing is called "Form 4A" and "Form 4B".

11.6 For all CPSM/WDM analytical runs the following documentation is printed and maintained for a period of not less than one year.

11.6.1 The SICP for one dioxin ion and one furan ion from each of the first four descriptors, and

11.6.2 The SICP for m/z 321.8936 and m/z 333.9339 demonstrating chromatographic resolution of the 2,3,7,8-TCDD isomer.

11.7 For all 2,3,7,8-TCDF CPSM analytical runs the following documentation is printed and maintained for a period of not less than one year.

11.7.1 The SICP for m/z 305.8987 demonstrating chromatographic resolution of the 2,3,7,8-TCDF isomer.

11.8 For all toluene instrument blanks the following documentation is printed and maintained for a period of not less than one year.

11.8.1 The Selected Ion Current Profile (SICP) for each ion in the blank run demonstrating that the analytical system is free from contaminating interferences.

11.9 For all analytical and laboratory Quality Control samples the following documentation is printed and maintained for a period of not less than one year (actual retention of analytical data is determined by the contract guidelines, but shall not be less than one year).

11.9.1 The Selected Ion Current Profile (SICP) for each ion in the analytical run, including any manual integrations,

11.9.2 Listing of retention times and peak areas for all target analytes in the analytical run,

11.9.3 Listing of the calculated concentrations of all target analytes, percent recovery of the $^{13}C_{12}$-labelled quantitation standards, and Estimated Detection Limits (EDLs) in the analytical run, and

11.9.4 Compiled data report in either the GERG standard format or other customized format requested by the client. The compiled data report includes forms calculating Relative Percent Difference (RPD) between duplicate analyses and calculating percent recovery of native analytes in LBS and MS/MSD QC samples.

Table 1. Chlorinated Dibenzo-*p*-Dioxins and Furans Determined by Isotope Dilution High Resolution Gas Chromatography (HRGC)/High Resolution Mass Spectrometry (HRMS).

CDDs/CDFs[a]	CAS Registry	Labeled Analog	CAS Registry
2,3,7,8-TCDD	1746-01-6	$^{13}C_{12}$-2,3,7,8-TCDD	76523-40-5
		$^{37}Cl_4$-2,3,7,8-TCDD	85508-50-5
Total TCDD	41903-57-5	----	----
2,3,7,8-TCDF	51207-31-9	$^{13}C_{12}$-2,3,7,8-TCDF	89059-46-1
Total-TCDF	55722-27-5	----	----
1,2,3,7,8-PeCDD	40321-76-4	$^{13}C_{12}$-1,2,3,7,8-PeCDD	109719-79-1
Total-PeCDD	36088-22-9	----	----
1,2,3,7,8-PeCDF	57117-41-6	$^{13}C_{12}$-1,2,3,7,8-PeCDF	109719-77-9
2,3,4,7,8-PeCDF	57117-31-4	$^{13}C_{12}$-2,3,4,7,8-PeCDF	116843-02-8
Total-PeCDF	30402-15-4	----	----
1,2,3,4,7,8-HxCDD	39227-28-6	$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	109719-80-4
1,2,3,6,7,8-HxCDD	57653-85-7	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	109719-81-5
1,2,3,7,8,9-HxCDD	19408-74-3	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	109719-82-6
Total HxCDD	34465-46-8	----	----
1,2,3,4,7,8-HxCDF	70648-26-9	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	114423-98-2
1,2,3,6,7,8-HxCDF	57117-44-9	$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	116843-03-9
1,2,3,7,8,9-HxCDF	72918-21-9	$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	116843-04-0
2,3,4,6,7,8-HxCDF	60851-34-5	$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	116843-05-1
Total-HxCDF	55684-94-1	----	----
1,2,3,4,6,7,8-HpCDD	35822-46-9	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	109719-83-7
Total-HpCDD	37871-00-4	----	----
1,2,3,4,6,7,8-HpCDF	67562-39-4	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	109719-84-8
1,2,3,4,7,8,9-HpCDF	55673-89-7	$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	109719-94-0
Total-HpCDF	38998-75-3	----	----
OCDD	3268-87-9	$^{13}C_{12}$-OCDD	114423-97-1
OCDF	39001-02-0	not used	

[a] Chlorinated dibenzo-*p*-dioxins and chlorinated dibenzofurans

TCDD = Tetrachlorodibenzo-*p*-dioxin
PeCDD = Pentachlorodibenzo-*p*-dioxin
HxCDD = Hexachlorodibenzo-*p*-dioxin
HpCDD = Heptachlorodibenzo-*p*-dioxin
OCDD = Octachlorodibenzo-*p*-dioxin

TCDF = Tetrachlorodibenzofuan
PeCDF = pentachlorodibenzofuran
HxCDF = Hexachlorodibenzofuran
HpCDF = Heptachlorodibenzofuran
OCDF = Octachlorodibenzofuran

Table 2. Retention Time References, Quantitation References, Relative Retention Times, and Minimum Levels for CDDs and CDFs.

CDD/CDF	Retention time and quantitation reference	Relative retention time[c]	Minimum level[a] Water (pg/L; ppq)	Solid (pg/g; ppt)	Extract (pg/µL; ppb)
Compounds using $^{13}C_{12}$-1-2,3,4-TCDD as the injection internal standard					
2,3,7,8-TCDF	$^{13}C_{12}$-2,3,7,8-TCDF	0.999-1.003	10	1	0.5
2,3,7,8-TCDD	$^{13}C_{12}$-2,3,7,8-TCDD	0.999-1.002	10	1	0.5
1,2,3,7,8-PeCDF	$^{13}C_{12}$-1,2,3,7,8-PeCDF	0.999-1.002	50	5	2.5
2,3,4,7,8-PeCDF	$^{13}C_{12}$-2,3,4,7,8-PeCDF	0.999-1.002	50	5	2.5
1,2,3,7,8-PeCDD	$^{13}C_{12}$-1,2,3,7,8-PeCDD	0.999-1.002	50	5	2.5
$^{13}C_{12}$-2,3,7,8-TCDF	$^{13}C_{12}$-1,2,3,4-TCDD	0.923-1.103			
$^{13}C_{12}$-2,3,7,8-TCDD	$^{13}C_{12}$-1,2,3,4-TCDD	0.976-1.043			
$^{37}Cl_4$-2,3,7,8-TCDD	$^{13}C_{12}$-1,2,3,4-TCDD	0.989-1.052			
$^{13}C_{12}$-1,2,3,7,8-PeCDF	$^{13}C_{12}$-1,2,3,4-TCDD	1.000-1.425			
$^{13}C_{12}$-2,3,4,7,8-PeCDF	$^{13}C_{12}$-1,2,3,4-TCDD	1.011-1.526			
$^{13}C_{12}$-1,2,3,7,8-PeCDD	$^{13}C_{12}$-1,2,3,4-TCDD	1.000-1.567			
Compounds using $^{13}C_{12}$-1,2,3,7,8,9-HxCDD as the injection internal standard					
1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	0.999-1.001	50	5	2.5
1,2,3,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	0.997-1.005	50	5	2.5
1,2,3,7,8,9-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	0.999-1.001	50	5	2.5
2,3,4,6,7,8-HxCDF	$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	0.999-1.001	50	5	2.5
1,2,3,4,7,8-HxCDD	$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	0.999-1.001	50	5	2.5
1,2,3,6,7,8-HxCDD	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	0.998-1.004	50	5	2.5
1,2,3,7,8,9-HxCDD	[b]	1.000-1.019	50	5	2.5
1,2,3,4,6,7,8-HpCDF	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	0.999-1.001	50	5	2.5
1,2,3,4,7,8,9-HpCDF	$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	0.999-1.001	50	5	2.5
1,2,3,4,6,7,8-HpCDF	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	0.999-1.001	50	5	2.5
OCDF	$^{13}C_{12}$-OCDD	0.999-1.008	100	10	5.0
OCDD	$^{13}C_{12}$-OCDD	0.999-1.001	100	10	5.0
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.944-0.970			
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.949-0.975			

Table 2. Cont.

CDD/CDF	Retention time and quantitation reference	Relative retention time[c]	Minimum level[a] Water (pg/L; ppq)	Solid (pg/g; ppt)	Extract (pg/µL; ppb)
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.977-1.047			
$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.959-1.021			
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.977-1.000			
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.981-1.003			
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	1.043-1.085			
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	1.057-1.151			
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	1.086-1.110			
$^{13}C_{12}$-OCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	1.032-1.311			

[a]The Minimum Level (ML) for each analyte is defined as the level at which the entire analytical system must give a recognizable signal and acceptable calibration point. It is equivalent to the concentration of the lowest calibration standard, assuming that all method-specific sample weights, volumes, and cleanup procedures have been employed.

[b]The retention time reference for 1,2,3,7,8,9-HxCDD is $^{13}C_{12}$-1,2,3,6,7,8-HxCDD, and 1,2,3,7,8,9-HxCDD is quantified using the averaged responses for $^{13}C_{12}$-1,2,3,4,7,8-HxCDD and $^{13}C_{12}$-1,2,3,6,7,8-HxCDD.

[c]Relative retention time criteria apply to reporting for EPA Method 1613. Unless EPA Method 1613 is specifically required, the -1 to + 3 second RT criteria are used (Section 3.2.1.1).

Table 3. Concentration of PCDDs and PCDFs in Calibration and Calibration Verification Solutions.

CDD/CDF	CS1 (pg/µL)	CS2 (pg/µL)	VER CS3 (pg/µL)	CS4 (pg/µL)	CS5 (pg/µL)
2,3,7,8-TCDD	0.5	2	10	40	200
2,3,7,8-TCDF	0.5	2	10	40	200
1,2,3,7,8-PeCDD	2.5	10	50	200	1000
1,2,3,7,8-PeCDF	2.5	10	50	200	1000
2,3,4,7,8-PeCDF	2.5	10	50	200	1000
1,2,3,4,7,8-HxCDD	2.5	10	50	200	1000
1,2,3,6,7,8-HxCDD	2.5	10	50	200	1000
1,2,3,7,8,9-HxCDD	2.5	10	50	200	1000
1,2,3,4,7,8-HxCDF	2.5	10	50	200	1000
1,2,3,6,7,8-HxCDF	2.5	10	50	200	1000
1,2,3,7,8,9-HxCDF	2.5	10	50	200	1000
2,3,4,6,7,8-HxCDF	2.5	10	50	200	1000
1,2,3,4,6,7,8-HpCDD	2.5	10	50	200	1000
1,2,3,4,6,7,8-HpCDF	2.5	10	50	200	1000
1,2,3,4,7,8,9-HpCDF	2.5	10	50	200	1000
OCDD	5.0	20	100	400	2000
OCDF	5.0	20	100	400	2000
$^{13}C_{12}$-2,3,7,8-TCDD	100	100	100	100	100
$^{13}C_{12}$-2,3,7,8-TCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8-PeCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8-PeCDF	100	100	100	100	100
$^{13}C_{12}$-2,3,4,7,8-PeCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	100	100	100	100	100
$^{13}C_{12}$-OCDD	200	200	200	200	200
Cleanup Standard					
$^{37}Cl_4$-2,3,7,8-TCDD	0.5	2	10	40	200
Internal Standards					
$^{13}C_{12}$-1,2,3,4-TCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	100	100	100	100	100

Table 4. Descriptors, Exact m/z's, m/z Types, and Elemental Compositions of the CDDs and CDFs.

Descriptor	Exact m/z[a]	m/z type	Elemental Composition	Substance[b]
1	292.9825	Lock	$C_7 F_{11}$	PFK
	303.9016	M	$C_{12} H_4 {}^{35}Cl_4 O$	TCDF
	305.8987	M+2	$C_{12} H_4 {}^{35}Cl_3 {}^{37}Cl O$	TCDF
	315.9419	M	$^{13}C_{12} H_4 {}^{35}CL_4 O$	TCDF[c]
	317.9389	M+2	$^{13}C_{12} H_4 {}^{35}Cl_4 {}^{37}Cl_3 O$	TCDF[c]
	319.8965	M	$C_{12} H_4 {}^{35}Cl_4 O_2$	TCDD
	321.8936	M+2	$C_{12} H_4 {}^{35}Cl_3 O_2$	TCDD
	327.8847	M	$C_{12} H_4 {}^{37}Cl_4 O_2$	TCDD[d]
	330.9792	QC	$C_7 F_{13}$	PFK
	331.9368	M	$^{13}C_{12} H_4 {}^{35}Cl_4 O_2$	TCDD[c]
	333.9339	M+2	$^{13}C_{12} H_4 {}^{35}Cl_3 {}^{37}Cl O_2$	TCDD[c]
	375.8364	M+2	$C_{12} H_4 {}^{35}Cl_5 {}^{37}Cl O$	HxCDPE
2	339.8597	M+2	$C_{12} H_3 {}^{35}Cl_4 {}^{37}Cl O$	PeCDF
	341.8567	M+4	$C_{12} H_3 {}^{35}Cl_3 {}^{37}Cl_2 O$	PeCDF
	351.9000	M+2	$^{13}C_{12} H_3 {}^{35}Cl_4 {}^{37}Cl O$	PeCDF
	353.8970	M+4	$^{13}C_{12} H_3 {}^{35}Cl_3 {}^{37}Cl_2 O$	PeCDF[c]
	354.9792	Lock	$C_9 F_{13}$	PFK
	355.8546	M+2	$C_{12} H_3 {}^{35}Cl_4 {}^{37}Cl O_2$	PeCDD
	357.8516	M+4	$C_{12} H_3 {}^{35}Cl_3 {}^{37}Cl_2 O_2$	PECDD
	367.8949	M+2	$^{13}C_{12} H_3 {}^{35}Cl_4 {}^{37}Cl O_2$	PeCDD[c]
	369.8919	M+4	$^{13}C_{12} H_3 {}^{35}Cl_3 {}^{37}Cl_2 O_2$	PeCDD[c]
	409.7974	M+2	$C_{12} H_3 {}^{35}Cl_6 {}^{37}Cl O$	HpCDPE
3	373.8208	M+2	$C_{12} H_2 {}^{35}Cl_5 {}^{37}Cl O$	HxCDF
	375.8178	M+4	$C_{12} H_2 {}^{35}Cl_4 {}^{37}Cl_2 O$	HxCDF
	383.8639	M	$^{13}C_{12} H_2 {}^{35}Cl_6 O$	HxDDF[c]
	385.8610	M+2	$^{13}C_{12} H_2 {}^{35}Cl_5 {}^{37}Cl O$	HxCDF[c]
	389.8157	M+2	$C_{12} H_2 {}^{35}Cl_5 {}^{37}Cl O_2$	HxCDD
	391.8127	M+4	$C_{12} H_2 {}^{35}Cl_4 {}^{37}Cl_2 O_2$	HxCDD
	392.9760	Lock	$C_9 F_{15}$	PFK
	401.8559	M+2	$^{13}C_{12} H_2 {}^{35}Cl_5 {}^{37}Cl O_2$	HxCDD[c]
	403.8529	M+4	$^{13}C_{12} H_2 {}^{35}Cl_4 {}^{37}Cl_2 O_2$	HxCDD[c]
	430.9729	QC	$C_9 F_{17}$	PFK
	445.7555	M+4	$C_{12} H_2 {}^{35}Cl_6 {}^{37}Cl_2 O$	OCDPE

Table 4. Cont.

Descriptor	Exact m/z[a]	m/z type	Elemental Composition	Substance[b]
4	407.7818	M+2	C_{12} H $^{35}Cl_6$ ^{37}Cl O	HpCDF
	409.7789	M+4	C_{12} H $^{35}Cl_5$ $^{37}Cl_2$ O	HpCDF
	417.8253	M	$^{13}C_{12}$ H $^{35}CL_7$ O	HpCDF[c]
	419.8220	M+2	$^{13}C_{12}$ H $^{35}Cl_6$ ^{37}Cl O	HpCDF[c]
	423.7766	M+2	C_{12} H $^{35}Cl_6$ ^{37}Cl O_2	HpCDD
	425.7737	M+4	C_{12} H $^{35}Cl_5$ $^{37}Cl_2$ O_2	HpCDD
	430.9729	Lock	C_9 F_{17}	PFK
	435.8169	M+2	$^{13}C_{12}$ H $^{35}Cl_6$ ^{37}Cl O_2	HpCDD[c]
	437.8140	M+4	$^{13}C_{12}$ H $^{35}Cl_5$ $^{37}Cl_2$ O_2	HpCDD[c]
	479.7165	M+4	C_{12} H $^{35}Cl_7$ $^{37}Cl_2$ O	NCDPE
5	441.7428	M+2	C_{12} $^{35}Cl_7$ ^{37}Cl O	OCDF
	442.9728	Lock	C_{10} F_{17}	PFK
	443.7399	M+4	C_{12} $^{35}Cl_6$ $^{37}Cl_2$ O	OCDF
	457.7377	M+2	C_{12} $^{35}Cl_7$ ^{37}Cl O_2	OCDD
	459.7348	M+4	C_{12} $^{35}Cl_6$ $^{37}Cl_2$ O_2	OCDD
	469.7779	M+2	$^{13}C_{12}$ $^{35}Cl_7$ ^{37}Cl O_2	OCDD[c]
	471.7750	M+4	$^{13}C_{12}$ $^{35}Cl_6$ $^{37}Cl_2$ O_2	OCDD[c]
	513.6775	M+4	C_{12} $^{35}Cl_8$ $^{37}Cl_2$ O	DCDPE

a Nuclidic masses used:

H =	1.007825	C =	12.00000	^{13}C =	13.003355	F =	18.9984
O =	15.994915	^{35}Cl =	34.968853	^{37}Cl =	36.965903		

b
TCDD = Tetrachlorodibenzo-*p*-dioxin
PeCDD = Pentachlorodibenzo-*p*-dioxin
HxCDD = Hexachlorodibenzo-*p*-dioxin
HpCDD = Heptachlorodibenzo-*p*-dioxin
OCDD = Octachlorodibenzo-*p*-dioxin
HxCDPE = Hexachlorodiphenyl ether
OCDPE = Octachlorodiphenyl ether
DCDPE = Decachlorodiphenyl ether
TCDF = Tetrachlorodibenzofuran
PeCDF = Pentachlorodibenzofuran
HxCDF = Hexachlorodibenzofuran
HpCDF = Heptachlorodibenzofuran
OCDF = Octachlorodibenzofuran
HpCDPE = Heptachlorodibenzofuran
NCDPE = Nonachlorodiphenyl ether
PFK = Perfluorokerosene

c Labeled compound

d There is only one m/z for $^{37}Cl_4$-2,3,7,8-TCDD (cleanup standard)

Table 5. GC Retention Time Window Defining Solution and Isomer Specificity Test Standard (Section 3.1.2).

DB-5 Column GC Retention-Time Window Defining Solution

CDD/CDF	First Eluted	Last Eluted
TCDF	1,3,6,8-	1,2,8,9-
TCDD	1,3,6,8-	1,2,8,9-
PeCDF	1,3,4,6,8-	1,2,3,8,9-
PeCDD	1,2,4,7,9-	1,2,3,8,9-
HxCDF	1,2,3,4,6,8-	1,2,3,4,8,9
HxCDD	1,2,4,6,7,9-	1,2,3,4,6,7
HpCDF	1,2,3,4,6,7,8-	1,2,3,4,7,8,9
HpCDD	1,2,3,4,6,7,9-	1,2,3,4,6,7,8-

DB-5 Column TCDD Specificity Test Standard

1,2,3,7+1,2,3,8-TCDD
2,3,7,8-TCDD
1,2,3,9-TCDD

DB-225 Column TCDF Isomer Specificity Test Standard

2,3,4,7-TCDF
2,3,7,8-TCDF
1,2,3,9-TCDF

Table 6. Theoretical Ion Abundance Ratios and QC Limits

Number of Chlorine Atoms	m/z's Forming Ratio	Theoretical Ratio	QC Limit[a] Lower	QC Limit[a] Upper
4[b]	M/(M+2)	0.77	0.65	0.89
5	(M+2)/(M+4)	1.55	1.32	1.78
6	(M+2)/(M+4)	1.24	1.05	1.43
6[c]	M/(M+2)	0.51	0.43	0.59
7	(M+2)/(M+4)	1.05	0.88	1.20
7[d]	M/(M+2)	0.44	0.37	0.51
8	(M+2)/(M+4)	0.89	0.76	1.02

[a] QC limits represent ±15% windows around the theoretical ion abundance ratios.
[b] Does not apply to $^{37}Cl_4$-2,3,7,8-TCDD (cleanup standard).
[c] Used for $^{13}C_{12}$-HxCDF only.
[d] Used for $^{13}C_{12}$-HpCDF only.

Table 7. QC Acceptance Criteria for Performance Tests for PCDDs and PCDFs[a].

CDD/CDF	Test Conc (pg/µL)	IPR[b,c] s (pg/µL)	IPR[b,c] X (pg/µL)	OPR (pg/µL)	VER or Continuing Calibration (pg/µL)
2,3,7,8-TCDD	10	2.8	8.3-12.9	6.7-15.8	7.8-12.9
2,3,7,8-TCDF	10	2.0	8.7-13.7	7.5-15.8	8.4-12.0
1,2,3,7,8-PeCDD	50	7.5	38-66	35-71	39-65
1,2,3,7,8-PeCDF	50	7.5	43-62	40-67	41-60
2,3,4,7,8-PeCDF	50	8.6	36-75	34-80	41-61
1,2,3,4,7,8-HxCDD	50	9.4	39-76	35-82	39-64
1,2,3,6,7,8-HxCDD	50	7.7	42-62	38-67	39-64
1,2,3,7,8,9-HxCDD	50	11.1	37-71	32-81	41-61
1,2,3,4,7,8-HxCDF	50	8.7	41-59	36-67	45-56
1,2,3,6,7,8-HxCDF	50	6.7	46-60	42-65	44-57
1,2,3,7,8,9-HxCDF	50	6.4	42-61	39-65	45-56
2,3,4,6,7,8-HxCDF	50	7.4	37-74	35-78	44-57
1,2,3,4,6,7,8-HpCDD	50	7.7	38-65	35-70	43-58
1,2,3,4,6,7,8-HpCDF	50	6.3	45-56	41-61	45-55
1,2,3,4,7,8,9-HpCDF	50	8.1	43-63	39-69	43-58
OCDD	100	19	89-127	78-144	79-126
OCDF	100	27	74-146	63-170	63-159
$^{13}C_{12}$-2,3,7,8-TCDD	100	37	28-134	20-175	82-121
$^{13}C_{12}$-2,3,7,8-TCDF	100	35	31-113	22-152	71-140
$^{13}C_{12}$-1,2,3,7,8-PeCDD	100	39	27-184	21-227	62-160
$^{13}C_{12}$-1,2,3,7,8-PeCDF	100	34	27-156	21-192	76-130
$^{13}C_{12}$-2,3,4,7,8-PeCDF	100	38	16-279	13-328	77-130
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	100	41	29-147	21-193	85-117
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	100	38	34-122	25-163	85-118
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	100	43	27-152	19-202	76-131
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	100	35	30-122	21-159	70-143
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	100	40	24-157	17-205	74-135
$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	100	37	29-136	22-176	73-137
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	100	35	34-129	26-166	72-138
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	100	41	32-110	21-158	78-129
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	100	40	28-141	20-186	77-129
$^{13}C_{12}$-OCDD	200	95	41-276	26-397	96-415
$^{37}Cl_4$-2,3,7,8-TCDD	10	3.6	3.9-15.4	3.1-19.1	7.9-12.7

a All specifications are given as concentration in the final extract, assuming a 20-µL volume.

b s = standard deviation of the concentration

c X = average concentration
IPR = Initial Precision and Recovery; four diluted aliquots of the precision and recovery used initially to establish acceptable precision and accuracy. It is also used after any method or instrumental modification to verify precision and accuracy.
OPR = Ongoing Precision and Recovery standard; an LBS analyzed like a sample to assure laboratory results remain within specified precision and recovery limits.

Table 8. QC Acceptance Criteria for EPA Method 1613 Labeled Compound Spike Solution (LCSS) Recovery in Samples When PCDDs and PCDFs Are Tested.

Compound	Test Conc (pg/µL)	Labeled Compound Recovery (pg/µL)[a]	(%)
$^{13}C_{12}$-2,3,7,8-TCDD	100	25-164	25-164
$^{13}C_{12}$-2,3,7,8-TCDF	100	24-169	24-169
$^{13}C_{12}$-1,2,3,7,8-PeCDD	100	25-181	25-181
$^{13}C_{12}$-1,2,3,7,8-PeCDF	100	24-185	24-185
$^{13}C_{12}$-2,3,4,7,8-PeCDF	100	21-178	21-178
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	100	32-141	32-141
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	100	28-130	28-130
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	100	26-152	26-152
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	100	26-123	26-123
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	100	29-147	29-147
$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	100	28-136	28-136
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	100	23-140	23-140
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	100	28-143	28-143
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	100	26-138	26-138
$^{13}C_{12}$-OCDD	200	34-313	17-157
$^{37}Cl_4$-2,3,7,8-TCDD	10	3.5-19.7	35-197

[a] Specification given as concentration in the final extract, assuming a 20-µL volume.

Table 9. Concentration of Stock and Spiking Solutions Containing PCDDs and PCDFs Labeled Compounds.

CDD/CDF	Labeled Compound Spiking Solution (LCSS) (pg/µL)	PAR Spiking Solution (pg/µL)
2,3,7,8-TCDD	----	40
2,3,7,8-TCDF	----	40
1,2,3,7,8-PeCDD	----	200
1,2,3,7,8-PeCDF	----	200
2,3,4,7,8-PeCDF	----	200
1,2,3,4,7,8-HxCDD	----	200
1,2,3,6,7,8-HxCDD	----	200
1,2,3,7,8,9-HxCDD	----	200
1,2,3,4,7,8-HxCDF	----	200
1,2,3,6,7,8-HxCDF	----	200
1,2,3,7,8,9-HxCDF	----	200
2,3,4,6,7,8-HxCDF	----	200
1,2,3,4,6,7,8-HpCDD	----	200
1,2,3,4,6,7,8-HpCDF	----	200
1,2,3,4,7,8,9-HpCDF	----	200
OCDD	----	400
OCDF	----	400
$^{13}C_{12}$-2,3,7,8-TCDD	100	----
$^{13}C_{12}$-2,3,7,8-TCDF	100	----
$^{13}C_{12}$-1,2,3,7,8-PeCDD	100	----
$^{13}C_{12}$-1,2,3,7,8-PeCDF	100	----
$^{13}C_{12}$-2,3,4,7,8-PeCDF	100	----
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	100	----
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	100	----
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	100	----
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	100	----
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	100	----
$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	100	----
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	100	----
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	100	----
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	100	----
$^{13}C_{12}$-OCDD	200	----

		Concentration (pg/µL)
Cleanup Standard	$^{37}Cl_4$-2,3,7,8-TCDD	0.8
Internal Standards	$^{13}C_{12}$-1,2,3,4,-TCDD	200
	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	200

SOP-9724
PURIFICATION OF EXTRACTS BY GEL PERMEATION CHROMATOGRAPHY PRIOR TO THE ANALYSIS OF POLYNUCLEAR AROMATIC HYDROCARBONS (PAH) AND ORGANOCHLORINE PESTICIDES (OC)/POLYCHLORINATED BIPHENYLS (PCB) ANALYSIS

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURES

SOP-9724

PURIFICATION OF EXTRACTS BY GEL PERMEATION CHROMATOGRAPHY PRIOR TO THE ANALYSIS OF POLYNUCLEAR AROMATIC HYDROCARBONS (PAH) AND ORGANOCHLORINE PESTICIDES (OC)/POLYCHLORINATED BIPHENYLS (PCB) ANALYSIS

This document presents the procedures used in the performance of the above laboratory activities.

Quality Assurance Manager

5/2/97
Date

Author/Revision By: W. Keeney-Kennicutt

PURIFICATION OF EXTRACTS BY GEL PERMEATION CHROMATOGRAPHY PRIOR TO THE ANALYSIS OF POLYNUCLEAR AROMATIC HYDROCARBONS (PAH) AND ORGANOCHLORINE PESTICIDES (OC)/POLYCHLORINATED BIPHENYLS (PCB) ANALYSIS

1.0 PURPOSE

The document provides the procedures used for the purification of extracts by gel permeation chromatography techniques by the staff of the Geochemical and Environmental Research Group (GERG) of the College of Geosciences and Maritime Studies at Texas A&M University prior to the analysis of PAHs, OCs, and PCBs.

1.1 Summary of the Procedure

Gel permeation chromatography (GPC) is an extract purification step which uses a chromatographic column to separate components in a complex mixture based upon their molecular size or shape. The size exclusion column used for this procedure separates lipids and high molecular weight components from target chlorinated hydrocarbons and aromatic hydrocarbons in tissue extracts.

A tissue subsample is extracted and purified by column chromatography. Further purification of the concentrated sample extracts is then provided by size exclusion chromatography using an high performance liquid chromatograph (HPLC) equipped with an ultraviolet detector (254 nm), an autosampler, and an isocratic pump. Extracts are processed through a guard column in series with two size exclusion columns. Fractions are collected by a fraction collector. The purified extracts are then analyzed for PAHs or OCs/PCBs using the appropriate instrumental procedures.

1.2 Applicability

1.2.1 Matrix

This procedure is applicable to extracts from all biological tissues and may also be used on other extracts when required.

2.0 SAFETY

The hazards, toxicity or carcinogenicity of each compound or reagent used in GERG's standard operating procedures have not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory maintains Material Safety Data Sheets (MSDS) which contain information regarding the safe handling of chemicals used at GERG's facilities. A reference file of MSDS is available to all personnel involved with these materials. All laboratory personnel should direct any questions regarding safety issues to their supervisors or the Safety Officer.

3.0 QUALITY CONTROL

3.1 Process all quality control samples in a manner identical to actual samples.

3.2 The calibration is verified before each extraction set or after every 12 hours, as explained in Sections 5.2 and 5.3. The calibration consists of analyzing three GPC/HPLC calibration solutions and one blank. Adjustment and/or recalibration is performed until the criteria are met. Only after the criteria are met are samples purified.

3.2.1 The compounds contained in the HPLC standard are biphenyl, perylene and 4,4'-dibromooctafluorobiphenyl (DBOFB). DBOFB elutes first at approximately 13 minutes, followed by biphenyl and perylene at approximately 14 and 16 minutes, respectively for the Spectra-Physics/Gilson system. The retention times are approximately 15, 16, and 18 minutes, respectively, on the Hewlett-Packard 1050 system.

3.2.2 During calibration and calibration verification, the retention times of the three peaks should not vary more than ±5%, or approximately 0.6 minutes between the three chromatograms.

3.2.3 During calibration and calibration verification, the area count of the DBOFB peak should be >1,000,000 for the Spectra-Physics/Gilson HPLC system and >500,000 for the Hewlett-Packard 1050 HPLC system.

3.3 During purification of the sample extraction batch, HPLC blanks are run after every 5 samples, or more often if the extracts have a high lipid content or are highly colored (See Sections 5.6.4 and 5.7.4). A continuing calibration standard (CCS) is required after every 10 samples.

3.4 Each HPLC extraction batch must end with a blank and a CCS.

3.5 Note any unusual occurrences in the "Comments" section of the Laboratory Sample Logbook.

3.5.1 When using the Spectra-Physics/Gilson HPLC system, if the autosampler skips samples or combines collection extract waste (S-1) with the collection of the sample fraction (S-2), document the error and notify the Extraction Laboratory Manager.

3.6 Use standard laboratory practice when filling out all paperwork. Use black waterproof ink. When correcting an entry, strike a single line through the incorrect entry, then date and initial the change.

4.0 APPARATUS AND MATERIALS

4.1 Apparatus

Spectra-Physics/Gilson HPLC System:

Autosampler, Gilson model 231-401 or equivalent
HPLC isocratic pump, Spectra-Physics 8000 or equivalent.
Two size-exclusion columns connected in series, 22.5 x 250 mm Phenogel 100 A columns, or equivalent, Phenomenex; Cat. #OOG-0642-PO.
Phenogel 100 A guard column, 7.8 x 50 mm or equivalent; Phenomenex; Cat. #03D-2090-KO.
UV Absorbance detector, Waters 440 or equivalent, 254 nm
Hewlett-Packard 3396 integrator
Fraction collector, LBK 2211 Super Rac or equivalent

Hewlett-Packard (HP) 1050 HPLC System:

HP autosampler
HP pump system.

Two size-exclusion columns connected in series, 22.5 x 250 mm Phenogel 100 A columns, or equivalent
Phenogel 100 A guard column, 7.8 x 50 mm or equivalent
HP UV Absorbance detector, 254 nm
HP 3396 integrator
Foxy fraction collector, or equivalent

Electrobalance, Cahn or equivalent, with an accuracy of 0.0001 mg

Nitrogen gas evaporation unit

Water bath, heated to 60-70°C

4.2 Labware

Boiling chips: Teflon, methylene chloride rinsed

Concentrator tubes, glass, 25 mL

Fraction collector vials, 25 x 100 mm, with glass funnel tops

HPLC tear drop 1 mL vials, screw-cap, glass with Teflon lined methylene chloride rinsed caps, Rainin or equivalent

HPLC tear drop 1 mL vials, crimp-top, glass with Teflon lined methylene chloride rinsed caps, National Scientific Company or equivalent.

Pasteur pipettes: 1 mL, glass, disposable

Sample vials, 2 mL, amber borosilicate glass with Teflon lined methylene chloride rinsed caps

As described in GERG SOP 9416, all glassware is washed and either rinsed with solvents or combusted at 440°C for 4 hours prior to use. All volumetric glassware is washed and solvent rinsed.

4.3 Chemicals

Biphenyl, 99% purity, Aldrich Cat. B3,465-6 or equivalent

4,4'-Dibromooctafluorobiphenyl (DBOFB), 99% purity, Aldrich Cat. #10,199-0 or equivalent

Perylene, 99% purity, Aldrich Cat. #P1,120-4 or equivalent

Methylene chloride, Burdick and Jackson Cat. #300-4; pesticide grade or equivalent, lot tested

Helium, 99.98% purity

4.4 Stock Solutions: Prepared in Methylene Chloride

4.4.1 Biphenyl (0.25 mg/mL)

Weigh 6.25 mg of biphenyl to three significant figures in a 25 mL ground glass stoppered volumetric flask and fill to the mark with methylene chloride. After the biphenyl is dissolved, transfer to a clean, labeled bottle with a Teflon-lined cap.

4.4.2 Perylene (0.125 mg/mL)

Weigh 3.125 mg of perylene to three significant figures in a 25 mL ground glass stoppered volumetric flask and fill to the mark with methylene chloride. After the perylene is dissolved, transfer to a clean, labeled bottle with a Teflon-lined cap.

4.4.3 4,4'-Dibromooctafluorobiphenyl (DBOFB) (0.125 mg/mL)

Weigh 3.75 mg of DBOFB to three significant figures in a 25 mL ground glass stoppered volumetric flask and fill to the mark with methylene chloride. After the DBOFB is dissolved, transfer to a clean, labeled bottle with a Teflon-lined cap.

4.5 GPC/HPLC Calibration Standards

4.5.1 Prepare GPC/HPLC calibration standards by adding 1 mL of each of the stock solutions described in Section 4.4 to a 250 mL ground glass stoppered volumetric flask and filling to the mark with methylene chloride. The resulting concentration of the GPC/HPLC calibration standard is shown below:

Biphenyl:	1 µg/mL
Perylene:	0.5 µg/mL
DBOFB:	0.5 µg/mL

4.6 Storage and Stability of Solutions

4.6.1 When not being used, all solid, stock, and diluted standards are stored in the dark at 0°C in the freezer. The solutions are brought to room temperature prior to use.

4.6.2 With proper storage, standard solutions are stable for 12 months.

5.0 PROCEDURE FOR GPC SAMPLE PURIFICATION

GPC purification is based upon size exclusion chromatography, using an HPLC equipped with an ultraviolet detector (254 nm), autosampler, and an isocratic pump. The sample extracts are purified by processing through a Phenogel 8x50 m m guard column in series with two size exclusion columns (22.5 x 250 mm Phenogel 100 A). Fractions are collected by the fraction collector. The mobile phase solvent, methylene chloride degassed with purified helium, is pumped for a total of 25 minutes per sample extract, at a flow rate of 7 mL/minute.

The X Chrom software, described in Section 5.1, is used for data acquisition, and provides both a hard copy and electronic copy of the calibration and extract purification sequence. See Sections 5.6 and 5.7 for the documentation requirements for the HPLC Maintenance and Analytical Batch Logbooks.

5.1 The X Chrom Sample Sequence and Data Acquisition Program

The following section describes the process for setting up the data acquisition using the X Chrom software. This must be completed before starting the calibration process so that all data is acquired onto the computer system when the GPC/HPLC instrument is started.

5.1.1 To log onto the Digital Computer in the GC/HPLC lab (GERGL2 system), use the following steps:

5.1.1.1 The first screen will appear:
"Start Session on GERGL2"

Enter the username, then press return
Enter the password, then press return

5.1.1.2 The second screen will appear:
"Session Application Options Help"
Under the APPLICATIONS menu, select X Chrom.

5.1.1.3 The third screen will appear:
"FileView X Chrom"
Close this screen by selecting Close.

5.1.1.4 The fourth screen will appear:
"X Chrom Main Window" (this will be your main screen for X Chrom)
Under the FILE menu, select Copy.

5.1.1.5 The Fifth Screen will appear:
"X Chrom Data Selection". The screen will ask which file to copy.
In the file menu (which lists files), find and select **lcmaster.**
The screen will then ask for a destination and a filename for the new file which is to be created. At the bottom of the window, under Data i.d. (e.g., Wong, Ch8.___), with the arrow click after the period and enter in the new page number or file name that you wish to "store the copy as" (e.g., Wong, Ch8.M1543). Channel 8 (Ch8) is the directory for all files analyzed using the Spectra-Physics/Gilson system and Channel 7 (Ch7) is the directory for all files analyzed using the Hewlett-Packard system. Hit return on the keyboard twice.

5.1.2 To set-up the run sequence, use the following steps:

5.1.2.1 Once the file is copied, go back to the X Chrom main window and under FILE, select **Open Analysis**
Click on the page number that was created in 5.1.1.4-5.1.1.5 to open the analysis file.

5.1.2.2 The Sixth Screen will appear with two sections to this display: Analysis Header and Sample Sequence, e.g., Analysis (Wong, ch. 8.M1543,0,0;1)
In the Analysis Header section, type in Analyst name, then press return.
Using the arrow keys on the keyboard to move the cursor to Analysis name. Enter the Analysis name (e.g., M1543), and then press return. Move the cursor to the Comments section, enter the project name (e.g., Fish and Wildlife) and press return.

5.1.2.3 Click on the "SAMPLE SEQUENCE" box at the bottom portion of the screen. This menu provides an outline of the GPC/HPLC calibration and analytical batch sequence. The first four calibration samples (3 standards and 1 blank) have already been specified. Continue typing in your sample sequence in the Sample ID column, starting with the QC samples and samples interspersed with HPLC blanks and calibration standards (see Section 3.3).
Under the SAMPLE ID column, enter the GERG I.D. number and press return.
Under SAMPLE NAME column, enter the client descriptor and press return.

5.1.2.4 In the sample TYPE column, HPLC standards should read "standard", HPLC blanks should read "Blank" and all samples (including QC) should read "sample". The entries can be changed to read either "sample", "blank", or "standard" by selecting the entry and then clicking on the middle button of the mouse.

5.1.2.5 Remove any of the unused rows by highlighting the row number, going to the EDIT menu and selecting **Delete Rows**.
To save the completed page, go to FILE menu and select **Save**.

5.1.2.6 Once the run sequence is saved, go to the FILE menu, and select **Exit** (you will return to the fourth screen, X Chrom main window).

5.1.3 To print a copy of your sample sequence:

5.1.3.1 Go to the PROCESS menu, and select **Report**. Make sure that the page to report is selected, listed in the Data ID entry and click OK.

5.1.3.2 Select **Sample Sequence** from the list of reporting types in the report menu and then click OK. Select the correct printer by clicking on "Change Printer", select "Portable 2 Post Script" and click on OK.

5.1.4 To prepare the computer for data acquisition:

5.1.4.1 Once the report is printing, go to the ACQUIRE menu, and select **Start Run**.

5.1.4.2 Select the correct analysis file to use as a template. Click on the box labeled START ANALYSIS, enter the file name (e.g., M1543) of your run and press return.

5.1.4.3 To make sure that the computer is ready to acquire data for your HPLC set, go to ACQUIRE menu again, and select **Status**.

5.1.4.4 The Acquisition Status screen should appear. Scroll down the menu and select **#8-CH. 8** (for Spectra-Physics/Gilson system) or **#7-CH. 7** (for Hewlett-Packard system). Make sure that the status reads "**wait inject**" and the correct analysis file is listed.

5.1.4.5 To close the Acquisition Status screen, go to the FILE menu, and select **Exit**.

5.1.6 To end the computer session:

5.1.6.1 Go to the FILE menu of the X Chrom Main window and select **Exit X Chrom**.

5.1.6.2 When the "Confirm" prompt appears, click on CONFIRM.

5.1.6.3 Under the "Session" Menu, select **End Session**. To confirm this command, click the YES when the "Confirm" prompt appears.

5.1.7 To print out a report after the analytical batch is complete:

5.1.7.1 Log onto the computer. Refer to Section 5.1.1.1-5.1.1.4.

5.1.7.2 On the X Chrom Main window screen under the Process menu, select **Detect**. Make sure that the correct file that you wish to report is selected and highlighted.

5.1.7.3 Once the "Detect" process is complete, under the Process menu select **Report** again. Make sure that the correct printer is selected ("Portable 2 Postscript").

5.1.7.4 Select "Chromatograph" and then "peak data-named peaks" from the list of data reports and then click OK.

5.1.7.5 Once your report is finished printing, you may log off as described in Section 5.1.6.

5.2 Spectra-Physics/Gilson HPLC System - Calibration

Prior to processing analytical extracts through the GPC/HPLC, the system performance is checked and the GPC/HPLC is calibrated. The calibration of the GPC/HPLC verifies the instrument performance based upon the retention time and area of the calibration standards. The time to start collecting the fractions for the actual samples is then determined by subtracting one minute from the retention time of DBOFB.

For this test, three GPC/HPLC calibration solution and a blank are used to verify the performance criteria. Adjustment and/or recalibration is performed until the criteria are met. Only after the criteria are met may samples be purified.

5.2.1 Before each calibration and/or extraction batch run, locate the GPC/HPLC calibration standard which is stored in the freezer at 0°C. The standard solution must be brought to room temperature prior to use.

5.2.2 Calibration standard vial preparation:

5.2.2.1 Approximately 20 vials are prepared in advance and are stored in the refrigerator until needed.

5.2.2.2 Using a disposable Pasteur pipette, transfer approximately 1 mL of the GPC/HPLC calibration solution into the Rainin tear-drop vials.

5.2.2.3 Seal the tear drop vials using screw caps with red dual-sided Teflon-lined septa pre-rinsed with methylene chloride.

5.2.2.4 Label the vials with an "S" to indicate standard and store in the designated refrigerator until needed.

5.2.3 Blank vial preparation:

5.2.3.1 Approximately 20 vials are prepared in advance and are stored in the refrigerator until needed.

5.2.3.2 Prepare blanks by filling tear drop vials with methylene chloride. Blanks are used during instrument calibration and in the sample extract sequence.

5.2.3.3 Seal the tear drop vials using screw caps with red dual-sided Teflon lined septa pre-rinsed with methylene chloride.

5.2.3.4 Label the vials with a "B" to indicate blank and store in the designated refrigerator until needed.

5.2.4 Prior to starting the Spectra-Physics/Gilson HPLC analytical batch, check the following:

5.2.4.1 Verify that the HPLC solvent reservoir is filled with methylene chloride;

5.2.4.2 Verify that the rinse reservoir for the autosampler is filled with methylene chloride.

5.2.4.3 Verify that the waste bottle for the HPLC is empty.

5.2.5 Start the Spectra-Physics pump by pushing the **RUN** button. The PSI should read between 600 to 800 psi. The LCD display on the pump should read *READY # PSI FLOW 7.00* when the pump has stabilized.

5.2.5.1 If the pressure is lower than 600 psi, check that the flow switching valve is not in the backflush position.

5.2.5.2 If the pressure is greater than 800 psi, the source of the increased pressure will need to be identified and corrected.

5.2.6 To backflush the guard column (to wash away the particles accumulated at the head of the guard column):

5.2.6.1 Turn the pump off by pressing **STOP**. Begin backflushing the guard column by turning the switching valve to the backflush position.

5.2.6.2 Loosen and disconnect the solvent inlet fitting which connects the guard column to the switching valve. Place the solvent inlet end of the guard column into a glass waste beaker.

5.2.6.3 Start the Spectra-Physics pump by pressing **RUN**. Let the guard column backflush into the beaker for approximately 30 min.

5.2.6.4 After 30 minutes, stop the pump by pressing **STOP**. Discard the backflushed solvent in the laboratory Waste Solvent container.

5.2.6.5 Reattach the guard column to the switching valve and turn the switching valve to the forward flow position.

5.2.6.6 Restart the pump and check for leaks at the fittings between the switching valve and the guard column.

5.2.6.7 Once the pump reads *READY*, the pump pressure will range from 600-800 psi. If the pressure exceeds 800 psi, stop the pump and identify the source of the elevated pressure.

5.2.7 Setup for GPC/HPLC calibration standards and blanks:

5.2.7.1 Remove 3 screw cap tear-drop vials of GPC/HPLC calibration standards and 1 tear-drop vial of the blank from the designated refrigerator and allow them to warm to room temperature.

5.2.7.2 Reset the LKB collector by pushing the **RETURN** button. The digital window display will read *RDY*.

5.2.7.3 Make sure there is an asterisk (*) on the HP integrator printout.

5.2.7.4 Place the tear-drop vials containing the GPC/HPLC calibration standards and the blank in the rectangular white sample tray of the Gilson autosampler.

5.2.7.4.1 Place the first tear-drop vial in the upper right corner, with consecutive vials placed to the left of the first vial. The calibration standards are placed in the first three positions and the blank vial is placed in the fourth position of the tray.

5.2.7.5 Label six fraction collection vials with an "S" to indicate standard and label two collection vials with a "B" to indicate calibration blank. Two collection vials are used for each calibration standard, blank, sample or continuing calibration standard. The first vial will collect waste only. The second vial will collect the standard fraction.

5.2.7.6 Position the gray 14-space collection tray so the white front is facing the analyst and place the first two standard collection vials into the collector tray.

5.2.7.7 Place the first collection vial (waste) for the standard in the upper left position and the second vial (standard) into the left second position on the tray. Place the remaining collection vials in sequential order on the collection tray. The calibration standard vials are placed in the first six positions and the blank vials are placed in the last two positions in the collection tray.

5.2.7.8 Place the tray on the left side of the black mat of the fraction collector with the white front facing the analyst and push the tray all the way to the back of the black mat.

5.2.7.9 After placing all of the standards in the tray and all of the standard collection vials in the collector rack, **review the HPLC checklist placed on the front of the HPLC.** If all items have been completed, continue to the next step to initiate the calibration run.

5.2.8 Programming and executing the calibration run:

5.2.8.1 Press the **EDIT/SAVE/FILE** button on the Gilson sample controller keypad until FILE 13 appears on the LCD screen.

5.2.8.2 Press **ENTER** three times until the words *FILE STORED* appear.

5.2.8.3 Press **START.** The following prompts will appear on the LCD screen.

5.2.8.4 When *SAMPLE NB* appears, enter the number of samples that are to be run (i.e., press **4 ENTER** to run 3 calibration standards and 1 blank).

5.2.8.5 When *TUBE#* appears, enter the position of the first collection vial on the collector tray (i.e., press **1 ENTER** for position #1).

5.2.8.6 For *INJECTION VOLUME*, input **1000 ENTER** to inject 1000 mL.

5.2.8.7 For *ELUTION TIME=*, input **2500 ENTER** (for a total runtime of 25 minutes).

5.2.8.8 When *TIME A=* appears, enter the time to start the fraction collection.

5.2.8.8.1 **For calibration standards, input 1300 ENTER to start collecting fractions 13 minutes into the HPLC run-time.**

5.2.8.9 When *TIME B* appears, input the total collect time by pressing **730 ENTER** to collect for 7 minutes, 30 seconds. The injection sequence will then begin.

5.2.8.9.1 As soon as the time for *TIME B* is entered, the Gilson autosampler will start the injection sequence; the LBK fraction collector will advance to the position of the first waste fraction to be collected; and the digital readout on the fraction collector will read *"00.00"*.

5.2.8.10 Record the date, HPLC pressure, and your initials in the HPLC Run Logbook.

5.2.8.11 Record the GPC/HPLC calibration standards run in the HPLC Run Logbook.

5.2.8.12 Check the level of methylene chloride in the HPLC solvent reservoir and in the autosampler rinse reservoir. Refill if necessary.

5.2.8.13 Check the level of waste in the HPLC waste bottle and empty, if necessary.

5.2.8.14 At the end of the autosampler sequence the pump and autosampler will automatically stop. The autosampler keypad will read *HOME*.

5.2.9 Assessing the calibration run:

5.2.9.1 The HPLC standard contains biphenyl, perylene, and DBOFB. Locate the three separate standard peaks on the three chromatograms. DBOFB elutes first at approximately 13 minutes, followed by biphenyl at approximately 14 minutes, and perylene at approximately 16 minutes (See example chromatogram).

5.2.9.2 The retention times of the three peaks should not vary more than 5%, or approximately 0.6 minutes between the three chromatograms.

5.2.9.3 The area count of the DBOFB peak should be >1,000,000.

5.2.9.4 If the calibration is verified, the system performance is acceptable and the analysis of samples may begin. If the retention times fall outside of the calibration verification range or if the area count of the DBOFB peak is too small, the system performance is unacceptable. The problem must be corrected and the system recalibrated.

5.2.9.5 Time A, the start collection time for sample extracts, is determined by the retention time of DBOFB. Time A is defined as one minute prior to the retention time of DBOFB. If the retention time of DBOFB is 13.75 minutes, Time A is 12.75 minutes. Record Time A in HPLC run notebook.

5.3 Hewlett-Packard HPLC System - Calibration

Prior to processing analytical extracts through the GPC/HPLC, the GPC/HPLC is calibrated and the system performance is checked. The calibration of the GPC/HPLC verifies the instrument performance based upon the retention time and area of the calibration standards. The time to start collecting the fractions for the actual samples is then determined by subtracting one minute from the retention time of DBOFB.

For this test, three GPC/HPLC calibration solution and a blank are used to verify the performance criteria. Adjustment and/or recalibration is performed until the criteria are met. Only after the criteria are met may samples be purified.

Notes on the Hewlett-Packard system: To turn the pump on or off, or to change the parameters such as flow rate, the control panel on the pump system should be used. The sample sequence, injection volume, run time, and start/stop of the entire HPLC system are executed on the autosampler. Collection time is specified on the Foxy fraction collector.

5.3.1 Before each calibration and/or extraction batch run, locate the GPC/HPLC calibration standard which is stored in the freezer at 0°C. The standard solution must be brought to room temperature prior to use.

5.3.2 Calibration standard vial preparation:

5.3.2.1 Approximately 20 vials are prepared in advance and are stored in the refrigerator until needed.

5.3.2.2 Using a disposable Pasteur pipette, transfer approximately 1 mL of the GPC/HPLC calibration solution into the crimp-top tear-drop vials.

5.3.2.3 Seal the tear drop vials using crimp caps with Teflon-lined septa.

5.3.2.4 Label the vials with an "S" to indicate standard and store in the designated refrigerator until needed.

5.3.3 Blank vial preparation:

5.3.4.1 Approximately 20 vials are prepared in advance and are stored in the refrigerator until needed.

5.3.4.2 Prepare blanks by filling crimp cap tear drop vials with methylene chloride. Blanks are used during instrument calibration and in the sample extract sequence.

5.3.3.3 Seal the tear drop vials using crimp caps with a Teflon lined septa.

5.3.3.4 Label the vials with an "B" to indicate blank and store in the designated refrigerator until needed.

5.3.4 Prior to starting the Hewlett-Packard HPLC run, check the following:

5.3.4.1 Verify that the HPLC solvent reservoir is filled with methylene chloride;

5.3.4.2 Verify that the rinse vial (vial #20 in the sample tray) is filled with methylene chloride.

5.3.4.3 Verify that the waste bottle for the HPLC is empty.

5.3.5 Start the Hewlett-Packard pump by pushing the **PUMP ON/OFF** button and **ENTER** button on the HP pump control panel. The LCD display on the pump will show the flow rate and pressure of the system: *Flow 7.00 7.00, (Press ###).* The normal operating pressure at a flow rate of 7 mL/min is between 1300 - 1600 psi.

5.3.5.1 If the pressure is lower than 1300 psi, check for leaks in the HPLC system. The source of the leak should be identified and corrected.

5.3.5.2 If the pressure is greater than 1600 psi, the source of the increased pressure should be identified and corrected.

5.3.5.3 Document all corrective actions in the HPLC maintenance logbook.

5.3.6 To backflush the guard column (to wash away the particles accumulated at the head of the guard column):

5.3.6.1 Turn the pump off by pressing **PUMP ON/OFF** and **ENTER**.

5.3.6.2 Disconnect the guard column from both ends and reverse the guard column so that the column outlet is connected to the line from the autosampler. Leave the inlet of the guard column disconnected and direct the inlet into a glass beaker.

5.3.6.3 Turn the pump on by pressing **PUMP ON/OFF** and **ENTER**. Backflush the guard column into the beaker for approximately 30 min.

5.3.6.4 After 30 minutes, stop the pump by pressing the **PUMP ON/OFF** and **ENTER** buttons. Discard the backflushed solvent into the laboratory Waste Solvent container.

5.3.6.5 Disconnect the guard column outlet from the autosampler line and reconnect to the guard column in the original orientation with the inlet side connected to the autosampler and the outlet connected to the two size exclusion columns.

5.3.6.6 Restart the pump and check for leaks at both ends of the guard column.

5.3.7 Setup for GPC/HPLC calibration standards and blanks:

5.3.7.1 Remove 3 crimp cap tear-drop vials of GPC/HPLC calibration standards and 1 crimp cap tear-drop vial of the blank from the designated refrigerator and let warm to room temperature.

5.3.7.2 Place the tear-drop vials containing the blank and the GPC/HPLC calibration standards in the sample tray, starting from the #1 position in the tray for the three standards.

5.3.7.3 Label three collection vials with a "S" to indicate the standard and one collection vial with a "B" to indicate the calibration blank. One collection vial for each calibration standard, blank, sample or continuing calibration standard is used to collect the fractions on the Hewlett-Packard HPLC system.

5.3.7.4 Place the first collection vial for the standard in position #1 in the first row at the front, left position. Collection will proceed from the first row at the front from left to right, then right to left in the second row and then left to right at the third row, following a zig-zag route. One collection bottle is needed for each sample or standard. The calibration standard vials are in the first three positions and the blank vial is in the fourth position for the calibration run.

5.3.8 Programming and executing the calibration run:

5.3.8.1 Press **VIAL #** button on the autosampler.

5.3.8.2 Press **Æ** button once to move the cursor on the LCD display to the field of *First vial*. Press **1** if the first vial to be injected is at position #1 of the sample tray.

5.3.8.3 Press **Æ** again to move the cursor to the field of *Last vial* on the LCD display. Press the number of the last vial position to be injected in the sample tray. Then press **ENTER**.

5.3.8.4 To set the collection time:

5.3.8.4.1 On the FOXY fraction collector, press **STOP** twice to move the collection arm to the back

of the rack. The LCD display should display *Ready Run A Edit A Load Quick review.*

5.3.8.4.2 Press **B** to select *Edit A*, and then press **B** to select *Peak.*

5.3.8.4.3 Press **ENTER**. The LCD display should read *window 1 of 2 ## to ###*, with the first field flashing. Enter the collection starting time. For example, if the starting collection time is 13 minutes and 25 seconds, press 1325 (displayed as 13:25). After the start collection time is inputted, press **ENTER** and the end collection time will flash. Enter the end collection time and press **ENTER**. Then press **C** to exit.

Note: The time value in the LCD display of the FOXY fraction collector is in min:sec format. For example, 13:45 is 13 minutes and 45 seconds. The retention time display on the integrator chromatogram and on the autosampler LCD display is in decimal units, i.e., 13.75 is 13 minutes and 45 seconds. When determining the start collect time on the fraction collector, the chromatogram time needs to be converted from the min:decimal format to the min:sec format.

5.3.8.4.4 Reset the Foxy fraction collector by pressing the **STOP** button twice and then the **RUN** button once. The collection arm should be at the front of the collector at this time and the LCD display should read *waiting for external to (re)start #1.*

5.3.8.5 Before starting a run, make sure the pump is on.

5.3.8.6 To start the run, press **START** on the autosampler. The LCD should display *First # Last ##*, which should

correspond to the first and last samples previously entered (see Sections 5.3.8.2 and 5.3.8.3). Press **ENTER** on the autosampler and the autosampler will start the injection.

5.3.8.7 Record the date, HPLC pressure, and your initials in the HPLC logbook.

5.3.8.8 Record the GPC/HPLC calibration standards run on the HPLC in the HPLC Run Logbook.

5.3.8.9 Check the level of methylene chloride in the HPLC solvent reservoir. Refill if necessary.

5.3.8.10 Check the level of waste in the HPLC waste bottle and empty if necessary.

5.3.9 Assessing the calibration run:

5.3.9.1 The HPLC standard contains biphenyl, perylene, and DBOFB. Locate the three separate standard peaks on the three chromatograms. DBOFB elutes first at approximately 15 minutes, followed by biphenyl at approximately 16 minutes, and perylene at approximately 18 minutes (See example chromatogram).

5.3.9.2 The retention times of the three peaks should not vary more than 5%, or approximately 0.6 minutes between the three chromatograms.

5.3.9.3 The area count of the DBOFB peak should be >500,000.

5.3.9.4 If the calibration is verified, the system performance is acceptable and the analysis of samples may begin. If the retention times fall outside of the calibration verification range or if the area count of the DBOFB peak is too small, the system performance is unacceptable. The problem must be corrected and the

system recalibrated. Document all corrective actions in the HPLC maintenance logbook.

5.3.9.5 The start collection time for sample extracts is determined by the retention time of DBOFB. The start collection time is defined as one minute prior to the retention time of DBOFB. For example, if the retention time of DBOFB is 13.75 minutes, the start collection time becomes 12.75 minutes (12:45 on the fraction collector). Record the start collection time in the HPLC logbook.

5.4 Spectra-Physics/Gilson HPLC System - Preparation of Sample Extracts

5.4.1 After the extraction of the tissue samples and purification of the extracts using column chromatography, the sample extracts are concentrated to a final volume of 0.5 mL methylene chloride.

5.4.2 Label the appropriate number of Rainin HPLC screw cap tear-drop vials with the sample logbook benchsheet number, sample number, and the sample ID.

5.4.3 Transfer the sample extracts from the 25 mL concentrator tubes to labeled HPLC vials using the disposable Pasteur pipettes.

5.4.4 Rinse the concentrator tubes at least once with approximately 0.3 mL methylene chloride and transfer the rinse into the HPLC tear drop vials containing the original extract. The total volume should not exceed the middle yellow line on the vials.

5.4.4.1 Extremely viscous extracts or extracts with high lipid content (greater than 500 mg or other macro-molecular contaminants) can be diluted in the concentrator tubes with methylene chloride as needed and may be divided into multiple tear drop vials. In this case, fractions collected from the HPLC are concentrated and combined prior to the addition of the GC internal standards (Section 6.0).

5.4.4.2 The tear drop vials may be centrifuged to separate particulates, if necessary. After centrifugation, transfer the extract to a new, properly labeled vial.

5.4.5 Seal the tear drop vials using screw caps with red double sided Teflon cap liners pre-rinsed with methylene chloride.

5.5 Hewlett-Packard 1050 HPLC System - Preparation of Sample Extracts

5.5.1 After the extraction of the tissue samples and purification of the extracts using column chromatography, the extracts are concentrated to a final volume of 0.5 mL methylene chloride.

5.5.2 Label the appropriate number of HPLC crimp top tear drop vials with the sample logbook benchsheet number, sample number, and the sample ID.

5.5.3 Transfer the sample extracts from the 25 mL concentrator tubes to labeled HPLC vials using disposable Pasteur pipettes.

5.5.4 Rinse the concentrator tubes at least once with approximately 0.3 mL methylene chloride and transfer the rinse into the HPLC tear drop vials containing the original extract. The total volume should be no more than 1 mL.

5.5.4.1 Extremely viscous samples or samples with high lipid content (greater than 500 mg or other macro-molecular contaminants) can be diluted in the concentrator tubes with methylene chloride as needed and may be divided into multiple tear drop vials. In this case, fractions collected from the HPLC are concentrated and combined prior to the addition of the GC internal standards (Section 6.0).

5.5.4.2 The tear drop vials may be centrifuged to remove particulates, if necessary. After centrifugation, transfer the extract to a new, properly labeled vial.

5.5.5 Seal the tear drop vials using crimp caps with Teflon lined septa.

5.6 Spectra-Physics/Gilson HPLC System - Required Logbook Documentation and GPC Run Sequence

5.6.1 Record in the HPLC instrument run logbook the extraction batch(es), project identification, the date, the associated calibration standards, and your initials. See Section 5.1 for information on how to set-up the GPC/HPLC run sequence using the X Chrom software. Record the following information in the HPLC Instrument Run Logbook:

- the date extracts are injected on the HPLC;
- the project identification;
- benchsheet number;
- time A (start collect time, DBOFB retention time minus one minute);
- time B (total collect time; use 730 to represent 7.5 minutes);
- instrument pressure;
- list of the sequence (see Section 5.6.4) of extracts, blanks and continuing calibration standards ;
- any comments (i.e., highly colored), and
- initials of the analyst.

5.6.2 Verify that the IDs on the tear drop extract vials correspond to the sample IDs listed in the Laboratory Sample Logbook benchsheet.

5.6.3 For each extract, blank and standard to be analyzed, label two collection vials with the sample number on the benchsheet, benchsheet number, and sample ID.

5.6.4 Any extraction batch(es) run on the HPLC will follow the run sequence described in this section.

5.6.4.1 The initial calibration standards (ICS) and calibration blank must be run according the Section 5.2 to determine Time A (see Section 5.2.9.5) for sample collection.

5.6.4.2 The QC samples for an extraction batch follow the initial calibration at the beginning of the sequence (i.e., blank, matrix spike, matrix spike duplicate, SRM), followed by the individual sample extracts interspersed with HPLC blanks and HPLC continuing calibration standards.

NOTE: If more than one extraction batch is included in a GPC/HPLC run, the run sequence should complete all extracts for one extraction batch, followed by an HPLC blank and an HPLC continuing calibration sample, prior to starting the run sequence for a different extraction batch.

5.6.4.3 HPLC blanks are interspersed throughout the sequence, usually after every five extracts.

NOTE: When extracts are highly viscous, or if the lipids mass is above 500 mg (e.g., fat and blubber samples), or samples are suspected to be high in waxy materials (e.g., lichens), then extracts may need to be diluted and split into sub-fractions to avoid overloading the GPC column. In this situation, HPLC blanks may have to be run after each sample. Collected fractions from sub-fractions are concentrated and combined after the completion of the GPC/HPLC run.

NOTE: If extracts are colored, blanks may need to be interspersed more frequently within the extract sequence, possibly between each extract.

5.6.4.4 A continuing calibration standard (CCS) is required after 10 extracts. The 10 extracts include QC samples as well as sample extracts.

5.6.4.5 Each GPC/HPLC sequence must end with a blank and a CCS.

5.6.5 Turn the pump back on by pressing **RUN** on the pump and reset the fraction collector by pressing **RETURN** on the LBK collector.

5.6.6 Place the HPLC tear drop vials in the autosampler tray starting from the upper right position and placing the next vial in the sequence in the tray to the left of the first vial. Vials #1-5 are placed in row 1 at the top of the autosampler tray, from right to left. Vials #6-10 are placed in row 2, also from right to left and the remaining vials are placed in the subsequent rows, from right to left.

5.6.7 For each extract, blank and standard to be run, place the 2 labeled collection vials in the gray sample collection trays, making sure the white front is facing the analyst. The first collection vial collects the waste and the second collection vial collects the extract fraction containing polyaromatic hydrocarbons (PAHs) and organochlorine compounds.

5.6.7.1 Seven extracts (i.e., 14 collection vials) are collected in each tray.

5.6.7.2 Two extra vials, labeled E for extra, are added after the final standard in the event that the collector skips a vial.

5.6.7.3 Place the collection trays on the black mat of the LKB collector, with the first tray on the left side of the mat and additional trays to the right of each previous tray.

5.6.7.4 All trays are placed in the collector with the white front facing the analyst.

5.6.7.5 Verify that the sequence of the extract vials placed in the autosampler tray corresponds with the correctly labeled collection vials in the collector trays.

5.7 Hewlett-Packard 1050 HPLC System - Required Logbook Documentation and GPC Run Sequence

5.7.1 See Section 5.1 for information on how to setup the GPC/HPLC run sequence using the X Chrom software. Record the following information in the HPLC Instrument Run Logbook:

- the date extracts are injected on the HPLC;
- the project identification;
- benchsheet number;
- start collection time (DBOFB retention time minus one minute);
- total collection time,
- instrument pressure;
- list of the sequence (see Section 5.5.4) of extracts, blanks and continuing calibration standards ;
- any comments (i.e., highly colored), and
- initials of the analyst.

5.7.2 Verify that the IDs on the tear drop extract vials correspond to the sample IDs listed in the Laboratory Sample Logbook benchsheet.

5.7.3 For each extract, blank and standard to be run, label one collection vial with the sample number on the benchsheet, benchsheet number, and sample ID.

5.7.4 Any extraction batch(es) run on the HPLC will follow the run sequence described in this section.

5.7.4.1 The initial calibration standards (ICS) and calibration blank must be run according the Section 5.3 to determine the starting collection time (see Section 5.3.9.5).

5.7.4.2 The QC samples for an extraction batch follow the initial calibration at the beginning of the sequence (i.e., blank, matrix spike, matrix spike duplicate, SRM), followed by the the individual sample extracts

interspersed with HPLC blanks and HPLC continuing calibration standards.

NOTE: If more than one extraction batch is included in a GPC/HPLC run, the run sequence should complete all extracts for one extraction batch, followed by an HPLC blank and an HPLC continuing calibration sample, prior to starting the run sequence for a different extraction batch.

5.7.4.3 HPLC blanks are interspersed throughout the sequence, usually after every five extracts.

NOTE: When extracts are highly viscous, or if the lipid mass is above 500 mg (e.g., fat and blubber samples), or samples are suspected to be high in waxy materials (e.g., lichens), then extracts may need to be diluted and split into sub-fractions to avoid overloading the GPC column. In this situation, HPLC blanks may have to be run after each sample. Collected fractions from sub-samples are concentrated and combined after the completion of the GPC/HPLC run.

NOTE: If extracts are colored, blanks may need to be run more frequently within the extract sequence, possibly between each extract.

5.7.4.4 A continuing calibration standard (CCS) is required after 10 extracts. The 10 extracts, include QC samples as well as sample extracts.

5.7.4.5 Each GPC/HPLC sequence must end with a blank and a CCS.

5.7.5 Turn the pump on by pressing **PUMP ON/OFF** and **ENTER** buttons on the pump control panel. Reset the fraction collector by pressing **STOP** twice and **RUN** once on the FOXY collector.

5.7.6 Place the HPLC tear drop vials in the autosampler tray starting from the #1 position.

5.7.7 For each extract, blank and standard to be run, place one labeled collection vial in the sample collection rack, starting from left to right at the front row, then from right to left at the second row, and then from left to right in the third row, following a zig-zag pattern.

5.7.7.1 Verify that the sequence of the extract vials placed in the autosampler tray corresponds with the correctly labeled collection vials in the collector tray.

5.8 Spectra-Physics/Gilson HPLC System - Starting the Sample Run

5.8.1 Programming the autosampler controller:

5.8.1.1 Press **EDIT/SAVE/FILE** until the LCD display reads *FILE 13*. Press **ENTER** three times until the display reads *FILE STORED*.

5.8.1.2 Press **START**.

5.8.1.3 The LCD display will read *SAMPLE NB*. Input the total number of vials to be injected (input #) and press **ENTER**.

5.8.1.4 The LCD will then display *TUBE #*. Input the collection vial number to start collecting the fraction (input **1 ENTER** for position #1).

5.8.1.5 For *INJECT VOLUME*, input **1000 ENTER**.

5.8.1.6 For *ELUTION TIME*, input **2500 ENTER**.

5.8.1.7 *TIME A* will be the retention time of the first standard, DBOFB, minus 1.0 min (i.e., if the retention time of DBOFB is 13.376 min, input **1238 ENTER**).

5.8.1.8 Input **730 ENTER** for *TIME B*, total collection time.

5.8.1.9 Upon entering time B, the autosampler will initiate the run.

5.8.2 Initial system checks:

5.8.2.1 Make sure that the methylene chloride reservoir is full and the autosampler rinse reservoir is filled with methylene chloride.

5.8.2.2 Check that the waste bottle for the HPLC is empty.

NOTE: During the HPLC run, consumption of the methylene chloride in the HPLC solvent and rinse reservoirs, and filling of the waste collection bottle will occur. Prior to leaving at the end of the work period, it is essential to refill the sample/rinse reservoirs and empty the waste bottle so that there is adequate solvent and waste capacity for the overnight run.

5.8.2.3 Periodically, throughout the run, check that the ID of the extract that is injected corresponds with the collection vial and that the pressure of the system is maintained.

5.8.4 After the end of the injection sequence, verify the system operation by performing the following activities:

5.8.4.1 Check the collector vials to make sure that all extracts are in the correct vials and the correct number of samples have been collected.

5.8.4.1.1 Extracts should be collected in every second vial (i.e., the first collection vial is for waste, the second for purified extract).

5.8.4.2 Check the HPLC vials to ensure that the entire extract has been injected. The HPLC vials should be empty.

5.8.4.2.1 If there is extract remaining in the HPLC vials, the volume of the extract may be brought up to 1 mL with methylene chloride and reinjected into the HPLC system.

5.8.4.2.2 The reinjected fraction collected is then combined with the original collected fraction.

5.8.4.3 Check the chromatograms for the GPC/HPLC run sequence.

5.8.4.3.1 If the continuing calibration retention times do not agree within 5% of the initial calibration or if the area of DBOFB IS < 1,000,000, the problem must be corrected and the HPLC must be recalibrated. In this case, immediately notify the Laboratory Manager who will contact the PA for the project(s). The affected extraction batch may need to be re-extracted.

5.8.4.4 If the above checks indicate problems with the GPC/HPLC run, then these occurrences must be recorded in the HPLC maintenance logbook and in the Sample Logbook. The Laboratory Manager must be notified, so that appropriate action can be taken.

5.8.4.5 The analytical date and analyst initials are recorded on the Laboratory Sample Logbook extraction benchsheet.

5.9 Hewlett-Packard 1050 HPLC System - Starting the Sample Run

5.9.1 Programming the autosampler controller:

5.9.1.1 Press **VIAL #** button and → button. Enter the position of the first vial in the autosampler tray (generally 1).

5.9.1.2 Press → button and enter the position of the last vial in the autosampler tray.

5.9.1.3 Press **ENTER**. The LCD display on the autosampler will display the first vial number and the last vial number that were inputted in Sections 5.9.1.1 and 5.9.1.2.

5.9.1.4 Set the collection time (see Section 5.3.8.4).

5.9.1.5 Press START on the autosampler.

5.9.2 Initial and periodic system checks:

5.9.2.1 Make sure that the methylene chloride reservoir is filled with methylene chloride.

5.9.2.2 Check that the waste bottle for the HPLC is empty.

NOTE: During the HPLC run, consumption of the methylene chloride in the HPLC solvent reservoir and filling of the waste collection bottle will occur. Prior to leaving at the end of the work period, it is essential to refill the HPLC solvent reservoir and empty the waste bottle so that there is adequate solvent and waste capacity for the overnight run.

5.9.2.3 Check that the pressure on the pump LCD display is between 1300 and 1600 psi.

5.9.2.4 Periodically, throughout the run, check that the ID of the extract that is injected corresponds with the collection vial.

5.9.3 After the end of the injection sequence, verify system operation by performing the following checking activities:

5.9.3.1 Check the collector vials to make sure that all extracts are in the correct vials and the correct number of extracts have been collected.

5.9.3.2 Check the HPLC vials to ensure that the entire extracts has been injected. The HPLC vials should be empty.

5.9.3.2.1 If there is extract remaining in the HPLC vials, the volume of the extract may be brought up to 1 mL with methylene chloride and reinjected into the HPLC system.

5.9.3.2.2 The reinjected fraction collected is combined with the original collected fraction.

5.9.3.3 Check the chromatograms for the GPC/HPLC run sequence.

5.9.3.3.1 If the continuing calibration retention times do not agree within 5% of the initial calibration or if the area of DBOFB IS <500,000, the problem must be corrected and the HPLC must be recalibrated. In this case, immediately notify the Laboratory Manager who will contact the PA for the project(s). The affected extraction batch may need to be re-extracted.

5.9.3.4 If the above checks indicate problems with the GPC/HPLC run, then these occurrences must be recorded in the HPLC maintenance logbook and in the Sample Logbook. The Laboratory Manager must be notified, so that appropriate action can be taken.

5.9.3.5 The analytical date and analyst initials are recorded on the Laboratory Sample Logbook extraction benchsheet.

6.0 EXTRACT CONCENTRATION AND PREPARATION FOR ANALYSIS

6.1 Concentration of the Purified Extracts

6.1.1 Place the filled collector vials in vial racks and cover with methylene chloride rinsed aluminum foil. Place the racks in the

designated area in the extraction laboratory and allow to allow the contents of the collection vials to partially evaporate at ambient temperature.

6.1.2 When the extracts in the collector vials have evaporated to approximately 45 mL, add one clean boiling chip and concentrate to approximately 5-10 mL on a 60-70°C water bath.

6.1.2.1 Quantitatively transfer the extract to a 25 mL concentrator tube labeled "HPLC", with sample number, benchsheet number and Sample ID. Rinse the collection vials at least three times with methylene chloride and add the rinses to the concentrator tube.

6.1.2.2 Add one clean boiling chip and, using a water bath (60-70°C), concentrate the purified extract as required for the appropriate analysis. **Check the benchsheet for the required final solvent and extract volume.**

6.1.2.3 Typically, both PAH and pesticide/PCB extracts are solvent exchanged to hexane for instrumental analysis. To change the solvent to hexane, concentrate the methylene chloride extract to about 2 mL, then add approximately 1 mL of hexane. The extract will stop boiling when all of the methylene chloride has evaporated from the sample extract. Adjust the final volume of the concentrated hexane extract to approximately 0.9 mL.

6.1.2.4 Cap the concentrator tubes with ground glass stoppers.

6.1.3 Place the sample extract in the designated area so that the the required internal standard solutions for PAH or pesticide/PCB analysis can be added.

6.1.4 Date and initial the appropriate Laboratory Sample Logbook extraction benchsheet in the third box labeled "concentration".

7.0 GPC/HPLC RUN HARDCOPY DELIVERABLES

7.1 The X Chrom software is used to print labeled chromatograms from the GPC/HPLC run. If X Chrom is not available, you must hand label each chromatogram from the sample number and the extraction benchsheet number.

7.2 The Laboratory Sample Logbook extraction benchsheet is photocopied and the copy is attached to the chromatograms, and filed along with a copy of the sample sequence from the X Chrom Data System.

8.0 EXAMPLE FORMS

8.1 Example HPLC Run Logbook

8.2 Example HPLC Maintenance Logbook

8.3 Example Laboratory Sample Logbook Extraction Benchsheet

8.4 Example of a Sample Chromatogram

HPLC #1 Instrument Log

BOOK 6
PAGE 101

Date	Project Directory	Analysis Filename	HPLC Run File #	Sequence Number	Collect Start Time	Collect Total Time	Pressure	GERG Sample #	Client Sample #	Description	Initials

1994-20 HPLC Instrument Log

8.1 Example HPLC Run Logbook

8

10/24/95 Restart HPLC P - 650 psi

10/12/95 Replaced seals, cleaned plungers
The squicking noise is gone.

10/13/95 Lubricated motor, checked the condition of all the moving part in the motor, piston, cam, etc.
Cleaned the motor, cam, driving wheel.
Everything is in good condition.
Check seals in pump head in six months.

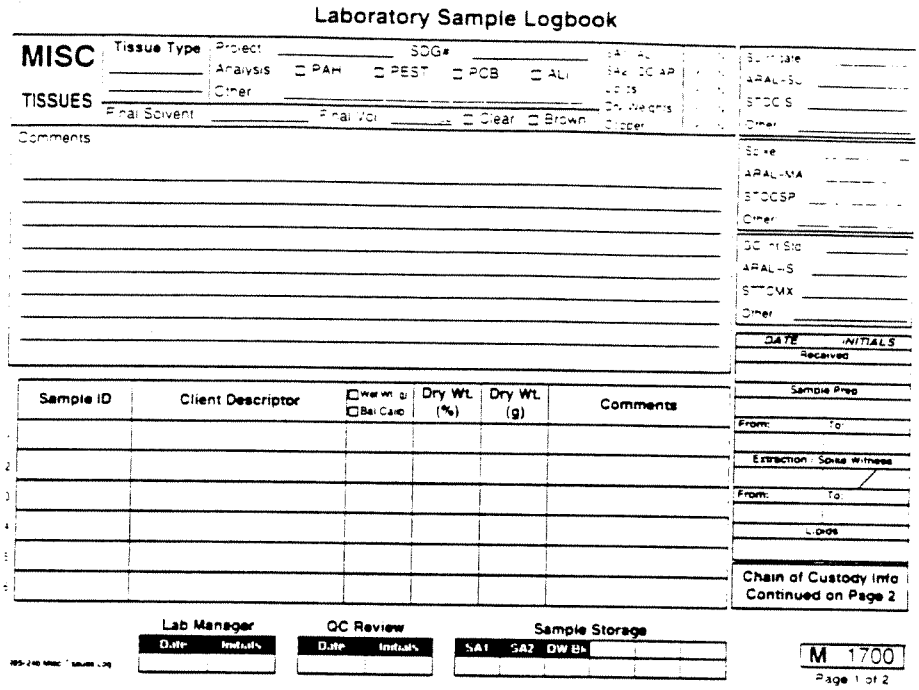
10/[illegible]/95 PRESSURE 650

12/8/95 Pump Motor has grounding noise during the run
Diagnosis is the motor axis is grinding
check oil seal

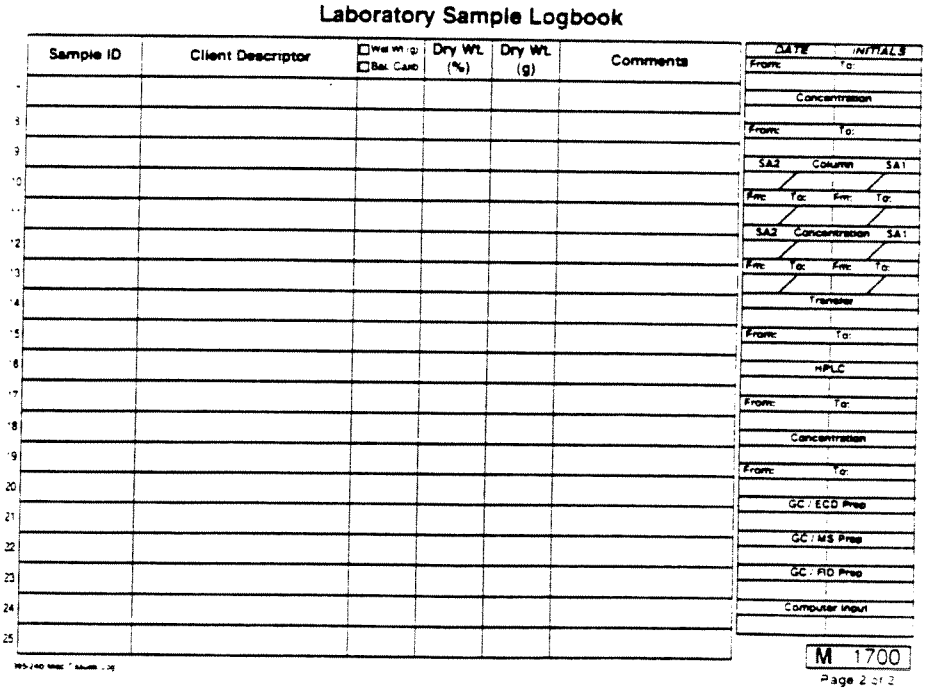
Sent to Thermoseparation on 12/21/95
pump for repairs

Returned 1/8/96 - still noisy by Thermoseparation says its OK

8.2 Example HPLC Maintenance Logbook

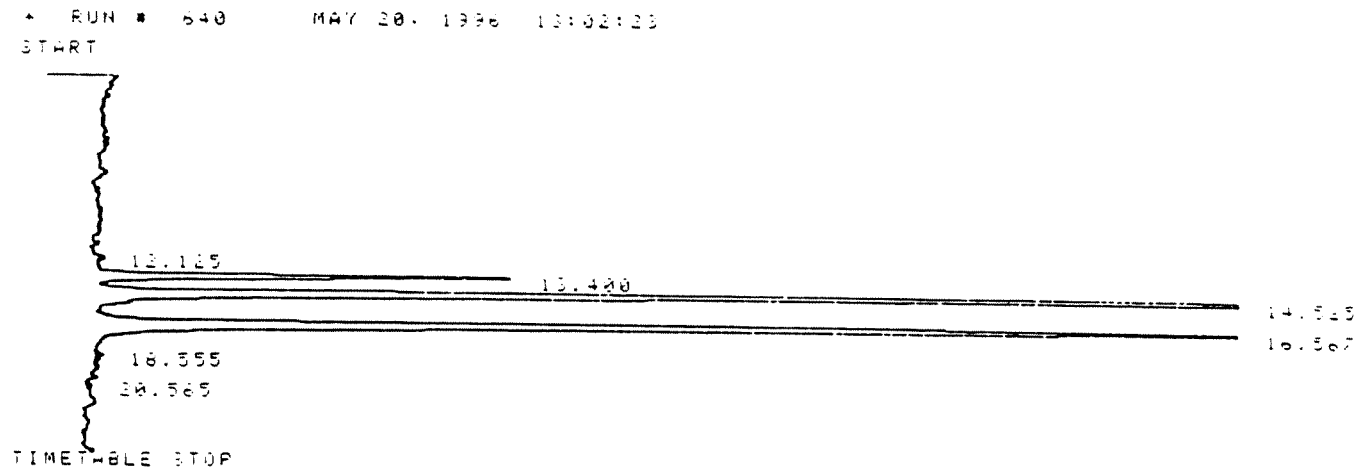

Laboratory Sample Logbook

MISC
TISSUES
Tissue Type
Project
SDG#
Analysis ☐ PAH ☐ PEST ☐ PCB ☐ ALI
Other
Final Solvent
Final Vol
☐ Clear ☐ Brown
Dry Weights
Comments
Sample ID
Client Descriptor
Wet Wt (g)
Bal Card
Dry Wt. (%)
Dry Wt. (g)
Comments
Lab Manager
Date
Initials
QC Review
Date
Initials
Sample Storage
SA1
SA2
DW Bk
DATE
INITIALS
Received
Sample Prep
From:
To:
Extraction : Spike Witness
Chain of Custody Info
Continued on Page 2
M 1700
Page 1 of 2


Laboratory Sample Logbook

Sample ID
Client Descriptor
Wet Wt (g)
Bal Card
Dry Wt. (%)
Dry Wt. (g)
Comments
DATE
INITIALS
From:
To:
Concentration
SA2
Column
SA1
SA2 Concentration SA1
Transfer
HPLC
GC / ECD Prep
GC / MS Prep
GC / FID Prep
Computer Input
M 1700
Page 2 of 2

8.3 Example Laboratory Sample Logbook Extraction Benchsheet



RUN# 640 MAY 20, 1996 13:02:23

AREA%

RT	AREA	TYPE	WIDTH	AREA%
12.125	12200	PV	.091	.07385
13.400	1647095	VV	.291	9.96989
14.525	7606973	VV	.289	46.04509
16.567	7218176	VV	.370	43.69171
18.555	24917	PV	.176	.15082
20.565	11342	PV	.090	.06865

TOTAL AREA=1.6521E+07
MUL FACTOR=1.0000E+00

8.4 Example of a Sample Chromatogram