Quality Assurance Project Plan
for

"Understanding the Cumulative Affects of Environmental and Psycho-social Stressors that Threaten the *Pohlik-lah* and *Ner-er-ner* Lifeway: The Yurok Tribe's Approach"

Conducted by the Yurok Tribe Environmental Program
In Collaboration with the Humboldt State University
California Center for Rural Policy
Institute for Spatial Analysis
With funding provided by the U.S. Environmental Protection Agency,
National Center for Environmental Research
Science to Achieve Results Grant # RD-83370801-0

FY 2008 – 2012

Prepared by:
Suzanne Fluharty, Ph.D.
Kathleen Sloan, Ph.D.
Yurok Tribe Environmental Program

February 2010

YUROK TRIBE
QUALITY ASSURANCE PROGRAM PLAN (QAPP)

ORGANIZATION: Yurok Tribe

APPROVALS:

Yurok Tribe

Name: Thomas P. O'Rourke, Sr.
Title: Chairperson, Yurok Tribal Council
Signature: T. P. O'Rourke
Date: 3-17-10

Yurok Tribe Environmental Program

Name: Kathleen Sloan, Ph.D.
Title: Quality Assurance Officer and Principal Investigator
Signature: Kathleen Sloan
Date: 3-10-10

Humboldt State University Sponsored Programs Foundation

Name: Steven Steinberg, Ph.D.
Sheila Steinberg, Ph.D.
Title: Co-Principal Investigators - HSU
Signature:
Date: 10 MARCH 2010
Signature: Sheila L. Steinberg
Date: 10 March 2010

U.S. Environmental Protection Agency

Name: Maggie Breville
Title: Project Officer, National Center for Environmental Research
U.S. Environmental Protection Agency
Signature:
Date: April 26, 2010

Table of Contents

1.0 Project Management **8**

1.1 QAPP Distribution List

1.2 Project Organization Chart

1.3 Project Team Personnel

1.4 Problem Statement and Background

1.4.1 Phase 1: Preliminary Assessment and Planning

1.4.2 Phase 2: Year 2 & 3: Primary Data Collection

1.4.3 Phase 3: Year 4: Analysis and Outputs

1.5 Project Description

1.6 Data Quality Objectives and Criteria

1.7 Data Collection and Analysis QA/ QC Components

1.8 Records, Documentation, and Reporting

1.8.1 Records and Documentation

1.8.1.1 Sample Collection Records
1.8.1.2 Sample Identification/ Chain of Custody Tag
1.8.1.3 Chain-of-custody Records
1.8.1.4 Quality Control (QC) Sample Records
1.8.1.5 General Field Procedures

1.8.2 Reporting

2.0 Data Collection, Analysis and Quality Assurance **24**

2.1 Experimental Sampling Design

2.1.1 Secondary Data

2.1.2 Water and Tissue Sampling

2.1.3 Geographic Information Systems (GIS)

2.1.4 Participatory Community Research: Public Participation Geographic Information (PPGI)

2.1.5 Tribal Member Health

2.2 Sampling, Collection, Analysis, and Quality Control Methods

2.2.1 Secondary Data: Collection, Validation, & QA/QC

2.2.1.1 Secondary Data: Water Quality & Contaminants Research
2.2.1.2 Secondary Data: Aquatic Toxicology Studies
2.2.1.3 Secondary Data: Ethnographic Studies
2.2.1.4 Secondary Data: Tribal Qualitative Research

2.2.1.5 Secondary Data: Tribal Health and Census
2.2.1.6 Secondary Data: Potential Environmental Contaminants Inventory
2.2.1.7 Secondary Data: Pesticide and Chemical Release Data
2.2.1.8 Secondary Data: GIS Data
2.2.1.9 Secondary Data: Quality Assurance

2.2.2 Water Sampling, Analysis, & QA/QC

2.2.2.1 Water Sampling Design
2.2.2.2 Water Sampling Method
2.2.2.3 Water Sample Collection
2.2.2.4 Water Sample Documentation, Records, and Data Storage
2.2.2.5 Water Samples: Analytical Method
2.2.2.6 Water Sampling and Analysis: Quality Assurance/Quality Control
2.2.2.7 Water Sampling: Special Training/Certification

2.2.3 Tissue Sampling, Analysis, & QA/QC

2.2.3.1 Tissue Sampling Design
2.2.3.2 Tissue Sampling Method
2.2.3.3 Tissue Sample Collection
2.2.3.4 Tissue Sample Storage and Shipping
2.2.3.5 Tissue Sample Documentation, Records, and Data Storage
2.2.3.6 Tissue Samples: Analytical Method
2.2.3.7 Tissue Sampling & Analysis: Quality Assurance/Quality Control
2.2.3.8 Tissue Sampling Special Training/Certification

2.2.4 GIS Data: Collection, Analysis, & QA/QC

2.2.4.1 GIS Data Design
2.2.4.2 GIS Data Generation Methods
2.2.4.3 GIS Data Collection Methods
2.2.4.4 GIS Documentation, Records, and Data Storage
2.2.4.5 GIS Analytical Methods
2.2.4.6 GIS Quality Assurance/Quality Control
2.2.4.7 Special Training/Certification

2.2.5 PPGIS Data: Collection, Analysis, & QA/QC

2.2.4.1 PPGIS Data: Sampling Design
2.2.4.2 PPGIS Data: Sampling Method
2.2.4.3 PPGIS Data: Sample Collection
2.2.4.4 PPGIS Data: Documentation, Records, and Data Storage
2.2.4.5 PPGIS Data: Analytical Methods
2.2.4.6 PPGIS Data: Quality Assurance/Quality Control
2.2.4.7 PPGIS Data: Special Training/Certification

3.0 Data Validation and Review 61

Figures

Figure 1: Project Organization Chart

Figure 2: Yurok Ancestral Territory and Reservation Map

Table 1: Distribution List

Table 2 Project Responsibility and Work Chart

Table 3: Data Quality Objectives and Performance Criteria

Table 4: Water Sampling Strategy

Table 5: Traditional Yurok Seasonal Resource Use

Table 6: Table 6: Species Selected for Analysis by Trophic Classification

Table 7: Tier One Tissue Sampling Strategy

Table 8: Examples of Possible Sampling Strategies for Tier Two

Appendices

Appendix A: Sample Forms

1. Alteration of Sample Size Form
2. Chain of Custody Form
3. Field Data Sheet for Eels/ Shellfish
4. Field Data Sheet for Fish
5. Field Data Sheet for Sea Lettuce
6. Field Data Sheet for Water
7. Public Participation Informed Consent Form
8. Sample Identification Label

Appendix B: Field Sampling Standard Operating Procedures (SOP)

1. Tier One Sampling File
    - Clams SOP
    - Lamprey SOP
    - Mussels SOP
    - Sea lettuce SOP
    - Sturgeon SOP
    - Surf fish SOP
    - Whole fish SOP
    - Water Sampling SOP

2. Tier Two Sampling Files
    - Clams SOP
    - Lamprey SOP
    - Mussels SOP
    - Sea lettuce SOP
    - Sturgeon SOP
    - Surf fish SOP
    - Whole fish SOP

Appendix C: Lab Documents

1. Lab Analysis SOPs File
    - Dioxin_furan_890-Fish SOP
    - Mercury analysis EPA 1631e
    - Mercury in tissue SOP revision_02-24-09_kp
    - Mercury in water SOP
    - Mercury_EPA 1631e Modifications
    - Microcystin SOP_WPCL#65
    - OC Pesticides in water_WPCL-AC-001
    - OPS IN WATER SOPv9_WPCL#52
    - PAH Extraction and Cleanup v1 8

PAH Water Extraction v1.0
PCB' in water_WPCL-AC-002
SO_TISSUE_SEDIMENT SOPv10 01-28-09
Trace metal and SO_SOP_MPSL-105

2. Chain of Custody_DFG_Jan 04
3. Field Collection SOP_MPSL_102a
4. Lab Receipt of Samples SOP_REV_Aug 07
5. SOP_Sample Management_REV_Aug 07
6. WPCL MDL_RL_Feb 17_10
7. WPCL QA Manual July 2009

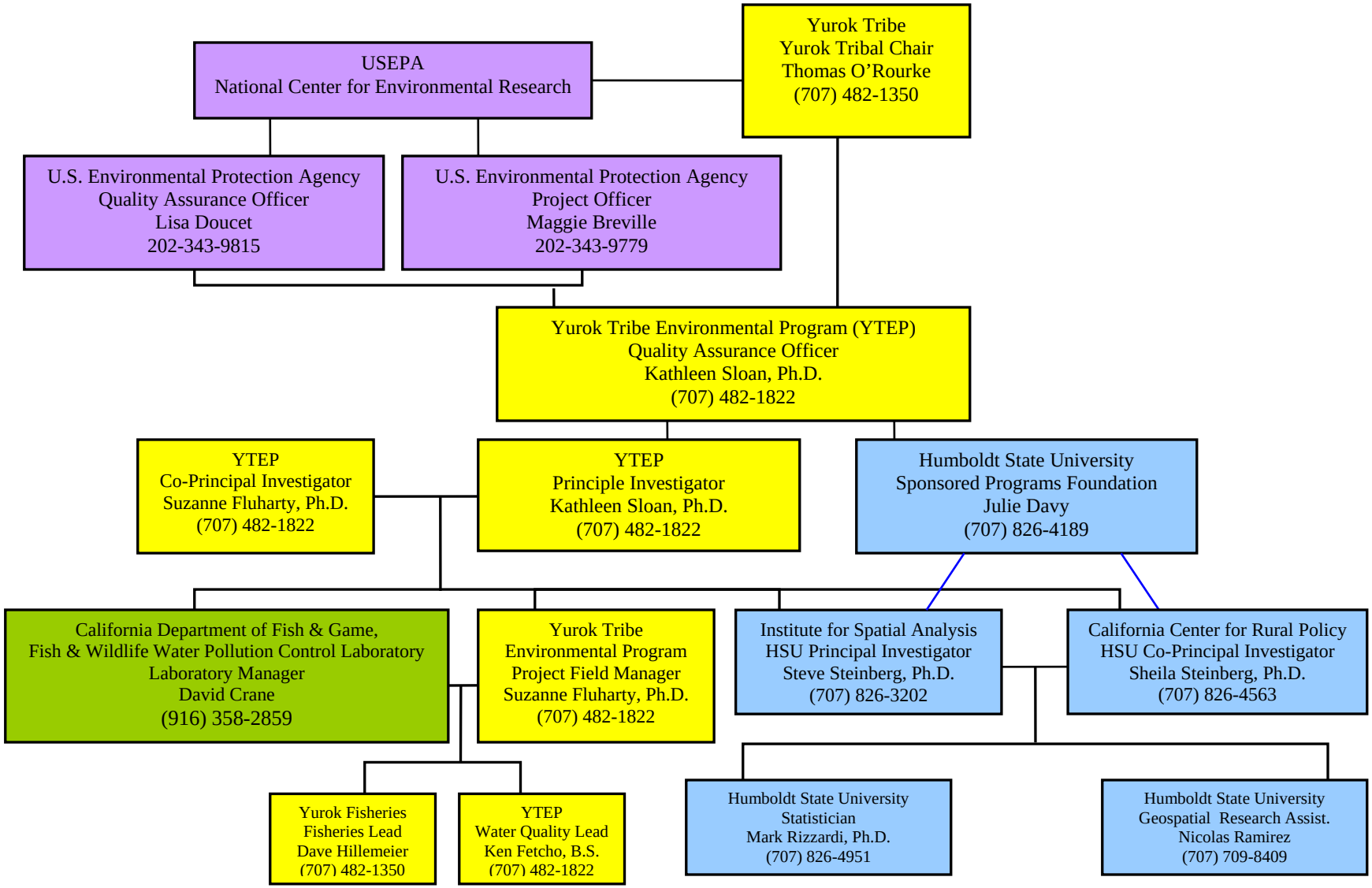
1.0 PROJECT MANAGEMENT

1.1 QAPP Distribution

Table 1: Distribution List

Title	Name	Affiliation	Telephone
Project Officer	Maggie Breville	National Center for Environmental Research, U.S. Environmental Protection Agency	(202) 343-9779
Quality Assurance Manager	Lisa Doucet	National Center for Environmental Research, U.S. Environmental Protection Agency	(202) 343-9815
Tribal Chair	Thomas O'Rourke, Sr.	Yurok Tribe	(707) 482-1350
Project Quality Assurance Manager	Kathleen Sloan, Ph.D.	Quality Assurance Officer, Yurok Tribe Environmental Program	(707) 482-1822
Project Team			
Principal Investigator	Kathleen Sloan, Ph.D.	Director, Yurok Tribe Environmental Program	(707) 482-1822
Co-PI/Project Field Manager	Suzanne Fluharty, Ph.D.	Environmental Health Specialist, Yurok Tribe Environmental Program	(707) 482-1822
Water Quality Lead	Ken Fetcho, B.S.	Assistant Director, Water Division Yurok Tribe Environmental Program	(707) 482-1822
Fisheries Program Lead	Dave Hillemeier	Fisheries Program Manager Yurok Tribe	(707) 482-1822
Laboratory Manager	David Crane	California Department of Fish and Game Fish & Wildlife Water Pollution Control Laboratory	(916) 358-2859
HSU Co- Principal Investigator, Social Science and Policy Analyst	Sheila Lakshmi Steinberg, Ph.D.	Professor, Dept. of Sociology Director, Community Research California Center for Rural Policy, Humboldt State University	(707) 826-4563
HSU Principal Investigator, Geographic Information Systems Analyst	Steven J. Steinberg, Ph.D.	Professor, Environmental & Natural Resource Sciences Director, Institute for Spatial Analysis Director, Klamath Watershed Institute Humboldt State University	(707) 826-3202
Statistician	Mark Rizzardi, Ph.D.	Professor of Statistics Mathematics Department, Humboldt State University	(707) 826-4951
Geospatial Research Assistant	Nicolas Ramirez	Graduate Student, Humboldt State University	(707) 709-8409

1.2 Project Organization Chart



1.3 Project Team Personnel

1.3.1 US Environmental Protection Agency (EPA)

- Maggie Breville, Project Officer
  US EPA National Center for Environmental Research, Washington, DC

- Lisa Doucet, Quality Assurance Officer
  US EPA National Center for Environmental Research, Washington, DC

1.3.2 Yurok Tribe

- Thomas P. O'Rourke, Sr., Yurok Tribal Chair
  Thomas O'Rourke is the current Tribal Chair for the Yurok Tribe, the chief executive officer of the Tribe, and in that capacity holds the authority to execute contracts and agreements on behalf of the Yurok Tribe.

- Kathleen Sloan, Project QA Manager & Principal Investigator
  Dr. Sloan is the Director of the Yurok Tribe Environmental Program (YTEP) and Quality Assurance Officer for the Department. She is serving as the Quality Assurance Manager and Principal Investigator (PI) for this project. Dr. Sloan will be responsible for project and grants management, including sub contracts to Humboldt State University and California Department of Fish and Game for their participation and contributions to this study. Dr. Sloan will oversee all aspects of the project and report to EPA and the Yurok Tribal Council on project progress and findings.

- Suzanne Fluharty, Yurok Tribe Co- PI & Project Field Manager
  The Project Field Manager, Dr. Fluharty, will serve as the primary staff to oversee all aspects of collection and processing of field samples up to transport to the appropriate laboratory. Additional responsibilities will include completion of tasks required to accomplish objectives identified in the grant, as determined by the PI including grant reporting, data entry, data analysis, interaction and coordination between research partners (HSU and Lab) and the tribal community. As Co-PI for the Yurok Tribe Environmental Program, Dr. Fluharty will help advize and direct project partners in meeting grant objectives.

- Ken Fetcho, Water Quality Lead
  Ken Fetcho serves as an Assistant Director in YTEP and oversees the Tribe's Water Quality Program. YTEP conducts water quality sampling on the Lower Klamath River and tributaries with EPA funding and EPA-approved sampling methods. Mr. Fetcho will serve as the lead for water quality sampling events conducted under the supervision of the Project Field Manager. He will also serve as the lead for obtaining fresh water mussel samples for testing.

- Dave Hillemeier, Fisheries Lead
  Dave Hillemeier is the Yurok Fisheries Program Manager and will serve as the lead for coordinating the procurement of fish (salmon and sturgeon) and pacific lamprey (eel) samples to be tested. Mr. Hillemeier will also assist in the coordination of PPGIS sessions with Yurok fishers during tribal fisheries meetings conducted in years 2 and 3 of the project by HSU researchers and YTEP staff.

1.3.3 Humboldt State University

- Julie Davy, Interim Director HSU Sponsored Programs Foundation
  The foundation's primary mission is to provide the Humboldt State University with professional pre- and post-award grant and contract services. Ms. Davy while acting within her official capacities as Interim Director will oversee and administer legal and fiduciary commitments as specified and agreed upon within the HSU sub-award contract. This is not limited to, but includes guarantee that all information shared or gathered during the project is deemed the intellectual, proprietary information of the Yurok Tribe and is to be treated and handled in a manner that protects the confidentiality and proprietary nature of Tribal data including: final disposition of all data and files used during the project; and enforcement of restricted and approved access to information, security and management of data housed, or utilized at HSU or their facilities or equipment.

- Steven Steinberg, HSU Principal Investigator
  Dr. Steinberg serves as full-time faculty and the Director of the Institute of Spatial Analysis. He will serve as the PI for the HSU sub-award for this project. Dr. Steinberg will oversee and supervise all aspects of GIS analysis conducted for this project, including directing the work of approved graduate research assistants.

- Sheila Steinberg, HSU Co-Principal Investigator
  Dr. Steinberg serves as full-time faculty and the Director of Community Research for the California Center for Rural Policy. She will serve as the Co-PI for the HSU sub-award. Dr. Steinberg will coordinate and direct sociospatial data analysis of existing data and primary data to be collected using Public Participation Geographic Information Systems sessions (PPGIS) with tribal members and analyze socio-cultural data on relationships between tribal members and environmental health.

- Mark Rizzardi, HSU Statistician
  Dr. Rizzardi will serve as a consultant to the Project Team and provide statistical analysis and recommendations for sampling and data analysis conducted throughout the project.

- Nick Ramirez, Geospatial Research Assistant
  Nick Ramirez is a graduate student at the Institute for Spatial Analysis working directly under the supervision of Dr. Steven Steinberg. Mr. Ramirez is a Master's candidate in Natural Resources with an emphasis in Geographic Information Systems (GIS) and will collect existing GIS data for use in the project. Mr. Ramirez is creating a GIS Ecotoxicological Assessment tool for this project as part of his Master's thesis work.

1.3.4 California Department of Fish & Game, Fish and Wildlife Water Pollution Control Laboratory

- David Crane, Lab Manager
  Dr. Crane serves as the Manager for the California Department of Fish & Game, Fish and Wildlife Water Pollution Control Laboratory, one of three collaborative facilities that comprise the Marine Pollution Studies Laboratory (MPSL); the lab selected for water and tissue analysis to be conducted for this project. Dr. Crane will provide the project team with QA documents and SOPs required for specific samples and analytes collected, as needed and insure that all samples will be processed and analyzed to EPA standards where applicable.

1.3.5 California Tribal Epidemiology Center (CTEC)

Negotiations are ongoing between the Yurok Tribe and the CTEC to engage their staff as epidemiological consultants. As such, their staff under the direction of Dr. Kristal Chichlowska, the center's Director will assist the Project Team in identifying possible negative health outcomes associated with specific chemicals, compilation and analysis of tribal health data, and interpretation of any health trends or patterns for the purposes of identifying potential risks and possible health outcomes of concern to tribal members.

.

1.4 Problem Statement and Background*

* A brief summary is offered here, see Grant Proposal RD-83370801-0, pages 2-4 for a more in depth background.

Yurok People have resided on the Lower Klamath River and territories along the Pacific Coasts since time immemorial. Those who reside along the River are traditionally known as *Pohlik-lah* and Yurok who live along the coast as *Ner-er-ner*. The Yurok Reservation lies within the Lower Klamath Sub-Basin and extends one mile on each side of the lower 45 miles of the Klamath River to its confluence with the Pacific Ocean. In addition to lands along the river, ancestral lands include a strip along the ocean together with all usual and customary off-shore fishing areas (Yurok Tribe, 1993) (see Figure 2). Currently the Yurok People maintain cultural, economic, and spiritual ties to these ancestral lands through subsistence use and management of traditional resources.

Figure 2 Yurok Ancestral Lands Showing Reservation

The distinctive location of the Yurok lands makes them a final catchment for the many resides used throughout the entire Klamath River watershed that encompasses 15,751 square miles. Recent increases in adverse health conditions of Tribal members along with declines in the Klamath River fisheries have intensified interest and concern among tribal members about the general condition of the Klamath River and their ancestral territories

The Yurok Tribal Council and membership have identified the health of the Klamath River, its fishery, and the continued dependence on key subsistence species (including salmon, sturgeon, and pacific lamprey) as a primary concern for the Tribe and its future (Yurok Tribal Council 1996, 2001; Yurok Tribe 2006a). The goal of this project is to collect data required to answer questions about the environmental health of the Lower Klamath River and the aquatic species that

are critical to the continuation of Yurok subsistence, traditions, ceremonies and lifeways. Furthermore, it expands existing data on Klamath River water quality and aquatic health.

Given these concerns the following hypotheses have been developed:

- The Yurok Tribe's subsistence use of river and coastal resources, combined with the Yurok Tribe's position within the Lower Klamath Basin exposes Tribal Members to a great array of chemical stressors.

- The cumulative affects of chemical stressors, particularly endocrine disrupting chemicals, and the nutritional and psycho-social consequences of diminished or depleted, and potentially contaminated subsistence, cultural and spiritual resources act synergistically to increase prevalence of diabetes and other diseases within the Tribe.

- Chemical exposures resulting from subsistence and cultural activities are compounded by other kinds of exposures (e.g. burning plastic in backyard burn barrels). Exposures with no cultural significance could be reduced or eliminated, which would reduce cumulative exposures.

The results from this study will inform Tribal policy and may benefit Tribal members' health by helping remove general anxiety and uncertainty regarding the Klamath River and its resources by providing individual members personal choice in both where and which species to harvest as well as increase the general knowledge of the river environment, the levels of contaminants (if any) in subsistence resources, and any variations in the levels of detectable toxins in resources at specific local harvesting areas.

The original hypotheses, identified in the project proposal have been refined during the planning phase to four primary research questions:

1. What are the chemical stressors known to be used in the Klamath River watershed, their pathways, and known adverse health outcomes associated with those chemical stressors and contaminants?

2. What contaminants are currently detectable in the river and key aquatic subsistence resources?

3. Is there a relationship between environmental health as reflected by resource health and community health?

4. How can this study and the data produced from this study be used to identify and reduce risk and improve tribal member and resource health?

1.5 Project Description

This research project examines the health, geospatial, and social relationships between subsistence resources within Yurok Ancestral Lands and Yurok People, including coastal and riverine environments. Information on known contaminants and the current conditions of specific aquatic species vital to Yurok culture, subsistence and lifeways will be collected to assist the Tribe in its ongoing efforts in monitoring and protecting Klamath River water quality. This project will rely upon Yurok cultural geography and traditional ecological knowledge about resource health, abundance, and management to identify sampling species and locations, as well as to record information on changes in resource health over time. This information will also assist the Team in assessing issues and patterns in Tribal health outcomes.

This study will be conducted in a four year, phased approach. Objectives, activities, responsibility, and deliverables have been identified, target dates for completion of specific tasks or phases have been set with a scheduled completion date of September 31, 2012, and are described below and can be referenced in Table 2 Project Responsibility and Work Chart.

1.5.1 Phase 1: Year 1: Preliminary Assessment and Planning

The first phase, the planning phase, will involve the completion of a final research design through the preparation of a Quality Assurance Project Plan (QAPP), a literature review of comparable toxicological studies, and the compilation, review, and gap analysis of existing tribal and outside agency data (ethnographic information, GIS data, and information pesticides, and chemicals known to be used or to occur (historically or contemporarily) within the Klamath River watershed;

1.5.2 Phase 2: Year 2 & 3: Primary Data Collection

The second phase, the data collection phase, will consist of primary data collection through 1) a two tiered water and tissue sampling design, 2) Public Participation GIS (PPGIS), and 3) the screening of Yurok health data.

This project is designed to examine both Klamath River water and select aquatic subsistence resources by identifying the presence or absence of contaminants known to be used (historically or contemporarily) in the Klamath watershed. Contaminants will be further selected for screening based upon the severity & likelihood of specific adverse health outcomes, intensity of use in the watershed, and suitability for testing in selected species. The purpose of conducting tissue sampling and analysis is to produce information about the physical health of species relied upon for subsistence by Yurok People for their own knowledge and uses.

Table 2 Project Responsibility and Work Chart

Phase	Objectives	Activities	Responsibility	Deliverables
One: Preliminary Assessment and Planning	A. Finalize research design	1. Review literature and archival documents to determine the types, locations, and amounts of chemicals used in the Klamath watershed and coastal region, merge data into GIS system and determine target analytes for screening. 2. Review literature & documents; consult with Tribal elders, members, and Council to determine the types & locations of aquatic substance foods. 3. Compile, review, perform gap analysis, & QA existing tribal and outside agency data (ethnographic information, GIS data, and pesticide information) to determine utility of existing data sets and needs for the project. 4. Synthesis of activities 1-3 and prepare Quality Assurance Project Plan (QAPP).	1. YTEP staff and HSU 2. YTEP staff 3. YTEP staff and HSU 4. YTEP staff	1. List of target analytes for screening. 2. List of targeted subsistence species for sampling and site locations. 3. Summary report of gap analysis on needed information and selection of appropriate data sets for project. 4. Completed QAPP sent to EPA for review.
Phase	**Objectives**	**Activities**	**Responsibility**	**Deliverables**
Two: Primary Data Collection	A. Physical & biological sampling	1. Tier One: broad screening for contaminants a. Water Sampling from Klamath b. Tissue Sampling from suite of aquatic subsistence species 2. Tier Two: target screening based on results of tier one screening to correlate species with positive toxin levels to harvesting sites	1 & 2. YTEP staff and CA Dept of Fish & Game, Fish and Wildlife Water Pollution Control Laboratory	1. Summary report of contaminants detected in subsistence species 2. Summary report of screening results differentiating levels of contaminants in subsistence species between various harvesting sites
	B. Public Participation GIS (PPGIS).	1. Convene public meetings for Participatory Community Research and incorporation of interactive GIS focusing on health, quality, and quantity of subsistence resources.	1. YTEP staff and HSU	1. GIS based maps and geospacial information for tribal members' feedback. 2. Summary report of environmental & resource health issues and perceptions
	C. Yurok health patterns	1. Collect and evaluate general health data on Yurok Tribe with focus on health problems associated with chronic exposure to target contaminants. 2. Review and GIS acceptable tribal health data, US and Tribal Census data, to facilitate correlation of possible locational patterns obtained through analysis of existing data	1& 2. YTEP, HSU, and CTEC staff	1. Summary report of health issues and trends associated with common mechanism groups (CMG), & pathways involving aquatic subsistence resources 2. GIS existing Yurok health data obtained through record analysis.
Phase	**Objectives**	**Activities**	**Responsibility**	**Deliverables**
Three: Analysis and Outputs	A. Interpretation	1. Return and ground-truth preliminary results through PPGIS 2. Using GIS, the water, subsistence resource, & health data, analyze and identify intersections of exposures and outcomes in space and time between environmental conditions, subsistence resource contamination.	1. YTEP staff, & HSU 2. YTEP, HSU, and CTEC staff	1. Final report of community experiences and perceptions of project results 2. GIS Eco-Toxicological Assessment Tool
	B. Inform Tribal policy.	1. Meet with Tribal Council and staff to determine how the findings can best be used to inform Tribal Policy, reduce Tribal Members' exposure and reduce the degradation or decline of Yurok subsistence resources.	1. YTEP Staff	1. Summary report with policy recommendations for Tribal Council
	C. Sharing Results	1. Determine how results may be applied within the Klamath basin and for other Tribes that rely on subsistence resources. 2. Analyze methods (not tribal-specific confidential data) on study design and for other potential applications. 3. Compile & review project; write results and recommendations	1. YTEP, HSU, and CTEC staff 2. YTEP, HSU, and CTEC staff 3. YTEP Staff	1. Educational materials, presentations & publications 2. Educational materials, presentations & publications 3. Final report to EPA

The sampling data will supplement the generation of an interactive collection of information, self reported from tribal members, and gathered using Public Participation GIS (PPGIS). Humboldt State University Co-Principal Investigators will conduct hands-on working sessions with tribal members to help identify relationships between resources, locations, and perceptions of resource health. The information generated from these sessions will be mapped in a GIS, shared back with community members for validation and ground-truthing of the findings, and incorporated into educational materials.

Tier one water and tissue data collection will be conducted in year two and the tier two sampling in year three. Tier one screening will test for a range of contaminants of concern in both Klamath River water and from composite samples of select subsistence species collected from across their habitats within Yurok Ancestral Lands. Tier one tissue sampling and analysis will be completed in order to identify presence/ absence/ levels of contaminants associated with ten targeted species and to inform Tier two sampling. Results from water and tissue sampling will be analyzed in relation to comparable toxicological studies and known health outcomes. Of primary concern are endocrine disruptors or other toxins with potential cumulative effects from chronic exposure to combinations of contaminants.

Tier two testing will be conducted in year three and consist of tissue sampling and analysis from those subsistence species that had detectable levels of toxins in the tier one samples. Tier two screening will be limited to those toxins identified in tier one, correlated to the specific species that confirmed the toxin presence. This second tier of sampling will focus on discriminating if different levels of contamination exist at various sites where traditional subsistence harvesting occurs. For those species (salmon, steelhead, lamprey, and sturgeon) not tied to specific locales, tier two screening will consist of intensifying sampling in order to better quantify the range of contaminants within the species population. Tier two sampling is designed to provide data for individual Tribal members' harvesting options that may help lower possible contamination risk. Analytes, species, and sites will be determined by tier one analysis.

Resulting data from both tier 1 and tier 2 sampling will be compared to general health data of the Yurok Tribe obtained from the United Indian Health Service and the California Area Tribal Epidemiology Center, both part of the California Rural Indian Health Board. A GIS focus will be for occurrences of specific diseases associated with known exposures (both acute and chronic) of specific contaminants and chemicals in order to examine possible associations between the health of the subsistence resource and the health of the Yurok population.

HSU Co-Principal Investigators will conduct hands-on working sessions with tribal members to help identify relationships between resources, locations, and perceptions of resource health. The information generated from these

sessions will be mapped in a GIS and shared back with community members for validation and ground-truthing of the findings. While no direct causality can be made, this information may assist in risk assessment and more importantly, the development of risk prevention methods. These will then be disseminated among tribal members and subsistence practitioners to reduce risks of exposures from any contaminants identified in the analysis.

1.5.3 Phase 3: Year 4: Analysis and Outputs

The final phase in year four will involve analyses of data generated during Phases 1 & 2 to examine relationships between environmental conditions, subsistence resource quantity, quality, and purity, and patterns in tribal member health. PPGIS will take back this project's findings to the community for final ground-truthing. The final analysis will take a general approach to consideration of all pertinent data, both quantitative and qualitative.

Outputs will include the development and production of a final EPA Final Project Report, GIS based maps and geospacial information for tribal members, the creation of a GIS Eco-Toxicological Assessment Tool, educational materials in print and electronic media, as well as presentations and publication(s) on study methods. The goal is to develop and disseminate materials that may help traditional tribal members and subsistence practitioners in general identify and minimize risk.

No specific tribal information or findings may be published without the expressed, written approval from the Yurok Tribe, however it is expected that the project team will co-author paper(s) for peer-review publication and present at one EPA conference on study methods and findings.

1.6 Data Quality Objectives and Criteria

Data Quality Objectives (DQO) have been developed for this project using the *Guidance on Systematic Planning using the Data Quality Objectives Process EPA QA/G-4* (U.S. EPA 2006) and are presented below in Table 3. The overall QA objectives are to develop and implement procedures for obtaining and evaluating data so that field measurements, sampling procedures, and analytical data provide information that is comparable and representative. EPA guidance defines the following criteria for a QAPP:

- Accuracy - the degree of agreement of a measurement with an accepted standard reference.

- Precision - a measure of mutual agreement among individual measurements of the same property, usually under prescribed similar conditions. Generally expressed in terms of the standard deviation.

- Completeness - the amount of valid data obtained from a measurement system compared to amount that was expected to meet the project goals.

- Comparability - expresses the confidence with which one data set can be compared to another.

- Representative - refers to a sample or group of samples that reflects the actual characteristic of the environmental matrix that is being tested and how well the sampling point represents the actual parameter variations.

Table 3: Data Quality Objectives and Performance Criteria

Task	**Data Quality Objective**	**Performance Criteria**
Compile existing data on contaminants within Klamath watershed	Verify accuracy of environmental data sets re: the Klamath River watershed for use in GIS	Source validation of all data sets utilized for Project Creation of metadata for all data set used in GIS
Collect water and tissue samples in the field.	Collect comparable, defensible and accurate data and samples in the field.	Completion of field forms for all samples collected. Adherence to approved SOPs for sample collection and handling. Adherence to protocols required by Lab for all samples to be analyzed.
Conduct Laboratory Analysis	Analyze accurate samples for comparable results.	Adherence to EPA standards. Adherence to Lab QA/QC protocols and standards.
Conduct PPGIS	Accurate capture of information provided by tribal member participants Informed consent obtained from all participants	Data validation of PPGIS sessions through content analysis of notes taken by both HSU & YTEP staff. Production of interactive maps during PPGIS sessions. Signed Informed Consent forms obtained from all participants.
Collect existing data on Yurok health patterns	Obtain accurate and anonymous medical data on general health patterns in Yurok Population	Signed agreements with health institutions on data requested and provided and allowable uses of data. Data printouts from medical databases (anonymous)
Conduct GIS Analysis of project data	Accurate analysis of geospatial relationships of data generated during project.	GIS of all data sets collected with supporting metadata. Creation of maps and GIS of study findings to share with tribal membership.
Develop Risk Assessment and Risk Prevention Tools	Identify Risk Prevention strategies.	Creation of interactive GIS Eco-Toxicological Assessment Tool with metadata. Production of print and electronic Risk Prevention educational materials for tribal membership.

1.7 Data Collection, Analysis, and QA/ QC Components

Due to the inter-disciplinary nature of this project, several components require QA protocols to assure accurate and reliable data collection and analysis. QA/QC components and procedures for data collection will include the:

1. Surface water sampling and analysis for target metals, chemical analytes, and the algal microcystins;

2. Tissue sampling and analysis for target chemical analytes and microcystins;

3. Community Based Participatory Research (CBPR) PPGIS methodology;

4. Tribal health data;

5. Geospatial data collection, inputs, analysis, and outputs and

6. Secondary data for collection, validation, and analysis

Detailed procedures to assure quality data will be described for each data collection and analysis activity. When incorporating previously collected data and laboratory procedures, QA/ QC procedures used to collect those datasets will be referenced. Specific SOPs for the collection and handling of samples in the field and in the lab are detailed in the appropriate sections. Further refinements or adjustments to these SOPs will be developed if needed, in consultation with the Project QA Manager, the EPA Project Officer, and the EPA QA Manager.

1.8 Records, Documentation, and Reporting

1.8.1 Records and Documentation

YTEP utilizes quality documents and records at program and project levels, as well as the laboratory and field levels. Specific sampling protocols for water and tissue sample collection in the field are detailed in specific Standard Operating Protocols (SOP) and will become part of the formal documentation process for biological and water samples collected for analysis as part of this study.

All data, records and information collected during this study will be kept by the Yurok Tribe Environmental Program and remain the property of the Yurok Tribe and may not be used, presented, or published without the written approval of the Yurok Tribe. All field and data collection activities will be documented and records will be kept on file by the Yurok Tribe Environmental Program offices in Klamath, CA. All tribal-specific data

shared or generated through the course of this study with Humboldt State University (HSU), its faculty, researchers, students or sub-contractors is subject to the restrictions outlined in the Contract between the Yurok Tribe and HSU's Sponsored Program Foundation for this project. All documents and data provided or generated by HSU for the purposes of this project are the property of the Yurok Tribe and will be returned to the Yurok Tribe upon completion of the project.

Records kept regarding the information shared between partners and all project data will be warehoused with YTEP in a secure location and stored on a secure server and backup systems. Upon completion of the project, all tribal data shared with and/or generated through the course of this project will be returned to the Yurok Tribe, care of the Environmental Program and stored on a secure server of the Yurok Tribe. All tribal data shared and generated by HSU, as part of the project, will be returned to the Yurok Tribe and no copies will be made or kept by HSU faculty, students, or participants in this project.

Specific details for records keeping and management during the field sampling phases of the project will follow standard scientific reporting formats and are presented below.

1.8.1.1 Sample Collection Records

Sample collection records document that established sampling protocol was followed in the field. This documentation includes the names of persons conducting activities, sample number, sample collection points, maps and diagrams, equipment/method used, climatic conditions, unusual observations, water parameter data for water samples, and all required information for specific tissue samples, as well as observations and references to changes or events in planned activities.

The U.S. EPA Standard Field Record Form has been modified for use for surface water sampling and tissue sampling for this project. An appropriate Field Record Form will be completed at each sampling location for each sampling event. Information from the entire list below will be included on tissue sample Field Record Forms and the italicized list will be included for surface water sampling.

Sample Field Record Forms are in Appendix A. Information to be collected for these forms is listed below, as applicable for sample type (water or tissue). Items in *italic* will be recorded for both water and tissue samples.

- *Geographic location using Global Positioning System*
- Species name
- *Date and time*
- *Method of collection*

- *Station number*
- *Sample identification number / numbers*
- Composite sample number
- *Weather conditions (e.g., cloud cover, rain or shine, windy)*
- Evidence of hatchery markings (e.g., fin clips, tags
- Total organism length (in millimeters)
- Total weight (to nearest gram) for fish and lamprey
- Sex of organisms if relevant and possible to determine
- *Sampling crew names*
- External marks or gross physiological abnormalities
- *Notes*

In addition, any field observations, unusual activities or problems encountered in the collection or handling of samples will be recorded in the Notes field on the Field Record form. It will also include a record of any photographs taken in the field. All forms and field notes associated with sample collection will be kept and maintained by the Project Field Manager and stored at the Yurok Tribe Environmental Program Offices.

1.8.1.2 Sample Identification Label

A waterproof Sample Identification/ Chain of Custody Label will be completed in indelible ink and attached to each water sample, as well as to each individual whole salmon, steelhead, and lamprey specimen and taped to each aluminum-foil-wrapped group of specimens before placing the specimen(s) in a plastic bag in the field. This tag will include the project name/code, station location/ number, sampling date and time, species name, sample number, organism length and weight, and the name, phone number, and signature of the sampler.

If a tissue sample tag is lost during shipment or a tag is never created, the sampler and/or field activities manager will write a statement detailing how the sample was collected, stored, and transferred to the laboratory. The statement will include all pertinent information regarding the sample, whether the sample was in the sample collector's physical possession or in a locked compartment until transported to the laboratory, etc.

1.8.1.3 Chain-of-Custody Records

Chain-of-custody records document the progression of samples as they travel from the original sampling location to the laboratory and finally to the disposal location. Chain-of-custody records will be kept and the CDFG lab record will be used (see Appendix A and C).

1.8.1.4 Quality Control (QC) Sample Records

QC sample records document the generation of field blanks and replicate samples. They also record information on sample integrity and preservation and include calibration and standards' traceability documentation capable of providing a reproducible reference point. QC sample records will contain information on the frequency, conditions, level of standards, and instrument calibration history. These records will be created and kept for each sampling event.

1.8.1.5 General Field Procedures

General field procedures are detailed in specific SOPs for water and tissue sampling (collection, processing, handling and transporting of samples from the field to the Lab). The specific field sampling methods are detailed in the following section.

1.8.1.5 Corrective Action Reports

Corrective action reports show what methods were used in cases where general field practices or other standard procedures were violated and include the methods used to resolve noncompliance. Corrective action reports will be completed and kept on file, as necessary.

1.8.2 Reporting

Informal summary reports will be submitted to the Principal Investigator by primary personnel responsible for the completion of specific tasks, as tasks are completed. Annual reports will be completed by the Principal Investigator within 90 days of the project anniversary date (effective date of signed grant agreement, July 15, 2008) and submitted to the EPA Project Officer. A final report will be prepared by the Principal Investigator and HSU Co-Principal Investigators at the close of Phase 3 and submitted to Yurok Tribal Council and EPA, National Center for Environmental Research as part of final project deliverables.

2.0 Data Collection, Analysis, and Quality Assurance

The research and sampling design for this study is intended to collect data on four key components of the study as outlined below.

1. Types and locations of chemical stressors within Yurok Ancestral Lands

    a. Known historic or contemporary contaminants or chemicals used by agriculture, forestry, and industry within Yurok Ancestral Lands (location, distribution, intensity/dose, duration and quantity)

    b. Contaminants of concern; those groups of chemicals either known or suspected to adversely impact health outcomes in humans or aquatic species.

2. Environmental contaminants in Yurok environment and aquatic subsistence resources will be determined following methods and protocols, MDL and RL established by Laboratory in accordance with standards set by EPA for each specific contaminant.

    a. Water sample taken within the lower Klamath River (Absence or Presence and detectable levels)

    b. Tissue samples of select aquatic subsistence species (Absence or Presence and detectable levels)

3. Relationship between resource contamination and adverse health patterns in Yurok tribal members

    a. General health outcomes resulting from exposure to known contaminants as identified through literature review

    b. Possible pathways and routes of exposure by subsistence practitioners

    c. Occurrences of specific diseases known to be associated with specific chemical exposures

4. Geospatial data and analysis to assist in the development of Ecotoxicological Assessment tools and the gathering of data to inform, protect, and improve tribal member and resource health.

    a. Types and locations of chemical stressors known to be used or occur within the Klamath River watershed;

    b. Results from previous water and tissue data collected by other agencies sampling for same geographic area and chemical stressors;

c. Yurok subsistence fishing and harvesting locations and types of practices;

d. Yurok Tribal demographics and health data;

e. Water and tissue sampling locations;

f. Water and tissue sampling findings;

g. Analysis to make possible connections between source and environmental stressor,

h. Development of an interactive GIS Eco-toxicological Assessment Tool.

2.1 Experimental Sampling Design

The project involves the use of pre-existing or secondary data surveys, archival documents, ethnographic interviews, GIS databases, and environmental data on pesticide use. The project also involves the collection of primary or new data generated through interviews and public participation geographic information system sessions and chemical screening and analysis of surface water and targeted species. The project adopts a sociospatial perspective (Steinberg and Steinberg 2006) that actively considers space, place, and social indicators in a holistic fashion. The approach is unique because it is integrated, multi-method, and relies on quantitative and qualitative data. “A sociospatial model is multi-dimensional, incorporating both social and environmental factors (especially geographic or spatial) that may impact an issue or problem under study.” (Steinberg and Steinberg 2009:100).

The data collection methods used in this project are outlined in this QAPP and are intended to maximize and build upon current and ongoing research on environmental and resource health and its importance to the health and vitality of Yurok people. The following sampling design outlines how data will be collected, evaluated, utilized and managed for use in this project.

2.1.1 Secondary Data

Pre-existing GIS, survey, health, toxicological and ethnographic data will be reviewed by the Principle Investigators for potential use in the project and will be selected based on their relevance or usefulness to assist in meeting project objectives and answering research questions specific to this project:

1. Is it geo-referenced or capable of being geo-referenced?
2. Does it contain data on tribal subsistence practices?

3. Does it contain data on tribal health or occurrences of certain diseases on concern?
4. Does it contain data on consumption of resources of concern?
5. Does it contain perceptions on environmental conditions impacting subsistence resources?
6. Can it be used in a manner that does not disclose personally-identifiable information?

All secondary data with qualitative components will only be used if the integrity of information is verified from multiple supporting documents and compiled from reputable, known sources. Additionally, all secondary data sets with quantitative components must meet the following guidelines:

- geographic references match validated and verified locations through field-truthing;
- data meet standards for verifiability and inclusion of metadata;
- collection methods are documented and follow QA protocols and meet EPA, USGS, and YTEP protocols standards.

Secondary data selected for use in the project will be incorporated into the GIS being developed by HSU. Where possible, spatial analysis will be used to synthesize, organize, present, and allow for the analysis of a range of tribal and environmental data on Yurok subsistence resources and practices throughout Yurok Ancestral Lands.

2.1.2 Water and Tissue Sampling and Analysis

Primary data collection for this study includes the sampling and analysis of ten key Yurok subsistence species for a range of chemicals of concern and is intended as a screening test. In general, screening tests are designed to be sensitive to allow detection of many possible targets at low concentrations. The goal is to identify all contaminants that might be present in aquatic subsistence resources when it is expected that no specific concentration of any single toxic substance exceeds regulatory or recommended standards or action levels. Once identified, this will allow the possibility of health outcomes associated with the various toxins to be further tested in more specific ways such as future risk assessments.

Those chemicals of concern include both natural and synthetic toxins and residues that have a high probability of bioaccumulating in selected species as well as chemicals known to have been used historically or contemporarily within the Klamath River basin. Also included for screening will be those known or suspected to be linked to specific adverse health outcomes such as diseases of the endocrine system, the immune system, or specific organs such as liver, kidneys, pancreas, or lungs.

Samples will be collected from locations and times associated with subsistence activities and will be determined through both previous Community-Based-Participatory-Research (CPBR) (Yurok Tribe 2006a, Sloan and McConnell 2007, 2009 and 2009a) and planned CPBR conducted by YTEP and HSU with tribal subsistence practitioners. Species have been identified for sampling based on previous CBPR (Yurok Tribe 2006, Sloan and McConnell 2007, 2009 and 2009a). Ten populations of key subsistence species utilized for foods by the Yurok will be sampled:

- Fall Coho (*Oncorhynchus kisutch*),
- Winter Steelhead (*Oncorhynchus mykiss*),
- Surf fish/ smelt (*Hypomesus pretiosus),*
- Green Sturgeon (*Acipenser medirostris*),
- Pacific Lamprey (*Lampetra tridentata*),
- Fresh Water Mussels (*Gonidea spp*.),
- Ocean Mussels (*Mytilus californianus*),
- Sea lettuce (*Ulva lactuca*),
- Razor Clams (*Siliqua patula),* and
- Washington Clams (*Saxidomus giganteus*).

Traditional Yurok subsistence relies on riverine and coastal/ marine resources. The sampling design has been developed to focus on key subsistence resources that many Yurok continue to rely upon and resource procurement locations historically and contemporarily used to harvest specific resources. Sampling events will be timed to coincide with peak harvest times associated with each resource so that a snapshot of resource health during periods of use can be obtained.

Tier one screening of water samples will be conducted during year two. Tier one screening of tissue samples will be conducted in year two to identify target chemicals of concern. Tier two testing to be conducted in year three, focusing on those species with detectable contaminants as determined in tier 1 screening. Tier 1 tissue sampling will be conducted using composite samples of single species comprised of several individuals of similar size and weight from across harvest locations for each species.

Tier two sampling will be conducted using composite samples comprised of several individuals of similar size and weight from single discrete harvest locations for each species in order to better identify the range of variation between harvesting areas.

All field collection methods, sampling protocols, processing and handling requirements will be identified by the lab and followed during project implementation. All samples will be collected, processed and transported to the lab for analysis by qualified YTEP staff, overseen and under the direction of the Project Field Manager to insure that each collected sample retain its

original physical form and chemical composition through collection to final disposal.

Prior to sampling, field team members will be familiar with:

- The responsibilities of each member of the field team
- Study objectives for this project
- Collection requirements
- Site locations
- Collection equipment and supplies needed at each site
- Proposed sampling dates and species of interest for each site location
- Composite sample size for each species and sample type
- Handling procedures and storage requirements.

Both water and tissues sample analysis and reporting will be conducted at the Marine Pollution Studies Laboratory (MPSL), a collaborative institution between research scientists of Moss Landing Marine Laboratories (MLML), the California Department of Fish and Game (DFG), and the University of California, Davis (UC Davis). They perform the most current state of science in pollution investigation and have built a reputation for research excellence. Their facilities and capabilities cover a broad spectrum of environmental pollution research and analysis that complements this projects research design. In addition, they currently manage the database for the California state-wide Surface Waters Ambient Monitoring Program (SWAMP); a centralized database developed using standardized data transfer protocols (SDTP) for data exchange and Data Entering/Editing Forms for field data and observations, making them eminently qualified to assist with this project's analysis phase.

The principal contact at MPSL for this project is Dr. Dave Crane, Director of the California Department of Fish and Game's Water Pollution Control Laboratory (DFG-WPCL) in Rancho Cordova, California. This is WPSL's main facility that performs analyses on water, sediment, and tissue samples and they are experienced with both state and federal EPA requirements and protocols. Specific protocols, QA/ QC procedures as identified by EPA or the lab, will be utilized for each species being collected and analytes being tested for. All samples will be processed according to established and approved EPA and lab QA/ QC standards and protocols. Specific SOPs for the collection, handling, processing, and transporting of samples to the lab are provided in Appendix B and C.

2.1.3 Geographic Information Systems (GIS)

Secondary data selected for use in the project will be incorporated into the GIS being developed by HSU. The GIS will synthesize, organize, present and allow for the analysis of a range of tribal and environmental data on Yurok subsistence resources and practices throughout Yurok Ancestral lands. All Yurok Tribe GIS data sets provided by the Yurok Tribe will be analyzed by

HSU Principal Investigator, Dr. Steven Steinberg for integrity and potential use in the Project. Any data sets lacking adequate metadata or possessing other data quality issues will be eliminated for use in the project. Dr. Steinberg will oversee all GIS data collection, input, and analysis to assure data quality and integrity throughout the completion of the Project. No copies will be made and the final GIS and all associated data will be turned over to the Yurok Tribe Environmental Program upon completion of the Project along with a User Manual for the GIS created for this Project. In addition, all files associated with this project will then be deleted and wiped from any and all instruments and computers.

GIS data will also be generated as a result of this study. PPGIS and other CPBR and environmental data will be collected by YTEP and HSU in Years 2 and 3 through additional research, interactive community workshops, planning sessions and discussions with tribal members, Tribal Council, tribal departments, and committees. Information generated will be integrated into the GIS being developed by HSU for this project. Newly generated data utilized in the GIS will be subject to the same standards of data quality, metadata, and data verification as secondary data selected for use in the project.

GIS metadata documenting methodological procedures such as transformations, conversions, the processing of data layers will be developed, stored and documented following Federal Geographic Data Committee (FDGC) standards and stored in digital form where feasible. Data stored in physical (paper form) will be stored in a secure cabinet in a secure facility accessible only by authorized project staff and will be delivered to the Yurok Tribe upon completion of the project. GIS or other digital data collected or carried to the field will be maintained on laptop computers and/or handheld GPS units for only the time required for completion of field work and then data will be downloaded and removed from field computers and GPS units upon return to the office and stored on a secure server and backup systems until completion of this project when all copies will be returned to the Yurok Tribe and all files deleted from all instruments and computers which will in turn be wiped to prevent rebuilding or recovery of deleted data and files.

2.1.4 Public Participation Geographic Information

The project will work with local tribal and community members in a group setting using Public Participation GIS community mapping sessions. Participants will be asked various questions about their knowledge of the natural resources and how they have changed over time. Participants will mark key features of resource quality and change over time on maps of the local region. Additionally, participants will share their knowledge about the locations and clustering of various health maladies experienced by the tribe as a whole. We will ask people to indicate places that they feel are no longer safe from an environmental health standpoint. Detailed notes will be taken

during the meetings by both HSU and YTEP staff. Sessions will not be recorded.

Notes will later be compiled and compared to assure accurate collection of information provided by PPGIS participants. Data collected from PPGIS sessions will be analyzed and mapped in a GIS to be shared with participants upon completion for validation and dissemination of project results. At the completion of the project, all recordings, notes, transcriptions, or generated reports will be turned over to the Yurok Tribe and any digital or computer files will be deleted and instruments and computers wiped.

2.1.5 Tribal Member Health Data

During this study we do not propose the collection of any individually identifiable health data on specific tribal members, nor do we expect that any specific health trends or outcomes can be extrapolated through the cursory analysis of the tribal health data collected in this project. Rather, the purpose of collecting data on tribal health, occurrences, and rates of specific adverse health outcomes is to serve as a starting point for evaluating the prevalence of health problems associated with chronic low level toxic exposures among the general Tribal Membership and within vulnerable sub-populations.

Some tribal members have articulated and identified a link between the health of the Klamath River, their Ancestral Lands, key subsistence and cultural resources, the health of the Tribe, and its individual members' health (Yurok Tribe 2006, Sloan and McConnell 2006, 2007, 2009 and 2009a). One of this study's goals to collect preliminary environmental data and existing tribal health data in an attempt to help define questions that address concerns about the relationship between tribal health and environmental health, as well as concerns about the condition and health of specific subsistence resources important to Yurok people and culture.

Anonymous health data will be requested from California Tribal Epidemiology Center (CTEC) on Yurok tribal members, their general health patterns, and the rates of occurrence of specific diseases associated with exposures to select contaminants of concern. Health data will be requested in a format with all personal identifiers removed prior to release to project staff and dissemination in public meetings in order to prevent the identification of individuals. Furthermore, any Yurok members' health data, self reported or inferred, that may be revealed during PPGIS sessions is incidental to the project goals. Any such information obtained through interviews or PPGIS workshops will have all personally identifying information removed prior to any inclusion into GIS, reporting, and analysis.

The principal source for health data will be the CTEC that began in 2006 as a collaboration of 21 tribal health organizations and 51 tribes that joined together to address the need for epidemiological services for Indians in

California by signing Health Data Sharing Agreements. They have since developed the capacity to collect and analyze existing health databases to determine the health and health status of American Indian and Alaskan Natives in Indian communities in California by partnering with the California Area Office of the Indian Health Service, the California Center for Health Statistics, and the University of California San Francisco, Institute for Health Policy Studies.

2.2 Sampling, Collection, Analysis, and Quality Control Methods

This study will involve the collection and analysis of both existing (available from other sources or previously collected) and new data (generated as a result of this study) for the following study components:

1. Secondary data and archival documents
2. Water quality sampling and analysis (new);
3. Tissue sampling and analysis (new);
4. GIS data collection (existing) and generation (new);
5. Qualitative data collection (PPGIS) and analysis.

The following section details the types of sampling and analysis methods to be used for the collection of data to be used in the final project analysis. Because each data set requires specific data collection and analysis and QA/ QC methods, the following section presents the information by data set type (ie, water, tissue, GIS, PPGIS, & health). Where applicable detailed SOPs are found in the appendices. Furthermore, all data collection, handling and management will be conducted under the supervision and direction of the Project Quality Assurance Manager, Dr. Kathleen Sloan. Specific QA procedures for the handling and management of all quantitative, qualitative, GIS, and other electronic data will be followed under the direction and supervision of the respective researchers for each area:

- Dr. Suzanne Fluharty: Aquatic Toxicology Data
- Dr. Steven Steinberg: GIS data
- Dr. Sheila Steinberg: Ethnographic, Survey, PPGIS, and Interview data
- Mark Rizzardi: Statistician, and
- The California Tribal Epidemiology Center, Dr. Kristal Chichlowska, Director

2.2.1 Secondary Data and QA/ QC

There have been several projects conducted by the Yurok Tribe, as well as other agencies that form the foundation from which this current research project builds. This includes water quality, fish tissue sampling, ethnographic studies, Tribal survey and census data collection, community consultation and outreach, pesticide monitoring, source pollutant location identification, and

geospatial information. Below is a brief discussion of this work, however, it should not be deemed exhaustive.

A complete citation of these data sources will be complied and listed in the references section of the final report so that these can be reviewed later if necessary. All QA methods shall be discussed in detail or referenced in the Final Report. All data will be warehoused with the Yurok Tribe and compiled in secure databases. If data is being transferred from its point of origin into a database, project staff will follow applicable QA/ QC methods for data transfer and management.

2.2.1.1 Secondary Data: Water Quality & Contaminants Research

Water quality data and associated geospacial information provides a clear understanding of water quality issues within the Yurok Reservation and will inform sampling locations and times for this project, particularly for microcystins analysis. The Yurok Tribe is a leader in producing water quality data for the Lower Klamath River since 2001. This includes water quality physical and chemical parameter data, (Yurok Tribe 2004, 2004a, 2005; Hiner 2006, 2006a, 2007, 2007a), as well as monitoring for certain phytoplankton, particularly MSAE and the resulting microcystins since 2005 (Fetcho 2006, 2007, 2008). All data included in this project has been collected according to an U.S. EPA approved QAPP (YTEP 2001) and/or Sampling Analysis Plans (SAPs) (YTEP 2003, 2008).

The U.S. Geological Survey (USGS) has also collected a significant amount of water quality data at over 400 stations within the Klamath Basin with the most historic sampling event occurring in 1950 (USGS…[updated 2009]). This information primarily includes physical and chemical parameter data, as well as metals analysis. Preliminary data synthesis indicates that mercury data will be informative, although further analysis of the data is needed (May, et al. 2005).

Records from other organizations that collect data on the presence of MSAE and microcystins for waters within the Klamath Basin will also be utilized. Specifically, the Karuk Tribe Department of Natural Resources in association with Jacob Kann at Aquatic Ecosystem Sciences LLC has collected MSAE and microcystins data from both Copco and Iron Gate Reservoirs (Kann and Corum 2006, 2007; Kann 2004, 2005, 2005a, 2006, 2007). QA/QC procedures for much of this data collection and lab analysis are included in the *Data Collection for Physical and Biological Characterizations of Klamath River Reservoirs* QAPP (Aquatic Ecosystem Sciences, LLC, 2005). In addition, PacifiCorp, owner of the KHP has also conducted MSAE sampling at various locations in the reservoirs, mainstem, and tributaries within the Basin since 2001 and the collection methods used are well documented (PacifiCorp…[updated 2009]). Likewise, there are other Tribes, federal and state agencies, and private

groups that have also collected MSAE and microcystins data throughout the Klamath Basin that may be used as supplemental data.

Beyond standard water quality monitoring within the Klamath River, YTEP also conducts pesticide water sampling at various timber spray locations within the Reservation that may be adjacent to tributaries of the Klamath River or drinking water intakes of Tribal members (YTEP 2001a, 2002, 2005) as per U.S. EPA approved SAP (YTEP 2002a). This work has been done independently some seasons, as well as conducted as a collaborative effort. The California Department of Pesticide Regulation (DPR), in cooperation with U.S. EPA Region IX and YTEP, conducted preliminary investigations concerning forestry herbicide migration in surface waters within the Klamath River watershed during spring and fall of 1999 as well as the spring of 2000 with an ISCO auto sampler. Sample sites were selected based on proximity to herbicide treated areas. Herbicide monitoring points were set in the creek or stream as close as possible down slope from the spray unit (California Department of Pesticide Regulation 2008; YTEP 1999, 2000). This collaboration continued to conduct a study of herbicide detection certain plants used by Tribal members for basketry because of the concern of pesticide transfer during material collection, preparation, and use (Wofford, *et al.* 2003). DPR has additionally conducted water sampling for a similar purpose in Karuk and Hupa Tribal territories, which are included within the Klamath Basin (Jones, *et al.* 2000).

Similarly, the North Coast Regional Water Quality Control Board (NCRWQCB) has been monitoring aerial applications of herbicides on private timberlands of the North Coast periodically since 1974. Of the water samples collected during 1991 approximately 26% contained a detectable amount of herbicide as analyzed by traditional gas chromatography (Wright-Shacklett, 1995). Water samples were collected by respective timber companies and analyzed for 2,4-Dichlorophenoxyacetic acid (2,4-D), Garlon (triclopyr), and Hexazinone. Between 1974 -1994, some 2,541 water samples were analyzed by the NCRWQCB, with only 2% exceeding the 10 ppb operational standard (*Ibid*.). It should be noted, however, that some of these samples were taken outside of the Klamath Basin and underscored that this was during aerial herbicide application, which ceased within the Reservation in 2001.

2.2.1.2 <u>Secondary Data: Aquatic Toxicology Studies</u>

Initial investigation for data analysis of fish tissue sampling for environmental contaminants has garnered results from several agencies and programs who have conducted sampling at various locations throughout the Klamath Basin, as well as along the coast of Yurok Ancestral lands. This data is used in this project to select which toxins to screen and analyze. Toxicology reports include documentation from the

US Fish and Wildlife Service (Bettaso and Goodman 2008), California Surface Water Monitoring Program (CALSWAMP), California State Mussel Watch Program (Phillips 1988), California Toxic Substances Monitoring Program (Jones et al. 2000), and National Oceanic and Atmospheric Administration Center for Coastal Monitoring and Assessment (1987). All of these programs have detected reportable levels of a variety of chemicals in several different local food sources, such as California mussel (*Mytilus californianus*). This data supports the hypothesis that chemical contaminants are bioaccumulating in Yurok subsistence riverine and coastal resources.

A similar hypothesis is also drawn in regards to the presence of microcystins in Yurok food sources. Microcystin toxins were added to the Clean Water Act Section 303(d) List on May 29, 2008, by the U.S. EPA. This is due in part to the data collected by or in association with the Yurok Tribe and Karuk Tribes. Fetcho (2006) found trace levels of microcystins in steelhead livers in 2005. The Karuk Tribe and Jacob Kann have also detected this hepatoxin in several species sampled from the Copco I Reservoir, at the Iron Gate Hatchery, Iron Gate Reservoir, and several locations along the mainstem of the Klamath River (Kann 2008).

2.2.1.3 Secondary Data: Ethnographic Studies

There are many published ethnographic accounts of Yurok life from early contact to contemporary times. Anthropologist A.L. Kroeber had an interesting fascination with Yurok people and subsequently, there are several texts documenting everyday life and beliefs that he published independently, as well as with Robert Spott, a renown Yurok and friend of Kroeber (Kroeber 1928, 1934, 1976; Spott and Kroeber 1942). Of particular interest to this study is work conducted by a student of Kroeber's, T.T. Waterman who in 1909 documented a very detailed cultural geography of Yurok territory, including some of the usual and accustomed fishing locations, which will complement Yurok contemporary ethnography and fishing locations (Waterman 1920) and inform this project.

The Yurok Tribe has conducted several ethnographic projects to document a variety of aspects of Yurok culture, including the importance of the Klamath River, as well as the reliance and use of traditional subsistence resources. This includes a Yurok Coastal Resources Oral History Project (Sloan and McConnell 2006) and a Yurok Coastal Resources Inventory Project (Sloan and McConnell 2009), which involved conducting ethnographic interviews with Tribal elders regarding Yurok uses of coastal and marine resources, identification of resource use locations, traditional harvesting techniques, and observations of changes in the resource(s) over time. Additionally, tribal member surveys have collected important ethnographic information about Yurok traditional

resource uses of the Klamath River, tribal member health, and changes in the resource base over time (Yurok Tribe 2006, Sloan and McConnell 2006, 2007, 2009 and 2009a). Other ethnographic studies similar in scope include an investigation into the use, harvesting techniques, and observed changes of Pacific lamprey *(Lampetra tridentata)* more commonly referred to as eels; eulachon *(Thaleicthys pacificus)* populations in the River; presence, locations, and importance of Yurok marine resources; and discussions with renowned fishermen within the Tribe (Larson and Belchick 1998).

2.2.1.4 Secondary Data: Tribal Qualitative Research Data

There have been several qualitative surveys conducted among the Tribal membership that inform this project, including the Health Effects Interviews (YTEP 2001b), Healthy River, Healthy People Traditional Foods Survey (Yurok Tribe 2006), Yurok Coastal Resources Oral History Project (Sloan and McConnell 2006), Yurok Coastal Resources Inventory Project (Sloan and McConnell 2009a), the Yurok Tribe Klamath River Beneficial Uses Study (Sloan and McConnell 2007), and Environmental Justice and Beneficial Uses Tribal Member Survey (Sloan, et al. 2008, Sloan and McConnell 2009a).

Information collected in these studies includes frequency trends on the use of subsistence riverine and coastal resources, changes in resources and subsistence over time, importance of subsistence resources, self-reported health data, and resource harvesting locations. These studies and the primary data collected during the studies have been analyzed by HSU researchers for quality, integrity and potential use in the project. Data sets that met HSU researcher criteria for inclusion will be incorporated into study design, GIS development, and the development of PPGIS workshops to be conducted in Phase 2.

2.2.1.5 Secondary Data: Tribal Census and Health Data

This project has a particular focus on chronic diseases associated with endocrine disruptors and their impact on hormonal changes including diabetes, birth defects, reduced birth size, infant mortality, increased spontaneous abortions and premature births, neurological conditions, thyroid and metabolic disorders, excessive weight gain, obesity, cardiovascular diseases, and cancers.

The California Tribal Epidemiology Center (CTEC) will analyze existing electronic state health surveillance databases to determine whether health conditions that have been linked generally to target toxins or contaminants have occurred or have an increased incidence or prevalence for Yurok tribal members in the Yurok reservation or Klamath area when compared to other populations.

Specifically, using electronic California birth and death databases CTEC will examine the incidence of adverse birth outcomes that have been generally linked to environmental hazards and exposures. These databases include vital statistics birth and fetal/infant death databases that have linked maternal and infant hospitalizations for 1998 to 2008. For 1988 to 1998 databases restricted to birth and fetal/infant deaths only would have to be used.

We will link electronic birth records to Yurok tribal enrollment records to identify records of births to Yurok men and Yurok women. From the remaining birth records other American Indian and non-Indian populations will be selected for comparison. The chief factors in establishing comparison groups will be likelihood of exposure to the agents in question.

In an attempt to capture time trends with small populations and rare events, we will roll 3 year averages in the incidence/prevalence of the birth and cancer outcome indicators in order to increase statistical power, however the project team will set up a health data analysis work group to meet monthly by phone for the first 6 months of Phase Two: Year 2 to consult and come up with most appropriate techniques for the analysis. Spatial areas for analysis will be zip code aggregation that best represents three community level areas:

1. tribal voting districts for those living on reservation
2. nearest towns for those living off reservation but within the Klamath area,
3. remote areas, a combined category for those living outside the Klamath area.

We will analyze the incidence and prevalence of adverse birth outcomes including: very low weight births, preterm births, congenital anomalies (birth defects), small for gestational age births (intrauterine growth retardation), fetal, neonatal and infant deaths, prenatal hospitalizations, and birth sex ratio for all groups, after adjustment for potentially confounding factors not related to hazard or exposure to the agents. We will test for significant clusters of individual types, or aggregated groups of adverse birth outcomes. We will calculate and present the 95% confidence intervals for rates and ratios used as outcome measures.

The CTEC will also obtain permission analyze the California Cancer Registry (CCR) for the incidence and prevalence of cancers potentially linked to toxins or contaminants. The CCR is one of state registries that was linked with the Indian Health Service registry in 2007 and therefore

has federally recognized American Indians flagged in the database. Again a further linkage of the CCR with Yurok tribal enrollment records will be done to determine cancers in Yurok tribal members. Again appropriate study and comparison groups will be constructed and the incidence of cancer over time and area for the groups will be analyzed.

The establishment of significant clusters, rates or ratios of these adverse health conditions will not prove that toxins or contaminants produced the poor health, but will help determine whether further investigation is warranted.

CTEC servers are inside the Indian Health Service/Health and Human Services Wide Area Network and behind their firewall. Additionally, they are in a secured area of CRIHB with minimal access except to specially authorized IT staff. Access to the server data is limited to the CTEC and EPI Research staff after CRIHB Institutional Review Board approval of the project. The CRIHB Institutional Review Board is chaired by the Corporate Compliance Officer who is also the HIPAA Privacy Officer (Susan Dahl).

2.2.1.6 Secondary Data: Yurok Reservation Potential Contaminant Inventory

The Yurok Tribe Environmental Program initiated a Community Based Participatory Research project in 2007 under a grant from the Department of Health and Human Services, Administration for Native Americans. The work conducted under this grant included extensive scoping and consultations with the tribal membership to identify possible locations of perceived and actual potential contaminant sources within the Yurok Reservation. Community members were asked to identify on USGS topographic maps the locations of: known or suspected illegal dump sites, burn piles, chemical spills, lumber mills and mill ponds, etc. Additional archival research was conducted to identify other historical sources such as gold mines and their locations on the Klamath and Trinity Rivers. This information has been entered into a GIS data base. YTEP is currently conducting the final phase of this project: field-truthing of reported locations and finalizing the GIS based upon these findings. This information and the associated GIS and maps will be shared with the project team and utilized, as applicable, in the GIS component conducted by HSU and also used in the PPGIS sessions with the tribal membership. Validated and verified locations will be integrated into the GIS used for this study, specifically in the locations of potential point and non-point sources of contamination within the Yurok Reservation.

2.2.1.7 Secondary Data: Pesticide and Chemical Release Data

Due to historic and contemporary pesticide applications on the Yurok Reservation and within the entire Klamath Basin, there is well-documented concern among tribal members about the long term impacts of these applications on tribal resources and tribal health. To-date there has been little coherent monitoring or compiling of this pesticide data for the basin or reservation, except for what has been reported by industry and what has been collected by the California Indian Basketweavers' Association and the Yurok Tribe Environmental Program.

As indicated in previous sections, existing data sets must be validated prior to use in GIS for this project and HSU analyzed the quality of pesticide and GIS data provided by YTEP for possible use in this project during Year 1 Preliminary Analysis and Planning. HSU researchers (experts in GIS and data quality issues in GIS) determined that this tribal pesticide data set does not meet the QA/QC standards for metadata to support using this data in this project. Efforts to collect pesticide use data for the entire basin will be done at a sub-basin level to estimate inputs into the Lower Klamath Sub-Basin (which comprises the portions of the Klamath River within Yurok Ancestral Lands). Aggregate information on historic pesticide applications will be collected from the California Department of Pesticide Regulation (DPR) and is aggregated to public land survey sections (~1 sq mi) and will be utilized in the project as applicable to attempt to reconstruct historic inputs and analyze current levels of contaminants entering the watershed for the targeted chemicals identified for analysis in this study.

2.2.1.8 Secondary Data: GIS Data

Pre-existing GIS data is being collected and analyzed for use in the project by HSU researchers during Phase 1: Preliminary Assessment and Planning and Phase 2: Primary Data Collection. All pre-existing data sets used in the project will be approved by Dr. Steven Steinberg prior to inclusion for use in the project. Any GIS data deemed as substandard in terms of data quality, verifiability or metadata will be excluded for use in the project.

One of the primary datasets to be used in the project is the National Hydrography Dataset Plus (NHDPlus). The NHDPlus was developed by the U.S. Geological Survey (USGS) and published by the U.S. Environmental Protection Agency (EPA) and is intended to support a variety of water-related applications. It is an integrated suite of application-ready geospatial data products incorporating many of the best features of the National Hydrography Dataset (NHD), the National Elevation Dataset (NED), and the National Watershed Boundary Dataset (WBD). It includes a stream network based on a static copy of the 1:100,000 scale NHD.

NHDPlus improves upon the original NHD by including enhanced capabilities for upstream and downstream navigation, analysis, and modeling. The NHDPlus contains extensive documentation on data quality information, metadata reference information, as well as contact information. Since it was developed by the EPA, it is assumed that this dataset meets the quality and metadata standards for our project and may be utilized.

2.2.1.9 Secondary Data: Quality Assurance

To incorporate any of these existing or secondary datasets into the project, sufficient QA/QC methodology must have been used or sufficient metadata must accompany data to provide confidence in the data. The Lead researcher for each subsection of this project will review any particular data set that may apply to their section and will determine if the existing data set meets the QA/QC standards for inclusion and use in the project. These include Dr. Steven Steinberg: GIS data, and Dr. Sheila Steinberg: Qualitative and Survey data. Additionally, Dr. Suzanne Fluharty, will conduct the literature review and will assist the Principal Investigator and Project Team in determining what previous related studies will be utilized in the study.

2.2.2 Water Sampling, Analysis, and QA/ QC

The Yurok Tribe conducts water quality monitoring on the Klamath River and its tributaries following EPA approved standards. Water samples are collected following rigorous SOPs and transported to the California Department of Fish & Game, Fish and Wildlife Water Pollution Control Laboratory (Lab), Rancho Cordova, CA. Upon receipt by the Lab, established QA and QC procedures and standards are implemented and followed for all samples and analytes.

2.2.2.1 Water Sampling Design

The surface water sampling design will incorporate a judgmental sampling approach as discussed in *Guidance on Choosing a Sampling Design for Environmental Data Collection (EPA QA/G-5S*) (U.S. EPA 2002). This approach has been selected because the interest is to identify the presence or absence and detectable levels of the chemicals of interest in the River within the same time and space as the species tissue samples are collected. This way two environmental media can be compared within a similar temporal and spatial scope for any given analyte.

2.2.2.2 Water Sampling Method

Using a judgmental sampling approach, surface water will be collected from 3 resource procurement locations on the Lower Klamath River. Water quality sampling will occur at the same time as tissue sampling. Specific sampling locations will be determined based upon reported locations most

utilized for the harvest of specific aquatic subsistence resources. No coastal sites will be included in the water sampling as there are comparability issues with analyzing water with high salinity rates.

Table 4: Water Sampling Strategy

Water Sampling					
Analyte	River Stations	Replicates	Field Blanks	Sampling Events	Total Samples
Organochlorines, organophospates, PCB's, PAH's, PBDE's, PCP/TCP'S, microcystins, carbamates, dioxin/furans, triazines, mercury, & trace elements	3	1/event	1/event	2 (late Spring and early Fall)	10

A total of 2 water quality sampling events in year 2 will be conducted for Tier 1 screening of the project. Sampling events will occur during the summer months of May to June and the Fall/Winter months of October to January, to coincide with peak subsistence uses of the River. All water samples will be tested for the full range of selected target contaminants of concern. Temporal information related to the date of collection will be strictly recorded to ensure there is clear documentation regarding the relationship between sample and date for data analysis.

2.2.2.3 Water Sample Collection

An isokinetic depth-integrated sampler will be used to ensure a representative sample within the water column at the sampling location in the river. Ambient water quality data should be collected prior to sample collection using a YSI 6600 multi-parameter water quality monitoring device.

The US D-95™ suspended-sediment and water-quality sampler has been selected based on the USGS *Report QQ: A Guide to the Proper Selection and Use of Federally Approved Sediment and Water-Quality Samplers* (Davis 2005). It meets the protocols for water quality sampling as outlined in the *USGS National Field Manual for the Collection of Water-Quality Data* (Wilde *et al.* 1998). The sampler uses a 1-liter bottle, and a US D-77 cap and nozzle. The bottle, cap, and nozzle are available in plastic and Teflon, however, Teflon is desirable for the project purposes to avoid sample cross-contamination given the environmental contaminants of interest. Detailed instructions on the use of the US D-95™ will be strictly adhered (Federal Interagency Sedimentation Project 2000).

YTEP's Field Sampling Procedures for Collection of Surface Water Samples willl be strictly adhered to in order to meet relevant requirements for the collection, processing and transporting of samples to the Lab for analysis and is provided in Appendix B.

2.2.2.4 Water Sample Documentation, Records, and Data Storage

All documentation will be completed in indelible ink. Three separate preprinted sample tracking forms will be used for each sampling site to document field activities from the time the sample is collected through processing and preservation until the sample is delivered to the laboratory. These are available in Appendix A and include:

1. Field Record Form
2. Sample Identification Label
3. Chain-of-Custody (COC) Form

2.2.2.5 Water Samples: Analytical Method

The analytical method used to detect potential environmental contaminants will conform to U.S. EPA methods for each target analyte. California Department of Fish and Game (DFG)'s Fish and Wildlife Water Pollution Control Laboratory, has been selected to perform all lab analysis because of their capabilities and demonstrated expertise in related studies including the California Surface Water Monitoring Program (CALSWAMP), the State Mussel Water (SMW) Program, and ongoing microcystins analysis conducted for YTEP. Analytical methods will meet the QA and QC standards of the Lab and the EPA.

For each analyte, the limit of detection (LOD), defined as the lowest concentration level that can be determined to be statistically different form a blank, and the limit of quantification (LOQ), defined as the level above which quantitative results may be obtained with a specified degree of confidence will be determined. The MDL and RL for each analyte will be determined by the lab and is documented in Appendix C.

For each set of samples, the Lab will analyze one sample in duplicate for each analyte. In addition, one spike recovery test will be run for each set of twenty samples for each analyte on each matrix type analyzed by the lab. For method validation, each matrix will be fortified with analyte at the reporting limit as specified in the appropriate SOPs found in Appendix C.

2.2.2.6 Water Sampling and Analysis: Quality Assurance/Quality Control

To ensure proper QA during field collection, approved SOPs will be followed. All members involved in field data collection will be trained in those guidelines and procedures by qualified staff prior to data collection.

Full QA/QC methods are detailed in the field SOP for water sample collection in Appendix B, tier one sampling.

The USGS Clean Hands, Dirty Hands (CH/DH) method will also be used during surface water data collection activities. "Clean" sampling procedures, including CH/DH techniques, are required when collecting inorganic samples for metals and other trace elements. Clean sampling procedures are recommended for all other sampling, to the extent that is reasonable, but particularly when the target analyte could be subject to contamination from field or laboratory procedures at a level that could exceed DQOs for reporting and interpretation. CH/DH techniques separate field duties and dedicate one individual as "clean hands" to tasks related to direct contact with the sample.

Precision will be based on the relative percent difference (RPD) between the primary sample and replicate sample. RPD will be determined by:

$$\% RPD = \left(\frac{|X1 - X2|}{\bar{X}} \right) \times 100\%$$

Where X1 = concentration of primary sample
X2 = concentration of replicate sample

The representativeness of the data will be maintained by following appropriate and consistent procedures for sampling using the selected isokinetic depth-integrated sampler, selecting a well mixed sampling point, by conducting sampling at locations heavily relied upon by Yurok Tribal members, and by following relevant SOPs. Field QA/QC samples will be used to provide information on the representativeness of the field sampling event, including replicates and field blanks.

Completeness will be based on percentage of samples collected in comparison with the sampling strategy. Some of the identified issues that may impact complete collection include collection methods, quantity needed for composite sample, preservation and shipping, and laboratory error. Percent completeness should be >90% and will be determined by:

$$\% \text{ Completeness} = \frac{\text{\# of valid results}}{\text{\# of samples taken}}$$

Accuracy of the lab's methods will be evaluated by calculating the percent recovery of target analytes or isotope-labeled target compounds in samples spiked by lab personnel at the lab using the following:

$$(C_s - C_u) \times 100/C_n$$

Where C_s = concentration of spiked sample

C_u = concentration of unspiked sample

C_n = nominal concentration (spiked concentration of spiked sample)

Percent recovery is typically 70-100% to be deemed normal, however, acceptance criteria will likely be structured on laboratory standards based on prior laboratory performance and be compound specific.

The comparability of the data will be ensured by the use of standard analytical methods and by reporting all values in consistent units. For example, no mixtures of English and metric units will be reported for depths, distances, elevations, etc. Related analytical data will be reported in consistent units; solids in milligrams or micrograms per kilogram (mg/kg or μg/kg), fluids in milligrams or micrograms per liter (mg/L or μg/L), or the units given in an approved reference methodology. Results of standard and non-standard analyses will not be compared without explicit presentation of the differences in the methods and their expected effect on the comparability of the data. For instance, comparability may be an issue with inclusion of secondary data in measurements of PCDDs/PCDFs and toxic, dioxin-like, PCBs due to new and improved methods for analysis versus those likely used in previous data analysis. The QA objectives outlined in the secondary data section will be evaluated in conjunction with the data validation process to ensure sufficient data comparability.

Other QC methods performed by the analytical lab will also be used to ensure data precision, accuracy, representativeness, completeness, and comparability as provided by the lab. Written SOPs for receipt of samples, tracking of custody, sample preparation and analysis, use of equipment and instrumentation, and data exchange procedures shall also be followed. These SOPs shall include use of standard data logging formats, logbook/worksheet entry procedures, and other written or printed documents relevant to the samples and will be provided by the lab, as well as lab SOPs for data reception and analysis (See Appendix B / C).

2.2.2.6 Water Sampling: Special Training/ Certification

Staff involved in the collection of water samples, physical measurements, and any other data pertaining to the assessment and monitoring activities will be trained based on U.S. EPA specified field sampling techniques and procedures. YTEP's Water Division conducts water sample monitoring within the Lower Klamath Basin and have been trained according to U.S. EPA specified field sampling techniques and procedures. The Project Water Quality Lead will lead the collection, processing, handling and transporting of all water samples collected for this study under the direct supervision and oversight of the Project Field Manager.

2.2.3 Tissue Sampling, Analysis, and QA/ QC

This project is considered a screening study (U.S. EPA 2000) to help identify the presence, absence, and concentration of a range of chemicals of concern within key subsistence populations. The main goal is to examine the relationships between water quality, resource contamination and quality, and tribal member health patterns and outcomes. The objective of tissue sampling is to collect and provide validated scientific information to the tribal membership on the overall purity of key subsistence resources that may help identify potential risks and routes of exposure, and to develop educational programs and materials designed to reduce, minimize, or prevent risks of exposures to any contaminants identified through sampling and analysis conducted during this study.

2.2.3.1 Tissue Sampling Design

Aquatic organisms may bioaccumulate environmental stressors at more than 1,000,000 times the concentrations detected in the water column (U.S. EPA1992, 1992b). It is for this reason, as well as the cultural connections that the Yurok Tribe maintains with the Klamath River that this project seeks to collect data on key aquatic subsistence species that are utilized by Yurok people. If chemicals of concern do exist within the water column, the potential for bioaccumulation and biomagnification in the aquatic resources is a likely outcome. Testing and analysis of tissue samples collected from key Yurok subsistence resources at traditional harvesting locations will help answer questions about both environmental and resource health.

Tissue sampling design will incorporate a judgmental sampling approach as discussed in *Guidance on Choosing a Sampling Design for Environmental Data Collection (EPA QA/G-5S*) (U.S. EPA 2002) because the interest is in those locations where subsistence activities are occurring. The area of interest for this project is key subsistence harvesting areas within Yurok Ancestral Lands. Sampling locations will be selected on the Lower Klamath River within the Yurok Reservation and the along the Pacific Coast of Humboldt and Del Norte counties. Exact locations will be determined based upon community input through surveys, ethnographic interviews, and Community-Based-Participatory-Research (CBPR).

Additional PPGIS will be used to confirm the selection of the river mouth location for the collection of coho salmon, steelhead, green sturgeon, and pacific lamprey and to select additional fishing locations as well as a minimum of two coastal sites each for the collection of surf fish/ smelt, mussels, clams and sea lettuce. When samples will be collected is to be based on traditional Yurok seasonal resource use for each selected species, as well as natural availability. Previous studies (Sloan and

McConnell 2006, 2007 and 2009) indicate in which months fishing, eeling, and shellfish activities traditionally occur (Table 5).

Table 5: Traditional Yurok Seasonal Resource Use (Sloan and McConnell 2007)

Subsistence Activities	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
Fishing	X	X	X	X	X	X	X	X	X	X	X	*
Eeling	X	X	X	X	X							*
Shell fish harvest	X	X	X	X						X	X	*
Seaweed collection				X	X	X						*

* Indicates the month when it is traditionally not culturally appropriate to gather any species.

Although each species has different lifecycles and durations of harvest time, there will be only a single sampling event for each tier of sampling for each species to coincide with times of peak substance harvesting. The general rule is that the fall season is the most desirable sampling period (Phillips, 1980) because the lipid content of the target species is generally highest at this time and water levels are typically lower, simplifying collection. Ultimately, sampling will occur when target species are most available and will be scheduled to coincide with peak harvesting cycles for each subsistence resource to reflect the time Yurok people are likely harvesting the resource for subsistence purposes. Additionally, California mussels, fresh water mussels, clams, and sea lettuce will be collected during months when there are no harvest restrictions and at low tide.

2.2.3.2 Tissue Sampling Method

All tissue testing will be conducted as a screening study for the absence/ presence and concentration of select contaminants of concern in aquatic subsistence food species utilized by the Yurok. The sampling strategy and methods to be utilized for the collection and analysis of tissue samples will be based upon US EPA guidance and standards as well as those standards, SOPs and QA/ QC procedures and protocols identified by the lab conducting the analysis of samples collected by the Yurok Tribe Environmental Program.
U.S. EPA recommends sampling aquatic environments with species from different positions in the food chains across trophic levels; for example, 1 bottom-feeder and 1 predator species for inland fresh waters and 1 shellfish and 1 fish species (or 2 fish species, 1 being a bottom-feeder) for estuarine/marine environments (*Ibid*.). While this standard methodology was considered, it was modified to meet the needs of this project and expanded to include the requirement that target species should be members of a suite of Yurok utilized subsistence species.

Bottom-feeders are important species indicators because they may accumulate high chemical concentrations from direct physical contact with

contaminated sediment and/or by consuming benthic invertebrates and epibenthic organisms that reside in contaminated sediment. Likewise, predator species are good indicators because of those persistent pollutants that can biomagnify through several trophic levels. California mussels (*Mytilus californianus*) are also known to bioaccumulate a variety of environmental contaminants and is considered a good indicator species for this type of study (Phillips 1980; Phillips 1988; National Oceanic and Atmospheric Administration (NOAA) 1987), as well as being an important traditional Yurok food source. The final list of five river species and five coastal species was identified for sampling from across trophic levels and from the suite of key subsistence species as determined through ethnographic
interviews, tribal member surveys, and discussions with the Yurok Fisheries Program Manager and Yurok Tribal Council (See Tables 6 & 7).

The sampling strategy for tissue collection incorporates information on subsistence harvesting practices identified through previous ethnographic research (Sloan and McConnell, 2006, 2007 and 2009). The strategy is based upon the timing and location of traditional subsistence activities associated with each resource (See Table 7). Although salmon, steelhead, eels, and sturgeon are anadromous, living part of their lives in the river and part in the oceans, for this project they will be considered a river resource as they are predominately harvested at the mouth of the Klamath River.

Table 6: Species Selected for Analysis by Trophic Classification

Classification	Species
Predator	Surf fish (*Hypomesus pretiosus)* Fall Coho (*Oncorhynchus kisutch*) Winter Steelhead (*Oncorhynchus mykiss*)
Bottom-feeder, Predator	Green sturgeon (*Acipenser medirostris*)
Filter feeder, Parasite, Predator	Pacific lamprey (*Lampetra tridentata*)
Filter feeder	California mussel (*Mytilus californianus*) Razor Clams (*Siliqua patula)* Washington Clams (*Saxidomus giganteus*) Fresh Water Mussels (*Gonidea spp*.)
Marine Producer	Sea lettuce (*Ulva lactuca*)

Tissue sampling and testing will be conducted during years two and three of the study. U.S. EPA recommends using composite samples of fish fillets and edible portions of shellfish for screening studies (1987; 1989). Tier one tissue samples will be ten composite samples from discrete organisms of each target species collected from across sites that correspond to peak times and use areas for Tribal Member harvesting. Individual organisms included in composites will be sampled with a focus on the larger mature individuals that are commonly harvested for subsistence with the target size clearly tracked on the specific species Field Sampling SOPs. Furthermore, because the concentrations in some

organisms for some pollutants (e.g., PCBs and mercury) have been shown to increase with age and size, an attempt will be made to collect organisms that represent the larger individuals being caught at the sampling site during the sampling period.

This goal may not be possible if species populations are low. In those cases where the above goal is unattainable during the time scheduled for sampling, available organisms will be used and the target size of collected specimens will be altered to reflect the average catch. Regardless, species should satisfy any legal requirements of harvestable size or weight, or if there are no legal harvest requirements, be of consumable size. Both the pre-determined target sizes for individuals being sampled for each species and any alteration to new sample size range will respect the guideline that each sample within each composite will be of similar size with the smallest individual being no less than 75% of the total length (size) of the largest individual (U.S. EPA 1990).

Table 7: Tier One Tissue Sampling Strategy

Analyte	**Coastal Resources**	**Sites**	**Sampling Events**	**Total Samples**
Organochlorines, organophospates, PCB's, PAH's, PBDE's, PCP/TCP'S, microcystins, carbamates, dioxin/furans, triazines, mercury & trace elements	Surf fish (*Hypomesus pretiosus)* Ocean Mussels (*Mytilus californianus*), Sea lettuce (*Ulva lactuca*) Razor Clams (*Siliqua patula)* Washington Clams (*Saxidomus giganteus*)	Composite from subsistence harvesting areas	1 per species population	5
Analyte	**Anadromous & Riverine Resources**	**Sites**	**Sampling Events**	**Total Samples**
Organochlorines, organophospates, PCB's, PAH's, PBDE's, PCP/TCP'S, microcystins, carbamates, dioxin/furans, triazines, mercury & trace elements	Fall Coho (Oncorhynchus kisutch) Winter Steelhead (Oncorhynchus mykiss) Green Sturgeon (Acipenser medirostris) Pacific Lamprey (Lampetra tridentata) Fresh Water Mussels (*Gonidea spp*.)	Composite from subsistence harvesting areas	1 per species population	5

Changes from the target size to an acceptable size range will be calculated in the field, recorded on standardized Alteration of Sample Size Forms, and kept by the Field Project Manager. The new size range will be consistently maintained during both tier one and tier two sampling to insure representativeness of the sampled individuals and comparability of any resulting data. In all cases, the total length and weight of each

individual in the composite sample will be recorded and maintained on the appropriate Field Data Sheets.

Specific protocols for the collection of each type of species sample will be identified by the lab. The collection, handling, and processing of samples will be followed by field personnel while collecting and processing the samples for delivery to the lab for analysis. Where possible, whole organisms will be collected and shipped to the lab for processing and analysis. The processing of organisms in the field will be avoided unless it is required by the laboratory prior to the acceptance of samples to limit possible contamination. For example, shipping whole sturgeon that may range between five to seven feet in length, when only 200 grams of tissue is required for screening is unpractical and wasteful.

Tier One: Composite Tissue Sampling and Screening

Tissue samples will be analyzed for the presence/ absence and concentration of a broad range of contaminants to identify possible inputs for cumulative risk analysis and to inform Tier Two sampling:Year Three tissue sampling and analysis. The first tier of screening will help limit the numbers of site specific sampling and eliminate costly tests for chemicals that may not be present in all targeted species. This will enable the second tier tissue screening to be focused and narrowed to only those contaminants that had detectable levels and correlated to specific species based upon the results of tier one screening.

Tier Two: Targeted Composite Tissue Sampling and Screening

Based upon tier one testing results, tier two testing will continue to use composite samples from individual species comparable in size and number set to those collected during tier one sampling. However, tier two will have targeted screening correlated and limited to 1) those specific species that had detectable contaminant levels and 2) the identified contaminant(s) associated with the specific species as identified in tier one screening. Table 8 offers an example of a possible sampling strategy for Tier Two.

Furthermore, tier two sampling of individuals for compositing will be collected within smaller discrete harvesting sites than tier one sampling. This will provide more detailed species information on the concentrations of identified chemicals of concern from different harvesting areas to provide possible options for Tribal Members to manage their harvesting and possibly reduce their risk should contaminants be detected in any of the screened subsistence species.

Table 8 Examples of Possible Sampling Strategies for Tier Two

Analyte	Species	Sites	Sampling Events	Replicate Samples	Total Samples
Dependent upon Tier 1 results	Surf Fish (if screening results in detectable contaminant)	1) Luffenholtz 2) Stone Lagoon	1	2	6
Dependent upon Tier 1 results	Ocean Mussels (if screening results in detectable contaminant)	1) Trinidad Bay 2) Clam Beach 3) Mussel Point	1	2	9
Dependent upon Tier 1 results	Sea lettuce (if screening results in detectable contaminant)	1) Trinidad Bay 2) Damnation Ck 3) Flint Rk Head	1	2	9

2.2.3.3 Tissue Sample Collection

EPA guidelines, standards and QA/ QC procedures identified by the lab will be utilized and followed in the collecting of all field samples used for analysis. Specific SOPs have been developed for the collection and handling of each species and are provided in Appendix B. Composite sampling methods will be used and because the goal of this project is to analyze those species utilized as subsistence foods, sampling will be performed on edible portions of individual organisms to reflect what is being consumed. Based upon the fact that in general, subsistence fishers eat what they collect (i.e. both males and females), samples of fish will not target either sex for collection. Also, whole-body samples will be collected because Yurok tribal members may consume several fish parts in addition to the fillet. Fish in general will be processed and sampled with their skin and for this project, a separate screening from whole salmon heads will be analyzed as these would likely be consumed by elderly Yurok People. In the case of shellfish, this will include the entire fleshy portions, excluding the shell. The entire blade will be sampled for the sea lettuce excluding the holdfast.

Composites must be collected during the same sampling event, ideally no more than 1 week apart. Sufficient numbers will be collected to provide a 200 g composite homogenate sample of edible tissue. The planned number of individuals per composite will vary for different species in order to meet both EPA recommendations and lab requirements. However, the same number of individuals will be included in each composite sample for each target species. Using the same number of individuals for a composite makes statistical methods for analyzing composite samples easier in that the arithmetic average of the replicate composite

measurements is an unbiased estimator of the population mean. For salmon, steelhead, and eels six whole organisms will be collected; for sturgeon six cross sections will be taken from six individuals, ten inches in length from the caudal regions to avoid organs and viscera; for surf fish/ smelt 54 total individuals will be collected: for mussels approximately 36 total organisms will be collected depending on species size; for clams between 36 to 108 total organisms will be collected depending on species size; and for sea lettuce approximately 70 centiliters. In all cases both the size and number collected at each site will be recorded and maintained across future tier two sampling for each species. However, the tissue mass may be reduced in the Tier 2 sampling if a limited number of specific analytes of concern have been identified.

The YTEP Project Field Manager will be present at all times when samples are collected in order to assure sample integrity. Sample integrity requires that aquatic organisms be handled in a manner that prevents loss of contaminants already in the organism and prevents extraneous tissue contamination. Loss of contaminants already in the fish tissue will be prevented in the field by ensuring that the skin on fish specimens has not been lacerated by the sampling gear. Sources of extraneous tissue contamination include contamination from dirty hands, sampling gear, greasy cables, spilled engine fuel, engine exhaust, dust, ice chests, and ice used for cooling the samples.

The YTEP Project Field Manager will identify all potential sources of contamination in the field and take appropriate steps to minimize or eliminate them. The following practices (and others as indicated by professional judgment) will be followed:

- Polyethylene gloves should always be worn when handling samples
- Caught organisms will only be placed on clean surfaces, such as aluminum foil.
- Ice chests will be cleaned prior to any sampling activities.
- Samples will be placed in waterproof plastic bags and double bagged to avoid contamination from melting ice.
- Sampling equipment, such as gillnets and dipnets, will be free from contaminants such as oils, grease and fuels.
- All utensils or equipment used directly in handling fish (e.g., such as fish hooks, measuring boards, and fish nets) will be cleaned prior to each field sampling effort and placed in aluminum foil.
- The field collection team will clean this equipment between sampling sites by rinsing with distilled water and rewrapping in aluminum foil.

2.2.3.4 Tissue Sample Storage and Shipping

The required protocols established by the lab for the storage and shipping of samples for analysis will be followed at all times. Specific steps required are determined by the species being sampled, size and type of sample, and lab QA/QC requirements. Preservation of tissue samples from time of collection to delivery at the processing laboratory follow applicable recommendations by U.S. EPA (2000, pg. 6-63) and the QA/QC requirements of the lab.

Organisms collected for tissue sampling will be inspected, measured, weighed, and the appropriate information recorded. The sampler will then immediately wrap the sample in clean, heavy-duty aluminum foil with the dull side in contact with the sample or place the sample in clean glass jars depending on the species and SOP. It will then be labeled according to established protocol; placed in a clean plastic bag; packed with ice contained in separate sealed bags (preferably dry ice) to ensure that ice melt does not come into direct contact with samples; and placed in clean ice chests to start cooling down the sampled organisms.

Because tissue will be dissected, composited, and homogenized by the lab, whole specimens will be transported and stored in a freezer at the Yurok Tribe Environmental Program lab until shipment to the lab. The freezer must have a temperature less than or equal to -20°C and must be secured at all times. Specimens will be completely frozen prior to shipping at which time they will placed in a ice chest with dry ice. Chain of Custody forms will be enclosed in plastic and taped to the inside lid of the chest. The ice chest will be closed and fiber tape will be wrapped completely around it so that it must be broken when the cooler is opened. Shipping will use Federal Express overnight delivery and will only be initiated between Monday and Wednesdays, no latter than 2:30 pm to allow for acceptance by the lab during working hours.

The contact person for the lab will be given sample ID numbers, number of ice chests being sent and species being sent in each mailing. Upon arrival at the laboratory, fish tissue may be distributed immediately to a technician for processing. If they are not processed immediately, they must be stored in a freezer at -20°C until they are removed for processing.

2.2.3.5 Tissue Sample Documentation, Records, and Data Storage

All documentation will be completed in indelible ink. Three separate preprinted sample tracking forms will be used for each sampling site to document field activities from the time the sample is collected through processing and preservation until the sample is delivered to the laboratory. These are available in Appendix A and include:

1. Field Data Sheet
2. Sample Identification Label
3. Chain-of-Custody (COC) Form.

2.2.3.6 Tissue Samples: Analytical Method

A final list of targeted chemicals for tier one screening has been identified during Phase One: Preliminary Assessment and Planning and are listed below:

- Microcystins
- Organochloriness
- Organophosphates
- Polychlorinated Biphenyls (PCBs)
- Polycyclic aromatic hydrocarbons (PAHs)
- Polybrominated diphenylethers (PBDEs)
- Phenols
- Dioxins and Furans
- Pyrethroids/Pyrethrins
- Triazines
- Carbamates
- Mercury
- Trace elements

These will be further limited for tier two screening and selected based on tier one results. Screening methods and detection limits for each analyte have been determined by the lab and are included in Appendix C.

2.2.3.7 Tissue Sampling & Analysis: Quality Assurance/Quality Control

EPA guidelines and standards in addition to QA/QC procedures and protocols identified by the lab will be followed for all phases of this study. All samples collected by YTEP for transport to the lab for tissue analysis will be collected following the SOPs identified by the lab for each species. Detailed SOPs for each type of sample are provided in Appendix B (Water, Whole Fish, Surf Fish/ Smelt, Pacific Lamprey, Mussels, Clams, and Sea lettuce). This QAPP will be followed during all phases of the project and incorporates any specific requirements from the lab for the collection, handling and processing of all tissue samples collected for analysis. The lab will follow all established EPA and lab QA/QC requirements to assure sample integrity, data quality and analysis of all tissue samples received for analysis in this project.

The Project Quality Assurance Manager will oversee all aspects of QA/QC throughout the project including the collection of samples for analysis. The Project QA Manager will not participate directly in the handling or processing of samples collected in the field. The Project Field Manager

will supervise and oversee all aspects of sample collection conducted for this study and will be the primary person implementing QA/QC requirements in the collection, handling, processing, and transport of samples to the lab for analysis. Once at the lab, qualified lab personnel will follow all established EPA and lab QA/QC procedures for each sample and test conducted for this study.

2.2.3.8 Tissue Sampling Special Training/Certification

All field staff involved in the collection, processing or transporting of samples to the lab will be trained in approved SOPs and QA/QC requirements as set by the EPA and the lab conducting the analysis prior to entry into the field for sample collection. All processing and testing of samples by the lab will be conducted per approved lab and EPA QA/QC standards and be conducted by qualified personnel.

2.2.4 Geospatial Information Systems (GIS) Data: Collection, Analysis, and QA/ QC

2.2.4.1 GIS Data Design

The geographical extent of locational data to be acquired will be limited to the ancestral lands of the Yurok tribe within the Lower Klamath River basin and adjacent coastal regions. Typical locational data will include the location of traditional subsistence gathering sites, the location of the source for various tissue samples, and the location, extent, and history of pesticide and herbicide use within the Klamath watershed and Yurok ancestral territory. Locational data will be provided by the Yurok Tribe, agencies in charge of tissue and water sampling, and public resources such as the California Pesticide Information Portal (CALPIP). All sources of data will be documented using FDGC metadata standards and evaluated based on relevance, resolution, logical consistency, and accuracy.

2.2.4.2 GIS Data Generation Methods

A spatial model will be developed in order to understand the movement of chemical stressors though the study area. The programming code will be written, documented, and developed using programming style standards used by the Humboldt State University Department of Computing Science, such as code appearance, naming conventions, and logical looping and control structures. Validation of assumptions, functions, and algorithms will be done through peer review and statistical testing. The model will be periodically updated and validated as new information is discovered, changes in the environment occur, or the need arises.

2.2.4.3 GIS Data Collection Methods

Existing GIS data will be obtained from a number of sources that report data on chemical releases, water or biological monitoring within the Klamath River basin and watershed in order to collect spatial data on the potential inputs into the Klamath River at the sub-basin level.

Existing and relevant environmental GIS and spatial data will be collected from outside agencies and entities on water quality and/or tissue studies conducted within the Klamath River basin and watershed.

Using GIS researchers will analyze the water, fish, shellfish, and public participation data to identify intersections of exposures and outcomes in space and time to reveal any clusters that could indicate sites of harmful harvesting sites and possible adverse health outcomes. Sampling locations, results of analysis of existing tribal health trends or patterns, and environmental pathways of the contaminants will be mapped to identify relationships and possible interactions between these factors. Where applicable, data will be incorporated into the GIS model development for the Eco-Toxicological Assessment Tool, and final analysis.

2.2.4.4 GIS Documentation, Records, and Data Storage

All GIS metadata documenting methodological procedures such as transformations, conversions, the processing of data layers will be stored and documented following Federal Geographic Data Committee (FDGC) standards and stored in digital form where feasible. Data stored in physical (paper form) will be stored in a secure cabinet in a secure facility accessible only by authorized project staff. GIS or other digital data collected or carried to the field will be maintained on laptop computers and/or handheld GPS units for only the time required for completion of field work and then data downloaded and removed from field computers and GPS units upon return to the office and stored on a secure server and backup systems.

Copies of GIS data sets will be transferred through a secure ftp or hand delivered by a project team member. Any hard copies that serve as the primary source materials for GIS data will be transferred in the custody of project team leaders and stored in a secure location in a secure file.

GIS data will be stored until completion of the project. During this time data will be stored on a secure server and backed up in real time using a secure RAID array accessible only to project staff. Offsite backups will be maintained on a weekly basis using multiple external hard drives on a rotational basis to maintain at least three weeks of backups at any one time. At the time of grant closure, all associated files, recordings, notes, transcriptions, or generated reports and copies will be turned over to the Yurok Tribe and any digital or computer files will be deleted and all

instruments and computers wiped in compliance with secure data deletion standards specified by Department of Defense 5220.22-M.

2.2.4.5 GIS Analytical Methods

The elements of GIS and remote sensing data such as positional accuracy; attribute accuracy; logical consistency; time; lineage; resolution accuracy; completeness of coverage, classification will be evaluated and documented based on how it pertains to the project goals, how current the data in regards to the project timeline, how relevant it will be in the final analysis, and how well the data meets FDGC metadata standards. This data will also be evaluated based on the source or agency providing the data as well as the detail of information provided in the metadata.

The most recent versions of following software for GIS data will be used: ArcMap and ArcCatalog published by the Environmental Systems Research Institute (ESRI) as components of ArcGIS, Excel published by Microsoft, Erdas Imagine published by ERDAS Inc., TerraSync and Pathfinder Office published by Trimble. We plan to use the Trimble Juno ST and Trimble GeoXT hand held GPS units. Model development will be conducted using scripting or programming tools available within the ArcGIS environment including Python, Arc Macro Language, Java, C++ and/or Visual Basic as appropriate to the specific requirements on the model. Statistical programming using the R language for statistical computing and graphics may also be incorporated into the model.

2.2.4.6 GIS Quality Assurance/Quality Control

The logical consistency, positional accuracy, and resolution of acquired data sets will be checked between different geospatial databases based on geographical accuracy when overlaid one on top of another using landmarks, benchmark, and when possible, groundtruthing. The accuracy and completeness of multivariate data set attributes will be screened by statistical tests to evaluate characteristic such as the distribution of data, missing data, and statistical parameters.

Any limitation of the data and the extent that these limitations are known will be documented using FDGC metadata standards.

2.2.4.7 Special Training/Certification

Dr. Steven Steinberg is the project lead for the geospatial data collection, analysis and reporting. He holds a Ph.D. in Forestry with emphasis in Geospatial Analysis. He has over 15 years of professional experience in research and teaching of geospatial analysis and is a Certified Geographic Information Systems Professional (GISP #59102). He serves as the Director of the Institute for Spatial Analysis at Humboldt State University.

Nicolas Ramirez the geospatial research assistant handling GIS data has completed the coursework required for the Humboldt State University Certificate of Study in GIS and Remote Sensing. He is enrolled in and has completed all coursework for advancement to candidacy for an M.S. in Natural Resources with emphasis in GIS and Remote Sensing. He is fully proficient with the software and data handling processes.

2.2.5 Public Participation Geographic Information Systems (PPGIS) Data Collection, Analysis, and QA/ QC

2.2.5.1 PPGIS Data: Sampling Design

The goal of this approach is to capture the community's knowledge and experience of the local aquatic resources in a geographic context. This approach facilitates dialogue among tribal members regarding their experiences with the environment and how it has changed over time. Environmental information to be included as a part of this exercise include data on pesticides and other forms of potential environmental contamination, aquatic subsistence harvesting sites, and tribal member's experiences and perceptions of local aquatic subsistence resource health, quality, and quantity. Social data that will be shared back include aggregated data on tribal members' perceptions about the health of the aquatic subsistence resources from those living both on and off the reservation as well as those who once lived on the reservation but now live in a variety of other places/locations. This will include water resources and tribal members' interaction with the river and its fisheries. It will allow tribal members to be aware of how they are using the resource and to share any experiences and observations about the health of the river not previously captured.

2.2.5.2 PPGIS Data: Sampling Method

A purposive sampling method will be employed. We will work with the Yurok Tribe to invite tribal membership to participate in the sessions from both on and off the reservation. Sessions will be conducted at the beginning of the project to gather data, in the middle of the project to groundtruth data, and at the end of the project to share findings back with the community. In totally we project public participation sessions occurring at three distinct time periods within the study. We will work with the Yurok tribe to determine the best times and location for these PPGIS sessions.

We use the term purposive sampling because we want to ensure especially that older residents with extensive knowledge about the local natural resources and traditions attend these sessions. All research participants will be provided information about the study and sign an informed consent form prior to participation (see Appendix A).

The public participation GIS methodology consists of:

1) Collecting data from tribal members in a group setting. Sessions will be held at multiple locations (both off and on reservation) to maximize opportunities for participation. Small groups of 4 -10 people will work with large scale maps of the area showing key locations and features. Participants will be asked to indicate on the maps the location of natural resources whose quality and or quantity has changed over time. In these sessions participants will note locations on poster size maps where they have seen natural resource changes, health changes for their population and potential sources of environmental contamination that we may not have noted. In addition to data annotations made directly on the maps, written notes and voice recordings will be collected for each group.

2) Groundtruth existing secondary data (environmental and social science data). Maps showing the pesticide use and other activities on and around the reservation will be shared with tribal members as indicated on maps. Additionally, PPGIS sessions will examine data that was collected by the tribe from two previous studies the Yurok Coastal Resources Oral History Project (Sloan and McConnell 2006) and the Health and Beneficial Uses Survey Data (Sloan and McConnell 2009). This will provide an opportunity to ground truth the data that was already collected.

Study participants will be asked to indicate any additional knowledge that they have of pesticides or other potential environmental contamination within or surrounding the reservation that may not be present on the maps.

Time Table:

First PPGIS Session (Spring 2010)

- In this session existing secondary data on natural resource use in the coastal areas will be presented and groundtruthed. Additionally, primary data will be collected on natural resource change in other tribal land area beside the coast resources area. Data on potential sources of environmental contamination will also be presented and groundtruthed. Furthermore, data on tribal member observations related to potential environmental contamination will also be collected.

Second PPGIS Session (Spring 2011)

- In this session primary data collected from the first PPGIS sessions in 2010 will be presented and groundtruthed. Additionally, any findings from research to date will be shared and ground truthed as well.

Third PPGIS Session (Spring 2012)

- In this session, final project analysis will be presented to the tribal community including any patterns or trends in secondary health data collected from CTEC and will be shared and groundtruthed.
- . Feedback will be sought on the findings.

2.2.5.3 PPGIS Data: Sample Collection

PPGIS sessions are planned to involve between 4 -10 tribal members from various geographic locations (on reservation and off-reservation). PPGIS sessions will involve groups of people sharing their knowledge and experiences with the natural environment and health of aquatic and subsistence resources, including experiences with pesticides or other environmental contaminants. Subsistence resources refer to any natural resource which members of the tribe use for subsistence purposes.

The methodological approach used in the PPGIS sessions is based on Sociospatial Grounded Theory (Steinberg and Steinberg 2006) which outlines a series of steps to follow to draw on local and indigenous knowledge. These steps are:

1. Determine a topic of interest;
2. Determine geographic location of interest;
3. Collect the data (qualitative, spatially linked social data);
4. Geocode the data;
5. Ground truth the data;
6. Analyze the data and look for spatial and social patterns;
7. Generate Theory.

The approach is called Sociospatial Grounded Theory because it allows for local knowledge and experience to help define the issue under study using local knowledge, observation, and experience.

In this case, we will be mapping and identifying key patterns in aggregate form from the existing secondary data. The secondary data consists of tribal collected ethnographic data on coastal resources that has been analyzed and had responses aggregated and geographically portrayed related to natural resource changes.

Primary data will be collected through PPGIS sessions consisting of small groups (4 -10 people) who sit around a map. In these sessions participants will note places on various poster size maps where they have seen potential environmental contamination as well as changes in aquatic subsistence resource health, quality, and/ or quantity. During the middle and end of the project primary data collected from the PPGIS sessions will be portrayed geographically and shared back with tribal community members in follow-up PPGIS sessions in order to groundtruth the data.

In total there will be three different types of maps for people to draw on: 1) known and potential environmental contamination maps, 2) traditional harvesting sites and current sites for harvesting traditional resources; and 3) the findings of tribal health data. Maps 1 and 2 will be presented during Phase 2: Years 2 -3 and map 3 will be presented in Year 4 as part of Phase 3. Notes will be taken at each group session.

2.2.5.4 PPGIS Data: Documentation, Records, and Data Storage

The PPGIS will be documented through voice recording of small group discussions around the PPGIS mapping exercise and written notes of public comments and statements recorded by the researchers during these sessions. To ensure confidentiality, data will be gathered in aggregate form and recorded as such. Individual names will not be associated with any comments or data gathered. All data collected will be stored in a locked filing cabinet in a secure facility accessible only by authorized project staff.

We are using existing interview and survey data gathered in a previous study by the Yurok Tribe, and have detailed metadata on the data collection procedures used. Physical copies of original data will be kept in a locked filing cabinet. As data is coded and entered into the computer (a secure computer, in a locked room that only project staff has access to) methods will be diligently tracked.

Copies of all Public Participation GIS data will be transferred through a secure ftp or hand delivered by a project team member. Hard copies of primary data will be handed to project team leaders and stored in secure facility in a locked filing cabinet until grant closure when it will be turned over to the Yurok Tribe.

Data will be stored on a secure server and backed up in real time using a secure RAID array accessible only to project staff. Offsite backups will be maintained on a weekly basis using multiple external hard drives on a rotational basis to maintain at least three weeks of backups at any one time. All data and copies (including physical and digital documents and storage media) collected during the project will be will be turned over to the Yurok Tribe at the time of grant closure and any digital or computer files will be deleted and all instruments and computers, both primary and backups will be wiped in compliance with secure data deletion standards specified by Department of Defense 5220.22-M.

2.2.5.5 PPGIS Data: Analytical Methods

The most recent version of Statistical Package for the Social Sciences (SPSS) will be used to analyze quantitative Yurok Beneficial Uses Survey

(Sloan and McConnell 2009). The qualitative data analysis program Hyper Research will be used to analyze narrative data to be collected during PPGIS sessions.

Dr. Mark Rizzardi, Professor and Chair of the Department of Mathematics and Computer Science at Humboldt State University, will serve as a statistical consultant along to guide statistical analysis and tests used in analysis of the secondary data. This process will occur in consultation with Dr. Sheila Steinberg, who will oversee the data coding, entry and analysis. Statistical analysis will include the use of spatial statistics to identify sociospatial patterns within the primary data collected using PPGIS and the secondary data that has already been collected by the tribe. The analysis will focus on conditions identified by the Tribal Members as pressing concerns and will also identify any other significant locational patterns of disease that may help in the determination of the existence and extent of possible environmental pathways of contaminants.

2.2.5.6 PPGIS Data: Quality Assurance/Quality Control

Data storage: Hard copies of all data will be stored in a locked filing cabinet accessible only to analysts working the project.

Data Entry: Data will be entered into Hyper Research program on a secure computer involving password protection. Only members of the project will have access to the data. The quality assurance for the PPGIS data coding and entry will involve two people; one to enter and one to double check the work and flag errors. Dr. Sheila L. Steinberg, Director, Community Research California Center for Rural Policy and Professor, Dept. of Sociology will oversee this process. Researchers will follow the procedures for careful data handling and analysis as outlined by Babbie (2009), *Practice of Social Research 12th Edition.* The data coding, entry and analysis process will be carefully documented and overseen.

2.2.5.7 PPGIS Data: Special Training/Certification

The lead investigator for the social science data collection, analysis and reporting holds a Ph.D. in Rural Sociology, with emphasis on research methods, especially community based participatory research with rural communities. She has over 17 year of professional experience in research and teaching social science research methods and analysis.

The project research assistants handling the social science data have extensive experience in survey and statistical analysis and research methods. They are fully proficient with SPSS software and Hyper Research 2.8 software and data handling processes and procedures.

3.0 Data Validation and Review

Each of the lead researchers in each of the research teams (Social Science and Policy; Geographic Information Systems Analyst; Epidemiology; Laboratory Manager; and Field Manger) will review the data collected specific to their fields to determine if it was collected and analyzed under this project's specified quality control procedures and if it meets the project guidelines for quality assurance. Reports summarizing their team's results along with their determination specifying any limitations on the use of the data, qualifiers, and recommendations, will be sent to the Project QA Officer.

One hundred percent of laboratory-generated data will be checked on receipt by the Project Manager for consistency, including whether blanks, spikes and duplicates are within specified targets and meet DQOs. Once data are merged or entered into a database, charting tools will be used to further check for data anomalies or errors. Any unusual values outside the range of norm will be flagged and all aspects of field data sheets, shipping handling and laboratory handling and testing will be reviewed. Water temperature, conductivity, pH and dissolved oxygen are measured in the field when samples are collected and values of these hand-held measurements can be used to check field conditions at the time of sampling.

The Project QA Officer will then provide an assessment and evaluation of the data validation reports considering both content and reasonableness. All validated data will be assessed by the Project QA Officer to determine if the data meets the goals and objectives of the project plan. Final usability of qualified and validated data will be determined by the Tribal Council, however the assessment of validated data will be reported in the Final Report for the project.

References Cited:

Aquatic Ecosystem Sciences, LLC. 2005. Data Collection for Physical and Biological Characterizations of Klamath River Reservoirs. Ashland, OR. Prepared for the U.S. Environmental Protection Agency Region IX.

Babbie, E.R., 2009. Practice of Social Research, 12th Edition. Wadsworth, Cengage Learning, Inc. Belmont, California.

Bettaso, J. and D. H. Goodman. 2008. Mercury contamination in two long-lived filter feeders in the Trinity River basin: a pilot project. U. S. Fish and Wildlife Service, Arcata Fish and Wildlife Office, Arcata Fisheries Technical Report Number TR2008-09 Arcata, California.

California State Water Resources Control (SWRB) State Mussel Watch and Toxic Substances Monitoring Database http://www.swrcb.ca.gov/programs/smw/ accessed Dec. 11 2006.

California Department of Fish and Game Fish and Wildlife Water Pollution Control Laboratory 2007. Standard Operating Procedure for the Management of Samples Received for Chemical Analysis. Rancho Cordova, CA. August.

2008. State of California Department of Fish and Game Office of Spill Prevention and Response Laboratory Quality Assurance Project Plan. Rancho Cordova, CA.

California Department of Pesticide Regulation. 2008. California Pesticide Information Portal. Version 2008.12. http://calpip.cdpr.ca.gov/cfdocs/calpip/prod/main.cfm accessed 2008.

Davis, Broderick E. 2005. USGS Report QQ: A Guide to the Proper Selection and Use of Federally Approved Sediment and Water-Quality Samplers. Federal Interagency Sedimentation Project. Vicksburg, MS.

Environmental Systems Research Institute (ESRI). 2009. ArcGIS version 9.3.1. Redlands, CA.

ERDAS Imagine version 9.3.2 (2009). ERDAS, Inc., Norcross, GA

Federal Interagency Sedimentation Project 2000. Sampling with the US D-95 Depth-Integrating Suspended-Sediment Sampler. Sponsored by the U.S. Army Corps of Engineers, U.S. Geological Survey, U.S.D.A. Agriculture Research Service, U.S, Bureau of Reclamation, U.S.D.A. Forest Service, and U.S. Bureau of Land Management. Vicksburg, MS.

Fetcho, Ken 2006. Klamath River Blue-Green Algae Summary Report; Water Year 2005. Yurok Tribe Environmental Program, Klamath, CA. January.
2007. 2006 Klamath River Blue-Green Algae Summary Report. Yurok Tribe Environmental Program, Klamath, CA. March.

2008. Final 2007 Klamath River Blue-Green Algae Summary Report. Yurok Tribe Environmental Program, Klamath, CA. July.

Hiner, Monica 2006. Hydrologic Monitoring in the Lower Klamath Basin; Water Year 2005 Yurok Tribe Environmental Program, Klamath, CA. October

2006a. Seasonal Water Quality in the Klamath River Estuary and Surrounding Sloughs, 2001-2003. Yurok Tribe, Klamath, CA. March.

2007. Hydrologic Monitoring in the Lower Klamath Basin; Water Year 2006. Yurok Tribe Environmental Program, Klamath, CA. April.

2007a. Hydrologic Monitoring in the Lower Klamath Basin; Water Year 2007. Yurok Tribe Environmental Program, Klamath, CA. December.

Hyper Research version 2.8
Copyright ResearchWare, Inc., Randolph, MA.

Jones, D., N. Singhasemanon, D. Tran, J. Hsu, J. Hernandez, and H. Feng 2000. Surface Water Monitoring for Pesticides in the Hupa and Karuk Territories. California Department of Pesticide Regulation, Sacramento, CA. November.

Kann, J. 2004. Memo on Copco Lake Analysis. To Karuk Tribe of California from Aquatic Ecosystem Sciences, Ashland, OR.

2005. Memo on Copco/Iron Gate Reservoir Toxic Cyanobacteria Results: Follow-up. To the Karuk Tribe and State Water Resources Control Board. Aquatic Ecosystem Sciences, Ashland, OR.

2005a. Copco/Irongate Reservoir Toxic Cyanobacteria Results, Aug-Sep. Ashland, OR. Technical Memorandum to Karuk Tribe, North Coast Regional Water Quality Control Board and State Water Resources Control Board.

2006. Microcystis aeruginosa Occurrence in the Klamath River System of Southern Oregon and Northern California. Ashland, OR. Technical Memorandum Prepared for the Yurok Tribe Environmental and Fisheries Programs.

2007. Toxic Cyanobacteria Results for Copco/Iron Gate Reservoirs: October 29-30, 2007. Ashland, OR. Technical Memorandum prepared for Karuk Tribe, Orleans, CA.

2008. Technical Memorandum. Microcystins Bioaccumulation in Klamath River Fish and Freshwater Mussel Tissue: Preliminary 2007 Results. Prepared by Jacob Kann, PhD of Aquatic Ecosystem Sciences LLC. Prepared for Karuk Tribe of California.

Kann, J. and S. Corum. 2006. Summary of 2005 Toxic Microcystis aeruginosa

Trends in Copco and Iron Gate Reservoirs on the Klamath River, CA. Aquatic Ecosystem Sciences, Ashland, OR and the Karuk Tribe Department of Natural Resources, Orleans, CA. Prepared for the Karuk Tribe Department of Natural Resources.

2007. Summary of 2006 Toxic microcystins aeruginosa trends in Copco and Iron Gate Reservoirs, CA. Orleans (CA). Technical Memorandum Prepared for the Karuk Tribe of California.

2008. Technical Memorandum Microcystins Bioaccumulation in Klamath River Fish and Freshwater Mussel Tissue: Preliminary 2007 Results. AQUATIC ECOSYSTEM SCIENCES LLC., Ashland, OR. Prepared for the Karuk Tribe of California, Orleans, CA, April.

Kroeber, A.L. 1928. Law of the Yurok Indians. *International Congress of Americanists Proceedings 22nd Session held in Rome,* 1926:vol.2:511-516.

1934 Yurok and Neighboring Kin Term Systems. *University of California Publications in American Archaeology and Ethnology* 35(2):15-22.

1976 *Yurok Myths.* University of California Press, Berkeley, CA.

Larson, Zachary S. and Michael R. Belchik 1998. A Preliminary Status Review of Eulachon and Pacific Lamprey in the Klamath River Basin. Yurok Tribal Fisheries Program, Klamath, CA, April.

May, J. T., R. L. Hothem, and C. N. Alpers. 2005. Mercury concentrations in fishes from selected water bodies in Trinity County, California, 200-2002. U. S. Geological Survey Open-File Report 2005-1321, Sacramento, CA.

Microsoft Office Excel 2007 12.0 (2006) Microsoft Corporation, Redmond, WA.

National Hydrography Dataset Plus (NHDPlus) version 1.0. 2006. US EPA Office of Water, Washington, DC.

National Oceanic and Atmospheric Administration 1987. National Status and Trends Program for Marine Environmental Quality-Progress Report: A Summary of Selected Data on Chemical Contaminants in Tissues Collected During 1984, 1985, and 1986. NOAA Technical Memorandum NOS OMA 38. U.S. Department of Commerce, Rockville, MD.

Natural Resources Conservation Service. 2002. Klamath River Basin Land Use/Land Cover Map. accessed at: http://www.nrcs.usda.gov/feature/klamath/images/kb-landcover.jpg. Accessed April 29.

PacifiCorp. 2002. Lake Ewauna to Keno Reach Water Quality Summary. PacifiCorp, Portland, OR.

2009. [Internet]. [updated 2009]. PacifiCorp, Portland, OR; [cited April 13, 2009]. Available from http://www.pacificorp.com/Article/Article82800.html.

Phillips, D.J.H. 1980. Quantitative Aquatic Biological Indicators. Pollution Monitoring Series. Applied Science Publishers Ltd, London, England.

Phillips, P.T. 1988. California State Mussel Watch-Ten Year Data Summary (1977-1987) Water Quality Monitoring Report No. 87-3. State Water Resources Control Board, Division of Water Quality, Sacramento, CA.

R Foundation for Statistical Computing. 2009. R version 2.9.2. Free Software Inc., Boston, MA.

Sloan, Kathleen M. and Robert McConnell
2006. Yurok Coastal Resources Oral History Project. Unpublished data. Yurok Tribe Environmental Program, Klamath, CA.

2007. Yurok Tribe Cultural Uses of the Klamath River and Tributaries. Unpublished data. Yurok Tribe Environmental Program, Klamath, CA.

2009. Yurok Beneficial Uses Survey 2008. Yurok Tribe Environmental Program, Klamath, CA.

2009a. Yurok Coastal Resources Inventory Project. Unpublished data. Yurok Tribe Environmental Program, Klamath, CA.

Sloan, Kathleen M., Robert McConnell, and Ruthie Maloney. 2008. Yurok Beneficial Uses Pilot Survey. Unpublished data. Yurok Tribe Environmental Program, Klamath, CA.

Statistical Package for the Social Sciences (SPSS) version 17.0. SPSS Inc., Chicago IL.

Steinberg, Steven J. and Sheila L. Steinberg. 2006. *Geographic Information Systems (GIS) for the Social Sciences: Investigating Space and Place.* Thousand Oaks, Sage Publications.

Steinberg, Sheila Lakshmi and Steve Jud Steinberg. 2009. “A Sociospatial Approach to Globalization: Mapping Ecologies of Inequality,” Book Chapter in*: Understanding Global Environment,* Samir Dasgupta, Editor. New Delhi, Pearson Education.

Spott, Robert, and A. L. Kroeber 1942. Yurok Narratives. *University of California Publications in American Archaeology and Ethnology* 35(9):143-256.

Trimble. 2008. GPS Pathfinder Office 4.10, Trimble Navigation Limited, Sunnyvale, CA.

2008a. Terrasync 3.30, Trimble Navigation Limited, Sunnyvale, CA.

U.S. Environmental Protection Agency 1987. Bioaccumulation Monitoring Guidance: 5. Strategies for Sample Replication and Compositing. EPA-430/9-87-003. Office of Marine and Estuarine Protection, Washington, D.C.

1989. Assessing Human Health Risks from Chemically Contaminated Fish and Shellfish: A Guidance Manual. EPA-503/8-89-002. Office of Water Regulations and Standards, Office of Marine and Estuarine Protection, Washington, D.C.

1990. Environmental Monitoring and Assessment Program (EMAP) Near Coastal Program Laboratory Methods for Filleting and Compositing Fish for Organic and Inorganic Contaminant Analyses. Draft. Office of Research and Development, Environmental Research Laboratory, Narragansett, RI.

1992. National Study of Chemical Residues in Fish. Volume I. EPA-823/R-92-008a. Office of Science and Technology, Washington, D.C.

1992b. National Study of Chemical Residues in Fish. Volume I. EPA-823/R-92-008a. Office of Science and Technology, Washington, D.C.

Field Sampling Guidance Document (SOP) #1229:Trace Metal Clean Sampling of Natural Waters (1999). Region IX. San Francisco, CA.

2000. Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume I. Fish Sampling and Analysis. Third Edition: Washington, D.C., EPA 823-B-00-007.

2001. EPA Requirements for Quality Assurance Project Plans, QA/R-5. Washington, D.C., March.

2002. Guidance for Quality Assurance Project Plans, EPA QA/G-5. Washington, D.C., December.

2006. Guidance on Systematic Planning using the Data Quality Objectives Process EPA QA/G-4. Washington, D.C., February.
2007. Guidance on Satisfying EPA Quality Requirements for STAR Grants, NCER G-1 STAR. Washington, D.C., June 22.

2007b. Guidance for Preparing Standard Operating Procedures (SOPs) EPA QA/G-6. Washington, D.C., April.

U.S. Geological Survey 2009. [Internet]. [updated April 21, 2009]. Water Quality Samples for USA: Sample Data. National Water Information System: Web Interface. USGS. [cited April 21, 2009]. Available from http://nwis.waterdata.usgs.gov/usa/nwis/qwdata.

Waterman, T.T. 1920. Yurok Geography. University of California Publications in American Archaeology and Ethnology. 16(5): 177-314.

Wilde, F.D., Radtke, D.B., Gibs, J., and Iwatsubo, R.T., 1998. National Field Manual for the Collection of Water-Quality Data: U.S. Geological Survey Techniques of Water- Resources Investigations, Selection of Equipment for Water Sampling, book 9, chap. A28

Wofford, Pamela, Kean Goh, DeeAn Jones, Heather Casjens, Hsiao Feng, Jean Hsu, Duc Tran, John Medina, and Jane White 2003. Forest Herbicide Residues in Surface Water and Plants in the Tribal Territory of the Lower Klamath River Watershed of California. California Department of Pesticide Regulation, Sacramento, CA.

Wright -Shacklett, Christine 1998. Executive Officer's Summary Report: Staff Evaluation of 1997 Aerial and Ground Applications of herbicides on Private Timberlands on the North Coast. North Coast Regional Water Quality Control Board. February 6.

Yurok Tribal Council 1996. Opposition to the Use of Pesticides on the Yurok Reservation. Resolution of the Yurok Tribe 96-23. Klamath, CA. April, 25.

2001. Meeting Minutes of the Yurok Tribal Council. Eureka, CA. June, 18.

Yurok Tribe 1993. Constitution of the Yurok Tribe. Article 1 Section 1. Klamath (CA).

2004. Water Year 2002 (WY02) Report. Yurok Tribe Environmental Program, Klamath, CA. January.

2004a. Water Year 2003 (WY03) Report. Yurok Tribe Environmental Program, Klamath, CA. March.

2005. Water Year 2004 (WY04) Report. Yurok Tribe Environmental Program, Klamath, CA. December.

2006. Healthy River, Healthy People, Traditional Foods Survey unpublished data. Yurok Tribe, Klamath, CA.

2006a. "Comments of the Yurok Tribe Regarding the Draft Environmental Impact Statement for Hydropower License for the Klamath Hydroelectric Project FERC Project No. 2082-027." Yurok Tribe, Klamath, CA. December.

Yurok Tribe Environmental Program 1999. Pesticide Water Sampling Results. Unpublished data. Yurok Tribe, Klamath, CA.

2000. Pesticide Water Sampling Results. Unpublished data. Yurok Tribe, Klamath, CA.

2001. Quality Assurance Program Plan
(QAPP) for Water Quality Assessment and Monitoring. Prepared with Assistance from the Yurok Tribe Natural Resources Programs and Humboldt Water Resources. Yurok Tribe Environmental Program, Klamath, CA. April

2001a. Pesticide Water Sampling Results. Unpublished data. Yurok Tribe, Klamath, CA.

2001b. Health Effects Interviews. Unpublished data. Klamath, CA.

2002. Pesticide Water Sampling Results. Unpublished data. Yurok Tribe, Klamath, CA.

2002a. Surface Water Monitoring Sampling and Analysis Plan for Forestry Herbicides with Immunochemical Analysis. Yurok Tribe Environmental Program, Orick, CA. August.

2003. Sampling and Analysis Plan for Bedload and Sediment in the Lower Klamath Watershed Basin. Yurok Tribe, Orick, CA. May.

2005. Pesticide Water Sampling Results. Unpublished data. Yurok Tribe, Klamath, CA.

2008. Lower Klamath River Nutrient, Periphyton,
Phytoplankton, and Algal Toxin Sampling Analysis Plan. Prepared with Assistance from Kier Associates. Yurok Tribe Environmental Program, Klamath, CA. June.

2008a. Yurok Subsistence and Cultural Beneficial Uses Survey 2008. Yurok Tribe, Klamath, CA.

2008b. Forestry Pesticide Use Within Yurok Ancestral Lands. Unpublished Geodatabase. Yurok Tribe, Klamath, CA.

2009. ANA Yurok Reservation Environmental Inventory. Unpublished Geodatabase, Yurok Tribe, Klamath, CA.

Appendix A: Sample Forms

1. **Alteration of Sample Size Form**
2. **Chain of Custody Form**
3. **Field Data Sheet for Fish**
4. **Field Data Sheet for Sea Lettuce**
5. **Field Data Sheet for Shellfish**
6. **Field Data Sheet for Water**
7. **Informed Consent Form**
8. **Sample Identification Label**

ALTERATION OF SAMPLE TARGET SIZE
FIELD CALCULATION SHEETS
YTEP EPA Star Grant # RD-83370801- 0

SPECIES: _____ **TARGET SIZE:** _____ **x** _____

PROJECT FIELD MANAGER _____

Location ID: _____

Latitude: _____

Longitude: _____

Datum: _____

Site Info			Field Measurements			
Site ID	Date (mm/dd/yy)	Time	Individual Organisms	Total Weight (grams)	Total Length (cm)	
			#1			
			#2			
Notes:			#3			
			#4			
			#5			
			Average			
			RANGE		75% Calculation	Check if within 75%
			Heaviest			
			Lightest			
			Longest			
			Shortest			
			Does new range need to be set? (circle) **YES** **NO**			

If smallest individuals ARE within 75% of largest then TARGET SIZE becomes acceptable size range for sampling. If NOT, and the smallest individual is NOT within 75% of largest, then size range must be calculated before continuing with the sample and all individuals not within the range must be released.

TO CALCULATE A NEW SAMPLING RANGE-

Average length	multiplied by	110% =	upper limit
	X	110	

THEN TAKE-

upper limit	multiplied by	75% =	lower limit
	X	0.75	

SAMPLE SIZE RANGE

_____ **X** _____

lower limit **upper limit**

DFG REQUEST FOR ANALYSIS AND CHAIN OF CUSTODY RECORD

Page_____ of _____

Sampler	Ph #	Send Results To	Lab Number
Address		Address	Field Number
City	Zip CA	City Zip CA	Lab Storage
		Copies To	Spill Title
Date Required/Reason		Address	Suspect
Shipped Via		City Zip CA	Index-PCA

☐ Fish & Wildlife Loss Date:_____ Region:_____

☐ DFG Code Violation:_____

☐ Suspected or Potential Problem

☐ Routine Analysis

Water Temp: F or C pH: DO: mg/L Conductivity: *u* mhos/cm

Analysis Requested >>>

Sample Identification/Location (Draw map on separate sheet if necessary)	Collection Date	Collection Time	Petroleum Fingerprint	Trace Elements (Specify Below)	Pesticides (Specify Below)							Sample Type: Water	Sample Type: Filtered Water	Sample Type: Soil	Sample Type: Tissue	Number of Containers: Plastic	Number of Containers: Glass	Number of Containers: VOA Vial		Preservation: Temp	Preservation: Acid	

Problem Description

Suspect/Incident Location

Comments/Special Instructions

Pollution Action Kit: Yes☐ No☐

Glove Size: Large ☐ Medium ☐

Hazmat Shipper Requested: Yes☐ No☐

Samples Reliquished By (Signature)	Print Name	Date	Received By (Signature)	Print Name	Date

Pesticide Investigations Lab
1701 Nimbus Road
Rancho Cordova, CA 95670
(916) 358-2950

☐

Petroleum Chemistry Lab
1995 Nimbus Road
Rancho Cordova, CA 95670
(916) 358-2803

☐

Water Pollution Control Lab
2005 Nimbus Road
Rancho Cordova, CA 95670
(916) 358-2858

☒

LAB COPIES: WHITE, CANARY, PINK **SUBMITTER: GOLDENROD** FG 1000 (Rev. 9/01)

YTEP FIELD DATA SHEETS
EPA Star Grant # RD-83370801- 0

TISSUE SAMPLING: _____ (species)

SIZE RANGE:

Location ID: _____

Latitude: _____

Longitude: _____

Datum: _____

Collectors: _____

_____

_____

Field Info		**Field Measurements**			
Date (mm/dd/yyyy)	Time	Individual Organisms	Total Weight (grams)	Total Length (cm)	Any Gross Abnormalities
		#001			
Composite Sample ID		#002			
		#003			
Picture Taken (circle one) yes no		#004			
Description/ limits		#005			
		#006			
		#007			
		#008			
		#009			
Method of Collection		#010			
		#011			
		#012			

Notes:

YTEP FIELD DATA SHEETS
EPA Star Grant # RD-83370801- 0

FISH TISSUE SAMPLING: _____ (fish species)

SIZE RANGE:

Location ID: _____

Latitude: _____ **Collectors:** _____

Longitude: _____ _____

Datum: _____ _____

Field Info		Field Measurements						
Date (mm/dd/yyyy)	Time	Individual Organisms	Total Weight (grams)	Total Length (cm)	Fork Length (cm)	Sex	Water Depth (m)	Hatchery Markings
		#001						
Composite Sample ID		#002						
		#003						
Picture Taken (circle one) yes no		#004						
Description/ limits		#005						
		#006						
		#007						
		#008						
		#009						
Method of Collection:		#010						
		#011						
		#012						

Notes:

YTEP FIELD DATA SHEETS
EPA Star Grant # RD-83370801- 0

SEA LETTUCE

Site Information			Site Desciption/ Limits
Latitude _____			
Longitude _____			
Datum _____			
Location ID	Date (mm/dd/yy)	Time	
Picture Taken (circle)	yes	no	
Collectors			Sample Information

Bottle ID # (check as collected)			Notes:
	#01		Notes:
	#02		Notes:
	#03		Notes:

Site Information			Site Description/ Limits
Latitude _____			
Longitude _____			
Datum _____			
Location ID	Date (mm/dd/yy)	Time	
Picture Taken (circle)	yes	no	
Collectors			Sample Information

Bottle ID # (check as collected)			Notes:
	#01		Notes:
	#02		Notes:
	#03		Notes:

Site Information			Site Description/ Limits
Latitude _____			
Longitude _____			
Datum _____			
Location ID	Date (mm/dd/yy)	Time	
Picture Taken (circle)	yes	no	
Collectors			Sample Information

Bottle ID # (check as collected)			Notes:
	#01		Notes:
	#02		Notes:
	#03		Notes:

YTEP FIELD DATA SHEETS
EPA Star Grant # RD-83370801- 0

WATER SAMPLING

Location ID: _____ **Collectors:** _____

Latitude: _____ _____

Longitude: _____ _____

Datum: _____ _____

Site Info			Field Measurements					
Site Name	Date (mm/dd/yy)	Time	Tw (C)	DO (mg/l)	DO (%)	Cond (uS/cm)	pH	BGA (cells/mL)

Bottles Collected								
Sample ID #	nutrients OC	periphyton	phytoplankton OC	phytoplankton SG	microcystin LR (EPA Lab) OC	microcystin LR (EPA Lab) SG	microcystin variants & anatoxin-a (DFG Lab) OC	microcystin variants & anatoxin-a (DFG Lab) SG

Notes:

HUMBOLDT STATE UNIVERSITY
COMMITTEE FOR THE PROTECTION OF HUMAN SUBJECTS IN RESEARCH

FORM 5: CONSENT TO ACT AS RESEARCH SUBJECT

This study involves research conducted by the Yurok Tribe and the California Center for Rural Policy, a rural policy research center at Humboldt State University. The purpose of this research is to gather information about Yurok Tribal Members' perceptions of changing environmental resources and overall observation of changing tribal population health. The procedure that will be used is called Public Participation GIS. Researchers will present a series of maps and have people work in groups of 4-6 people. They will also draw on maps to indicate geographically where they've noticed various health issues for members of their population. The PPGIS session will take approximately 1 hour to complete. Groups will draw on maps to indicate where changes in local resources have occurred. Study participants will also be asked to discuss and tell stories around their maps about how the resources have changed and in their opinion how tribal member's health has changed. With study group permission these stories will be tape recorded and notes will be taken. All participants will be provided with a copy of the informed consent form to sign. Confidentiality will be maintained because names will not be associated with the data collected and it will be collected by groups of people in aggregate form. Each map will coded by number, and kept in a locked filing cabinet during the course of the study. The maps and tape recordings will be kept for 2 years after the project is completed and then will be destroyed. Final project results will be exist only in aggregate form to protect the information shared by each group of people. Data will only be shared with groups the tribe deems appropriate, including the funding agency.

I understand that the procedures described involve the following possible risks and/or discomforts and possible benefits. Risk: No possible risk. Benefit: Will provide a broad understanding of the changing health of the natural environmental for Yurok people and how this connects to health of its population. Ultimately, this information will be shared with tribal members to help improve health and educational services for tribal members The PPGIS session will be conducted on the reservation. I understand that I will receive a copy of this consent form.

If you have any questions about this research please contact:
Dr. Sheila Steinberg, Director of Community Research, California Center for Rural Policy
Humboldt State University, Arcata, CA 95521
Phone (707) 826-4563, E-Mail: ss51@humboldt.edu

I hereby agree to have the following person(s) be involved in the data collection process:
Dr. Steven Steinberg, Director, Institute for Spatial Analysis

This information was explained to me by _____.
I understand that he/she will answer any questions I may have concerning this investigation or the procedures at any time. I also understand that my participation in any study is entirely voluntary and that I may decline to enter this study or may withdraw from it at any time without jeopardy.

Compensation:

I understand that my payment for participation in this investigation is $
If I do not complete this study, I will receive:

X I am not receiving any compensation for participating in this study.

_____
Subject's Signature

_____Date

Sample Identification Label

Yurok Tribe EPA Star Grant: # RD-83370801-0	
Site Identification:	Date:
Site GPS Latitude: Longitude: Datum:	Collected by:
Species:	Time:
Specimen ID #	Composite Sample ID #

Appendix B: Field Sampling Standard Operating Procedures (SOP)

1. **Tier One Sampling File**
    - **Clams SOP**
    - **Lamprey SOP**
    - **Mussels SOP**
    - **Sea lettuce SOP**
    - **Sturgeon SOP**
    - **Surf fish SOP**
    - **Whole fish SOP**
    - **Water Sampling SOP**

2. **Tier Two Sampling Files**
    - **Clams SOP**
    - **Lamprey SOP**
    - **Mussels SOP**
    - **Sea lettuce SOP**
    - **Sturgeon SOP**
    - **Surf fish SOP**
    - **Whole fish SOP**

PROCEDURES FOR *TIER ONE* FIELD SAMPLING

CLAMS

Deleted: SHELLFISH

Formatted: Font: 14 pt, Underline

Formatted: Font: 14 pt

Formatted: Font: 14 pt, Underline

Formatted: Font: 14 pt, Not Bold, Underline

The following procedures describe techniques for collection of marine clams in preparation for shipping to laboratory for composite sampling.

*****SUMMARY*****

- MINIMIZE HANDLING OF SAMPLES
- COLLECT INDISCRIMINATELY FROM ACROSS ENTIRE SAMPLING SITE
- ALL SAMPLES ARE DOUBLE BAGGED
- WHOLE, LIVE CLAMS with UNDAMAGED SHELLS, should be collected.
- CLAMS SHOULD NOT BE OPENED IN THE FIELD
- **108 Razor** Clams **TOTAL** are needed
- **36-72 Washington** Clams **TOTAL** are needed

Deleted: UNDAMAGED,

Deleted: SHOULD BE COLLECTED

Formatted: Indent: Left: 54 pt

*****BEFORE LEAVING OFFICE AREA*****

1. Inspect and check equipment for
   - safety,
   - contaminants (oil, grease, organic tissue or fluids) and
   - damage (sharp edges or holes)

2. **ELIMINATE DAMAGED SAMPLING GEAR**

3. Verify that instruments have been cleaned
   - washed with a detergent solution,
   - rinsed with ambient water,
   - rinsed with a high-purity solvent (methanol or petroleum ether)
   - finally rinsed with MilliQ/ distilled water and
   - wrapped in aluminum foil and bagged

4. Double check list that ALL equipment is available and loaded into vehicle

- Camera, digital
- Clam shovels
- Data Sheets w/ clipboard
- Daypacks
- Dry Ice or Ice sealed in bags
- GPS
- Heavy Duty Aluminum Foil,
- Heavy Duty plastic bags, 30 g
- Labels, gummed waterproof
- Methanol *or* Petroleum Ether
- Micro Detergent
- Micrometer
- MilliQ: Type II/ distilled water
- Nylon Cable Ties, 7” long
- Permanent Marking Pen
- Plastic bucket, 3 gallon
- Plastic Ice Chests
- Polyethylene Gloves
- Teflon Wash Bottle, 500 Ml
- Wading gear
- Zipper-closure Polyethylene Bags, 4milx13”x18”

Deleted: prepared

Deleted: Clear

Deleted: allon

SHELLFISH

*****AFTER ARRIVAL SAMPLING SITE*****

1. RECORD all possible crew, location, and sample information **FIRST**
   - List crew members and date
   - Photo-document study reaches (non-critical)
   - Take and record location with GPS

2. Wear polyethylene gloves while digging and removing shellfish from substrate.

3. Examine and rinse off any sand, soil, etc using ambient water
   - **Clams of 20 to 30mm** in length are the ***target size***.
   - **First 5 clams** will be accessed by the Project Field Manager to determine the acceptable ***size range*.**
   - **ALL clams for the sample will be within the set range.**

4. COLLECT **36 Razor** Clams at each of three sites.
   6-24 Washington Clams at each of three sites.
   - **If you suspect a counting error, recount.**

5. RECORD sizes for all clams.
   - Lengths are measured **across the longest** part of each shell

6. Wrap in foil with the dull side exposed to the sample.

7. Re wrap in opposite direction so edges of foil run crosswise to first wrapping.

8. Label, place in first zipper bag immediately, **write specimen # on bag as well as label.**

9. Then place in second zipper bag; **write specimen # on bag, AGAIN**

10. CARE should be used NOT to break shells or damage samples.

11. Place in ice chest with ice immediately.

PRIOR TO LEAVING FOR NEXT SITE

- Instruments and equipment should be
  - washed with a detergent solution,
  - rinsed with ambient water,
  - rinsed with a high-purity solvent (methanol or petroleum ether)
  - finally rinsed with MilliQ/ distilled water
  - re-wrapped in aluminum foil and bagged.
- Waste detergent and solvent solutions must be collected and taken back to the office.
- **DOUBLE CHECK THAT DATA IS RECORDED FOR EACH SITE AND ALL SAMPLES**

AFTER RETURN FROM THE FIELD

- Place samples in freezer at -20°C, **LOCK** it, and complete sample log form
- Clean, repair, and replace all equipment and supplies

Deleted: ¶
Formatted: Indent: Left: 36 pt
Formatted: Font: Arial, Font color: Auto
Formatted: Font color: Black
Formatted: Indent: Left: 36 pt
Formatted: Font: Bold
Formatted: Indent: Left: 36 pt
Formatted: Font color: Black
Formatted: Indent: Left: 18 pt
Formatted: Font color: Black
Formatted: Indent: Left: 18 pt
Deleted: and
Formatted: Font: Not Bold, Font color: Black
Formatted: Indent: Left: 18 pt
Formatted: Font: Not Bold, Font color: Black
Deleted: N
Formatted: Indent: Left: 18 pt
Deleted: Place samples in their zipper-closure bags in cleaned nylon daypacks to carry out of collection site.
Formatted: Font color: Black
Formatted: Indent: Left: 18 pt
Deleted: laboratory
Formatted: Bulleted + Level: 1 + Aligned at: 18 pt + Tab after: 36 pt + Indent at: 36 pt
Deleted: c
Formatted: Font: Not Bold
Deleted: ¶
Deleted: ¶
C
Deleted: chain of custody

PROCEDURES FOR *TIER ONE* FIELD SAMPLING

LAMPREY EELS

The following procedures describe techniques for collection of eels in preparation for shipping to laboratory for composite sampling.

*****SUMMARY*****

- MINIMIZE HANDLING OF SAMPLES
- ALL SAMPLES ARE DOUBLE BAGGED
- WHOLE, UNDAMAGED, LIVE LAMPREY SHOULD BE COLLECTED
- **6 lamprey TOTAL** are needed

*****BEFORE LEAVING OFFICE AREA*****

1. Inspect and check equipment for
   - safety
   - contaminants (oil, grease, organic tissue or fluids) and
   - damage (sharp edges or holes)

2. **ELIMINATE DAMAGED SAMPLING GEAR**

3. Verify that instruments have been cleaned
   - washed with a detergent solution,
   - rinsed with ambient water,
   - rinsed with a high-purity solvent (methanol or petroleum ether)
   - finally rinsed with MilliQ/ distilled water and
   - wrapped in aluminum foil and bagged

4. Double check list that ALL equipment is available and loaded into vehicle

   - Camera, digital
   - Calculator
   - Data Sheets w/ clipboard
   - Dry Ice or Ice
   - Dip net, sm. mesh
   - Eel trap
   - GPS
   - Heavy Duty Aluminum Foil,
   - Labels, gummed waterproof
   - Methanol *or* Petroleum Ether
   - Micro Detergent
   - Millimeter ruler to 30cm
   - MilliQ: Type II/ distilled water
   - Permanent Marking Pen
   - Plastic buckets, 3 gallon
   - Plastic Ice Chests
   - Polyethylene Gloves
   - Teflon Wash Bottle, 500 Ml
   - Zipper-closure Polyethylene Bags, 4milx13”x18”

<u>*AFTER ARRIVAL SAMPLING SITE***</u>**

1. RECORD all possible crew, location, and sample information **FIRST**
    - List crew members and date
    - Photo-document study reaches (non-critical)
    - Take and record location with GPS

2. Wearing polyethylene gloves, place netted eels into bucket with ambient water to transport to shore

3. Examine and release any damaged eels.
    - **Lamprey of 40 to 60 cm** in length are the ***<u>target size</u>***.
    - **First 5 lamprey** will be accessed by the Project Field Manager to determine the acceptable ***<u>size range</u>*****.**
    - **ALL lamprey for the sample will be within the set range.**

4. COLLECT **2** lamprey at each of three sites. **If you suspect a counting error, recount.**

5. RECORD sizes for all lamprey.
    - Total lengths are measured
    - Release lamprey that do not meet the target size class.
    - Weigh those that are kept for sample.

6. Lamprey that meet the target size class will be subdued with the backpack electro-fisher.
    - The backpack shocker is operated by a trained person, making sure that all others helping follow appropriate safety rules.

7. Drain and <u>wrap individual lamprey</u> in foil with the dull side exposed to the sample.

8. Re wrap in opposite direction so edges of foil run crosswise to first wrapping.

9. Label and place in first zipper bag immediately, **write specimen # on bag as well label.**

10. Then place in second zipper bag; **write specimen # on bag, AGAIN**

11. Place in ice chest with ice immediately.

<u>PRIOR TO LEAVING FOR NEXT SITE</u>

Instruments and equipment should be
1) washed with a detergent solution,
2) rinsed with ambient water,
3) rinsed with solvent, and
4) finally rinsed with MilliQ/ distilled water.
5) re-wrapped in aluminum foil and bagged.

- Waste detergent & solvent solutions must be collected and taken back to the laboratory.
- **DOUBLE CHECK THAT DATA IS RECORDED FOR EACH SITE AND ALL SAMPLES**

<u>AFTER RETURN FROM THE FIELD</u>

- Place samples in freezer at -20°C, **LOCK** it, and complete sample log form
- Clean, repair, and replace all equipment and supplies

PROCEDURES for *TIER ONE* FIELD SAMPLING

MUSSELS

The following procedures describe techniques for collection of marine clams in preparation for shipping to laboratory for composite sampling.

*****SUMMARY*****

- MINIMIZE HANDLING OF SAMPLES
- COLLECT INDISCRIMINATELY FROM ACROSS ENTIRE SAMPLING SITE
- ALL SAMPLES ARE DOUBLE BAGGED
- WHOLE, UNDAMAGED, LIVE MUSSELS SHOULD BE COLLECTED
- MUSSELS SHOULD NOT BE OPENED IN THE FIELD
- **36 mussels TOTAL** are needed (depending on size)

*****BEFORE LEAVING OFFICE AREA*****

1. Inspect and check equipment for
   - safety issues
   - contaminants (oil, grease, organic tissue or fluids) and
   - damage (sharp edges or holes)

2. **ELIMINATE DAMAGED SAMPLING GEAR**

3. Verify that instruments have been cleaned
   - washed with a detergent solution,
   - rinsed with tap water,
   - rinsed with a high-purity solvent (methanol or petroleum ether)
   - finally rinsed with MilliQ and
   - wrapped in aluminum foil and bagged

4. Double check list that ALL equipment is available and loaded into vehicle

   - Camera, digital
   - Data Sheets w/ clipboard
   - Daypacks
   - Dry Ice or Ice
   - GPS
   - Heavy Duty Aluminum Foil, prepared
   - Heavy Duty plastic bags, Clear 30 gallon
   - Labels, gummed waterproof
   - Methanol *or* Petroleum Ether
   - Micro Detergent
   - Micrometer
   - MilliQ: Type II water
   - Nylon Cable Ties, 7/16” wide x 7” long
   - Permanent Marking Pen
   - Plastic bucket, 3 gallon
   - Plastic Ice Chests
   - Polyethylene Gloves
   - Stainless steel dive knives
   - Teflon Wash Bottle, 500 Ml
   - Wading gear
   - Zipper-closure Polyethylene Bags, 4milx13”x18”

<u>*AFTER ARRIVAL SAMPLING SITE***</u>**

1. RECORD all possible crew, location, and sample information **FIRST**
    - List crew members and date
    - Photo-document study reaches (non-critical)
    - Take and record location with GPS
2. Wearing polyethylene gloves, carefully pry mussels from substrate using dive knife.
3. Examine and rinse off any sand, soil, etc using ambient water
    - **Mussels of 10 to 15 cm** in length are the ***<u>target size</u>***.
    - **First 5 mussels** will be accessed by the Project Field Manager to determine the acceptable ***<u>size range</u>*****.**
    - **ALL clams for the sample will be within the set range.**
4. COLLECT 12 mussels from each of three sites
    - **If you suspect a counting error, recount.**
5. RECORD sizes for all mussels.
    - Lengths are measured across the longest part of each shell
6. Wrap in foil with the dull side exposed to the sample.
7. Re wrap in opposite direction so edges of foil run crosswise to first wrapping.
8. Label and place in plastic bag immediately; **write specimen # on bag as well as label.**
9. Tie securely
10. Then place in second bag so label is visible and tie securely
11. Place samples in their bags in cleaned nylon daypacks to carry out of collection site. CARE should be used NOT to break or damage samples.
12. Place in ice chest with ice immediately.

<u>PRIOR TO LEAVING FOR NEXT SITE</u>

- Instruments and equipment should be
    - washed with a detergent solution,
    - rinsed with ambient water,
    - rinsed with a high-purity solvent (methanol or petroleum ether)
    - finally rinsed with MilliQ
    - re-wrapped in aluminum foil and bagged.
- Waste detergent and solvent solutions must be collected and taken back to the laboratory.
- **DOUBLE CHECK THAT DATA IS RECORDED FOR EACH SITE AND ALL SAMPLES**

<u>AFTER RETURN FROM THE FIELD</u>

- Place samples in freezer at -20°c
- **LOCK** it
- Complete chain of custody form
- Clean, repair, and replace all equipment and supplies

PROCEDURES for *TIER ONE* FIELD SAMPLING

SEA LETTUCE

The following procedures describe techniques for collection of marine clams in preparation for shipping to laboratory for composite sampling.

*****SUMMARY*****

- MINIMIZE HANDLING OF SAMPLES
- COLLECT INDISCRIMINATELY FROM ACROSS ENTIRE SAMPLING SITE
- WHOLE, UNDAMAGED BLADES SHOULD BE COLLECTED
- SAMPLING BOTTLE SHOULD ONLY BE FILLED 1/2 FULL

*****BEFORE LEAVING OFFICE AREA*****

1. Inspect and check equipment for
   - safety,
   - contaminants (oil, grease, organic tissue or fluids) and
   - damage (sharp edges or holes)

2. **ELIMINATE DAMAGED SAMPLING GEAR**

3. Verify that instruments have been cleaned
   - washed with a detergent solution,
   - rinsed with ambient water,
   - rinsed with a high-purity solvent (methanol or petroleum ether)
   - finally rinsed with MilliQ and
   - wrapped in aluminum foil and bagged

4. Double check list that ALL equipment is available and loaded into vehicle

   - Bubble wrap
   - Camera, digital
   - Clear tape
   - Data Sheets w/ clipboard
   - Daypacks
   - Dry Ice or Ice
   - GPS
   - Labels, gummed waterproof
   - Methanol *or* Petroleum Ether
   - Micro Detergent
   - MilliQ: Type II water
   - Permanent Marking Pen
   - Plastic bucket, 3 gallon
   - Plastic Ice Chests
   - Polyethylene Gloves
   - Stainless Steal Knife
   - Teflon Wash Bottle, 500 Ml
   - Wading gear
   - Wide-mouthed, 16-ounce sample bottle
   - Zipper-closure Polyethylene Bags, 4milx13”x18”

<u>*AFTER ARRIVAL SAMPLING SITE***</u>**

1. RECORD all possible crew, location, and sample information **FIRST**
    - List crew members and date
    - Photo-document study reaches (non-critical)
    - Take and record location with GPS
2. Wearing polyethylene gloves, cut carefully to avoid or limit damage to blade/ leaf
3. Examine and rinse off any sand, soil, etc using ambient water
4. Place drained blade/ leaf into sample bottle until about 1/2 full.
    - **<u>Do not overfill</u>**
    - Be sure to leave an air space to accommodate expansion upon freezing.
5. Tighten cap securely.
6. Secure bottle tops with clear tape
7. Label
8. Place in zipper bag
9. Place samples in cleaned nylon daypacks to carry out of collection site. CARE should be used NOT to break or damage samples.
10. Wrap each bottle in bubble wrap.
11. COLLECT **1/2** bottle of sea lettuce from each of 3 sites
12. Place in ice chest with bubble wrap covering the bottom and sides of chest and ice immediately.

<u>PRIOR TO LEAVING FOR NEXT SITE</u>

- Instruments and equipment should be
    - washed with a detergent solution,
    - rinsed with ambient water,
    - rinsed with a high-purity solvent (methanol or petroleum ether)
    - finally rinsed with MilliQ
    - re-wrapped in aluminum foil and bagged.
- Waste detergent and solvent solutions must be collected and taken back to the laboratory.
- **DOUBLE CHECK THAT DATA IS RECORDED FOR EACH SITE AND ALL SAMPLES**

<u>AFTER RETURN FROM THE FIELD</u>

- Place samples in freezer at -20°c
- **LOCK** it
- Complete chain of custody form
- Clean, repair, and replace all equipment and supplies

PROCEDURES FOR *TIER ONE* FIELD SAMPLING

STURGEON

The following procedures describe techniques for collection of sturgeon in preparation for shipping to laboratory for composite sampling.

*****SUMMARY*****

- MINIMIZE HANDLING OF SAMPLED CROSS SECTION
- During lab dissection, a subsection of the cross section is removed, discarding any tissue exposed by field dissection and used for analysis.
- ALL SAMPLES ARE DOUBLE BAGGED
- WHOLE, UNDAMAGED, LIVE FISH SHOULD BE COLLECTED
- **6 fish TOTAL** are needed

*****BEFORE LEAVING OFFICE AREA*****

1. Inspect and check equipment for
   - safety,
   - contaminants (oil, grease, organic tissue or fluids) and
   - damage (sharp edges or holes)

2. **ELIMINATE DAMAGED SAMPLING GEAR**

3. Verify that equipment has have been cleaned
   - washed with a detergent solution,
   - rinsed with ambient water,
   - rinsed with a high-purity solvent (methanol or petroleum ether)
   - finally rinsed with MilliQ/ distilled water and
   - wrapped in aluminum foil and bagged

4. Double check list that ALL equipment is available and loaded into vehicle

   - Camera, digital
   - Data Sheets w/ clipboard
   - Daypacks
   - Dry Ice or Ice
   - Electroshocking Backback
   - Field weighing scale
   - Gill net
   - GPS
   - Heavy Duty Aluminum Foil,
   - Heavy Duty plastic bags, 30 g
   - Labels, gummed waterproof
   - Methanol *or* Petroleum Ether
   - Micro Detergent
   - Meter measuring tape
   - MilliQ: Type II/ distilled water
   - Nylon Cable Ties 7” long
   - Permanent Marking Pen
   - Plastic bucket, 3 gallon
   - Plastic Ice Chests
   - Polyethylene Gloves
   - Stainless Steel Knives
   - Teflon Wash Bottle, 500 Ml

<u>*AFTER ARRIVAL SAMPLING SITE***</u>**

1. RECORD all possible crew, location, and sample information **FIRST**
    - List crew members and date
    - Photo-document study reaches (non-critical)
    - Take and record location with GPS

2. Once a fish is caught, the gill net will be immediately pulled to the surface and the fish removed while wearing polyethylene gloves.

3. Examine and transport to the shore.
    - **Fish of 42 to 100 cm** in length are the ***<u>target size</u>***.
    - **The first fish** will be used by the Project Field Manager to determine the acceptable ***<u>size range</u>*.**
    - **ALL additional fish for the sample will be within the set range.**
    - Release fish that are not in size range.
    - Fish that **<u>do</u>** meet the size requirement will be subdued with knock to head.

4. RECORD sizes for all fish.
    - Lengths are measured for both total length <u>and</u> to tail fork.
    - Weigh fish that are kept for sample

5. Transport fish to shore and place on an aluminum covered board for initial dissection.

6. Cut a large, 25 cm cross section from behind the pectoral fins with a cleaned knife.
    - The internal organs are not cut into, to prevent contamination.
    - The knife is cleaned (micro, DI, methanol) between fish.
    - New aluminum foil is placed on cutting board for each fish.

7. Wrap removed section in clean foil with the dull side exposed to the sample, as soon as cut.

8. Re wrap in opposite direction so edges of foil run crosswise to first wrapping.

9. Label, place in first zipper bag immediately, **write specimen # on bag as well as label.**

10. Then place in second zipper bag; **write specimen # on bag, AGAIN**

11. Place in ice chest with ice immediately.

12. If possible COLLECT 6 FISH samples TOTAL

<u>PRIOR TO LEAVING</u>

- **DOUBLE CHECK THAT DATA IS RECORDED FOR ALL SAMPLES**

<u>AFTER RETURN FROM THE FIELD</u>

- Place samples in freezer at -20°C, **LOCK** it, and complete sample log form
- Clean, repair, and replace all equipment and supplies

PROCEDURES for *TIER ONE* FIELD SAMPLING

SURF FISH/ SMELT

The following procedures describe techniques for collection of surf fish/ smelt in preparation for shipping to laboratory for composite sampling.

*****SUMMARY*****

- MINIMIZE HANDLING OF SAMPLES
- ALL SAMPLES ARE DOUBLE BAGGED
- WHOLE, UNDAMAGED, LIVE FISH SHOULD BE COLLECTED
- BEST CAUGHT ON A FALLING HIGH TIDE IN MILD SURF
- **54 surf fish/ smelt TOTAL** are needed (depending on size)

*****BEFORE LEAVING OFFICE AREA*****

1. Inspect and check equipment for
   - safety,
   - contaminants (oil, grease, organic tissue or fluids) and
   - damage (sharp edges or holes)

2. **ELIMINATE DAMAGED SAMPLING GEAR**

3. Verify that instruments have been cleaned
   - washed with a detergent solution,
   - rinsed with tap water,
   - rinsed with a high-purity solvent (methanol or petroleum ether)
   - finally rinsed with MilliQ and
   - wrapped in aluminum foil and bagged

4. Double check list that ALL equipment is available and loaded into vehicle

   - Camera, digital
   - Data Sheets w/ clipboard
   - Daypacks
   - Dip Nets, mesh ½" to 3"
   - Dry Ice or Ice
   - Electroshocking Backback
   - GPS
   - Heavy Duty Aluminum Foil, prepared
   - Heavy Duty plastic bags, Clear 30 gallon
   - Labels, gummed waterproof
   - Methanol *or* Petroleum Ether
   - Micro Detergent
   - Millimeter ruler to 30cm
   - MilliQ: Type II water
   - Nylon Cable Ties, 7/16” wide x 7” long
   - Permanent Marking Pen
   - Plastic bucket, 3 gallon
   - Plastic Ice Chests
   - Polyethylene Gloves
   - Teflon Wash Bottle, 500 Ml
   - Wading gear
   - Zipper-closure Polyethylene Bags, 4milx13”x18”

<u>*AFTER ARRIVAL SAMPLING SITE***</u>**

1. RECORD all possible crew, location, and sample information **FIRST**
    - List crew members and date
    - Photo-document study reaches (non-critical)
    - Take and record location with GPS
2. Wearing polyethylene gloves, skim dip net through surf close to the substrate without dragging.
3. Once a fish is caught, the dip net will be pulled to the surface and the fish removed.
4. Only the fish species selected for the project will be retained and other species will be released.
5. Examine and rinse off any sand, soil, etc using ambient water and place in bucket to transport to the shore.
    - **Surf fish of 14 to 22 cm** in length are the ***<u>target size</u>***.
    - **First 5 surf fish** will be accessed by the Project Field Manager to determine the acceptable ***<u>size range</u>*****.**
6. COLLECT **18** surf fish from each of three sites.
    - **ALL surf fish for the sample will be within the set range**
    - **If you suspect a counting error, recount.**
7. RECORD sizes for all surf fish.
    - Lengths are measured TWICE
        1. from mouth to fin TIP
        2. from mouth to fin FORK
    - Release fish that do not meet the target size class.
8. Fish that do meet the target size class will be subdued with the backpack electro-fisher.
    - The backpack shocker is operated by a trained person,
    - Make sure that all others helping follow appropriate safety rules.
9. Drain and wrap collected group of fish in foil with the dull side exposed to the sample.
10. Re wrap in opposite direction so edges of foil run crosswise to first wrapping.
11. Label and place in first zipper bag immediately, **write specimen # on bag as well label.**
12. Then place in second zipper bag; **write specimen # on bag, AGAIN**
13. Place samples in their zipper-closure bags in cleaned nylon daypacks to carry out of collection site. CARE should be used NOT to break or damage samples.
14. Place in ice chest with ice immediately.

<u>PRIOR TO LEAVING FOR NEXT SITE</u>

- Instruments and equipment should be
    - washed with a detergent solution,
    - rinsed with ambient water,
    - rinsed with a high-purity solvent (methanol or petroleum ether)
    - finally rinsed with MilliQ
    - re-wrapped in aluminum foil and bagged.
- Waste detergent & solvent solutions must be collected and taken back to the laboratory.
- **DOUBLE CHECK THAT DATA IS RECORDED FOR EACH SITE AND ALL SAMPLES**

<u>AFTER RETURN FROM THE FIELD</u>

- Place samples in freezer at -20°C, **LOCK** it
- Complete chain of custody form
- Clean, repair, and replace all equipment and supplies

PROCEDURES FOR *TIER ONE* FIELD SAMPLING

WHOLE FISH/ SALMON & STEELHEAD

The following procedures describe techniques for collection of whole fish in preparation for shipping to laboratory for composite sampling.

*****SUMMARY*****

- MINIMIZE HANDLING OF SAMPLES
- ALL SAMPLES ARE DOUBLE BAGGED
- WHOLE, UNDAMAGED, LIVE FISH SHOULD BE COLLECTED
- **6 fish TOTAL** are needed

*****BEFORE LEAVING OFFICE AREA*****

1. Inspect and check equipment for
   - safety,
   - contaminants (oil, grease, organic tissue or fluids) and
   - damage (sharp edges or holes)

2. **ELIMINATE DAMAGED SAMPLING GEAR**

3. Verify that equipment has have been cleaned
   - washed with a detergent solution,
   - rinsed with ambient water,
   - rinsed with a high-purity solvent (methanol or petroleum ether)
   - finally rinsed with MilliQ/distilled water and
   - wrapped in aluminum foil and bagged

4. Double check list that ALL equipment is available and loaded into vehicle

   - Camera, digital
   - Data Sheets w/ clipboard
   - Dry Ice or Ice
   - Electroshocking Backback
   - Field weighing scale
   - Gill net
   - GPS
   - Heavy Duty Aluminum Foil
   - Heavy Duty plastic bags, 30 g
   - Labels, gummed waterproof
   - Methanol *or* Petroleum Ether
   - Micro Detergent
   - Meter measuring tape
   - MilliQ: Type II/ distilled water
   - Nylon Cable Ties 7” long
   - Permanent Marking Pen
   - Plastic bucket, 3 gallon
   - Plastic Ice Chests
   - Polyethylene Gloves
   - Teflon Wash Bottle, 500 Ml

<u>*AFTER ARRIVAL SAMPLING SITE***</u>**

1. RECORD all possible crew, location, and sample information **FIRST**
    - List crew members and date
    - Photo-document study reaches (non-critical)
    - Take and record location with GPS

2. Once a fish is caught, the gill net will be immediately pulled to the surface and the fish removed while wearing polyethylene gloves

3. Examine and place in large bucket to transport to the shore.
    - **Fish of 42 to 100 cm** in length are the ***<u>target size</u>***.
    - **First 5 fish** will be accessed by the Project Field Manager to determine the acceptable ***<u>size range</u>*****.**
    - **ALL fish for the sample will be within the set range.**

4. Fish that **<u>do</u>** meet the target size class will be subdued with the backpack electro-fisher.
    - The backpack shocker must be operated by a trained person; make sure that all others helping follow appropriate safety rules.

5. COLLECT **2** FISH TOTAL from each site. **If you suspect a counting error, recount.**

6. RECORD sizes and weight for all fish.
    - Lengths are measured **TWICE**; 1) mouth to fin TIP, 2) mouth to fin FORK.
    - Weigh fish that are kept for sample

7. Wrap individual fish in foil with the dull side exposed to the sample.

8. Re wrap in opposite direction so edges of foil run crosswise to first wrapping.

9. Label, place in first bag and seal with tie, **write specimen # on bag as well as label.**

10. Then place in second bag and seal with tie; **write specimen # on bag, AGAIN.**

11. Place in ice chest with ice immediately.

<u>PRIOR TO LEAVING</u>

- Instruments and equipment should be
    1. washed with a detergent solution,
    2. rinsed with ambient water,
    3. rinsed with a high-purity solvent (methanol or petroleum ether)
    4. finally rinsed with MilliQ/ distilled water
    5. re-wrapped in aluminum foil and bagged.
- Waste detergent & solvent solutions must be collected and taken back to the laboratory.
- **DOUBLE CHECK THAT DATA IS RECORDED FOR EACH SITE AND ALL SAMPLES**

<u>AFTER RETURN FROM THE FIELD</u>

- Place samples in freezer at -20°C, **LOCK** it, and complete sample log form
- Clean, repair, and replace all equipment and supplies

Field Sampling Procedures for Collection of Surface Water Samples

The following procedures describe techniques for collection of surface water samples in preparation for shipping to laboratory for analysis and have been adapted from the *USGS National Field Manual for the Collection of Water-Quality Data* Version 2.0 (9/2006).

Clean Hands/Dirty Hands techniques will be utilized for the collection of surface water samples and requires two or more people working together. At the field site, one person is designated as Clean Hands (*CH*) and a second person as Dirty Hands (*DH*). Although specific tasks are assigned at the start to *CH* or *DH*, some tasks overlap and can be handled by either, as long as the prescribed care is taken to prevent contaminating the sample. *CH* and *DH* wear appropriate disposable, powderless gloves during the entire sampling operation and change gloves frequently, usually with each change in task. (Wearing multiple layers of gloves allows rapid glove changes.) Gloves must be appropriate to withstand any acid, solvent, or other chemical substance that will be used or contacted.

***CH* takes care of all operations involving equipment that contacts the sample; for example, *CH*:**

- Handles the surface-water sampler bottle
- Handles the discharge end of the surface-water or ground-water sample tubing
- Handles the inner protective bag on the churn splitter
- Transfers sample to churn or cone splitter
- Prepares a clean work space (inside vehicle)
- Sets up processing and preservation chambers
- Places equipment inside chambers (for example, sample bottles, filtration and preservation equipment)
- Works exclusively inside chambers during collection/processing and preservation
- Changes chamber covers, as needed
- Sets up field-cleaning equipment and cleans equipment

***DH* takes care of all operations involving contact with potential sources of contamination; for example, *DH*:**

- Works exclusively exterior to processing and preservation chambers
- Prepares and operates sampling equipment, including pumps and discrete samplers, peristaltic pump switch, pump controller, manifold system
- Operates cranes, tripods, drill rigs, vehicles, or other support equipment
- Handles the compressor or other power supply for samplers
- Handles tools such as hammers, wrenches, keys, locks, and sample-flow manifolds
- Handles single or multiparameter instruments for field measurements
- Handles the churn carrier, including outer protective bags
- Handles stream-gaging or water-level equipment
- Sets up and calibrates field-measurement instruments
- Measures and records water levels and field measurements

Site selection
Sites should be sampled in the order of least to greatest potential for equipment fouling or contamination, if possible. The cleanest sites are often – although not always – those that are in pristine environments, in areas where concentrations of dissolved solids are low, or upstream or upgradient from known or suspected sources of contamination.

On-site preparations
When arriving at the field site, take the appropriate measures to avoid sample contamination, such as fumes from traffic or other sources and proper handling and care of sampling equipment. Only clean equipment should be transported to the field. Once field work has begun, and before samples are collected, the sample-wetted portions of most of the collection and processing equipment require a field rinse with native water. Field rinsing helps to condition, or equilibrate, sampling equipment to the sample environment. Rinsing also serves to ensure that all cleaning-solution residues have been removed.

The Clean Hands team member is responsible for field rinsing the equipment whenever *CH/DH* procedures are used. The use and field-rinsing procedures are summarized below for the isokinetic sampler and for sample-compositing and the churn splitter.

Churn splitter: The 14-Liter churn will be used to split the surface water samples. Churn lid requires a covered opening. To split a sample into subsamples for nonvolatile organic-compound analyses, a Teflon-coated churn will be used.

Equal-width-increment (EWI) method
The collection of isokinetic, depth-integrated samples will involve using an equal-width-increment (EWI) sampling method. The EWI method will result in a composite sample that represents the discharge-weighted concentrations of the stream cross section being sampled. The EWI method is used to divide a selected cross section of a stream into increments having a specified width. The term vertical refers to that location within the increment at which the sampler is lowered and raised through the water column. **EWI verticals are located at the midpoint of each width increment.**

For the EWI sampling method, the stream cross section is divided into a number of equal-width increments. Samples will be collected by lowering and raising the isokinetic depth-integrated sampler through the water column at the center of each increment. (This sampling location is referred to as the vertical.) The combination of the same constant transit rate used to sample at each vertical and the isokinetic property of the sampler results in a discharge-weighted sample that is proportional to total streamflow. The same size sampler container (Teflon bottle) and nozzle will be used at each of the sampling verticals. Collect samples using the same transit rate at each vertical during descent and ascent of the sampler. The transit rate must be constant and within the operational range of the sampler.. Composite subsamples will be composited from all verticals in a 14L Teflon coated churn splitter.

Step 1. Prepare for sampling

a. Upon arrival at the field site, set out safety equipment such as life vests and throw bags.

b. Assemble sampling equipment and set up a clean work space by laying out plastic sheeting over the floor of the boat and by having sampling equipment and sample bottles laid out with plastic sheeting covering them prior to use.

Step 2. Select the number and width of equal-width increments.

SITE DETERMINANTS & CONSTRAINTS

a. Visually inspect the stream from bank to bank and longitudinally, observing velocity, width, and depth distribution, and apparent distribution of sediment and aquatic biota along the cross section. Note and document the location of stagnant water, eddies, backwater, reverse flows, areas of faster than normal flow, and piers or other features along the cross section.

b. Determine stream width from a tagline or from distance markings on a bridge railing or cableway.

c. At sites with little sampling history, measure and record the cross-sectional variation of field measurements (such as specific electrical conductance, pH, temperature, and dissolved oxygen). Review the magnitude of the variations along the cross section.

d. Determine the width of the increment. To obtain the number of increments, divide the stream width by the increment width.

NOTE:

The number of increments must be a whole number. Increment width is based on study objectives, variation in field measurements and flow, and stream-channel characteristics along the cross section.

- Collect the subsample at the center of each equal-width increment (the vertical).
- If the subsample does not represent the mean value for that increment, decrease the increment width until the mean value for the increment is represented. This will increase the number of increments sampled.

e. Locate the first sampling vertical at a distance of one-half of the selected increment width from the edge of the water. Locate all the other verticals at the center of each remaining equal-width increment along the cross section.

Example:

- If a stream 56 ft wide has been divided into 14 increments of 4 ft each, the first sampling vertical would be 2 ft from the water's edge and subsequent verticals would be at 6, 10, 14 ft from the water's edge, and so forth.
- Even if streamflow is divided, as in a braided channel, equal-width increments must be identical from channel to channel, and the same constant transit rate must be used at each vertical.

f. Make slight adjustments to sampling locations, if necessary, to avoid sampling where the flow is affected by a pier or other obstruction.

EQUIPMENT CONSTRAINTS

Equipment limitations also constrain the number of increments selected.

Example:

- When using a 1-L bottle sampler at maximum depth with a 14-L churn splitter, EWI samples can be collected at no more than 14 to 17 verticals.

Step 3. Select the transit rate.

a. For determining the transit rates for collecting isokinetic, depth-integrated samples use the trial-and-error method to determine the minimum transit rate.

b. Locate the equal-width increment containing the largest discharge (largest product of depth times velocity) by sounding for depth and either measuring or estimating velocity. At the vertical for this increment, use of the minimum transit rate results in the maximum allowable filling of the sampler bottle during one vertical traverse.

c. Determine the minimum transit rate at this vertical for the US-D95 sampler, 1/4 nozzle, using a 1L sample bottle.

Approximate the mean velocity of the vertical in feet per second by timing a floating marker (such as a peanut) as it travels a known distance. (A known length of flagging tape tied to the cable where the sampler is attached often is used to measure the distance.) Divide the distance (in feet) by the time (in seconds) and multiply by 0.86. Make sure that the transit rate does not exceed the maximum allowable transit rate to be used at any of the remaining verticals along the cross section. This can be determined by sampling the slowest increment. Remember that you must keep the transit rate unidirectional, constant, and within the isokinetic transit range of the sampler when collecting isokinetic samples at each centroid. The descending and ascending transit rate must be constant in each direction and must be the same for each vertical along the cross section. The transit rate selected must be sufficiently rapid to keep from overfilling the sampler. The sampler is overfilled when the water surface in the sampler container is above the bottom edge of the nozzle when the sampler is held in the sampling position.

NOTE: The same size sampler nozzle and container must be used at all verticals along the cross section.

Step 4. Collect sample water.

The sample-collection procedure is the same whether you are wading or using the reel-and-cable suspension method. When sampling from a bridge, deploy the sampler from the upstream of the bridge, if possible, to avoid bridge-related contamination of the sample.

Use *CH/DH* techniques, as required.

a. Move to the first vertical (midpoint of first EWI near edge of water) and field rinse equipment. Collect the rinse water at the edge of the stream in a section of low stream velocity to minimize including suspended sediment.

<u>To field rinse a churn splitter:</u>

1. Put on gloves.
2. Pour 2 to 4 L of rinse water from the sampler into the churn splitter (churn) through the top funnel.
3. Remove the churn from the churn carrier, leaving the outer plastic bag inside the carrier.
4. Move the churn disk up and down several times to ensure that the inside of the churn is thoroughly wetted, then swirl the rinse water vigorously in the churn.
5. Pierce a hole through the inner plastic bag to expose the churn spigot and drain the rinse water through the spigot.

   NOTE: If sand is present, swirl water vigorously in the churn, open the plastic bag, and partially lift the churn cover to pour the rinse water out of the top of the churn. (Draining the rinse water through the spigot will not adequately remove sand.)

6. After draining the rinse water from the churn, rotate the churn in the plastic bag so that the spigot is not exposed. Place the inner plastic bag holding the churn into an outer plastic bag and place into the churn carrier.

b. Record start time and gage height.

c. Lower field-rinsed sampler at the predetermined constant transit rate until slight contact is made with the streambed. **Do not pause** upon contacting the streambed. Raise the sampler immediately at the same constant transit rate until sampler completes the vertical traverse.

- Take care not to disturb the streambed by bumping the sampler on it; bed material may enter the nozzle, resulting in erroneous data.
- Do not overfill the sampler container. Overfilling results in a sample that is not isokinetic and that could be enriched with heavy particulates because of secondary circulation of water through the sampler (from nozzle through air exhaust). This enrichment will result in an artificially increased sediment concentration and will bias particle-size distribution toward heavier and larger particulates.
- Do not underfill the sampler container. Underfilling will result in a sample that is not isokinetically collected because the maximum transit rate has been exceeded.

d. Inspect each subsample as it is collected, looking for overfilling or underfilling of the sampler container and (or) the presence of anomalously large amounts of particulates that might have been captured because of excessive streambed disturbance during sample collection.

NOTE: If you note any of these conditions, discard the sample, making sure there are no residual particulates left in the container, and resample.

e. Move sampling equipment to the next vertical. Maintain the selected transit rate. The volume of the subsample can vary considerably among verticals. Subsamples can be collected at several verticals before emptying the sampler container, as long as the maximum volume of sample in a bottle sampler has not been exceeded. If the container is overfilled, it is necessary to resample.

f. Continue to the next vertical until no more samples can be collected without overfilling the sampler container. Empty the subsample into a field-rinsed churn splitter and repeat sample collection in the same manner until subsamples have been collected at all the verticals.

- To ensure that all particulates are transferred with the sample, swirl the subsample gently to keep particulates suspended and pour the subsample quickly into the churn splitter.
- Sample EWI verticals as many times as necessary to ensure that an adequate sample volume is collected as required for analysis, ***but*** **sample at each vertical an equal number of times.** (The composite cross-sectional sample will remain proportional to flow at the time of sampling.)
- If flow is stable during sampling, then multiple samples can be collected at each vertical during a single traverse along the cross section. If flow is changing, however, study objectives should determine whether to collect multiple samples at each vertical during a single traverse or to collect one sample at each vertical during multiple traverses along the cross section. Document on field forms the method used.

g. Record the following information after all samples have been collected:

- Sampling end time.
- Ending gage height.
- All field observations and any deviations from standard sampling procedures

Step 5. Process Samples

Completely filling the churn allows all samples to be filled from one churn; thereby minimizing differences in water properties and quality between samples. Proper use of the churn guarantees the water is well mixed before the sample is collected. The churn should be stirred at a uniform rate by raising or lowering the splitter at approximately 9 inches per second. This mixing must continue while the bottles are being filled. If filling is stopped for some reason, the stirring rate must be resumed before the next sample is drawn from the churn. As the volume of water in the churn decreases, the round trip frequency increases as the velocity of the churn splitter remains the same. Care must be taken to avoid breaking the surface of the water as the splitter rises toward the top of the water in the churn.

Sample bottles and chemical preservatives used are provided by associated laboratories and were considered sterile prior to field usage. Sample bottles without chemical preservatives are rinsed with stream water from the churn 3 times before filling with sample water. In the case of bottles that contained chemical preservatives, bottles are not rinsed before sample collection and care is taken to avoid over-spillage that would result in chemical preservative loss. Collected samples are placed in coolers on wet ice with appropriate chain of custody forms filled out for transport to contracted laboratories for analysis.

Step 6. Collect Replicate and Blank bottle sets

To ensure laboratory and sampling accuracy and precision, one site every sampling period is randomly selected to receive three additional QA/QC bottle sets. These bottle sets contain replicate and blank water samples. Replicate and blank samples are disguised with unique sample site IDs and times so the lab does not know the difference between QA/QC samples and the primaries samples that have been submitted for anlaysis. Replicate samples are obtained using the same process as regular samples. These are used to assure the laboratory maintains precision within results.

Blank samples are utilized to assess accuracy of the analysis and verify that the handling and transportation of the samples in the field does not influence the results. Blank samples also evaluate the laboratory's sample handling and decontamination measures. Blank samples are collected by filling the sample bottles directly with distilled water. Sample bottles without chemical preservatives are rinsed with distilled water 3 times before filling with distilled water. In the case of bottles that contain chemical preservatives, bottles are not rinsed before being filled with distilled water and care will be taken to avoid over-spillage that would result in chemical preservative loss. All bottle sets are then placed on wet ice and are transported to the associated laboratories with appropriate chain of custody forms filled out. All grab samples are processed within known laboratory holding periods.

Step 7. Clean Equipment

a. If the sampler will not be reused during a field trip, rinse sampler components with deionized water before they dry and place them into a plastic bag for transporting to the office laboratory to be cleaned.
b. If the sampler will be reused during the field trip, rinse the components with DIW while still wet from sampling and then field-clean while at the sampling site using the prescribed procedures:

1. Fill churn splitter through the funnel with detergent solution.
2. Soak for 30 minutes.
3. Scrub interior and exterior surfaces with a soft brush, taking care not to abrade the surface.
4. Pay particular attention to cleaning the paddle and the area around the spigot.
5. Make sure spigot and funnel are free of sediment, including fine particulates (clay), organic matter, and stains.
6. Drain some of the cleaning solution through the spigot before discarding the remaining solution.
7. Fill churn through the funnel splitter about one-third full with tap water; swirl and shake churn vigorously to remove detergent residues. Allow tapwater to pass through the spigot.
8. Repeat rinse procedure until no bubbles remain in rinse water after the water is agitated.
9. Fill the churn splitter through the funnel with DIW to about one third full.
10. Swirl the DIW vigorously and pour it out of the top of the churn into the neutralization container.
11. Repeat the fill-and-swirl procedures of 1 and 2 above at least twice, checking the pH of the DIW after each swirl with narrow range pH indicator strips.
    NOTE: Pass a portion of the DIW through the spigot only after the DIW pH equals or is greater than either 6.0 or the pH of the DIW before acidification. Pour the rest of the DIW into the neutralization container.

Step 8. Store Equipment

1. Package clean, dry churn splitter in two new plastic bags and loosely tie or secure with a nonmetal clip. If a churn splitter must be packaged while wet, use within 1 to 3 days and (or) keep chilled to prevent bacterial growth.
2. Place entire package into the churn carrier.

Supply and Equipment Checklist:

- Calibrated Portable Water Quality instrument = YSI instrument
- Barometer
- US-D95 Isokinetic sampler
- ¼ inch nozzles
- Teflon bottles for US-D95
- B-reel
- funnel
- Plastic Sheeting
- Plastic Bags
- Ice (in bottles or packs)
- Sample Jars (from laboratory)
- Coolers
- 14L Teflon lined churn splitter
- Clip board
    - Data sheets
    - Sharpies
    - Pencils
    - Chain of Custody forms
    - Sampling Protocol
- Nitrile Powderless Gloves
- Digital Camera
- Cellular phone
- Watch
- Waders and boots
- Distilled Water- 10+ gallons
- First Aid Kit
- Life Vests
- Throw Bags

PROCEDURES FOR *TIER TWO* FIELD SAMPLING
CLAMS

The following procedures describe techniques for collection of marine clams in preparation for shipping to laboratory for composite sampling.

*****SUMMARY*****

- MINIMIZE HANDLING OF SAMPLES
- COLLECT INDISCRIMINATELY FROM ACROSS ENTIRE SAMPLING SITE
- ALL SAMPLES ARE DOUBLE BAGGED
- WHOLE, UNDAMAGED, LIVE CLAMS SHOULD BE COLLECTED
- CLAMS SHOULD NOT BE OPENED IN THE FIELD
- **108 Razor** Clams are needed from each site
- **36-72 Washington** Clams are needed from each site

*****BEFORE LEAVING OFFICE AREA*****

1. Inspect and check equipment for
   - safety,
   - contaminants (oil, grease, organic tissue or fluids) and
   - damage (sharp edges or holes)

2. **ELIMINATE DAMAGED SAMPLING GEAR**

3. Verify that instruments have been cleaned
   - washed with a detergent solution,
   - rinsed with ambient water,
   - rinsed with a high-purity solvent (methanol or petroleum ether)
   - finally rinsed with MilliQ and
   - wrapped in aluminum foil and bagged

4. Double check list that ALL equipment is available and loaded into vehicle

   - Camera, digital
   - Clam shovels
   - Data Sheets w/ clipboard
   - Daypacks
   - Dry Ice or Ice
   - GPS
   - Heavy Duty Aluminum Foil, prepared
   - Heavy Duty plastic bags, Clear 30 gallon
   - Labels, gummed waterproof
   - Methanol *or* Petroleum Ether
   - Micro Detergent
   - Micrometer
   - MilliQ: Type II water
   - Nylon Cable Ties, 7/16” wide x 7” long
   - Permanent Marking Pen
   - Plastic bucket, 3 gallon
   - Plastic Ice Chests
   - Polyethylene Gloves
   - Teflon Wash Bottle, 500 Ml
   - Wading gear
   - Zipper-closure Polyethylene Bags, 4milx13”x18”

<u>*AFTER ARRIVAL SAMPLING SITE***</u>**

1. RECORD all possible crew, location, and sample information **FIRST**
    - List crew members and date
    - Photo-document study reaches (non-critical)
    - Take and record location with GPS
2. Wearing polyethylene gloves while digging and removing clam from substrate.
3. Examine and rinse off any sand, soil, etc using ambient water
    - Clams of _____ **mm** in length are the acceptable ***<u>size range</u>*** as set by tier one sampling
    - **ALL clams for the sample will be within the set range.**
4. COLLECT **108 Razor Clams ~ 36-72 Washington Clams**
    - **If you suspect a counting error, recount.**
5. RECORD sizes for all clams.
    - Lengths are measured across the longest part of each shell
6. Wrap in foil with the dull side exposed to the sample.
7. Re wrap in opposite direction so edges of foil run crosswise to first wrapping.
8. Label and place in first zipper bag immediately.
9. Then place in second zipper bag
10. **COLLECT second set of samples for** Field Replicates (DO IT **ALL** AGAIN!)
11. Place samples in their zipper-closure bags in cleaned nylon daypacks to carry out of collection site. CARE should be used NOT to break or damage samples.
12. Place in ice chest with ice immediately.

<u>PRIOR TO LEAVING FOR NEXT SITE</u>

- Instruments and equipment should be
    - washed with a detergent solution,
    - rinsed with ambient water,
    - rinsed with a high-purity solvent (methanol or petroleum ether)
    - finally rinsed with MilliQ
    - re-wrapped in aluminum foil and bagged.
- Waste detergent and solvent solutions must be collected and taken back to the laboratory.
- **DOUBLE CHECK THAT DATA IS RECORDED FOR EACH SITE AND ALL SAMPLES**

<u>AFTER RETURN FROM THE FIELD</u>

- Place samples in freezer at -20°c
- **LOCK** it
- Complete chain of custody form
- Clean and replace all supplies and equipment

PROCEDURES FOR *TIER TWO* FIELD SAMPLING

LAMPREY EELS

The following procedures describe techniques for collection of marine mussels in preparation for shipping to laboratory for composite sampling.

*****SUMMARY*****

- MINIMIZE HANDLING OF SAMPLES
- ALL SAMPLES ARE DOUBLE BAGGED
- WHOLE, UNDAMAGED, LIVE LAMPREY SHOULD BE COLLECTED
- **6 lamprey TOTAL** are needed from each site

*****BEFORE LEAVING OFFICE AREA*****

1. Inspect and check equipment for
   - safety,
   - contaminants (oil, grease, organic tissue or fluids) and
   - damage (sharp edges or holes)

2. **ELIMINATE DAMAGED SAMPLING GEAR**

3. Verify that instruments have been cleaned
   - washed with a detergent solution,
   - rinsed with ambient water,
   - rinsed with a high-purity solvent (methanol or petroleum ether)
   - finally rinsed with MilliQ and
   - wrapped in aluminum foil and bagged

4. Double check list that ALL equipment is available and loaded into vehicle

   - Camera, digital
   - Data Sheets w/ clipboard
   - Daypacks
   - Dry Ice or Ice
   - Electroshocking Backback
   - Gaff hooks
   - GPS
   - Heavy Duty Aluminum Foil, prepared
   - Heavy Duty plastic bags, Clear 30 gallon
   - Labels, gummed waterproof
   - Methanol *or* Petroleum Ether
   - Micro Detergent
   - Micrometer
   - MilliQ: Type II water
   - Nylon Cable Ties, 7/16” wide x 7” long
   - Permanent Marking Pen
   - Plastic bucket, 3 gallon
   - Plastic Ice Chests
   - Polyethylene Gloves
   - Teflon Wash Bottle, 500 Ml
   - Wading gear
   - Zipper-closure Polyethylene Bags, 4milx13”x18”

<u>*AFTER ARRIVAL SAMPLING SITE***</u>**

1. RECORD all possible crew, location, and sample information **FIRST**
    - List crew members and date
    - Photo-document study reaches (non-critical)
    - Take and record location with GPS
2. Wearing polyethylene gloves, place gaffed eels into bucket with ambient water to transport to shore
3. Examine and and release any damaged eels.
    - LAMPREY of _____**-**_____ **mm** in length are the acceptable ***<u>size range</u>*** as set by tier one sampling
    - **ALL lamprey for the sample will be within the set range.**
4. COLLECT 6 LAMPREY
    - **If you suspect a counting error, recount.**
5. RECORD sizes for all lamprey.
    - Total lengths are measured
    - Release lamprey that do not meet the target size class.
    - Weigh those that are kept for sample.
6. Lamprey that <u>do</u> meet the target size class will be subdued with the backpack electro-fisher.
    - The backpack shocker is operated by a trained person, making sure that all others helping follow appropriate safety rules.
7. Drain and wrap individual lamprey in foil with the dull side exposed to the sample.
8. Re wrap in opposite direction so edges of foil run crosswise to first wrapping.
9. **Label** and place in first zipper bag immediately.
10. Then place in second zipper bag
11. **COLLECT second set of samples for** Field Replicates (DO IT **ALL** AGAIN!)
12. Place samples in their zipper-closure bags in cleaned nylon daypacks to carry out of collection site. CARE should be used NOT to break or damage samples.
13. Place in ice chest with ice immediately.

<u>PRIOR TO LEAVING FOR NEXT SITE</u>

- Instruments and equipment should be
    - washed with a detergent solution,
    - rinsed with ambient water,
    - rinsed with a high-purity solvent (methanol or petroleum ether)
    - finally rinsed with MilliQ
    - re-wrapped in aluminum foil and bagged.
- Waste detergent and solvent solutions must be collected and taken back to the laboratory.
- **DOUBLE CHECK THAT DATA IS RECORDED FOR EACH SITE AND ALL SAMPLES**

<u>AFTER RETURN FROM THE FIELD</u>

- Place samples in freezer at -20°c , **LOCK** it
- Complete chain of custody form
- Clean and replace all supplies and equipment

PROCEDURES FOR *TIER TWO* FIELD SAMPLING
MUSSELS

The following procedures describe techniques for collection of marine mussels in preparation for shipping to laboratory for composite sampling.

*****SUMMARY*****

- MINIMIZE HANDLING OF SAMPLES
- COLLECT INDISCRIMINATELY FROM ACROSS ENTIRE SAMPLING SITE
- ALL SAMPLES ARE DOUBLE BAGGED
- WHOLE, UNDAMAGED, LIVE MUSSELS SHOULD BE COLLECTED
- MUSSELS SHOULD NOT BE OPENED IN THE FIELD
- **36 Mussels TOTAL** are needed from each site

*****BEFORE LEAVING OFFICE AREA*****

1. Inspect and check equipment for
   - safety,
   - contaminants (oil, grease, organic tissue or fluids) and
   - damage (sharp edges or holes)

2. **ELIMINATE DAMAGED SAMPLING GEAR**

3. Verify that instruments have been cleaned
   - washed with a detergent solution,
   - rinsed with ambient water,
   - rinsed with a high-purity solvent (methanol or petroleum ether)
   - finally rinsed with MilliQ and
   - wrapped in aluminum foil and bagged

4. Double check list that ALL equipment is available and loaded into vehicle

   - Camera, digital
   - Clam shovels
   - Data Sheets w/ clipboard
   - Daypacks
   - Dry Ice or Ice
   - GPS
   - Heavy Duty Aluminum Foil, prepared
   - Heavy Duty plastic bags, Clear 30 gallon
   - Labels, gummed waterproof
   - Methanol *or* Petroleum Ether
   - Micro Detergent
   - Micrometer
   - MilliQ: Type II water
   - Nylon Cable Ties, 7/16” wide x 7” long
   - Permanent Marking Pen
   - Plastic bucket, 3 gallon
   - Plastic Ice Chests
   - Polyethylene Gloves
   - Teflon Wash Bottle, 500 Ml
   - Wading gear
   - Zipper-closure Polyethylene Bags, 4milx13”x18”

<u>*AFTER ARRIVAL SAMPLING SITE***</u>**

1. RECORD all possible crew, location, and sample information **FIRST**
    - List crew members and date
    - Photo-document study reaches (non-critical)
    - Take and record location with GPS
2. Wearing polyethylene gloves pry mussels off rocks with dive knives.
3. Examine and rinse off any sand, soil, etc using ambient water
    - Mussels of _____ **mm** in length are the acceptable ***<u>size range</u>*** as set by tier one sampling
    - **ALL mussels for the sample will be within the set range.**
4. COLLECT 36 MUSSELS
    - **If you suspect a counting error, recount.**
5. RECORD sizes for all mussels.
    - Lengths are measured across the longest part of each shell
6. Wrap in foil with the dull side exposed to the sample.
7. Re wrap in opposite direction so edges of foil run crosswise to first wrapping.
8. Label and place in first zipper bag immediately.
9. Then place in second zipper bag
10. **COLLECT second set of samples for** Field Replicates (DO IT **ALL** AGAIN!)
11. Place samples in their zipper-closure bags in cleaned nylon daypacks to carry out of collection site. CARE should be used NOT to break or damage samples.
12. Place in ice chest with ice immediately.

<u>PRIOR TO LEAVING FOR NEXT SITE</u>

- Instruments and equipment should be
    - washed with a detergent solution,
    - rinsed with ambient water,
    - rinsed with a high-purity solvent (methanol or petroleum ether)
    - finally rinsed with MilliQ
    - re-wrapped in aluminum foil and bagged.
- Waste detergent and solvent solutions must be collected and taken back to the laboratory.
- **DOUBLE CHECK THAT DATA IS RECORDED FOR EACH SITE AND ALL SAMPLES**

<u>AFTER RETURN FROM THE FIELD</u>

- Place samples in freezer at -20°c
- **LOCK** it
- Complete chain of custody form
- Clean and replace all supplies and equipment

PROCEDURES FOR *TIER TWO* FIELD SAMPLING

SEA LETTUCE

The following procedures describe techniques for collection of marine mussels in preparation for shipping to laboratory for composite sampling.

*****SUMMARY*****

- MINIMIZE HANDLING OF SAMPLES
- COLLECT INDISCRIMINATELY FROM ACROSS ENTIRE SAMPLING SITE
- WHOLE, UNDAMAGED BLADES SHOULD BE COLLECTED
- SAMPLING BOTTLE SHOULD ONLY BE FILLED 3/4 FULL

*****BEFORE LEAVING OFFICE AREA*****

1. Inspect and check equipment for
   - safety,
   - contaminants (oil, grease, organic tissue or fluids) and
   - damage (sharp edges or holes)

2. **ELIMINATE DAMAGED SAMPLING GEAR**

3. Verify that instruments have been cleaned
   - washed with a detergent solution,
   - rinsed with ambient water,
   - rinsed with a high-purity solvent (methanol or petroleum ether)
   - finally rinsed with MilliQ and
   - wrapped in aluminum foil and bagged

4. Double check list that ALL equipment is available and loaded into vehicle

- Bubble wrap
- Camera, digital
- Clear tape
- Data Sheets w/ clipboard
- Daypacks
- Dry Ice or Ice
- GPS
- Labels, gummed waterproof
- Methanol *or* Petroleum Ether
- Micro Detergent
- MilliQ: Type II water
- Permanent Marking Pen
- Plastic bucket, 3 gallon
- Plastic Ice Chests
- Polyethylene Gloves
- Stainless Steal Knife
- Teflon Wash Bottle, 500 Ml
- Wading gear
- Wide-mouthed, 16-ounce sample bottle
- Zipper-closure Polyethylene Bags, 4milx13”x18”

<u>*AFTER ARRIVAL SAMPLING SITE***</u>**

1. RECORD all possible crew, location, and sample information **FIRST**
    - List crew members and date
    - Photo-document study reaches (non-critical)
    - Take and record location with GPS
2. Wearing polyethylene gloves, cut carefully to avoid or limit damage to blade/ leaf
3. Examine and rinse off any sand, soil, etc using ambient water
4. Place drained blade/ leaf into sample bottle until about 1/2 to 2/3 full.
    - **<u>Do not overfill</u>**
    - Be sure to leave an air space to accommodate expansion upon freezing.
5. Tighten cap securely.
6. Secure bottle tops with clear tape
7. Label
8. Place in zipper bag
9. Place samples in cleaned nylon daypacks to carry out of collection site. CARE should be used NOT to break or damage samples.
10. Wrap each bottle in bubble wrap
11. Place in ice chest with bubble wrap covering the bottom and sides of chest and ice immediately.

<u>PRIOR TO LEAVING FOR NEXT SITE</u>

- Instruments and equipment should be
    - washed with a detergent solution,
    - rinsed with ambient water,
    - rinsed with a high-purity solvent (methanol or petroleum ether)
    - finally rinsed with MilliQ
    - re-wrapped in aluminum foil and bagged.
- Waste detergent and solvent solutions must be collected and taken back to the laboratory.
- **DOUBLE CHECK THAT DATA IS RECORDED FOR EACH SITE AND ALL SAMPLES**

<u>AFTER RETURN FROM THE FIELD</u>

- Place samples in freezer at -20°c
- **LOCK** it
- Complete chain of custody form
- Clean and replace all supplies and equipment

PROCEDURES FOR *TIER TWO* FIELD SAMPLING
STURGEON

The following procedures describe techniques for collection of sturgeon in preparation for shipping to laboratory for composite sampling.

*****SUMMARY*****

- MINIMIZE HANDLING OF SAMPLED CROSS SECTION
- During lab dissection, a subsection of the cross section is removed, discarding any tissue exposed by field dissection and used for analysis.
- ALL SAMPLES ARE DOUBLE BAGGED
- WHOLE, UNDAMAGED, LIVE FISH SHOULD BE COLLECTED
- **6 fish** are needed from each site

*****BEFORE LEAVING OFFICE AREA*****

1. Inspect and check equipment for
   - safety,
   - contaminants (oil, grease, organic tissue or fluids) and
   - damage (sharp edges or holes)

2. **ELIMINATE DAMAGED SAMPLING GEAR**

3. Verify that instruments have been cleaned
   - washed with a detergent solution,
   - rinsed with ambient water,
   - rinsed with a high-purity solvent (methanol or petroleum ether)
   - finally rinsed with MilliQ and
   - wrapped in aluminum foil and bagged

4. Double check list that ALL equipment is available and loaded into vehicle

- Camera, digital
- Bone Saw
- Data Sheets w/ clipboard
- Daypacks
- Dry Ice or Ice
- Electroshocking Backback
- Field weighing scale
- Gill net
- GPS
- Heavy Duty Aluminum Foil, prepared
- Heavy Duty plastic bags, Clear 30 gallon
- Labels, gummed waterproof
- Methanol *or* Petroleum Ether
- Micro Detergent
- Meter measuring tape
- MilliQ: Type II water
- Nylon Cable Ties, 7” long
- Permanent Marking Pen
- Plastic bucket, 3 gallon
- Plastic Ice Chests
- Polyethylene Gloves
- Stainless Steel Knives
- Teflon Wash Bottle, 500 Ml

<u>*AFTER ARRIVAL SAMPLING SITE***</u>**

1. RECORD all possible crew, location, and sample information **FIRST**
    - List crew members and date
    - Photo-document study reaches (non-critical)
    - Take and record location with GPS
2. Once a fish is caught, the gill net will be pulled to the surface and the fish removed while wearing **polyethylene gloves**
    - Wash the net with detergent and rinse with ambient water before using for next fish.
3. Once a fish is caught, the dip net will be pulled to the surface and the fish removed.
4. Only the fish species selected for the project will be retained and other species will be released.
5. Examine and rinse off any sand, soil, etc using ambient water and place in bucket to transport to the shore.
    - Fish of _____**-**_____ **mm** in length are the acceptable ***<u>size range</u>*** as set by tier one sampling
    - **ALL fish for the sample will be within the range set during tier one sampling.**
6. Transport fish to shore and place on an aluminum covered board for initial dissection.
7. Cut a large, 25 cm cross section from behind the pectoral fins with a cleaned bone saw or meat cleaver.
    - The internal organs are not cut into, to prevent contamination.
    - The bone saw is cleaned (micro, DI, methanol) between fish.
    - New aluminum foil is placed on cutting board for each fish.
8. Each section is individually wrapped in clean aluminum foil with the dull side exposed to the sample, as soon as it is cut.
9. Re wrap in opposite direction so edges of foil run crosswise to first wrapping.
10. **Label** and place in first zipper bag immediately.
11. Then place in second zipper bag
12. Place in ice chest with ice immediately.
13. COLLECT 6 FISH samples TOTAL
    - **If you suspect a counting error, recount.**
14. **COLLECT second set of samples for** Field Replicates (DO IT **ALL** AGAIN!)
15. CARE should be used NOT to break or damage samples.

<u>PRIOR TO LEAVING FOR NEXT SITE</u>

- Instruments and equipment should be
    - washed with a detergent solution,
    - rinsed with ambient water,
    - rinsed with a high-purity solvent (methanol or petroleum ether)
    - finally rinsed with MilliQ
    - re-wrapped in aluminum foil and bagged.
- Waste detergent and solvent solutions must be collected and taken back to the laboratory.
- **DOUBLE CHECK THAT DATA IS RECORDED FOR EACH SITE AND ALL SAMPLES**

<u>AFTER RETURN FROM THE FIELD</u>

- Place samples in freezer at -20°c , **LOCK** it, complete chain of custody form
- Clean and replace all supplies and equipment

PROCEDURES FOR *TIER TWO* FIELD SAMPLING

SURF FISH

The following procedures describe techniques for collection of marine mussels in preparation for shipping to laboratory for composite sampling.

*****SUMMARY*****

- MINIMIZE HANDLING OF SAMPLES
- ALL SAMPLES ARE DOUBLE BAGGED
- WHOLE, UNDAMAGED, LIVE FISH SHOULD BE COLLECTED
- BEST CAUGHT ON A FALLING HIGH TIDE IN MILD SURF
- **54 Surf fish TOTAL** are needed from each site

*****BEFORE LEAVING OFFICE AREA*****

1. Inspect and check equipment for
   - safety,
   - contaminants (oil, grease, organic tissue or fluids) and
   - damage (sharp edges or holes)

2. **ELIMINATE DAMAGED SAMPLING GEAR**

3. Verify that instruments have been cleaned
   - washed with a detergent solution,
   - rinsed with ambient water,
   - rinsed with a high-purity solvent (methanol or petroleum ether)
   - finally rinsed with MilliQ and
   - wrapped in aluminum foil and bagged

4. Double check list that ALL equipment is available and loaded into vehicle

   - Camera, digital
   - Data Sheets w/ clipboard
   - Daypacks
   - Dipnet, mesh ½" to 3"
   - Dry Ice or Ice
   - Electroshocking Backback
   - GPS
   - Heavy Duty Aluminum Foil, prepared
   - Heavy Duty plastic bags, Clear 30 gallon
   - Labels, gummed waterproof
   - Methanol *or* Petroleum Ether
   - Micro Detergent
   - Micrometer
   - MilliQ: Type II water
   - Nylon Cable Ties, 7/16” wide x 7” long
   - Permanent Marking Pen
   - Plastic bucket, 3 gallon
   - Plastic Ice Chests
   - Polyethylene Gloves
   - Teflon Wash Bottle, 500 Ml
   - Wading gear
   - Zipper-closure Polyethylene Bags, 4milx13”x18”

<u>*AFTER ARRIVAL SAMPLING SITE***</u>**

1. RECORD all possible crew, location, and sample information **FIRST**
    - List crew members and date
    - Photo-document study reaches (non-critical)
    - Take and record location with GPS
2. Wearing polyethylene gloves, skim dip net through surf close to the substrate without dragging.
3. Once a fish is caught, the dip net will be pulled to the surface and the fish removed.
4. Only the fish species selected for the project will be retained and other species will be released.
5. Examine and rinse off any sand, soil, etc using ambient water and place in bucket to transport to the shore.
    - Fish of _____**-**_____ **mm** in length are the acceptable ***<u>size range</u>*** as set by tier one sampling
    - **ALL fish for the sample will be within the range set during tier one sampling.**
6. COLLECT 18 SURF FISH
    - **If you suspect a counting error, recount.**
7. RECORD sizes for all fish.
    - Lengths are measured for both total length <u>and</u> to tail fork
    - Release fish that do not meet the target size class.
8. Fish that do meet the target size class will be subdued with the backpack electro-fisher.
    - The backpack shocker is operated by a trained person, making sure that all others helping follow appropriate safety rules.
9. Drain and wrap collected group of fish in foil with the dull side exposed to the sample.
10. Re wrap in opposite direction so edges of foil run crosswise to first wrapping.
11. **Label** and place in first zipper bag immediately.
12. Then place in second zipper bag
13. **COLLECT second set of samples for** Field Replicates (DO IT **ALL** AGAIN!)
14. Place samples in their zipper-closure bags in cleaned nylon daypacks to carry out of collection site. CARE should be used NOT to break or damage samples.
15. Place in ice chest with ice immediately.

<u>PRIOR TO LEAVING FOR NEXT SITE</u>

- Instruments and equipment should be
    - washed with a detergent solution,
    - rinsed with ambient water,
    - rinsed with a high-purity solvent (methanol or petroleum ether)
    - finally rinsed with MilliQ
    - re-wrapped in aluminum foil and bagged.
- Waste detergent and solvent solutions must be collected and taken back to the laboratory.
- **DOUBLE CHECK THAT DATA IS RECORDED FOR EACH SITE AND ALL SAMPLES**

<u>AFTER RETURN FROM THE FIELD</u>

- Place samples in freezer at -20°c , **LOCK** it
- Complete chain of custody form
- Clean and replace all supplies and equipment

PROCEDURES FOR *TIER TWO* FIELD SAMPLING

WHOLE FISH/ SALMON & STEELHEAD

The following procedures describe techniques for collection of whole fish in preparation for shipping to laboratory for composite sampling.

*****SUMMARY*****

- MINIMIZE HANDLING OF SAMPLES
- ALL SAMPLES ARE DOUBLE BAGGED
- WHOLE, UNDAMAGED, LIVE FISH SHOULD BE COLLECTED
- **6 fish** are needed from each site

*****BEFORE LEAVING OFFICE AREA*****

1. Inspect and check equipment for
   - safety,
   - contaminants (oil, grease, organic tissue or fluids) and
   - damage (sharp edges or holes)

2. **ELIMINATE DAMAGED SAMPLING GEAR**

3. Verify that instruments have been cleaned
   - washed with a detergent solution,
   - rinsed with ambient water,
   - rinsed with a high-purity solvent (methanol or petroleum ether)
   - finally rinsed with MilliQ and
   - wrapped in aluminum foil and bagged

4. Double check list that ALL equipment is available and loaded into vehicle

   - Camera, digital
   - Data Sheets w/ clipboard
   - Daypacks
   - Dry Ice or Ice
   - Electroshocking Backback
   - Field weighing scale
   - Gill net
   - GPS
   - Heavy Duty Aluminum Foil, prepared
   - Heavy Duty plastic bags, Clear 30 gallon
   - Labels, gummed waterproof
   - Methanol *or* Petroleum Ether
   - Micro Detergent
   - Meter measuring tape
   - MilliQ: Type II water
   - Nylon Cable Ties, 7” long
   - Permanent Marking Pen
   - Plastic bucket, 3 gallon
   - Plastic Ice Chests
   - Polyethylene Gloves
   - Teflon Wash Bottle, 500 Ml

*****AFTER ARRIVAL SAMPLING SITE*****

1. RECORD all possible crew, location, and sample information **FIRST**
    - List crew members and date
    - Photo-document study reaches (non-critical)
    - Take and record location with GPS
2. Once a fish is caught, the gill net will be pulled to the surface and the fish removed while wearing polyethylene gloves
3. Only the target fish species will be retained and all other species will be released.
4. Examine and rinse off any sand, soil, etc using ambient water and place in bucket to transport to the shore.
    - Fish of _____**-**_____ **mm** in length are the acceptable ***size range*** as set by tier one sampling
    - **ALL fish for the sample will be within the range set during tier one sampling.**
5. Fish that do meet the target size class will be subdued with the backpack electro-fisher.
    - The backpack shocker is operated by a trained person, making sure that all others helping follow appropriate safety rules.
6. COLLECT 6 FISH
    - **If you suspect a counting error, recount.**
    - Wash the net with detergent and rinse with ambient water between use for each fish before using for next fish.
7. RECORD sizes and weight for all fish.
    - Lengths are measured TWICE
        1. from mouth to fin TIP
        2. from mouth to fin FORK
    - Weigh fish that are kept for sample
8. Drain and wrap individual fish in foil with the dull side exposed to the sample.
9. Re wrap in opposite direction so edges of foil run crosswise to first wrapping.
10. **Label** and place in heavy duty plastic bag immediately.
11. Seal bag tightly with cable tie.
12. Place in ice chest with ice immediately.
13. **COLLECT second set of samples for** Field Replicates (DO IT **ALL** AGAIN!)
14. CARE should be used NOT to break or damage samples.

PRIOR TO LEAVING FOR NEXT SITE

- Instruments and equipment should be
    - washed with a detergent solution,
    - rinsed with ambient water,
    - rinsed with a high-purity solvent (methanol or petroleum ether)
    - finally rinsed with MilliQ
    - re-wrapped in aluminum foil and bagged.
- Waste detergent and solvent solutions must be collected and taken back to the laboratory.
- **DOUBLE CHECK THAT DATA IS RECORDED FOR EACH SITE AND ALL SAMPLES**

AFTER RETURN FROM THE FIELD

- Place samples in freezer at -20°c , **LOCK** it, complete chain of custody form
- Clean and replace all supplies and equipment

Appendix C: Lab Documents

1. **Lab Analysis SOPs File**
   - **Mercury analysis EPA 1631e**
   - **Mercury in tissue SOP revision_02-24-09_kp**
   - **Mercury in water SOP**
   - **Mercury_EPA 1631e Modifications**
   - **Microcystin SOP_WPCL#65**
   - **OC Pesticides in water_WPCL-AC-001**
   - **OPS IN WATER SOPv9_WPCL#52**
   - **PAH Extraction and Cleanup v1 8**
   - **PAH Water Extraction v1.0**
   - **PCB' in water_WPCL-AC-002**
   - **SO_TISSUE_SEDIMENT SOPv10 01-28-09**
   - **Trace metal and SO_SOP_MPSL-105**

2. **Chain of Custody_DFG_Jan 04**
3. **Field Collection SOP_MPSL_102a**
4. **Lab Receipt of Samples SOP_REV_Aug 07**
5. **SOP_Sample Management_REV_Aug 07**
6. **WPCL QA Manual Jun_08**
7. **WPC Laboratory MDL_RL**

Status : Final	**Approved by:**
	Bruce LaBelle, Ph.D. **Chief, ECL**
Cindy Dingman **QA Officer, ECL**	**Myrto Petreas, Ph.D.** **Supervisor**

SOP No. 890-S

Extraction, Cleanup and Lipid Determination of Fish for PCDD/F, Coplanar PCB and PBDE Analyses

1 SCOPE AND APPLICATION

This SOP applies to previously ground fish, whole body or otherwise, and covers its preparation for ultratrace (part per trillion) level analysis of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (PCBs) and trace level analysis of polybrominated diphenyl ethers (PBDEs).

2 SUMMARY

2.1 Ground fish tissue is freeze-dried, spiked with ^{13}C-labeled PCDD/F and coplanar PCB internal standards, and homogenized and extracted with 1:1 methylene chloride : hexane in the presence of anhydrous sodium sulfate. An aliquot of the extract is used to measure lipid content. Based on percent lipid, an appropriate small portion is removed for PBDE analysis.

2.2 The portion taken for PBDE analysis is spiked with a ^{13}C-labeled PBDE internal standard and cleaned up by gel permeation chromatography followed by a column of acid and neutral silica.

2.3 The remainder of the extract, for PCDD/F and coplanar PCB analysis, is passed through potassium silicate, silica gel and an AX21 carbon column. PCDD/Fs and coplanar PCBs, retained on the carbon column, are eluted with toluene and, after concentration and exchange to hexane, further cleaned up on a column of potassium silicate, acid silica and neutral silica. If it is determined that the sample originally had at least 2.0 grams of lipid, the extract is additionally cleaned by gel permeation chromatography (GPC).

2.4 After evaporation of solvent, the extract for each analysis is combined with an appropriate recovery standard in tetradecane, concentrated to 10 uL and submitted for analysis by HRGC/MS.

3 APPARATUS AND MATERIALS

Major/key equipment is listed here. For apparatus and materials not mentioned here or for greater detail, see SOP No. 892-S, Apparatus and Materials Used in Preparation of Samples for Trace Organohalogen Analyses.

3.1 Freeze dryer: VirTis Company Model 12SL Freezemobile.

3.2 Homogenizer, Brinkmann Polytron

3.3 Shaker, Burrell Wrist Action

3.4 Evaporation system, RapidVap N2

3.5 Carbon Column

3.5.1 An Ace glass column (1.1 cm ID x 6 cm) with ends that accept threaded Teflon fittings is generally used. A column can also be constructed from disposable thick walled glass tubing (12.5 mm OD x 15 cm) and 1/2" to 1/8" Swagelok reducing unions with Teflon ferrules.

3.5.2 The column is packed with 50 mg AX21 carbon dispersed on 500 mg of glass fibers. The fibers are obtained from glass fiber sheets (Nuclepore Corp.) cut into small pieces and shredded in 50 mL 1:1 methylene chloride : hexane with a Polytron homogenizer. Excess solvent is decanted and the AX21 carbon is mixed with the fibers and the resulting slurry packed into the column. Avoid packing too tightly. The packing requires only about 3 cm of a 1.1 cm ID column; the remaining volume is taken up with 11-mm glass fiber discs.

3.5.3 Flexible heating tape is wrapped around the carbon column. The carbon column is installed in one of several networks of manual valves and Teflon tubing that we've devised in our lab so that solvent, driven by compressed nitrogen gas, can flow from a reservoir to either the bottom or the top of the column.

3.6 Carbon and Kontes Column Network

A Kontes Chromaflex column, 4.8 cm x 30 cm (or 60 cm), and a washing reservoir (modified separatory funnel, air sample flask, or small Ace or Kontes column, 50-250 mL) are connected by 1/8" Teflon tubing to a four-port two-way valve using PTFE plugs. This is connected to a Hamilton 4-

port 4-opposing valve to allow either the larger Kontes sample column or washing reservoir to be in line with the 4-port 4-opposing valve. The 4-opposing valve has a carbon column attached at two of its ports, while the fourth port is left open for collection of eluate. The Kontes column and washing reservoir are equipped to allow connection to (filtered) low-pressure nitrogen gas by 1/8" Teflon tubing.

3.7 Fluid Management System (FMS) automated Gel Permeation Chromatograph (GPC), which includes a glass column containing a 2.5 cm diameter, 49 – 53 cm high bed of Envirobeads S-X3 Select (or BioRad BioBeads S-X3) in 1:1 methylene chloride : hexane.

3.8 Nitrogen evaporator, Organomation Associates Meyer N-Evap, Model 112, or equivalent, with a sand bath that can be heated to 40 ^{o}C.

4 PRE-EXTRACTION PROCEDURE

4.1 Thaw the sample by leaving it at room temperature several hours.

4.2 Mix the sample (previously ground) thoroughly using a spatula.

4.3 Into a tared 500 mL Teflon bottle, the weight of which has been recorded, weigh ~100 grams of the sample, recording the exact weight.

4.4 Put into the –20 degrees C freezer the Teflon bottle containing the weighed sample, as well as the original sample container (which may or may not still contain tissue).

4.5 When it is frozen, freeze-dry the sample in the Teflon bottle to constant weight using the Virtis freeze dryer and Virtis flasks. See Appendix at the end of this SOP for operation of the freeze dryer.

4.6 After freeze-drying, record the weight of the Teflon bottle containing the freeze-dried sample.

5 EXTRACTION

5.1 Add 300 ml of 1:1 methylene chloride : hexane to the freeze-dried sample in the Teflon bottle.

5.2 Spike the sample with ^{13}C-labeled PCDD/F and coplanar PCB internal standards (400 pg or 500 pg of each, ordinarily). After spiking, screw the lid tightly onto the bottle and swirl and invert it gently to distribute the standards.

5.3 Wrap the bottle in aluminum foil (to protect the sample from light) and let the

sample soak in the solvent overnight (at least 16 hours).

5.4 Add ~20 grams of anhydrous sodium sulfate to the contents of the Teflon bottle. Homogenize the sample in the solvent using the Brinkmann Polytron homogenizer with a large "probe", at a setting of '4' to '5', until the sample mass is broken into free-flowing particles, adding another ~20 grams of anhydrous sodium sulfate while doing so. Try to cut any skin, fins and head parts that resist the homogenizer into tiny (< 3 mm diameter) pieces with small scissors, and return them to the Teflon bottle.

5.5 Rinse the homogenizer "probe" with ~50 mL 1:1 methylene chloride : hexane, collecting the rinse in a beaker for later addition to the sample.

5.6 Let the solids in the bottle settle.

5.7 Decant the liquid in the bottle into a 500 mL graduated cylinder.

5.8 Stopper the 500 mL graduated cylinder and wrap it in foil.

5.9 Add to the solids remaining in the bottle the rinse of the "probe" collected previously and 200 mL of 1:1 methylene chloride : hexane.

5.10 Secure the lid tightly on the bottle and shake it on the Burrell Wrist Action shaker for 15 minutes, at a setting sufficient to achieve complete agitation of the sample.

5.11 Set up a Kontes column as follows. Place upright in the nitrogen driven network (see Apparatus and Materials) a 30 cm long Kontes Chromaflex column with a fitting securely installed at the bottom of it. Place a 4.7 cm diameter glass fiber filter disc into the Kontes column, so that the filter disc lies flat on the fitting. On top of the filter disc place anhydrous sodium sulfate to a depth of 1 – 2 cm. Set the two-way valve under the Kontes column so that it directs flow from the Kontes column to the "side arm" rather than to the carbon column.

5.12 Put ~100 mL of 1:1 methylene : hexane into the Kontes column (gently to avoid dislodging the anhydrous sodium sulfate and filter disc) and let it run out to a waste container until the anhydrous sodium sulfate is covered by only ~1 cm of solvent. (Lift up the Kontes column as necessary to start the flow.)

5.13 Install a 600 mL RapidVap vessel with a 2- or 3-mL stem (or, if evaporation will be by rotary evaporator, a 500 mL boiling flask) to collect the next output from the Kontes column.

5.14 Decant the liquid from the Teflon bottle into the Kontes column. See that it drains into the collecting vessel.

5.15 Add 250 mL of 1:1 methylene chloride : hexane to the solids remaining in the

bottle.

5.16 Secure the lid tightly on the bottle and again shake it on the Burrell Wrist Action shaker for 15 minutes, at a setting sufficient to achieve complete agitation of the sample.

5.17 Remove the vessel that has been collecting from the Kontes column and put in its place a new one.

5.18 Decant the liquid from the Teflon bottle into the Kontes column (third and final extract). See that it drains into the collecting vessel.

5.19 Add sufficient 1:1 methylene chloride : hexane (~30 mL) to the solids in the bottle to make a slurry and, after most or all of the decanted liquid has passed through it, transfer to the Kontes column all the contents of the bottle. Follow with two ~25 mL 1:1 methylene chloride : hexane rinses of the bottle.

5.20 Add to the Kontes column another 50 mL of 1:1 methylene chloride : hexane.

5.21 If there is sufficient remaining space in the collecting vessel, apply nitrogen gas to the Kontes column to drive out remaining liquid and collect that also.

5.22 As a check on extraction completeness, for at least one of the samples (randomly selected) per batch, after doing the above steps apply 100 mL of 1:1 methylene chloride : hexane to the Kontes column, collecting eluate in a separate, tared (weight recorded), clear glass vessel, such as a 16-oz jar. Leave foil-covered to air-dry or, if in a suitable vessel, RapidVap to remove all solvent. When dry, examine it for residue and weigh it. If there is any residue measurable on a top-loading balance, transfer it to a tared weigh pan and weigh it on an analytical balance. Record the findings.

5.23 Evaporate the contents of the two vessels that were used to collect from the Kontes column, until the sum of their volumes is less than 100 mL, using the Labconco RapidVap (vortex, with or without nitrogen, at up to 45 degrees C) or a rotary evaporator.

5.24 Transfer the contents of the collecting vessels to the 500 mL graduated cylinder that has been storing the first extract of the sample. Rinse each of the collecting vessels with a few mL of 1:1 methylene chloride : hexane, and transfer the rinses also to the 500 mL graduated cylinder. Repeat the rinsing at least once more.

5.25 Bring the volume of the 500 mL cylinder exactly to 500 mL with 1:1 methylene chloride : hexane, stopper and mix by inverting. Leave the cylinder foil-covered whenever there is a pause of more than a few hours in processing.

5.26 Determine % lipid using this procedure:

5.26.1 Be sure that each sample’s total extract volume is 500 mL (if not add

1:1 methylene chloride: hexane until it is) and that it has been thoroughly mixed (stoppered and inverted) after the last addition.

5.26.2 Label an aluminum weigh pan with a scratch mark at the bottom of the pan and rinse it with acetone/hexane.

5.26.3 Weigh the pan on an analytical balance (calibrated) and record the weight. Always use tweezers. Avoid touching the pan with ungloved hands.

5.26.4 Attach a pipet bulb to a 10 mL Class A volumetric pipet, immerse the tip of the pipet in the extract in the 500 mL cylinder, and draw the liquid above the graduation mark.

5.26.5 Dispense excess back into the 500 mL cylinder. Zero meniscus at top of graduation.

5.26.6 Touch the tip of the pipet to the cylinder wall to detach any hanging drop.

5.26.7 Dispense in vertical position with unrestricted flow to the pre-weighed aluminum pan.

5.26.8 Touch off last remaining drop to surface or side of the aluminum pan after free-flow ceases. Do not blow out. You may return the last drop to the cylinder containing the extract.

5.26.9 Repeat steps 4-8 for duplicate measurement.

5.26.10 Cover the aluminum pan with foil (avoid touching foil to the pan) and let it dry in a fume hood for at least 3-4 hours at room temperature.

5.26.11 After 3-4 hours weigh the pan on an analytical balance.

5.26.12 Repeat steps 10 -11 until the weight is constant.

5.26.13 Calculate % lipid by this formula:

$$\textbf{\% Lipid} = \frac{\textbf{[(wt. of residue + wt. of pan) - wt. of pan] x 50}}{\textbf{Wet wt. of sample}} \textbf{ x 100 \%}$$

5.27 In the case of the extract of a ~100 gram sample, pipet to an appropriately-sized graduated cylinder the following volume for PBDE analysis:

a. If measured % lipid is 2.4 % or more, remove 25 mL

b. If measured % lipid is < 2.4 %, remove 50 mL

In the case of the extract of a sample not ~100 grams (for instance, a reference material sample), calculate from the % lipid whether the entire extract contains 2.4 grams. If it contains that much or more, remove 25 mL. Otherwise, remove 50 mL.

Wrap completely in foil the cylinder containing the portion for PBDE analysis.

6 CARBON CLEANUP (for PCDD/Fs and coplanar PCBs)

6.1 Carbon Column Conditioning

6.1.1 Install a carbon column in the nitrogen driven network (see Apparatus and Materials).

6.1.2 Fill the reservoir with ~75 mL toluene, set the valve determining direction so that flow will be from bottom to top (“reverse”direction) through the column and turn on the nitrogen gas (less than 5 psi).

6.1.3 Once the flow of toluene starts, plug in the column’s (electric) heating tape.

6.1.4 Check for any leaks. Reverse the flow for a few minutes to the top of the column to ensure no leakage from the connector.

6.1.5 Once the toluene has passed through, unplug the heating tape and wait for the carbon column to cool.

6.1.6 Likewise, but without heating, rinse the column with ~50 mL methanol and then, changing the valve so that flow is in the “forward” direction, ~100 mL 1:1 methylene chloride : hexane.

6.2 Gently, to avoid disarraying the adsorbent bed, put ~200 mL of 1:1 methylene chloride: hexane into the Kontes column and let it run out to a waste container until the sodium sulfate is covered by only ~1 cm of solvent. (Lift up the Kontes column as necessary to start the flow.)

6.3 Switch the valve to direct flow from the Kontes to the carbon column.

6.4 With a container (preferably of 600 mL or greater capacity) in place to collect eluate, transfer to the Kontes column the remaining extract in the 500 mL cylinder. Include adequate rinses of the 500 mL cylinder.

6.5 Using nitrogen gas pressure, drive the extract through the Kontes column and

the carbon column at ~2 mL/minute, collecting eluate as Fr.1 (Fraction 1).

6.6 Replace the collection container with another if necessary to obtain space for the next eluate.

6.7 Before its bed goes dry, add 50 mL of 1:1 methylene chloride : hexane to the Kontes column. Drive the solvent through the bed at ~2 mL/minute, draining the bed of liquid. The eluate is the final part of Fr.1.

6.8 Wrap completely in foil the container(s) holding Fr.1, which is retained for possible future non-coplanar PCB or PBDE analysis. It may be evaporated and transferred to a light-occluding vial to conserve storage space.

6.9 Reverse the direction of flow through the carbon column and elute it with 75 mL toluene, applying heat by turning on the heating tape once the flow is established. Collect eluate in a round bottom flask as Fr.2.

7 SILICA COLUMN CLEANUP (for PCDD/Fs and coplanar PCBs)

7.1 Evaporate Fr.2 to residue by rotary evaporator.

7.2 Place a glass wool plug into the tip of a disposable 25 mL, 14 mm ID, graduated pipet (with the top cut off), and then pack the pipet with (from bottom to top) ~0.7 cc of anhydrous sodium sulfate, 2 cc of silica gel, ~0.7 cc of anhydrous sodium sulfate, 4 cc of acid silica (40% sulfuric acid by weight), ~0.7 cc of anhydrous sodium sulfate, 2 cc K silicate and ~0.7 cc of anhydrous sodium sulfate. Settle the packings by tapping.

7.3 Apply ~50 mL hexane (as rinse) to the column, discarding the outflowing hexane.

7.4 Bring the volume of Fr.2 to 1 mL with hexane and, when the last of the rinse hexane descends into the column, put a boiling flask under the column to collect eluate and pipet Fr.2 into the column. Follow with three 1 mL hexane rinses of the Fr.2 flask, then 120 mL hexane, not letting the column dry out until all of the liquid has passed through.

7.5 Evaporate the eluate by rotary evaporator to:

1 – 2 mL if the lipid determination shows the sample originally had less than 2.0 grams of lipid.

1 – 4 mL if the lipid determination shows the sample originally had 2.0 grams or more of lipid.

8 GPC CLEANUP for PCDD/Fs and coplanar PCBs (samples originally having 2.0 g or more of lipid)

8.1 Transfer the evaporated eluate from the silica column cleanup to a 10 mL graduated cylinder, including at least three 1:1 methylene chloride : hexane rinses of the eluate flask. Bring volume to exactly 8 mL with 1:1 methylene chloride : hexane.

8.2 Process through GPC as described in "Use of Fluid Management System's (FMS) Automated Gel Permeation Chromatograph (GPC) and Florisil Column in the Cleanup of Adipose Samples for PCB, PBDE and OCP Analyses" (SOP 887-S) for a sample having less than 80 mg fat, except without a Florisil column.

8.3 Evaporate the collected fraction in the RapidVap (vortex, with or without nitrogen, at up to 45 degrees C) to1 – 2 mL.

9 COMPLETION for PCDD/Fs and coplanar PCBs

9.1 Add to a 1 mL Reacti-Vial the volume of a ^{13}C-1,2,3,4-TCDD and ^{13}C-1,2,3,7,8,9-HxCDD standard solution that contains 300 pg of each compound (the recovery standard). Add additional tetradecane as necessary to bring the total volume of tetradecane to 10 uL.

9.2 Transfer to the Reacti-Vial the cleaned up, evaporated extract. Evaporate by nitrogen stream on the N-Evap evaporator. Rinse the container the extract is being transferred from three times with ~0.5 mL of 1:1 methylene chloride : hexane, adding the rinses to the Reacti-Vial as space becomes available in it. After all is transferred evaporate to 10 uL.

10 GPC with Acid Silica CLEANUP (for PBDEs)

10.1 Spike the portion for PBDE analysis, which has been reserved in a graduated cylinder, with 15 ng (ordinarily) of ^{13}C-labeled PBDE 77 (3,3',4,4'-tetrabromodiphenyl ether). Stopper, jostle and invert the cylinder to mix.

10.2 Re-wrap the cylinder in foil and allow particulates to settle for at least a few hours.

10.3 From the % lipid value and the original sample weight compute the weight of lipid in the portion, and evaporate it on the N-Evap to the volume indicated in the table below.

Amount of lipid the PBDE portion contains	Volume to concentrate it to
< 80 mg	8 mL
80-150 mg	16 mL
150-220 mg	24 mL
> 220 mg	32 mL

10.4 For each 8 mL of the PBDE portion prepare an acid silica column as follows. Install a 13 mm diameter glass fiber filter disc into each of two 1/2" to 1/8" Swagelok reducing-union fittings with Teflon ferrules. Put a glass wool plug into one end of a ~20 cm long section of thick-walled 0.8 cm ID glass tubing. Through its other end pack into it, successively, ~1 g of silica gel, 5.0 g of acid silica (40% sulfuric acid by weight) and ~1 g of silica gel. Settle the packings by tapping. Put a glass wool plug into the other end of the tubing, and slip a Swagelok fitting onto each end. Lightly tighten the fittings with wrenches.

10.5 As described in "Use of Fluid Management System's (FMS) Automated Gel Permeation Chromatograph (GPC) and Florisil Column in the Cleanup of Adipose Samples for PCB, PBDE and OCP Analyses" (SOP 887-S), prepare the GPC and acid silica column(s) and process the PBDE portion, 8 mL at a time, acid silica column(s) taking the place of Florisil column(s). Set 120 mL (or other amount as determined by calibration of the GPC) as the volume for Fraction 2 (also called the "Collect" fraction), containing the analytes.

10.6 Evaporate the "Collect" fraction(s) in the RapidVap vessel(s) in the RapidVap (vortex, with or without nitrogen, at up to 45 degrees C) to 1 – 2 mL.

10.7 Transfer the concentrated "Collect" fraction(s) to a single 4 mL silanized amber vial, including 1:1 methylene chloride : hexane rinses of each RapidVap vessel. (Combine the contents of multiple vessels if more than one vessel was needed for the sample.)

11 COMPLETION for PBDEs

11.1 Evaporate the content of the vial to ~0.5 mL on the N-Evap.

11.2 Add to a 1 mL Reacti-Vial the volume of a ^{13}C-PCB 128 and 178 standard solution that contains 10 ng of each compound (the recovery standard). Add additional tetradecane as necessary to bring the total volume of tetradecane to 10 uL.

11.3 Transfer the content of the 4 mL amber vial to the Reacti-Vial, and evaporate it on the N-Evap evaporator. Rinse the vial the extract is being transferred from three times with ~0.5 mL of 1:1 methylene chloride : hexane, adding the rinses to the Reacti-Vial as space becomes available in it. After all is transferred evaporate to 10 uL.

12 REFERENCES

The following ECL SOPs/Methods are used in conjunction with this SOP:

SOP 892-S: Apparatus and Materials Used in Preparation of Samples for Trace Organohalogen Analyses

SOP 882-S: Procedure for Washing Biological Glassware for PCDD/PCDF Analysis

SOP 887-S: Use of Fluid Management System's (FMS) Automated Gel Permeation Chromatograph (GPC) and Florisil Column in the Cleanup of Adipose Samples for PCB, PBDE and OCP Analyses

Method 880-M: Polychlorinated Dibenzo-p-Dioxins, Polychlorinated Dibenzofurans and Coplanar PCBs in Environmental and Biological Samples

APPENDIX

Freeze Dryer Operation

The instructions below serve as a quick reference to normal freeze dryer operation. For detailed information please refer to the instruction manual for The VirTis Company Model 12SL Freezemobile.

1. Start with clean, dry system (manifold, condenser and drain line).
2. Check level and clarity of vacuum pump oil.
3. Close all of the valves (knobs pointing upward) and the condenser door. Put a plug into the end of the condenser drain hose.
4. Turn on condenser. (Switch on "REFRIGERATE".) Wait until the condenser status light indicates "OK" before proceeding.
5. Turn on vacuum. Wait until the vacuum status light indicates "OK" before proceeding (~15 min.). See that the condenser door (with latch down) shows a good seal.
6. Freeze the samples (in Teflon bottles) by leaving them sufficient time in a freezer. (The –20 degree C freezer is appropriate.)

7. Put each Teflon bottle containing a frozen sample into a wide-mouth filter seal freeze-drying vessel.

8. Connect one vessel to a manifold valve. Apply vacuum by turning the valve's knob to the "open" position (pointing downward). Vacuum light will indicate "OK" for ~1 minute and then go "OFF". Wait for approx. 3-4 minutes for vacuum status light to indicate "OK" again.

9. When both status lights indicate "OK", an additional sample may be attached. Continue, adding another sample each time both status lights indicate "OK", until all of them are attached.

10. Samples are done when the vessel is no longer chilled and no moisture is on the outside of the vessel (usually in 72 hours).

11. Close each valve that has been connected to a sample (turn knob until pointing upward). This releases the vacuum in the vessel but keeps the freeze dryer under vacuum.

12. Open a valve that is unattached to a sample (removing one of the samples if necessary) by turning its knob until it points up and then immediately turn off vacuum.

13. Turn off condenser (refrigeration).

14. Remove the sample vessels.

15. Unplug the end of the condenser drain hose and put it into a container to collect water draining from the condenser.

16. To quickly restore the freeze dryer for re-use, turn on defrost.

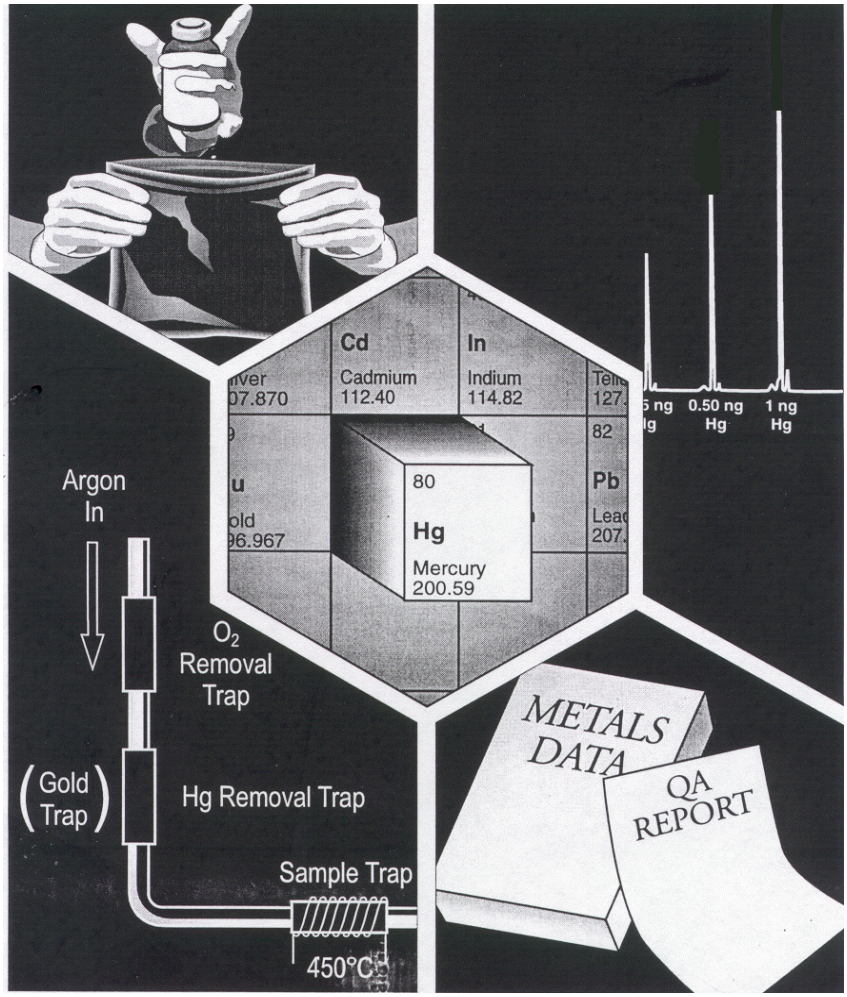
17. Clean the condenser.

18. Unplug the freeze dryer if it is not likely to be used for a few days.

United States
Environmental Protection
Agency

Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry

August 2002



United States Environmental Protection Agency
Office of Water 4303
EPA-821-R-02-019
August 2002

Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry

Acknowledgments

This Method was developed under the direction of William A. Telliard and Maria Gomez-Taylor of the Engineering and Analysis Division (EAD) within the U.S. Environmental Protection Agency's (EPA's) Office of Science and Technology (OST). EPA acknowledges contributions to this method by Frontier Geosciences, Inc., Albion Environmental, Battelle Marine Sciences Laboratory, STL-Canton, and Tekran Inc. Additional assistance in preparing the Method was provided by DynCorp Environmental and Interface, Inc.

Disclaimer

This Method has been reviewed and approved for publication by the Statistics and Analytical Support Branch within EPA's Engineering and Analysis Division. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Questions concerning this Method or its application should be addressed to:

W.A. Telliard
Statistics and Analytical Support Branch (4303T)
U.S. Environmental Protection Agency
Ariel Rios Building
1200 Pennsylvania Avenue, N.W.
Washington, DC 20460
Phone: 202/566–1061
Fax: 202/566–1053

Table of Contents

Introduction . . . . . *iii*

1.0 Scope and Application 1

2.0 Summary of Method 2

3.0 Definitions 3

4.0 Contamination and Interferences 3

5.0 Safety 6

6.0 Apparatus and Materials 8

7.0 Reagents and Standards 11

8.0 Sample Collection, Preservation, and Storage 13

9.0 Quality Control 14

10.0 Calibration and Standardization 22

11.0 Procedure 25

12.0 Data Analysis and Calculations 27

13.0 Method Performance 29

14.0 Pollution Prevention 29

15.0 Waste Management 30

16.0 References 30

17.0 Glossary 31

18.0 Tables and Figures 34

Introduction

Method 1631 (the "Method") supports technology-based and water quality-based monitoring programs authorized under the Clean Water Act (CWA; the "Act").

CWA Sections 301 and 306 require EPA to publish effluent standards that restrict the direct discharge of pollutants to the nations waters, and CWA Sections 307(b) and (c) require EPA to promulgate nationally applicable pretreatement standards which restrict pollutant discharges into sewers flowing to publicly owned treatment works (POTWs). The effluent limitations guidelines are published at CFR parts 401-503.

CWA Section 303 requires each State to set a water quality standard for each body of water within its boundaries. A State water quality standard consists of a designated use or uses of a water body or a segment of a water body, the water quality criteria that are necessary to protect the designated use or uses, and an antidegradation policy. CWA Section 304(a) requires EPA to publish water quality criteria that reflect the latest scientific knowledge concerning the physical fate of pollutants, the effects of pollutants on ecological and human health, and the effect of pollutants on biological community diversity, productivity, and stability. These water quality standards serve two purposes: (1) they establish the water quality goals for a specific water body, and (2) they are the basis for establishing water quality-based treatment controls and strategies beyond the technology-based controls required by CWA Sections 301(b) and 306.

In 1987, amendments to the CWA required States to adopt numeric criteria for toxic pollutants (designated in Section 307(a) of the Act) based on EPA Section 304(a) criteria or other scientific data, when the discharge or presence of those toxic pollutants could reasonably be expected to interfere with designated uses. Method 1631 was specifically developed to provide reliable measurements of mercury at EPA WQC levels.

In developing methods for determination of trace metals, EPA found that one of the greatest difficulties was precluding sample contamination during collection, transport, and analysis. The degree of difficulty, however, is highly dependent on the metal and site-specific conditions. Method 1631 is designed to preclude contamination in nearly all situations. It also contains procedures necessary to produce reliable results at the lowest WQC levels published by EPA. In recognition of the variety of situations to which this Method may be applied, and in recognition of continuing technological advances, Method 1631 is performance based. Alternative procedures may be used so long as those procedures are demonstrated to yield reliable results.

Requests for additional copies of this draft Method should be directed to:

U.S. EPA Sample Control Center
6101 Stevenson Avenue
Alexandria, VA 22304-3540
703/461–2100

Note: This Method is performance based. The laboratory is permitted to omit steps or modify procedures provided that all performance requirements in this Method are met. The laboratory must not omit or modify any procedure defined by the term “shall” or “must” and must perform all quality control tests.

Method 1631, Revision E

Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry

1.0 Scope and Application

1.1 Method 1631, Revision E (the "Method") is for determination of mercury (Hg) in filtered and unfiltered water by oxidation, purge and trap, desorption, and cold-vapor atomic fluorescence spectrometry (CVAFS). This Method is for use in EPA's data gathering and monitoring programs associated with the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act. The Method is based on a contractor-developed procedure (Reference 16.1) and on peer-reviewed, published procedures for the determination of mercury in aqueous samples, ranging from sea water to sewage effluent (References 16.2–16.5).

1.2 This Method is accompanied by Method 1669: *Sampling Ambient Water for Determination of Trace Metals at EPA Water Quality Criteria Levels* (Sampling Method). The Sampling Method guidance document is recommended to preclude contamination during the sampling process.

1.3 This Method is for determination of Hg in the range of 0.5–100 ng/L. Application may be extended to higher levels by selection of a smaller sample size or by calibration of the analytical system across a higher range. For measurement of blank samples, the Method may be extended to a lower level by calibration to a lower calibration point. Section 10.4 gives requirements for extension of the calibration range.

1.4 The ease of contaminating ambient water samples with mercury and interfering substances cannot be overemphasized. This Method includes suggestions for improvements in facilities and analytical techniques that should minimize contamination and maximize the ability of the laboratory to make reliable trace metals determinations. Certain sections of this Method contain suggestions and other sections contain requirements to minimize contamination.

1.5 The detection limit and minimum level of quantitation in this Method usually are dependent on the level of interferences rather than instrument limitations. The method detection limit (MDL; 40 CFR 136, Appendix B) for Hg has been determined to be 0.2 ng/L when no interferences are present. The minimum level of quantitation (ML) has been established as 0.5 ng/L. An MDL as low as 0.05 ng/L can be achieved for low Hg samples by using a larger sample volume, a lower BrCl level (0.2%), and extra caution in sample handling.

1.6 Clean and ultraclean—The terms "clean" and "ultraclean" have been applied to the techniques needed to reduce or eliminate contamination in trace metals determinations. These terms are not used in this Method because they lack an exact definition. However, the information provided in this Method is consistent with the summary guidance on clean and ultraclean techniques (References 16.6-16.7).

1.7 This Method follows the EPA Environmental Methods Management Council's "Guidelines and Format for Methods to Be Proposed at 40 CFR, part 136 or part 141."

1.8 This Method is "performance based." The laboratory is permitted to modify the Method to overcome interferences or lower the cost of measurements if all performance criteria are met. Section 9.1.2.1 gives the requirements for establishing method equivalency.

1.9 Any modification of this Method, beyond those expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.10 This Method should be used only by analysts experienced in the use of CVAFS techniques and who are trained thoroughly in the sample handling and instrument techniques described in this Method. Each laboratory that uses this Method must demonstrate the ability to generate acceptable results using the procedures in Section 9.2.

1.11 This Method is accompanied by a data verification and validation guidance document, *Guidance on the Documentation and Evaluation of Trace Metals Data Collected for CWA Compliance Monitoring* (Reference 16.8), that can be used for verification and validation of the data obtained.

1.12 This Method uses either a bubbler or flow-injection system for determination of mercury in water. Separate calibration, analysis, and calculation procedures are provided for a bubbler system (Sections 10.2, 11.2.1, and 12.2) and for a flow-injection system (Sections 10.3, 11.2.2, and 12.3).

2.0 Summary of Method

2.1 A 100- to 2000-mL sample is collected directly into a cleaned, pretested, fluoropolymer or glass bottle using sample handling techniques designed for collection of mercury at trace levels (Reference 16.9).

2.2 For dissolved Hg, the sample is filtered through a 0.45-µm capsule filter prior to preservation.

2.3 The sample is preserved by adding either pretested 12N hydrochloric acid (HCl) or bromine monochloride (BrCl) solution. If a sample will also be used for the determination of methyl mercury, it should be preserved according to procedures in the method that will be used for determination of methylmercury.

2.4 Prior to analysis, all Hg in a 100-mL sample aliquot is oxidized to Hg(II) with BrCl.

2.5 After oxidation, the sample is sequentially reduced with $NH_2OH{\cdot}HCl$ to destroy the free halogens, then reduced with stannous chloride ($SnCl_2$) to convert Hg(II) to volatile Hg(0).

2.6 The Hg(0) is separated from solution either by purging with nitrogen, helium, or argon, or by vapor/liquid separation. The Hg(0) is collected onto a gold trap (Figures 1, 2, and 3).

2.7 The Hg is thermally desorbed from the gold trap into an inert gas stream that carries the released Hg(0) to a second gold (analytical) trap. The Hg is desorbed from the analytical trap into a gas stream that carries the Hg into the cell of a cold-vapor atomic fluorescence spectrometer (CVAFS) for detection (Figures 2 and 3).

2.8 Quality is assured through calibration and testing of the oxidation, purging, and detection systems.

3.0 Definitions

3.1 Total mercury—all BrCl-oxidizable mercury forms and species found in an unfiltered aqueous solution. This includes, but is not limited to, Hg(II), Hg(0), strongly organo-complexed Hg(II) compounds, adsorbed particulate Hg, and several tested covalently bound organo-mercurials (e.g., CH_3HgCl, $(CH_3)_2Hg$, and $C_6H_5HgOOCCH_3$). The recovery of Hg bound within microbial cells may require the additional step of UV photo-oxidation. In this Method, total mercury and total recoverable mercury are synonymous.

3.2 Dissolved mercury—all BrCl-oxidizable mercury forms and species found in the filtrate of an aqueous solution that has been filtered through a 0.45-µm filter.

3.3 Apparatus—Throughout this Method, the sample containers, sampling devices, instrumentation, and all other materials and devices used in sample collection, sample processing, and sample analysis that come in contact with the sample and therefore require careful cleaning will be referred to collectively as the Apparatus.

3.4 Definitions of other terms used in this Method are given in the glossary (Section 17.0).

4.0 Contamination and Interferences

4.1 Preventing samples from becoming contaminated during the sampling and analysis process constitutes one of the greatest difficulties encountered in trace metals determinations. Over the last two decades, marine chemists have come to recognize that much of the historical data on the concentrations of dissolved trace metals in seawater are erroneously high because the concentrations reflect contamination from sampling and analysis rather than ambient levels. Therefore, it is imperative that extreme care be taken to avoid contamination when collecting and analyzing samples for trace metals.

4.2 Samples may become contaminated by numerous routes. Potential sources of trace metals contamination during sampling include: metallic or metal-containing labware (e.g., talc gloves that contain high levels of zinc), containers, sampling equipment, reagents, and reagent water; improperly cleaned or stored equipment, labware, and reagents; and atmospheric inputs such as dirt and dust. Even human contact can be a source of trace metals contamination. For example, it has been demonstrated that dental work (e.g., mercury amalgam fillings) in the mouths of laboratory personnel can contaminate samples directly exposed to exhalation (Reference 16.9).

4.3 Contamination Control

4.3.1 Philosophy—The philosophy behind contamination control is to ensure that any object or substance that contacts the sample is metal free and free from any material that may contain mercury.

4.3.1.1 The integrity of the results produced cannot be compromised by contamination of samples. This Method and the Sampling Method give requirements and suggestions for control of sample contamination.

4.3.1.2 Substances in a sample cannot be allowed to contaminate the laboratory work area or instrumentation used for trace metals measurements. This Method gives requirements and suggestions for protecting the laboratory.

4.3.1.3 Although contamination control is essential, personnel health and safety remain the highest priority. The Sampling Method and Section 5 of this Method give suggestions and requirements for personnel safety.

4.3.2 Avoiding contamination—The best way to control contamination is to completely avoid exposure of the sample to contamination in the first place. Avoiding exposure means performing operations in an area known to be free from contamination. Two of the most important factors in avoiding/reducing sample contamination are (1) an awareness of potential sources of contamination and (2) strict attention to work being done. Therefore, it is imperative that the procedures described in this Method be carried out by well-trained, experienced personnel.

4.3.3 Use a clean environment—The ideal environment for processing samples is a class-100 clean room. If a clean room is not available, all sample preparation should be performed in a class-100 clean bench or a nonmetal glove box fed by mercury-and particle-free air or nitrogen. Digestion should be performed in a nonmetal fume hood equipped with HEPA filtration and ideally situated in a clean room.

4.3.4 Minimize exposure—The Apparatus that will contact samples, blanks, or standard solutions should be opened or exposed only in a clean room, clean bench, or glove box so that exposure to an uncontrolled atmosphere is minimized. When not being used, the Apparatus should be covered with clean plastic wrap, stored in the clean bench or in a plastic box or glove box, or bagged in clean zip-type bags. Minimizing the time between cleaning and use will also minimize contamination.

4.3.5 Clean work surfaces—Before a given batch of samples is processed, all work surfaces in the hood, clean bench, or glove box in which the samples will be processed should be cleaned by wiping with a lint-free cloth or wipe soaked with reagent water.

4.3.6 Wear gloves—Sampling personnel must wear clean, non-talc gloves during all operations involving handling of the Apparatus, samples, and blanks. Only clean gloves may touch the Apparatus. If another object or substance is touched, the glove(s) must be changed before again handling the Apparatus. If it is even suspected that gloves have become contaminated, work must be halted, the contaminated gloves removed, and a new pair of clean gloves put on. Wearing multiple layers of clean gloves will allow the old pair to be quickly stripped with minimal disruption to the work activity.

4.3.7 Use metal-free Apparatus—All Apparatus used for determination of mercury at ambient water quality criteria levels must be nonmetallic, free of material that may contain metals, or both.

4.3.7.1 Construction materials—Only fluoropolymer or glass containers must be used for collection of samples that will be analyzed for mercury because mercury vapors can diffuse in or out of other materials, leading to results that are biased low or high. Polyethylene and/or polypropylene labware may be used for digestion and other purposes because the time of sample exposure to these materials is relatively short. All materials, regardless of construction, that will directly or

indirectly contact the sample, must be known to be clean and free of Hg at the levels specified in this Method before proceeding.

4.3.7.2 Serialization—It is recommended that serial numbers be indelibly marked or etched on each piece of reusable Apparatus so that contamination can be traced, and logbooks should be maintained to track the sample from the container through the labware to introduction into the instrument. It may be useful to dedicate separate sets of labware to different sample types; e.g., receiving waters vs. effluents. However, the Apparatus used for processing blanks and standards must be mixed with the Apparatus used to process samples so that contamination of all labware can be detected.

4.3.7.3 The laboratory or cleaning facility is responsible for cleaning the Apparatus used by the sampling team. If there are any indications that the Apparatus is not clean when received by the sampling team (e.g., ripped storage bags), an assessment of the likelihood of contamination must be made. Sampling must not proceed if it is possible that the Apparatus is contaminated. If the Apparatus is contaminated, it must be returned to the laboratory or cleaning facility for proper cleaning before any sampling activity resumes.

4.3.8 Avoid sources of contamination—Avoid contamination by being aware of potential sources and routes of contamination.

4.3.8.1 Contamination by carryover—Contamination may occur when a sample containing a low concentration of mercury is processed immediately after a sample containing a relatively high concentration of mercury. The Hg concentration at which the analytical system (purge, traps, detector) will carry greater than 0.5 ng/L of Hg into a succeeding bubbler or system blank must be determined by analyzing calibration solutions containing successively larger concentrations of Hg. This test must be run prior to first use of the analytical system and whenever a change is made that would increase the amount of carryover. When a sample contains ½ or greater of this determined Hg concentration, a bubbler blank (bubbler system) or system blank (flow injection system) must be analyzed to demonstrate no carryover at the blank criteria level. For the bubbler system, the blank must be run using the same bubbler and sample trap used to run the high concentration sample. Samples analyzed following a sample that has been determined to result in carryover must be reanalyzed. Samples that are known or suspected to contain the lowest concentration of mercury should be analyzed first followed by samples containing higher levels.

4.3.8.2 Contamination by samples—Significant laboratory or instrument contamination may result when untreated effluents, in-process waters, landfill leachates, and other undiluted samples containing concentrations of mercury greater than 100 ng/L are processed and analyzed. Samples known or suspected to contain Hg concentrations greater than 100 ng/L should be diluted prior to bringing them into the clean room or laboratory dedicated for processing trace metals samples.

4.3.8.3 Contamination by indirect contact—Apparatus that may not directly come in contact with the samples may still be a source of contamination. For example, clean tubing placed in a dirty plastic bag may pick up contamination from the bag and subsequently transfer the contamination to the sample. It is imperative that every piece of the Apparatus that is directly or indirectly used in the collection, processing, and analysis of water samples be thoroughly cleaned (Section 6.1.2).

4.3.8.4 Contamination by airborne particulate matter—Less obvious substances capable of contaminating samples include airborne particles. Samples may be contaminated by airborne dust, dirt, particles, or vapors from unfiltered air supplies; nearby corroded or rusted pipes, wires, or other fixtures; or metal-containing paint. Whenever possible, sample processing and analysis should occur as far as possible from sources of airborne contamination.

4.3.8.5 Contamination from reagents— Contamination can be introduced into samples from method reagents used during processing and analysis. Reagent blanks must be analyzed for contamination prior to use (see Section 9.4.3). If reagent blanks are contaminated, a new batch of reagents must be prepared (see Section 9.4.3.2).

4.4 Interferences

4.4.1 At the time of promulgation of this Method, gold and iodide were known interferences. At a mercury concentration of 2.5 ng/L and at increasing iodide concentrations from 30 to 100 mg/L, test data have shown that mercury recovery will be reduced from 100 to 0 percent. At iodide concentrations greater than 3 mg/L, the sample should be pre-reduced with $SnCl_2$ (to remove the brown color) and additional or more concentrated $SnCl_2$ should be added. To preclude loss of Hg, the additional $SnCl_2$ should be added in a closed vessel or analysis should proceed immediately. If samples containing iodide concentrations greater than 30 mg/L are analyzed, it may be necessary to clean the analytical system with 4N HCl after the analysis (Reference 16.10).

4.4.2 The potential exists for destruction of the gold traps if free halogens are purged onto them, or if they are overheated (>500 °C). When the instructions in this Method are followed, neither of these outcomes is likely.

4.4.3 Water vapor may collect in the gold traps and subsequently condense in the fluorescence cell upon desorption, giving a false peak due to scattering of the excitation radiation. Condensation can be avoided by predrying the gold trap. Traps that tend to absorb large quantities of water vapor should not be used.

4.4.4 The fluorescent intensity is strongly dependent upon the presence of molecular species in the carrier gas that can cause "quenching" of the excited atoms. The dual amalgamation technique eliminates quenching due to trace gases, but it remains the laboratory's responsibility to ensure high purity inert carrier gas and a leak-free analytical train.

5.0 Safety

5.1 The toxicity or carcinogenicity of each chemical used in this Method has not been precisely determined; however, each compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level.

5.1.1 Chronic mercury exposure may cause kidney damage, muscle tremors, spasms, personality changes, depression, irritability and nervousness. Organo-mercurials may cause permanent brain damage. Because of the toxicological and physical properties of Hg, pure standards should be handled only by highly trained personnel thoroughly familiar with handling and cautionary procedures and the associated risks.

5.1.2 It is recommended that the laboratory purchase a dilute standard solution of the Hg in this Method. If primary solutions are prepared, they shall be prepared in a hood, and a NIOSH/MESA-approved toxic gas respirator shall be worn.

5.2 This Method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a current file of OSHA regulations for safe handling of the chemicals specified in this Method. OSHA rules require that a reference file of material safety data sheets (MSDSs) must be made available to all personnel involved in these analyses (29 CFR 1917.28, Appendix E). It also is suggested that the laboratory perform personal hygiene monitoring of each analyst who uses this Method and that the results of this monitoring be made available to the analyst. Personal hygiene monitoring should be performed using OSHA or NIOSH approved personal hygiene monitoring methods. Additional information on laboratory safety can be found in References 16.11-16.14. The references and bibliography included in Reference 16.14 are particularly comprehensive in dealing with the general subject of laboratory safety.

5.3 Samples suspected to contain concentrations of Hg at µg/L or higher levels are handled using essentially the same techniques employed in handling radioactive or infectious materials. Well-ventilated, controlled access laboratories are required. Assistance in evaluating the health hazards of particular laboratory conditions may be obtained from certain consulting laboratories and from State Departments of Health or Labor, many of which have an industrial health service. Each laboratory must develop a safety program for handling Hg.

5.3.1 Facility—When samples known or suspected of containing high concentrations of mercury are handled, all operations (including removal of samples from sample containers, weighing, transferring, and mixing) should be performed in a glove box demonstrated to be leak-tight or in a fume hood demonstrated to have adequate airflow. Gross losses to the laboratory ventilation system must not be allowed. Handling of the dilute solutions normally used in analytical and animal work presents no inhalation hazards except in an accident.

5.3.2 Protective equipment—Disposable plastic gloves, apron or lab coat, safety glasses or mask, and a glove box or fume hood adequate for radioactive work should be used. During analytical operations that may give rise to aerosols or dusts, personnel should wear respirators equipped with activated carbon filters.

5.3.3 Training—Workers must be trained in the proper method of removing contaminated gloves and clothing without contacting the exterior surfaces.

5.3.4 Personal hygiene—Hands and forearms should be washed thoroughly after each manipulation and before breaks (coffee, lunch, and shift).

5.3.5 Confinement—Isolated work areas posted with signs, segregated glassware and tools, and plastic absorbent paper on bench tops will aid in confining contamination.

5.3.6 Effluent vapors—The effluent from the CVAFS should pass through either a column of activated charcoal or a trap containing gold or sulfur to amalgamate or react mercury vapors.

5.3.7 Waste handling—Good technique includes minimizing contaminated waste. Plastic bag liners should be used in waste cans. Janitors and other personnel must be trained in the safe handling of waste.

5.3.8 Decontamination

5.3.8.1 Decontamination of personnel—Use any mild soap with plenty of scrubbing action.

5.3.8.2 Glassware, tools, and surfaces—Sulfur powder will react with Hg to produce mercuric sulfide, thereby eliminating the possible volatilization of Hg. Satisfactory cleaning may be accomplished by dusting a surface lightly with sulfur powder, then washing with any detergent and water.

5.3.9 Laundry—Clothing known to be contaminated should be collected in plastic bags. Persons that convey the bags and launder the clothing should be advised of the hazard and trained in proper handling. If the launderer knows of the potential problem, the clothing may be put into a washer without contact. The washer should be run through a cycle before being used again for other clothing.

5.3.10 Wipe tests—A useful method of determining cleanliness of work surfaces and tools is to wipe the surface with a piece of filter paper. Extraction and analysis by this Method can achieve a limit of detection of less than 1 ng per wipe. Less than 0.1 µg per wipe indicates acceptable cleanliness; anything higher warrants further cleaning. More than 10 µg on a wipe constitutes an acute hazard and requires prompt cleaning before further use of the equipment or work space, and indicates that unacceptable work practices have been employed.

6.0 Apparatus and Materials

Disclaimer: The mention of trade names or commercial products in this Method is for illustrative purposes only and does not constitute endorsement or recommendation for use by the Environmental Protection Agency. Equivalent performance may be achievable using apparatus, materials, or cleaning procedures other than those suggested here. The laboratory is responsible for demonstrating equivalent performance.

6.1 Sampling equipment

6.1.1 Sample collection bottles-fluoropolymer or glass, 125- to 1000-mL, with fluoropolymer or fluoropolymer-lined cap.

6.1.2 Cleaning

6.1.2.1 New bottles are cleaned by heating to 65–75 °C in 4 N HCl or concentrated HNO_3 for at least 48 h. The bottles are cooled, rinsed three times with reagent water, and filled with reagent water containing 1% HCl. These bottles are capped and placed in a clean oven at 60-70°C overnight. After cooling, they are rinsed three more times with reagent water, filled with reagent water containing 0.4% (v/v) HCl, and placed in a mercury-free Class-100 clean bench until the outside surfaces are dry. The bottles are tightly capped (with a wrench), double-bagged in new polyethylene zip-type bags until needed, and stored in wooden or plastic boxes until use. The bottles may be shipped to the sampling site containing dilute HCl solution (e.g., 0.04%), containing reagent water, or empty.

6.1.2.2 Used bottles known not to have contained mercury at high (>100 ng/L) levels are cleaned as above, except for only 6–12 h in hot 4 N HCl.

6.1.2.3 Bottle blanks must be analyzed as described in Section 9.4.7. To verify the effectiveness of the cleaning procedures, bottle blanks must be demonstrated to be free of mercury at the ML of this Method.

6.1.2.4 As an alternative to cleaning by the laboratory, bottles may be purchased from a commercial supplier and each lot certified to be clean. Bottles from the lot must be tested as bottle blanks (Section 9.4.7) and demonstrated to be free of mercury at the ML of this Method. If mercury is present above this level in any bottle, either the lot must be rejected or the bottles must be re-cleaned.

6.1.3 Filtration Apparatus

6.1.3.1 Filter—0.45-µm, 15-mm diameter capsule filter (Gelman Supor 12175, or equivalent)

6.1.3.2 Peristaltic pump—115-V a.c., 12-V d.c., internal battery, variable-speed, single-head (Cole-Parmer, portable, "Masterflex L/S," Catalog No. 07570-10 drive with Quick Load pump head, Catalog No. 07021-24, or equivalent).

6.1.3.3 Tubing—styrene/ethylene/butylene/silicone (SEBS) resin for use with peristaltic pump, approx 3/8-in ID by approximately 3 ft (Cole-Parmer size 18, Catalog No. 06424-18, or approximately 1/4-in OD, Cole-Parmer size 17, Catalog No. 06424-17, or equivalent). Tubing is cleaned by soaking in 5–10% HCl solution for 8–24 h, rinsing with reagent water in a clean bench in a clean room, and drying in the clean bench by purging with metal-free air or nitrogen. After drying, the tubing is double-bagged in clear polyethylene bags, serialized with a unique number, and stored until use.

6.2 Equipment for bottle and glassware cleaning

6.2.1 Vat, 100–200 L, high-density polyethylene (HDPE), half filled with 4 N HCl in reagent water.

6.2.2 Panel immersion heater, 500-W, all-fluoropolymer coated, 120 vac (Cole-Parmer H-03053-04, or equivalent)

WARNING: *Read instructions carefully!! The heater will maintain steady state, without temperature feedback control, of 60–75°C in a vat of the size described. However, the equilibrium temperature will be higher (up to boiling) in a smaller vat. Also, the heater plate MUST be maintained in a vertical position, completely submerged and away from the vat walls to avoid melting the vat or burning out!*

6.2.3 Laboratory sink—in Class-100 clean area, with high-flow reagent water (Section 7.1) for rinsing.

6.2.4 Clean bench—Class-100, for drying rinsed bottles.

6.2.5 Oven—stainless steel, in Class-100 clean area, capable of maintaining ± 5°C in the 60–70°C temperature range.

6.3 Cold vapor atomic fluorescence spectrometer (CVAFS): The CVAFS system used may either be purchased from a supplier, or built in the laboratory from commercially available components.

6.3.1 Commercially available CVAFS—Tekran (Toronto, ON) Series 2600 CVAFS, Brooks-Rand (Seattle, WA) Model III CVAFS, Leeman Labs Hydra AF Gold*plus* CVAFS, or equivalent

6.3.2 Custom-built CVAFS (Reference 16.15). Figure 2 shows the schematic diagram. The system consists of the following:

6.3.2.1 Low-pressure 4-W mercury vapor lamp

6.3.2.2 Far UV quartz flow-through fluorescence cell—12 mm x 12 mm x 45 mm, with a 10-mm path length (NSG Cells, or equivalent).

6.3.2.3 UV-visible photomultiplier (PMT)—sensitive to < 230 nm. This PMT is isolated from outside light with a 253.7-nm interference filter (Oriel Corp., Stamford, CT, or equivalent).

6.3.2.4 Photometer and PMT power supply (Oriel Corp. or equivalent), to convert PMT output (nanoamp) to millivolts

6.3.2.5 Black anodized aluminum optical block—holds fluorescence cell, PMT, and light source at perpendicular angles, and provides collimation of incident and fluorescent beams (Frontier Geosciences Inc., Seattle, WA, or equivalent).

6.3.2.6 Flowmeter—with needle valve capable of reproducibly keeping the carrier gas flow rate at 30 mL/min

6.4 Hg purging system—Figure 2 shows the schematic diagram for the purging system. The system consists of the following:

6.4.1 Flow meter/needle valve—capable of controlling and measuring gas flow rate to the purge vessel at 350 ± 50 mL/min.

6.4.2 Fluoropolymer fittings—connections between components and columns are made using 6.4-mm OD fluoropolymer tubing and fluoropolymer friction-fit or threaded tubing connectors. Connections between components requiring mobility are made with 3.2-mm OD fluoropolymer tubing because of its greater flexibility.

6.4.3 Acid fume pretrap—10-cm long x 0.9-cm ID fluoropolymer tube containing 2–3 g of reagent grade, nonindicating, 8–14 mesh soda lime chunks, packed between wads of silanized glass wool. This trap is cleaned of Hg by placing on the output of a clean cold vapor generator (bubbler) and purging for 1 h with N_2 at 350 mL/min.

6.4.4 Cold vapor generator (bubbler)—200-mL borosilicate glass (15 cm high x 5.0 cm diameter) with standard taper 24/40 neck, fitted with a sparging stopper having a coarse glass frit that extends to within 0.2 cm of the bubbler bottom (Frontier Geosciences, Inc. or equivalent).

6.5 The dual-trap Hg(0) preconcentrating system

6.5.1 Figures 2 and 3 show the dual-trap amalgamation system (Reference 16.5).

6.5.2 Gold-coated sand traps—10-cm long x 6.5-mm OD x 4-mm ID quartz tubing. The tube is filled with 3.4 cm of gold-coated 45/60 mesh quartz sand (Frontier Geosciences Inc., Seattle, WA, or equivalent). The ends are plugged with quartz wool.

6.5.2.1 Traps are fitted with 6.5-mm ID fluoropolymer friction-fit sleeves for making connection to the system. When traps are not in use, fluoropolymer end plugs are inserted in trap ends to eliminate contamination.

6.5.2.2 At least six traps are needed for efficient operation, one as the "analytical" trap, and the others to sequentially collect samples.

6.5.3 Heating of gold-coated sand traps—To desorb Hg collected on a trap, heat for 3.0 min to 450–500 °C (a barely visible red glow when the room is darkened) with a coil consisting of 75 cm of 24-gauge Nichrome wire at a potential of 10-14 vac. Potential is applied and finely adjusted with an autotransformer.

6.5.4 Timers—The heating interval is controlled by a timer-activated 120-V outlet (Gralab, or equivalent), into which the heating coil autotransformer is plugged. Two timers are required, one each for the "sample" trap and the "analytical" trap.

6.5.5 Air blowers—After heating, traps are cooled by blowing air from a small squirrel-cage blower positioned immediately above the trap. Two blowers are required, one each for the "sample" trap and the "analytical" trap.

6.6 Recorder—Any multi-range millivolt chart recorder or integrator with a range compatible with the CVAFS is acceptable. By using a two-pen recorder with pen sensitivity offset by a factor of 10, the dynamic range of the system is extended to 10^3.

6.7 Pipettors—All-plastic pneumatic fixed-volume and variable pipettors in the range of 10 µL to 5.0 mL.

6.8 Analytical balance capable of weighing to the nearest 0.01 g

7.0 Reagents and Standards

Note: The quantities of reagents and the preparation procedures in this section are for illustrative purposes. Equivalent performance may be achievable using quantities of reagents and procedures other than those suggested here. The laboratory is responsible for demonstrating equivalent performance.

7.1 Reagent water—18-MΩ minimum, ultrapure deionized water starting from a prepurified (distilled, reverse osmosis, etc.) source. Water should be monitored for Hg, especially after ion exchange beds are changed.

7.2 Air—It is very important that the laboratory air be low in both particulate and gaseous mercury. Ideally, mercury work should be conducted in a new laboratory with mercury-free paint on the walls. A source of air that is very low in Hg should be brought directly into the Class-100 clean bench air intake. If this is not possible, air coming into the clean bench can be cleaned for mercury by placing a gold-coated cloth prefilter over the intake. Gold-coated cloth filter: Soak 2 m^2 of cotton gauze in 500 mL of 2% gold chloride solution at pH 7. In a hood, add 100 mL of 30% $NH_2OH{\cdot}HCl$ solution, and homogenize into the cloth with gloved hands. The material will turn black as colloidal gold is precipitated. Allow the mixture to set for several hours, then rinse

with copious amounts of deionized water. Squeeze-dry the rinsed cloth, and spread flat on newspapers to air-dry. When dry, fold and place over the intake prefilter of the laminar flow hood.

CAUTION: Great care should be taken to avoid contaminating the laboratory with gold dust. This could cause interferences with the analysis if gold becomes incorporated into the samples or equipment. The gilding procedure should be done in a remote laboratory if at all possible.

7.3 Hydrochloric acid—trace-metal purified reagent-grade HCl containing less than 5 pg/mL Hg. The HCl should be analyzed for Hg before use.

7.4 Hydroxylamine hydrochloride—Dissolve 300 g of $NH_2OH{\cdot}HCl$ in reagent water and bring to 1.0 L. This solution may be purified by the addition of 1.0 mL of $SnCl_2$ solution and purging overnight at 500 mL/min with Hg-free N_2. Flow injection systems may require the use of less $SnCl_2$ for purification of this solution.

7.5 Stannous chloride—Bring 200 g of $SnCl_2{\cdot}2H_2O$ and 100 mL concentrated HCl to 1.0 L with reagent water. Purge overnight with mercury-free N_2 at 500 mL/min to remove all traces of Hg. Store tightly capped.

7.6 Bromine monochloride (BrCl)—In a fume hood, dissolve 27 g of reagent grade KBr in 2.5 L of low-Hg HCl. Place a clean magnetic stir bar in the bottle and stir for approximately 1 h in the fume hood. Slowly add 38 g reagent grade $KBrO_3$ to the acid while stirring. When all of the $KBrO_3$ has been added, the solution color should change from yellow to red to orange. Loosely cap the bottle, and allow to stir another hour before tightening the lid.

WARNING: *This process generates copious quantities of free halogens (Cl_2, Br_2, BrCl), which are released from the bottle. Add the $KBrO_3$ slowly in a fume hood!*

7.7 Stock mercury standard—NIST-certified 10,000-ppm aqueous Hg solution (NIST-3133). This solution is stable at least until the NIST expiration date.

7.8 Secondary Hg standard—Add approx 0.5 L of reagent water and 5 mL of BrCl solution (Section 7.6) to a 1.00-L Class A volumetric flask. Add 0.100 mL of the stock mercury standard (Section 7.7) to the flask and dilute to 1.00 L with reagent water. This solution contains 1.00 µg/mL (1.00 ppm) Hg. Transfer the solution to a fluoropolymer bottle and cap tightly. This solution is considered stable until the NIST expiration date.

7.9 Working Hg Standard A—Dilute 1.00 mL of the secondary Hg standard (Section 7.8) to 100 mL in a Class A volumetric flask with reagent water containing 0.5% by volume BrCl solution (Section 7.6). This solution contains 10.0 ng/mL and should be replaced monthly, or longer if extended stability is demonstrated.

7.10 Working Hg Standard B—Dilute 0.10 mL of the secondary Hg standard (Section 7.8) to 1000 mL in a Class A volumetric flask with reagent water containing 0.5% by volume BrCl solution (Section 7.6). This solution contains 0.10 ng/mL and should be replaced monthly, or longer if extended stability is demonstrated.

7.11 Initial Precision and Recovery (IPR) and Ongoing Precision and Recovery (OPR) solutions—Using the working Hg standard A (Section 7.9), prepare IPR and OPR solutions at a

concentration of 5 ng/L Hg in reagent water. IPR/OPR solutions are prepared using the same amounts of reagents used for preparation of the calibration standards.

7.12 Nitrogen—Grade 4.5 (standard laboratory grade) nitrogen that has been further purified by the removal of Hg using a gold-coated sand trap.

7.13 Argon—Grade 5.0 (ultra high-purity, GC grade) argon that has been further purified by the removal of Hg using a gold-coated sand trap.

8.0 Sample Collection, Preservation, and Storage

8.1 Before samples are collected, consideration should be given to the type of data required (i.e., dissolved or total), so that appropriate preservation and pretreatment steps can be taken. An excess of BrCl should be confirmed either visually (presence of a yellow color) or with starch iodide indicating paper, using a separate sample aliquot, prior to sample processing or direct analysis to ensure the sample has been properly preserved.

8.2 Samples are collected into rigorously cleaned fluoropolymer bottles with fluoropolymer or fluoropolymer-lined caps. Glass bottles may be used if Hg is the only target analyte. It is critical that the bottles have tightly sealing caps to avoid diffusion of atmospheric Hg through the threads (Reference 16.4). Polyethylene sample bottles must not be used (Reference 16.15).

8.3 Collect samples using guidance provided in the Sampling Method (Reference 16.9). Procedures in the Sampling Method are based on rigorous protocols for collection of samples for mercury (References 16.4 and 16.15).

NOTE: *Discrete samplers have been found to contaminate samples with Hg at the ng/L level. Therefore, great care should be exercised if this type of sampler is used. It may be necessary for the sampling team to use other means of sample collection if samples are found to be contaminated using the discrete sampler.*

8.4 Sample filtration—For dissolved Hg, a sample is filtered through a 0.45-µm capsule filter (Section 6.1.3.1) in a mercury-free clean area prior to preservation. If the sample is filtered, it must be accompanied by a blank that has been filtered under the same conditions. The Sampling Method describes sample filtration procedures.

8.5 Preservation—Samples are preserved by adding either 5 mL/L of pretested 12N HCl or 5 mL/L BrCl solution to the sample bottle. If a sample will be used also for the determination of methyl mercury, it should be collected and preserved according to procedures in the method that will be used for determination of methyl mercury (e.g., HCl or H_2SO_4 solution). Preserved samples are stable for up to 90 days of the date of collection.

8.5.1 Samples to be analyzed for total or dissolved Hg only may be shipped to the laboratory unpreserved and unrefrigerated if they are collected in fluoropolymer or glass bottles and capped tightly. Samples must be either preserved or analyzed within 48 hours of collection. If a sample is oxidized in the sample bottle, the time to preservation can be extended to 28 days.

8.5.2 Samples that are acid-preserved may lose Hg to coagulated organic materials in the water or condensed on the walls (Reference 16.16). The best approach is to add BrCl directly to the sample bottle at least 24 hours before analysis. If other Hg species are to be analyzed, these aliquots must be removed prior to the addition of BrCl. If BrCl

cannot be added directly to the sample bottle, the bottle must be shaken vigorously prior to sub-sampling.

8.5.3 Handling of the samples in the laboratory should be undertaken in a mercury-free clean bench, after rinsing the outside of the bottles with reagent water and drying in the clean air hood.

NOTE: *Because of the potential for contamination, it is recommended that filtration and preservation of samples be performed in the clean room in the laboratory. However, if circumstances prevent overnight shipment of samples, samples should be filtered and preserved in a designated clean area in the field in accordance with the procedures given in Method 1669 (Reference 16.9). If filtered in the field, samples ideally should be filtered into the sample bottle.*

8.6 Storage—Sample bottles should be stored in clean (new) polyethylene bags until sample analysis.

8.7 Sample preservation, storage, and holding time requirements also are given at 40 CFR part 136.3(e) Table II.

9.0 Quality Control

9.1 Each laboratory that uses this Method is required to operate a formal quality assurance program (Reference 16.17). The minimum requirements of this program consist of an initial demonstration of laboratory capability, ongoing analysis of standards and blanks as a test of continued performance, and the analysis of matrix spikes (MS) and matrix spike duplicates (MSD) to assess precision and recovery. Laboratory performance is compared to established performance criteria to determine that the results of analyses meet the performance characteristics of the Method.

9.1.1 The laboratory shall make an initial demonstration of the ability to generate acceptable accuracy and precision. This ability is established as described in Section 9.2.

9.1.2 In recognition of advances that are occurring in analytical technology, the laboratory is permitted certain options to improve results or lower the cost of measurements. These options include automation of the dual-amalgamation system, single-trap amalgamation (Reference 16.18), direct electronic data acquisition, calibration using gas-phase elemental Hg standards, use of the bubbler or flow-injection systems, or changes in the detector (i.e., CVAAS) when less sensitivity is acceptable or desired. Changes in the determinative technique, such as the use of colorimetry, are not allowed. If an analytical technique other than the CVAFS technique specified in this Method is used, that technique must have a specificity for mercury equal to or better than the specificity of the technique in this Method.

9.1.2.1 Each time this Method is modified, the laboratory is required to repeat the procedure in Section 9.2 to demonstrate that an MDL (40 CFR part 136, Appendix B) less than or equal to one-third the regulatory compliance limit or less than or equal to the MDL of this Method (Table 1), whichever is greater, can be achieved. If the change will affect calibration, the instrument must be recalibrated according to Section 10.

Note: If the compliance limit is greater than the concentration of Hg in the OPR/OPR (5 ng/L), the acceptance criteria for blanks and the concentrations of mercury spiked into quality control samples may be increased to support measurements at the compliance limit. For example, if the compliance limit is 12

ng/L (National Toxics Rule, 40 CFR 131.36), the MDL must be less than or equal to 4 ng/L; concentrations of the calibration standards may be 5, 10, 20, 50 , and 100 ng/L; concentrations of the IPR/OPR samples may be 10 ng/L; spike concentrations and acceptance criteria for MS/MSD samples would remain as specified in Section 9.3; and an appropriate blank acceptance criterion would be 5 ng/L.

9.1.2.2 The laboratory is required to maintain records of modifications made to this Method. These records include the following, at a minimum:

9.1.2.2.1 The names, titles, addresses, and telephone numbers of the analyst(s) who performed the analyses and modification, and the quality control officer who witnessed and will verify the analyses and modification

9.1.2.2.2 A narrative stating the reason(s) for the modification(s)

9.1.2.2.3 Results from all quality control (QC) tests demonstrating the performance of the modified method, including the following:

(a) Calibration (Section 10)
(b) Initial precision and recovery (Section 9.2.2)
(c) Analysis of blanks (Section 9.4)
(d) Matrix spike/matrix spike duplicate analysis (Section 9.3)
(e) Ongoing precision and recovery (Section 9.5)
(f) Quality control sample (Section 9.6)
(g) Method detection limit (Section 9.2.1)

9.1.2.2.4 Data that will allow an independent reviewer to validate each determination by tracking the instrument output to the final result. These data are to include the following:

(a) Sample numbers and other identifiers
(b) Processing dates
(c) Analysis dates
(d) Analysis sequence/run chronology
(e) Sample weight or volume
(f) Copies of logbooks, chart recorder, or other raw data output
(g) Calculations linking raw data to the results reported

9.1.3 Analyses of MS and MSD samples are required to demonstrate the accuracy and precision and to monitor matrix interferences. Section 9.3 describes the procedure and QC criteria for spiking.

9.1.4 Analyses of blanks are required to demonstrate acceptable levels of contamination. Section 9.4 describes the procedures and criteria for analyzing blanks.

9.1.5 The laboratory shall, on an ongoing basis, demonstrate through analysis of the ongoing precision and recovery (OPR) sample and the quality control sample (QCS) that the system is in control. Sections 9.5 and 9.6 describe these procedures, respectively.

9.1.6 The laboratory shall maintain records to define the quality of the data that are generated. Sections 9.3.7 and 9.5.3 describe the development of accuracy statements.

9.1.7 Quality of the analyses is controlled by an analytical batch. An analytical batch is a set of samples oxidized with the same batch of reagents, and analyzed during the same 12-hour shift. A batch may be from 1 to as many as 20 samples. Each batch must be accompanied by 3 system blanks (Section 9.4.2 for the flow-injection system), a

minimum of 3 bubbler blanks (Section 9.4.1 for the bubbler system), 1 OPR sample at the beginning and end of the batch (Section 9.5), a QCS (Section 9.6), and at least 3 method blanks (Section 9.4.4). In addition, there must be 1 MS and 1 MSD sample for every 10 samples (a frequency of 10%). A typical analytical sequence would be:

(a) Three system blanks (Section 9.4.2) or a minimum of 3 bubbler blanks (Section 9.4.1)
(b) A minimum of five, non-zero calibration standards (Section 10.2.2.1)
(c) On-going precision and recovery (Section 9.5)
(d) Quality control sample (Section 9.6)
(e) Method blank (Section 9.4.4)
(f) Seven samples
(g) Method blank (Section 9.4.4)
(h) Three samples
(i) Matrix spike (Section 9.3)
(j) Matrix spike duplicate (Section 9.3)
(k) Four samples
(l) Method blank (Section 9.4.4)
(m) Six samples
(n) Matrix spike (Section 9.3)
(o) Matrix spike duplicate (Section 9.3)
(p) Ongoing precision and recovery (Section 9.5)

The above sequence includes calibration. If system performance is verified at the end of the sequence using the OPR, analysis of samples and blanks may proceed without recalibration (i.e., the analytical sequence would be entered at Step (d) above), unless more than 12 hours has elapsed since verification of system performance. If more than 12 hours has elapsed, the sequence would be initiated at Step (c) above.

9.2 Initial demonstration of laboratory capability

9.2.1 Method detection limit—To establish the ability to detect Hg, the laboratory shall achieve an MDL that is less than or equal to the MDL listed in Section 1.5 or one-third the regulatory compliance limit, whichever is greater. The MDL shall be determined according to the procedure at 40 CFR 136, Appendix B using the apparatus, reagents, and standards that will be used in the practice of this Method. This MDL shall be used for determination of laboratory capability only, and should be determined when a new operator begins work or whenever, in the judgment of the laboratory, a change in instrument hardware or operating conditions would dictate reevaluation of capability.

9.2.2 Initial precision and recovery (IPR)—To establish the ability to generate acceptable precision and recovery, the laboratory shall perform the following operations:

9.2.2.1 Analyze four replicates of the IPR solution (5 ng/L, Section 7.11) according to the procedure beginning in Section 11.

9.2.2.2 Using the results of the set of four analyses, compute the average percent recovery (X), and the standard deviation of the percent recovery (s) for Hg.

9.2.2.3 Compare s and X with the corresponding limits for initial precision and recovery in Table 2. If s and X meet the acceptance criteria, system performance is acceptable and analysis of samples may begin. If, however, s exceeds the

precision limit or X falls outside the acceptance range, system performance is unacceptable. Correct the problem and repeat the test (Section 9.2.2.1).

9.3 Matrix spike (MS) and matrix spike duplicate (MSD)—To assess the performance of the Method on a given sample matrix, the laboratory must spike, in duplicate, a minimum of 10% (1 sample in 10) from a given sampling site or, if for compliance monitoring, from a given discharge. Therefore, an analytical batch of 20 samples would require two pairs of MS/MSD samples (four spiked samples total).

9.3.1 The concentration of the spike in the sample shall be determined as follows:

9.3.1.1 If, as in compliance monitoring, the concentration of Hg in the sample is being checked against a regulatory compliance limit, the spiking level shall be at that limit or at 1–5 times the background concentration of the sample (as determined in Section 9.3.2), whichever is greater.

9.3.1.2 If the concentration of Hg in a sample is not being checked against a limit, the spike shall be at 1–5 times the background concentration or at 1-5 times the ML in Table 1, whichever is greater.

9.3.2 To determine the background concentration (B), analyze one sample aliquot from each set of 10 samples from each site or discharge according to the procedure in Section 11. If the expected background concentration is known from previous experience or other knowledge, the spiking level may be established *a priori*.

9.3.2.1 If necessary, prepare a standard solution to produce an appropriate level in the sample (Section 9.3.1).

9.3.2.2 Spike two additional sample aliquots with identical amounts of the spiking solution and analyze these aliquots as described in Section 11.1.2 to determine the concentration after spiking (A).

9.3.3 Calculate the percent recovery (R) in each aliquot using the following equation:

$$\% R = 100 \frac{(A-B)}{T}$$

where:

A = *Measured concentration of analyte after spiking*
B = *Measured concentration of analyte before spiking*
T = *True concentration of the spike*

9.3.4 Compare percent recovery (R) with the QC acceptance criteria in Table 2.

9.3.4.1 If results of the MS/MSD are similar and fail the acceptance criteria, and recovery for the OPR standard (Section 9.5) for the analytical batch is within the acceptance criteria in Table 2, an interference is present and the results may not be reported or otherwise used for permitting or regulatory compliance purposes. If the interference can be attributed to sampling, the site or discharge should be resampled. If the interference can be attributed to a method deficiency, the laboratory must modify the method, repeat the test required in Section 9.1.2, and repeat analysis of the sample and MS/MSD. However, during the development

of Method 1631, very few interferences have been noted in the determination of Hg using this Method. (See Section 4.4 for information on interferences.)

9.3.4.2 If the results of both the spike and the OPR test fall outside the acceptance criteria, the analytical system is judged to be not in control, and the results may not be reported or used for permitting or regulatory compliance purposes. The laboratory must identify and correct the problem and reanalyze all samples in the sample batch.

9.3.5 Relative percent difference (RPD)—Compute the RPD between the MS and MSD results according to the following equation using the concentrations found in the MS and MSD. Do not use the recoveries calculated in Section 9.3.3 for this calculation because the RPD is inflated when the background concentration is near the spike concentration.

$$RPD = 200 \times \frac{(|D1-D2|)}{(D1+D2)}$$

Where:
D1 = concentration of Hg in the MS sample
D2 = concentration of Hg in the MSD sample

9.3.6 The RPD for the MS/MSD pair must not exceed the acceptance criterion in Table 2. If the criterion is not met, the system is judged to be out of control. The problem must be identified and corrected, and the MS/MSD and corresponding samples reanalyzed.

9.3.7 As part of the QC program for the laboratory, method precision and recovery for samples should be assessed and records maintained. After analyzing five samples in which the recovery passes the test in Section 9.3.4, compute the average percent recovery (R_a) and the standard deviation of the percent recovery (s_r). Express the accuracy assessment as a percent recovery interval from R_a - $2s_r$ to R_a + $2s_r$. For example, if R_a = 90% and s_r = 10% for five analyses, the accuracy interval is expressed as 70–110%. Update the accuracy assessment regularly (e.g., after every five to ten new accuracy measurements).

9.4 Blanks—Blanks are critical to the reliable determination of Hg at low levels. The sections below give the minimum requirements for analysis of blanks. Analysis of additional blanks is recommended as necessary to pinpoint sources of contamination in, and external to, the laboratory.

9.4.1 Bubbler blanks—Bubbler blanks are analyzed to demonstrate that bubbler systems are free from contamination at levels that could affect data quality. At least three bubbler blanks must be run during calibration and with each analytical batch.

9.4.1.1 To analyze a bubbler blank, place a clean gold trap on the bubbler. Purge and analyze previously purged water using the procedure in Section 11, and determine the amount of Hg remaining in the system.

9.4.1.2 If the bubbler blank is found to contain more than 50 pg Hg, the system is out of control. The problem must be investigated and remedied, and the samples run on that bubbler must be reanalyzed. If the blanks from other bubblers contain less than 50 pg Hg, the data associated with those bubblers remain valid, provided that all other criteria in Section 9 also are met.

9.4.1.3 The mean result for all bubbler blanks (from bubblers passing the specification in Section 9.4.1.2) must be < 25 pg (0.25 ng/L) Hg with a standard deviation (n-1) of <10 pg (0.10 ng/L). If the mean is < 25 pg, the average peak area or height is subtracted from all raw data before results are calculated (Section 12.2).

9.4.1.4 If Hg in the bubbler blank exceeds the acceptance criteria in Section 9.4.1.3, the system is out of control. The problem must be resolved and the system recalibrated. Usually, the bubbler blank is too high for one of the following reasons:

(a) Bubblers need rigorous cleaning;
(b) Soda-lime is contaminated; or
(c) Carrier gas is contaminated.

9.4.2 System blanks— System blanks are analyzed to demonstrate that flow injection systems are free from contamination at levels that could affect data quality. Three system blanks must be run during calibration and with each analytical batch.

9.4.2.1 To analyze a system blank, analyze reagent water containing the same amount of reagents used to prepare the calibration standards.

9.4.2.2 If a system blank is found to contain ≥ 0.50 ng/L Hg, the system is out of control. The problem must be investigated and remedied, and the system recalibrated. If the blanks contain < 0.50 ng/L Hg, the data associated with the blanks remain valid, provided that all other criteria in Section 9 also are met.

9.4.2.3 The mean result for the three system blanks must be <0.5 ng/L Hg with a standard deviation (n-1) <0.1 ng/L. If the mean exceeds these criteria, the system is out of control, and the problem must be resolved and the system recalibrated. If the mean is <0.5 ng/L, the average peak height or area is subtracted from all raw data before results are calculated (Section 12.3).

9.4.3 Reagent blanks—Reagent blanks are used to demonstrate that the reagents used to prepare samples for Hg analyses are free from contamination. The Hg concentration in reagent blanks is determined by analyzing the reagent solutions using either the bubbler or flow-injection system. For the bubbler system, reagent may be added directly to previously purged water in the bubbler.

9.4.3.1 Reagent blanks are required when the batch of reagents (bromine monochloride plus hydroxylamine hydrochloride) are prepared. The amount of Hg in a reagent blank containing 0.5% (v/v) BrCl solution (Section 7.6) and 0.2% (v/v) hydroxylamine hydrochloride solution (Section 7.4) must be < 20 pg (0.2 ng/L).

9.4.3.2 The presence of more than 20 pg (0.2 ng/L) of Hg indicates a problem with the reagent solution. The purging of certain reagent solutions, such as $SnCl_2$ or NH_2OH, with mercury-free nitrogen or argon can reduce Hg to acceptable levels. Because BrCl cannot be purified, a new batch must be prepared and tested if the BrCl is contaminated.

9.4.4 Method blanks— Method blanks are used to demonstrate that the analytical system is free from contamination that could otherwise compromise sample results. Method blanks are prepared and analyzed using sample containers, labware, reagents, and analytical procedures identical to those used to prepare and analyze the samples.

9.4.4.1 A minimum of three method blanks per analytical batch are required for both the bubbler and flow-injection systems.

9.4.4.2 If the result for any method blank containing the nominal amount of reagent used to prepare a sample (Section 11.1.1) is found to contain ≥ 0.50 ng/L (50 pg) Hg, the system is out of control. Mercury in the analytical system must be reduced until a method blank is free from contamination at the 0.50 ng/L level. Samples associated with a contaminated method blank must be reanalyzed.

9.4.4.3 Because method blanks are analyzed using procedures identical to those used to analyze samples, any sample requiring an increased amount of reagent must be accompanied by at least one method blank that includes an identical amount of reagent.

9.4.5 Field blanks–Field blanks are used to demonstrate that samples have not been contaminated by the sample collection and transport activities.

9.4.5.1 Analyze the field blank(s) shipped with each set of samples (samples collected from the same site at the same time, to a maximum of 10 samples). Analyze the blank immediately before analyzing the samples in the batch.

9.4.5.2 If Hg or any potentially interfering substance is found in the field blank at a concentration equal to or greater than the ML (Table 1), or greater than one-fifth the level in the associated sample, whichever is greater, results for associated samples may be the result of contamination and may not be reported or otherwise used for regulatory compliance purposes.

9.4.5.3 Alternatively, if sufficient multiple field blanks (a minimum of three) are collected, and the average concentration (of the multiple field blanks) plus two standard deviations is equal to or greater than the regulatory compliance limit or equal to or greater than one-half of the level in the associated sample, results for associated samples may be the result of contamination and may not be reported or otherwise used for regulatory compliance purposes.

9.4.5.4 If contamination of the field blanks and associated samples is known or suspected, the laboratory should communicate this to the sampling team so that the source of contamination can be identified and corrective measures taken before the next sampling event.

9.4.6 Equipment blanks—Before any sampling equipment is used at a given site, the laboratory or cleaning facility is required to generate equipment blanks on all sampling equipment that will be used to demonstrate that the sampling equipment is free from contamination.

9.4.6.1 Equipment blanks are generated in the laboratory or at the equipment cleaning facility by processing reagent water through the sampling devices using the same procedures that are used in the field (see Sampling Method). Therefore, the "clean hands/dirty hands" technique used during field sampling should be followed when preparing equipment blanks at the laboratory or cleaning facility for low level mercury measurements. If grab samples are to be collected using any ancillary equipment, e.g., an extension pole or a dipper, an equipment blank

is generated by submersing this equipment into the reagent water and analyzing the resulting reagent water collected.

9.4.6.2 The equipment blank must be analyzed using the procedures in this Method. If mercury or any potentially interfering substance is detected in the blank at or above the level specified for the field blank (Section 9.4.5), the source of contamination or interference must be identified, and the problem corrected. The equipment must be demonstrated to be free from mercury and interferences before the equipment may be used in the field.

9.4.7 Bottle blanks— Bottles must be subjected to conditions of use to verify the effectiveness of the cleaning procedures. A representative set of sample bottles (Section 6.1.2) should be filled with reagent water acidified to pH <2 and allowed to stand for a minimum of 24 h. At least 5% of the bottles from a given lot should be tested, and the time that the bottles are allowed to stand should be as close as possible to the actual time that the sample will be in contact with the bottle. After standing, the water must be analyzed for any signs of contamination. If a bottle shows contamination at or above the level specified for the field blank (Section 9.4.5), the problem must be identified, the cleaning procedures corrected or cleaning solutions changed, and all affected bottles re-cleaned.

9.5 Ongoing precision and recovery (OPR)—To demonstrate that the analytical system is within the performance criteria of this Method and that acceptable precision and recovery is being maintained within each analytical batch, the laboratory shall perform the following operations:

9.5.1 Analyze the OPR solution (5 ng/L, Section 7.11) prior to the analysis of each analytical batch according to the procedure beginning in Section 11. An OPR also must be analyzed at the end of an analytical sequence or at the end of each 12-hour shift.

9.5.2 Compare the recovery with the limits for ongoing precision and recovery in Table 2. If the recovery is in the range specified, the analytical system is in control and analysis of samples and blanks may proceed. If, however, the concentration is not in the specified range, the analytical process is not in control. Correct the problem and repeat the ongoing precision and recovery test. All reported results must be associated with an OPR that meets the Table 2 performance criteria at the beginning and end of each batch.

9.5.3 The laboratory should add results that pass the specification in Section 9.5.2 to IPR and previous OPR data and update QC charts to form a graphic representation of continued laboratory performance. The laboratory also should develop a statement of laboratory data quality by calculating the average percent recovery (R_a) and the standard deviation of the percent recovery (s_r). Express the accuracy as a recovery interval from $R_a - 2s_r$ to $R_a + 2s_r$. For example, if $R_a = 95\%$ and $s_r = 5\%$, the accuracy is 85–105%.

9.6 Quality control sample (QCS) – The laboratory must obtain a QCS from a source different from the Hg used to produce the standards used routinely in this Method (Sections 7.7–7.10). The QCS should be analyzed as an independent check of system performance.

9.7 Depending on specific program requirements, the laboratory may be required to analyze field duplicates and field spikes collected to assess the precision and accuracy of the sampling, sample transportation, and storage techniques. The relative percent difference (RPD) between field duplicates should be less than 20%. If the RPD of the field duplicates exceeds 20%, the laboratory should communicate this to the sampling team so that the source of error can be identified and corrective measures taken before the next sampling event.

10.0 Calibration and Standardization

10.1 Calibration and standardization— Separate calibration procedures are provided for a bubbler system (Section 10.2) and flow-injection system (Section 10.3). Both systems are calibrated using standards traceable to NIST Standard Reference Materials. If system performance is verified at the end of an analytical batch using the OPR, analysis of samples and blanks may proceed without recalibration, unless more than 12 hours has elapsed since verification of system performance.

10.2 Bubbler system calibration

10.2.1 Establish the operating conditions necessary to purge Hg from the bubbler and to desorb Hg from the traps in a sharp peak. Further details for operation of the purge-and-trap, desorption, and analysis systems are given in Sections 11.2.1 and 11.2.2.

10.2.2 The calibration must contain a minimum of five non-zero points and the results of analysis of three bubbler blanks. The lowest calibration point must be at the Minimum Level (ML).

NOTE: *The purge efficiency of the bubbler system is 100% and is independent of volume at the volumes used in this Method. Calibration of this system is typically performed using units of mass. For purposes of working in concentration, the volume is assumed to be 100 mL.*

10.2.2.1 Standards are analyzed by the addition of aliquots of Hg working standard A (Section 7.9) and Hg working standard B (Section 7.10) directly into the bubblers. Add 0.50 mL of working standard B and 0.5 mL $SnCl_2$ to the bubbler. Swirl to produce a standard containing 50 pg of Hg (0.5 ng/L). Purge under the optimum operating conditions (Section 10.2.1). Sequentially follow with the addition of aliquots of 0.05, 0.25, 0.50 and 1.0 mL of working standard A to produce standards of 500, 2500, 5000, and 10,000 pg Hg (5.0, 25.0, 50.0 and 100.0 ng/L).

NOTE: *If calibration to the higher levels results in carryover (Section 4.3.8.1), calibrate the system across a narrower range (Section 10.4)*

10.2.2.2 Analyze the standards beginning with the lowest concentration and proceeding to the highest. Tabulate the height or area for each peak.

10.2.2.3 Prepare and analyze a minimum of 3 bubbler blanks. If multiple bubblers are used, there must be 1 bubbler blank per bubbler (to a maximum of 4 bubblers). Calculate the mean peak area or height for the bubbler blanks.

10.2.2.4 For each calibration point, subtract the mean peak height or area of the bubbler blanks from the peak height or area for each standard. Calculate the calibration factor (CF_x) for Hg in each of the five standards using the mean bubbler-blank-subtracted peak height or area and the following equation:

$$CF_x = \frac{(A_x) - (\overline{A}_{BB})}{(C_x)}$$

Where:

A_x = *peak height or area for Hg in standard*
$\overline{A}_{BB}$ = *mean peak height or area for Hg in bubbler blank*
C_x = *mass in standard analyzed (ng)*

10.2.2.5 Calculate the mean calibration factor (CF_m), the standard deviation of the calibration factor (SD; n-1), and the relative standard deviation (RSD) of the calibration factor, where RSD = 100 x SD/CF_m.

10.2.2.6 If RSD ≤ 15%, calculate the recovery for the lowest standard using CF_m. If the RSD ≤ 15% and the recovery of the lowest standard is in the range of 75-125%, the calibration is acceptable and CF_m may be used to calculate the concentration of Hg in samples. If RSD > 15% or if the recovery of the lowest standard is not in the range of 75-125%, recalibrate the analytical system and repeat the test.

10.2.2.7 Calculate the concentration of Hg in the bubbler blanks (Section 10.2.2.1) using CF_m. The bubbler blanks must meet the criteria in Section 9.4.1; otherwise, mercury in the system must be reduced and the calibration repeated until the bubbler blanks meet the criteria.

10.3 Flow-injection system calibration

10.3.1 Establish the operating conditions necessary to purge Hg from the gas-liquid separator and dryer tube and desorb Hg from the traps in a sharp peak. Further details for operating the analytical system are given in Section 11.2.1.

10.3.2 The calibration must contain a minimum of 5 non-zero points and the results of analysis of 3 system blanks. The lowest calibration point must be at the minimum level (ML).

10.3.2.1 Place 25-30 mL of reagent water and 250 µL of concentrated BrCl solution (Section 7.6) in each of 5 calibrated 50-mL autosampler vials. Prepare the 0.5 ng/L calibration standard by adding 250 µL of working standard B (Section 7.10) to the vial. Dilute to the mark with reagent water. Sequentially follow with the addition of aliquots of 25, 125, 250 and 500 µL of working standard A (Section 7.9) to produce standards of 5.0, 25.0, 50.0 and 100.0 ng/L, respectively. Cap the vials and invert once to mix.

10.3.2.2 Immediately prior to analysis, remove the caps and add 125 µL of NH_2OH solution (Section 7.4). Re-cap, invert once to mix, and allow to stand until the yellow color disappears. Remove all caps and place vials into the analysis rack.

10.3.2.3 Analyze the standards beginning with the lowest concentration and proceeding to the highest. Tabulate the height or area for the Hg peak.

10.3.2.4 Prepare and analyze a minimum of 3 system blanks and tabulate the peak heights or areas. Calculate the mean peak area or height for the system blanks.

10.3.2.5 For each calibration point, subtract the mean peak height or area of the system blanks (Section 9.4.2) from the peak height or area for each standard. Calculate

the calibration factor (CF_x) for Hg in each of the five standards using the mean reagent-blank-subtracted peak height or area and the following equation:

$$CF_x = \frac{(A_x) - (\overline{A}_{SB})}{(C_x)}$$

Where:

A_x = *peak height or area for Hg in standard*
$\overline{A}_{SB}$ = *mean peak height or area for Hg in calibration blanks*
C_x = *concentration of standard analyzed (ng/L)*

10.3.2.6 Calculate the mean calibration factor (CF_m), the standard deviation of the calibration factor (SD; n-1), and the relative standard deviation (RSD) of the calibration factor, where RSD = 100 x SD/CF_m.

10.3.2.7 If RSD ≤ 15%, calculate the recovery for the lowest standard (0.5 ng/L) using CF_m. If the RSD ≤ 15% and the recovery of the lowest standard is in the range of 75-125%, the calibration is acceptable and CF_m may be used to calculate the concentration of Hg in samples, blanks, and OPRs. If RSD > 15% or if the recovery of the lowest standard is not in the range of 75-125%, recalibrate the analytical system and repeat the test.

10.3.2.8 Calculate the concentration of Hg in the system blanks (Section 9.4.2) using CF_m. The system blanks must meet the criteria in Section 9.4.2; otherwise, mercury in the system must be reduced and the calibration repeated until the system blanks meet the criteria.

10.4 Calibration to a range other than 0.5 to 100 ng/L—This Method may be calibrated to a range other than 0.5 to 100 ng/L, provided that the following requirements are met:

(a) There must be a minimum of five non-zero calibration points.
(b) The difference between successive calibration points must be no greater than a factor of 10 and no less than a factor of 2 and should be approximately evenly spaced on a logarithmic scale over the calibration range.
(c) The relative standard deviation (RSD) of the calibration factors for all calibration points must be less than 15%.
(d) The calibration factor for any calibration point at a concentration greater than 100 ng/L must be within ±15% of the average calibration factor for the points at or below 100 ng/L.
(e) The calibration factor for any point <0.5 ng/L must be within 25% of the average calibration factor for all points.
(f) If calibration is to a higher range and this Method is used for regulatory compliance, the ML must be less than one-third the regulatory compliance limit

11.0 Procedure

NOTE: *The following procedures for analysis of samples are provided as guidelines. Laboratories may find it necessary to optimize the procedures, such as drying time or potential applied to the Nichrome wires, for the laboratory's specific instrument set-up.*

11.1 Sample Preparation

11.1.1 Pour a 100-mL aliquot from a thoroughly shaken, acidified sample, into a 125-mL fluoropolymer bottle. If BrCl was not added as a preservative (Section 8.5), add the amount of BrCl solution (Section 7.6) given below, cap the bottle, and digest at room temperature for a 12 h minimum.

11.1.1.1 For clear water and filtered samples, add 0.5 mL of BrCl; for brown water and turbid samples, add 1.0 mL of BrCl. If the yellow color disappears because of consumption by organic matter or sulfides, more BrCl should be added until a permanent (12-h) yellow color is obtained.

11.1.1.2 Some highly organic matrices, such as sewage effluent, will require high levels of BrCl (e.g., 5 mL/100 mL of sample) and longer oxidation times, or elevated temperatures (e.g., place sealed bottles in oven at 50 °C for 6 h). The oxidation must be continued until it is complete. Complete oxidation can be determined by either observation of a permanent yellow color remaining in the sample or the use of starch iodide indicating paper to test for residual free oxidizer. The sample also may be diluted to reduce the amount of BrCl required, provided that the resulting level of mercury is sufficient for reliable determination.

11.1.2 Matrix spikes and matrix spike duplicates—For every 10 or fewer samples, pour 2 additional 100-mL aliquots from a selected sample (see Section 9.3), spike at the level specified in Section 9.3, and process in the same manner as the samples. There must be a minimum of 2 MS/MSD pairs for each analytical batch of 20 samples.

11.2 Hg reduction and purging—Separate procedures are provided for the bubbler system (Section 11.2.1) and flow-injection (Section 11.2.2).

11.2.1 Hg reduction and purging for the bubbler system

11.2.1.1 Add 0.2-0.25 mL of NH_2OH solution to the BrCl-oxidized sample in the 125-mL sample bottle. Cap the bottle and swirl the sample. The yellow color will disappear, indicating the destruction of the BrCl. Allow the sample to react for 5 min with periodic swirling to be sure that no traces of halogens remain.

NOTE: *Purging of free halogens onto the gold trap will result in damage to the trap and low or irreproducible results.*

11.2.1.2 Connect a fresh trap to the bubbler, pour the reduced sample into the bubbler, add 0.5 mL of $SnCl_2$ solution, and purge the sample onto a gold trap with N_2 at 350 ± 50 mL/min for 20 min.

11.2.1.3 When analyzing Hg samples, the recovery is quantitative, and organic interferents are destroyed. Thus, standards, bubbler blanks, and small amounts of high-level samples may be run directly in previously purged water. After very high samples (Section 4.3.8.1), a small degree of carryover (<0.01%) may occur. Bubblers that contain such samples must be demonstrated to be clean prior to proceeding with low level samples. Samples run immediately following a sample that has been determined to result in carryover must be reanalyzed using a bubbler that is demonstrated to be clean as per Section 4.3.8.1.

11.2.2 Hg reduction and purging for the flow-injection system

11.2.2.1 Add 0.2-0.25 mL of NH_2OH solution (Section 7.4) to the BrCl-oxidized sample in the 125-mL sample bottle or in the autosampler tube (the amount of NH_2OH required will be approximately 30 percent of the BrCl volume). Cap the bottle and swirl the sample. The yellow color will disappear, indicating the destruction of the BrCl. Allow the sample to react for 5 minutes with periodic swirling to be sure that no traces of halogens remain.

NOTE: *Purging of free halogens onto the gold trap will result in damage to the trap and low or irreproducible results.*

11.2.2.2 Pour the sample solution into an autosampler vial and place the vial in the rack.

11.2.2.3 Carryover may occur after analysis of a sample containing a high level of mercury. Samples run immediately following a sample that has been determined to result in carryover (Section 4.3.8.1) must be reanalyzed using a system demonstrated to be clean as per Section 4.3.8.1.

11.3 Desorption of Hg from the gold trap

11.3.1 Remove the sample trap from the bubbler, place the Nichrome wire coil around the trap and connect the trap into the analyzer train between the incoming Hg-free argon and the second gold-coated (analytical) sand trap (Figure 2).

11.3.2 Pass argon through the sample and analytical traps at a flow rate of approximately 30 mL/min for approximately 2 min to drive off condensed water vapor.

11.3.3 Apply power to the coil around the sample trap for 3 minutes to thermally desorb the Hg (as Hg(0)) from the sample trap onto the analytical trap.

11.3.4 After the 3-min desorption time, turn off the power to the Nichrome coil, and cool the sample trap using the cooling fan.

11.3.5 Turn on the chart recorder or other data acquisition device to start data collection, and apply power to the Nichrome wire coil around the analytical trap. Heat the analytical trap for 3 min (1 min beyond the point at which the peak returns to baseline).

11.3.6 Stop data collection, turn off the power to the Nichrome coil, and cool the analytical trap to room temperature using the cooling fan.

11.3.7 Place the next sample trap in line and proceed with analysis of the next sample.

NOTE: *Do not heat a sample trap while the analytical trap is still warm; otherwise, the analyte may be lost by passing through the analytical trap.*

11.4 Peaks generated using this technique should be very sharp and almost symmetrical. Mercury elutes at approximately 1 minute and has a width at half-height of about 5 seconds.

11.4.1 Broad or asymmetrical peaks indicate a problem with the desorption train, such as improper gas flow rate, water vapor on the trap(s), or an analytical trap damaged by chemical fumes or overheating.

11.4.2 Damage to an analytical trap is also indicated by a sharp peak, followed by a small, broad peak.

11.4.3 If the analytical trap has been damaged, the trap and the fluoropolymer tubing downstream from it should be discarded because of the possibility of gold migration onto downstream surfaces.

11.4.4 Gold-coated sand traps should be tracked by unique identifiers so that any trap producing poor results can be quickly recognized and discarded.

12.0 Data Analysis and Calculations

12.1 Separate procedures are provided for calculation of sample results using the bubbler system (Section 12.2) and the flow-injection system (Section 12.3), and for method blanks (Section 12.4).

12.2 Calculations for the bubbler system

12.2.1 Calculate the mean peak height or area for Hg in the bubbler blanks measured during system calibration or with the analytical batch (A_{BB}; n = 3 minimum).

12.2.2 Calculate the concentration of Hg in ng/L (parts-per-trillion; ppt) in each sample according to the following equation:

$$[Hg]\ (ng/L) = \frac{A_s - \overline{A}_{BB}}{CF_m \times V}$$

where:
A_s = *peak height (or area) for Hg in sample*
$\overline{A}_{BB}$ = *peak height (or area) for Hg in bubbler blank*
CF_m = *mean calibration factor (Section* 10.2.2.5)
V = *Volume of sample (L)*

12.3 Calculations for the flow-injection system

12.3.1 Calculate the mean peak height or area for Hg in the system blanks measured during system calibration or with each analytical batch (A_{SB}; n = 3)

12.3.2 Calculate the concentration of Hg in ng/L in each sample according to the following equation:

$$[Hg]\ (ng/L) = \frac{(A_s - \overline{A}_{SB})}{CF_m} \times \frac{V_{std}}{V_{sample}}$$

where:
A_s = *peak height (or area) for Hg in sample*
$\overline{A}_{SB}$ = *mean peak height (or area) for Hg in system blanks*
CF_m = *mean calibration factor (Section* 10.3.2.6)
V_{std} = *volume (mL) used for standards – volume (mL) reagent used in standards*
V_{sample} = *volume (mL) of sample – volume (mL) reagent used in sample*

12.4 Calculations for concentration of Hg in method blanks, field blanks, and reagent blanks.

12.4.1 Calculate the concentration of Hg in the method blanks (C_{MB}), field blanks (C_{FB}), or reagent blanks (C_{RB}) in ng/L, using the equation in Section 12.2.2 (if bubbler system is used) or Section 12.3.2 (if flow injection system is used) and substituting the peak height or area resulting from the method blank, field blank, or reagent blank for A_s.

12.4.2 Determine the mean concentration of Hg in the method blanks associated with the analytical batch (a minimum of three). If a sample requires additional reagent(s) (e.g., BrCl), a corresponding method blank containing an identical amount of reagent must be analyzed (Section 9.4.4.3). The concentration of Hg in the corresponding method blank may be subtracted from the concentration of Hg in the sample per Section 12.5.2.

12.5 Reporting

12.5.1 Report results for Hg at or above the ML, in ng/L, to three significant figures. Report results for Hg in samples below the ML as <0.5 ng/L, or as required by the regulatory authority or in the permit. Report results for Hg in reagent blanks and field blanks at or above the ML, in ng/L, to three significant figures. Report results for Hg in reagent blanks, method blanks, or field blanks below the ML but at or above the MDL to two significant figures. Report results for Hg not detected in reagent blanks, method blanks, or field blanks as <0.2 ng/L, or as required by the regulatory authority or in the permit.

12.5.2 Report results for Hg in samples, method blanks and field blanks separately. In addition to reporting results for the samples and blank(s) separately, the concentration of Hg in the method blanks or field blanks associated with the sample may be subtracted from the results for that sample, or must be subtracted if requested or required by a regulatory authority or in a permit.

12.5.3 Results from tests performed with an analytical system that is not in control must not be reported or otherwise used for permitting or regulatory compliance purposes, but do not relieve a discharger or permittee of reporting timely results.

13.0 Method Performance

13.1 This Method was tested in 12 laboratories using reagent water, freshwater, marine water and effluent (Reference 16.19). The quality control acceptance criteria listed in Table 2 were verified by data gathered in the interlaboratory study, and the method detection limit (MDL) given in Section 1.5 was verified in all 12 laboratories. In addition, the techniques in this Method have been compared with other techniques for low-level mercury determination in water in a variety of studies, including ICES-5 (Reference 16.20) and the International Mercury Speciation Intercomparison Exercise (Reference 16.21).

13.2 Precision and recovery data for reagent water, freshwater, marine water, and secondary effluent are given in Table 3.

14.0 Pollution Prevention

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Many opportunities for pollution prevention exist in laboratory operation. EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address waste generation. When it is not feasible to reduce wastes at the source, the Agency recommends recycling as the next best option. The acids used in this Method should be reused as practicable by purifying by electrochemical techniques. The only other chemicals used in this Method are the neat materials used in preparing standards. These standards are used in extremely small amounts and pose little threat to the environment when managed properly. Standards should be prepared in volumes consistent with laboratory use to minimize the disposal of excess volumes of expired standards.

14.2 For information about pollution prevention that may be applied to laboratories and research institutions, consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's Department of Governmental Relations and Science Policy, 1155 16th Street NW, Washington DC 20036, 202/872–4477.

15.0 Waste Management

15.1 The laboratory is responsible for complying with all Federal, State, and local regulations governing waste management, particularly hazardous waste identification rules and land disposal restrictions, and for protecting the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required. An overview of requirements can be found in *Environmental Management Guide for Small Laboratories* (EPA 233-B-98-001).

15.2 Acids, samples at pH <2, and BrCl solutions must be neutralized before being disposed of, or must be handled as hazardous waste.

15.3 For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* and *Less is Better: Laboratory Chemical Management for Waste Reduction*, both available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington, DC 20036.

16.0 References

16.1 Bloom, Nicolas, Draft “Total Mercury in Aqueous Media,” Frontier Geosciences, Inc., September 7, 1994.

16.2 Fitzgerald, W.F.; Gill, G.A. "Sub-Nanogram Determination of Mercury by Two-Stage Gold Amalgamation and Gas Phase Detection Applied to Atmospheric Analysis," *Anal. Chem.* 1979, *15*, 1714.

16.3 Bloom, N.S; Crecelius, E.A. "Determination of Mercury in Sea water at Subnanogram per Liter Levels," *Mar. Chem.* 1983, *14*, 49.

16.4 Gill, G.A.; Fitzgerald, W.F. "Mercury Sampling of Open Ocean Waters at the Picogram Level," *Deep Sea Res* 1985, *32*, 287.

16.5 Bloom, N.S.; Fitzgerald, W.F. "Determination of Volatile Mercury Species at the Picogram Level by Low-Temperature Gas Chromatography with Cold-Vapor Atomic Fluorescence Detection," *Anal. Chim. Acta.* 1988, *208*, 151.

16.6 Guidance on Establishing Trace Metal Clean Rooms in Existing Facilities, U.S. EPA, Office of Water, Office of Science and Technology, Engineering and Analysis Division (4303), 401 M Street SW, Washington, DC 20460, January 1996, EPA 821-B-96-001.

16.7 Trace Metal Cleanroom, prepared by Research Triangle Institute for U.S. Environmental Protection Agency, 26 W. Martin Luther King Dr., Cincinnati, OH 45268, RTI/6302/04-02 F.

16.8 Guidance on the Documentation and Evaluation of Trace Metals Data Collected for Clean Water Act Compliance Monitoring, U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, Engineering and Analysis Division (4303), 401 M Street SW, Washington, DC 20460, July 1996, EPA 821-B-96-004.

16.9 Method 1669, "Method for Sampling Ambient Water for Determination of Metals at EPA Ambient Criteria Levels," U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, Engineering and Analysis Division (4303), 401 M Street SW, Washington, DC 20460, April 1995 with January 1996 revisions.

16.10 Correspondence from Nicolas Bloom, Frontier Geosciences, Inc. to Dale Rushneck, Interface, Inc., December 31, 1998.

16.11 "Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service. Centers for Disease Control. NIOSH Publication 77-206, Aug. 1977, NTIS PB-277256.

16.12 "OSHA Safety and Health Standards, General Industry," OSHA 2206, 29 CFR 1910.

16.13 "Safety in Academic Chemistry Laboratories," ACS Committee on Chemical Safety, 1979.

16.14 "Standard Methods for the Examination of Water and Wastewater," 18th ed. and later revisions, American Public Health Association, 1015 15th Street NW, Washington, DC 20005. 1-35: Section 1090 (Safety), 1992.

16.15 Bloom, N.S. "Trace Metals & Ultra-Clean Sample Handling," *Environ. Lab.* 1995, *7*, 20.

16.16 Bloom, N.S. "Influence of Analytical Conditions on the Observed 'Reactive Mercury,' Concentrations in Natural Fresh Waters," In *Mercury as a Global Pollutant*; Huckabee, J. and Watras, C.J., Eds.; Lewis Publishers, Ann Arbor, MI: 1994.

16.17 "Handbook of Analytical Quality Control in Water and Wastewater Laboratories," U.S. Environmental Protection Agency. Environmental Monitoring Systems Laboratory, Cincinnati, OH 45268, EPA-600/4-79-019, March 1979.

16.18 Liang, L.; Bloom, N.S. "Determination of Total Mercury by Single-Stage Gold Amalgamation with Cold Vapor Atom Spectrometric Detection," *J. Anal. Atomic Spectrom.* 1993, *8*, 591.

16.19 "Results of the EPA Method 1631 Validation Study," February, 1998. Available from the EPA Sample Control Center, 6101 Stevenson Avenue, Alexandria, VA, 22304; 703/461-2100.

16.20 Cossa, D.; Couran, P. "An International Intercomparison Exercise for Total Mercury in Sea Water," *App. Organomet. Chem.* 1990, *4*, 49.

16.21 Bloom, N.S.; Horvat, M.; Watras, C.J. "Results of the International Mercury Speciation Intercomparison Exercise," *Wat. Air. Soil Pollut.*, 1995, 80, 1257.

17.0 Glossary

The definitions and purposes below are specific to this Method, but have been conformed to common usage as much as possible.

17.1 **Ambient Water**—Waters in the natural environment (e.g., rivers, lakes, streams, and other receiving waters), as opposed to effluent discharges.

17.2 **Analytical Batch**—A batch of up to 20 samples that are oxidized with the same batch of reagents and analyzed during the same 12-hour shift. Each analytical batch must also include at least three bubbler blanks, an OPR, and a QCS. In addition, MS/MSD samples must be prepared at a frequency of 10% per analytical batch (one MS/MSD for every 10 samples).

17.3 **Bottle Blank**—The bottle blank is used to demonstrate that the bottle is free from contamination prior to use. Reagent water known to be free of mercury at the MDL of this Method is added to a bottle, acidified to pH <2 with BrCl or HCl, and allowed to stand for a minimum of 24 hours. The time that the bottle is allowed to stand should be as close as possible to the actual time that the sample will be in contact with the bottle. After standing, the water is analyzed.

17.4 **Bubbler Blank**—For this Method, the bubbler blank is specific to the bubbler system and is used to determine that the analytical system is free from contamination. After analysis of a standard, blank, or sample, the solution in the bubbler is purged and analyzed. A minimum of three bubbler blanks is required for system calibration.

17.5 **Equipment Blank**—Reagent water that has been processed through the sampling device at a laboratory or other equipment cleaning facility prior to shipment of the sampling equipment to the sampling site. The equipment blank is used to demonstrate that the sampling equipment is free from contamination prior to use. Where appropriate, the "clean hands/dirty hands" technique used during field sampling should be followed when preparing equipment blanks at the laboratory or cleaning facility.

17.6 **Field Blank**—Reagent water that has been transported to the sampling site and exposed to the same equipment and operations as a sample at the sampling site. The field blank is used to demonstrate that the sample has not been contaminated by the sampling and sample transport systems.

17.7 **Intercomparison Study**—An exercise in which samples are prepared and split by a reference laboratory, then analyzed by one or more testing laboratories and the reference laboratory. The intercomparison, with a reputable laboratory as the reference laboratory, serves as the best test of the precision and accuracy of the analyses at natural environmental levels.

17.8 **Matrix Spike (MS) and Matrix Spike Duplicate (MSD)**—Aliquots of an environmental sample to which known quantities of the analyte(s) of interest is added in the laboratory. The MS and MSD are analyzed exactly like a sample. Their purpose is to quantify the bias and precision caused by the sample matrix. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the MS and MSD corrected for these background concentrations.

17.9 **May**—This action, activity, or procedural step is allowed but not required.

17.10 **May not**—This action, activity, or procedural step is prohibited.

17.11 **Method blank**— Method blanks are used to determine the concentration of mercury in the analytical system during sample preparation and analysis, and consist of a volume of reagent water that is carried through the entire sample preparation and analysis. Method blanks are prepared by placing reagent water in a sample bottle and analyzing the water using reagents and procedures identical to those used to prepare and analyze the corresponding samples. A minimum of three method blanks is required with each analytical batch.

17.12 **Minimum Level (ML)**—The lowest level at which the entire analytical system must give a recognizable signal and acceptable calibration point for the analyte. It is equivalent to the concentration of the lowest calibration standard, assuming that all method-specified sample weights, volumes, and cleanup procedures have been employed. The ML is calculated by multiplying the MDL by 3.18 and rounding the result to the number nearest to (1, 2, or 5) x 10^n, where n is an integer (See Section 1.5).

17.13 **Must**—This action, activity, or procedural step is required.

17.14 **Quality Control Sample (QCS)**—A sample containing Hg at known concentrations. The QCS is obtained from a source external to the laboratory, or is prepared from a source of standards different from the source of calibration standards. It is used as an independent check of instrument calibration.

17.15 **Reagent blank**—Reagent blanks are used to determine the concentration of mercury in the reagents (BrCl, $NH_2OH{\cdot}HCl$, and $SnCl_2$) that are used to prepare and analyze the samples. In this Method, reagent blanks are required when each new batch of reagents is prepared.

17.16 **Reagent Water**—Water demonstrated to be free of mercury at the MDL of this Method. It is prepared from 18 MΩ ultrapure deionized water starting from a prepurified source. Reagent water is used to wash bottles, as trip and field blanks, and in the preparation of standards and reagents.

17.17 **Regulatory Compliance Limit**—A limit on the concentration or amount of a pollutant or contaminant specified in a nationwide standard, in a permit, or otherwise established by a regulatory authority.

17.18 **Shall**—This action, activity, or procedure is required.

17.19 **Should**—This action, activity, or procedure is suggested, but not required.

17.20 **Stock Solution**— A solution containing an analyte that is prepared from a reference material traceable to NIST, or a source that will attest to the purity and authenticity of the reference material.

17.21 **System Blank**— For this Method, the system blank is specific for the flow-injection system and is used to determine contamination in the analytical system and in the reagents used to prepare the calibration standards. A minimum of three system blanks is required during system calibration.

17.22 **Ultraclean Handling**— A series of established procedures designed to ensure that samples are not contaminated during sample collection, storage, or analysis.

18.0 Tables and Figures

Table 1

Lowest Ambient Water Quality Criterion for Mercury and the Method Detection Limit and Minimum Level of Quantitation for EPA Method 1631

Metal	Lowest Ambient Water Quality Criterion[1]	Method Detection Limit (MDL) and Minimum Level (ML)	
		MDL[2]	ML[3]
Mercury (Hg)	1.3 ng/L	0.2 ng/L	0.5 ng/L

1. Lowest water quality criterion for the Great Lakes System (Table 4, 40 CFR 132.6). The lowest Nationwide criterion is 12 ng/L (40 CFR 131.36).
2. Method detection limit (40 CFR 136, Appendix B)
3. Minimum level of quantitation (see Glossary)

Table 2

Quality Control Acceptance Criteria for Performance Tests in EPA Method 1631

Acceptance Criteria	Section	Limit (%)
Initial Precision and Recovery (IPR)	9.2.2	
Precision (RSD)	9.2.2.3	21
Recovery (X)	9.2.2.3	79-121
Ongoing Precision and Recovery (OPR)	9.5.2	77-123
Matrix Spike/Matrix Spike Duplicate (MS/MSD)	9.3	
Recovery	9.3.4	71-125
Relative Percent Difference (RPD)	9.3.5	24

Table 3

Precision and Recovery for Reagent Water, Fresh Water, Marine Water, and Effluent Water Using Method 1631

Matrix	*Mean Recovery (%)	*Precision (% RSD)
Reagent Water	98.0	5.6
Fresh Water (Filtered)	90.4	8.3
Marine Water (Filtered)	92.3	4.7
Marine Water (Unfiltered)	88.9	5.0
Secondary Effluent (Filtered)	90.7	3.0
Secondary Effluent (Unfiltered)	92.8	4.5

*Mean percent recoveries and RSDs are based on expected Hg concentrations.

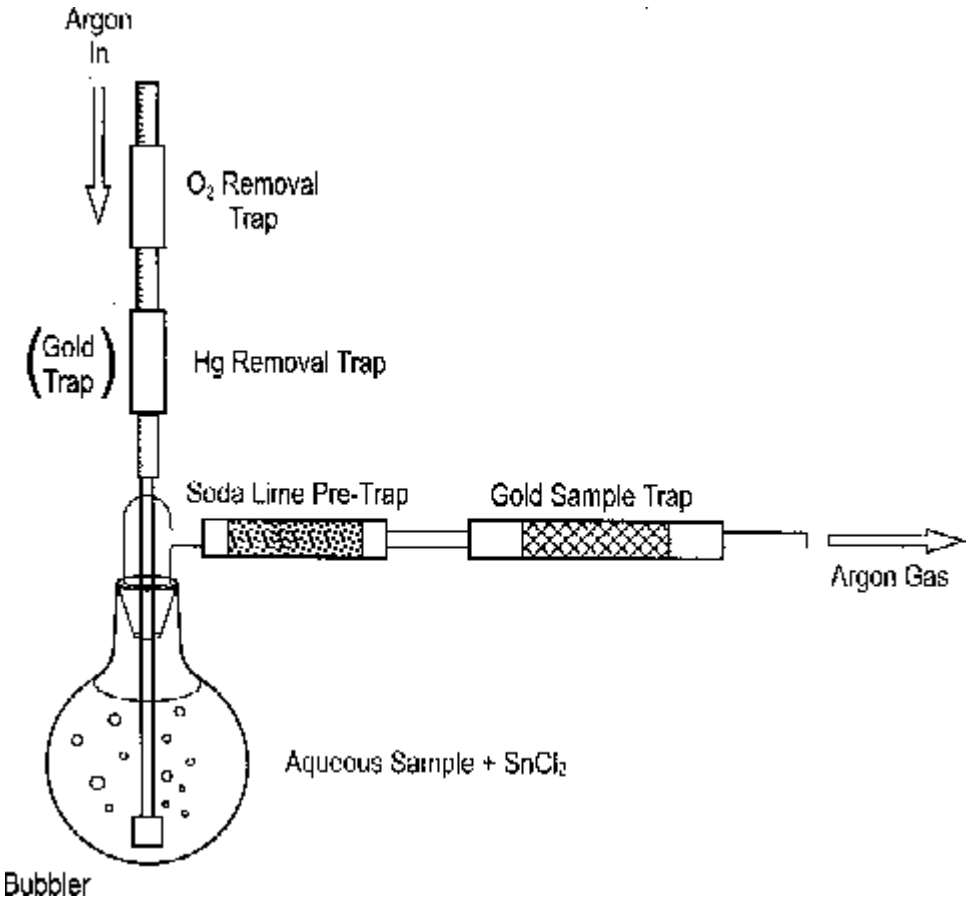


Figure 1. Schematic Diagram of Bubbler Setup

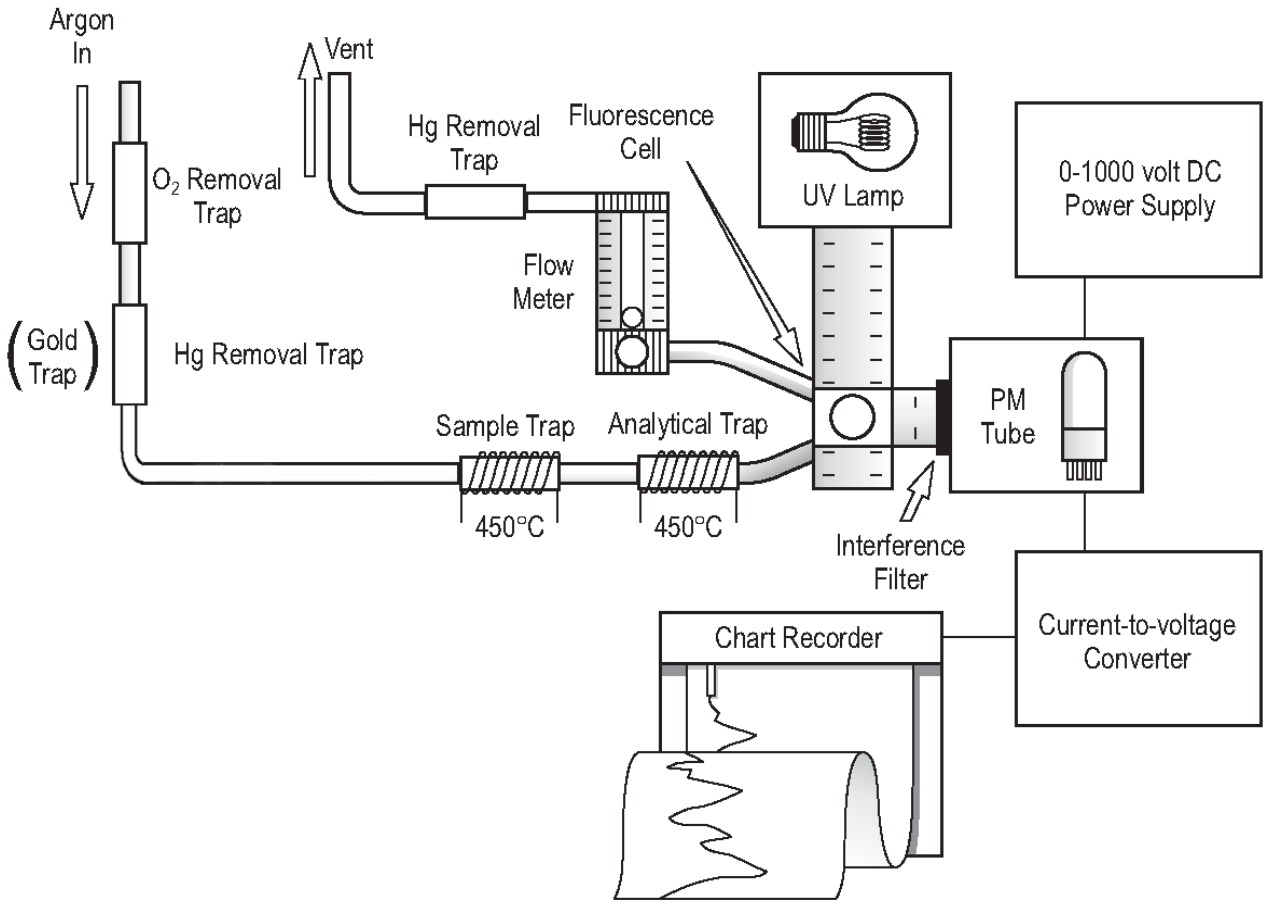


Figure 2. Schematic Diagram of the Bubbler, Purge and Trap, Cold Vapor Atomic Fluorescence Spectrometer (CVAFS) System

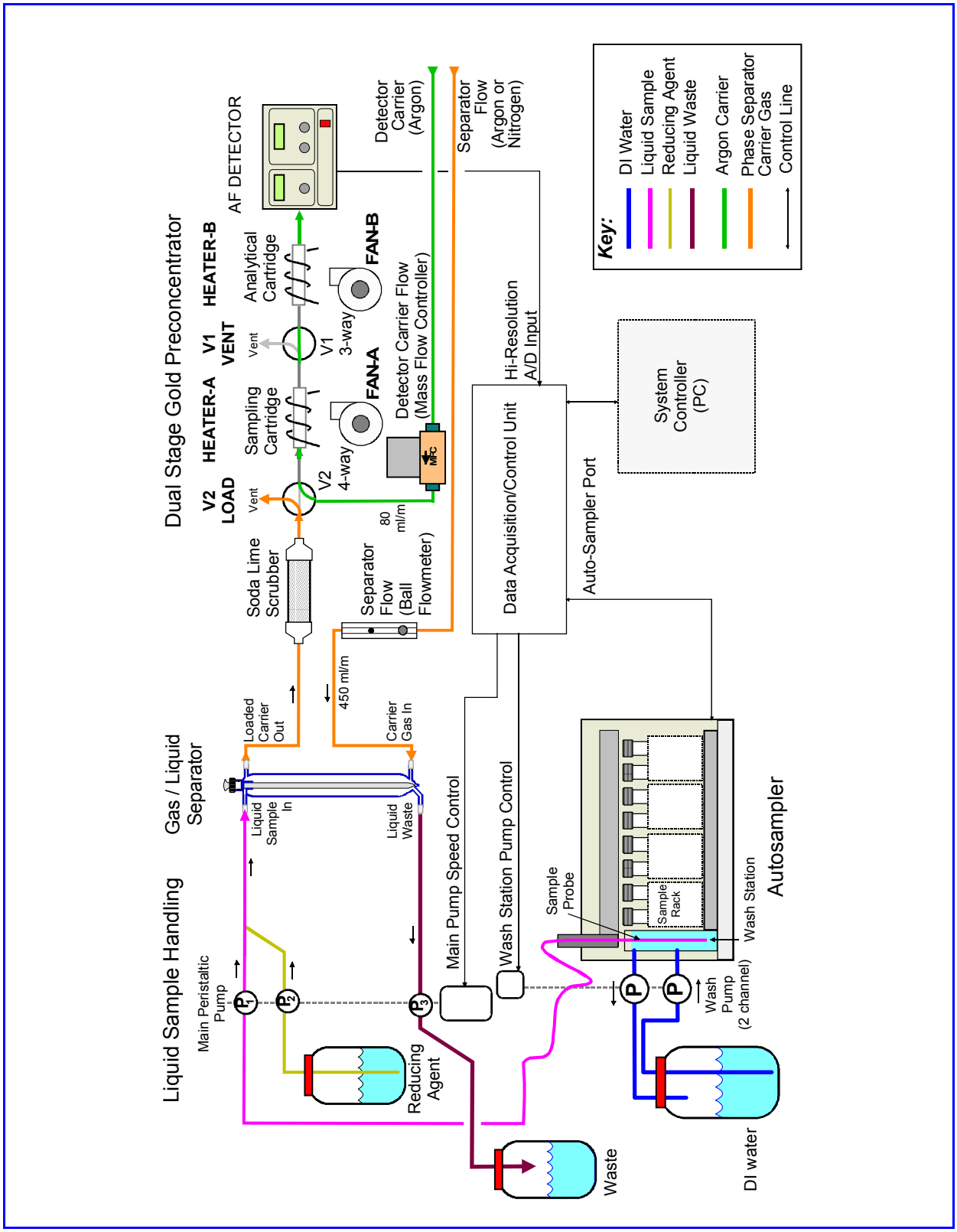


Figure 3. Schematic Diagram of the Flow-Injection, Cold Vapor Atomic Fluorescence Spectrometer (CVAFS) System

Date: February 24, 2009
Revision #: 5 KP
Prepared by: DFG-WPCL

CDFG FISH AND WILDLIFE WATER POLLUTION CONTROL LABORATORY STANDARD OPERATING PROCEDURE FOR THE DETERMINATION OF MERCURY IN FISH TISSUE AND BIRD EGG BY FLOW INJECTION MERCURY SYSTEM (FIMS)

1.0 SCOPE AND APPLICATION

This is an atomic spectroscopy method for the determination of mercury in fish tissue.

2.0 SUMMARY OF METHOD

2.1 Fish tissue is digested with concentrated nitric acid. The mercury ions are reduced to elemental mercury with stannous chloride. The mercury vapor is analyzed by cold vapor atomic spectroscopy.

2.2 The method detection limit for this method is approximately 0.005 µg/g (ppm) (fresh weight), 0.020 µg/g (dry weight) for flesh assuming 80% moisture content for a 1.0 g sample in 25 ml total extract volume.

3.0 INTERFERENCES

Certain volatile organic materials that absorb at this wavelength (253.7 nm) may cause interference. A preliminary run without reagents should determine if this type of interference is present. Chlorine causes severe interference.

4.0 APPARATUS AND MATERIALS

4.1 Atomic Spectroscopy Perkin Elmer equipped with: flow injection mercury system 400 (FIMS 400), data system, programmable autosampler (AS-90 series).

4.2 Compressed argon – instrument grade

4.3 Digestion tubes: polypropylene digestion vessels (Environmental Express)

4.4 Analytical balance with the capability of weighing to the nearest 0.0001g

4.5 Hot block for metals digestions - Cat. # SC154 from Environmental Express

4.6 Teflon spatulas

4.7 50 ml disposable polypropylene skirted centrifuge tubes with screw caps

4.8 Ribbed watch glass (Environmental Express)

4.9 100 mL volumetric flasks

4.10 Disposable filter funnels (VWR 60872-316)

4.11 Filter papers - Cat. # 1004 090 (Whatman) or (Fisherbrand P5 Cat.#09-801B)

5.0 REAGENTS

5.1 Type II water (MQ water)

5.2 Hydrochloric acid (HCl), concentrated, reagent grade.

5.3 Nitric acid (HNO_3), concentrated, reagent grade.

5.4 3.0% HCl carrier and rinse water – Partially fill a 1000 ml volumetric flask with MQ water. Add 30 ml concentrated HCl acid and bring up to volume with MQ water. Prepare fresh daily.

(Reagents Cont.)

5.5 Stannous chloride dihydrate, crystal ("Baker Analyzed" JT3980-11), 25% $SnCl_2 \cdot 2H_2O$ in 20% HCl. In a 1000 ml volumetric flask dissolve 250g $SnCl_2 \cdot 2H_2O$ in 200 ml HCl. Mix and allow to stand until $SnCl_2 \cdot 2H_2O$ has dissolved and solution is clear. Bring to volume with MQ water. Approximately 800 ml needed for the set of 32 samples, QC and standard curve. Prepare fresh daily.

6.0 PREPARATION OF CALIBRATION AND QC STANDARDS

6.1 Stock calibration and spike standard solution – purchased 1000 ppm $HgCl_2$

6.2 Intermediate calibration standard - 1.0 ppm in 1.0% nitric acid. Partially fill a 100 ml volumetric flask with MQ water. Add 0.100 ml of 1000 ppm stock $HgCl_2$. Bring to volume with MQ water. Expires 6 months.

6.3 Working calibration standards (in 25 mL total volume): Partially fill each standard tube with MQ water, add 10 ml concentrated nitric acid (40% acid) and let cool. Add the appropriate volume of $HgCl_2$ standard according to the table below and bring to volume with MQ water. Mix well. Prepare fresh daily.

Standard Concentration (ppb)	Volume of Stock Std in 25 ml
25.0	0.625 ml of 1.0 ppm $HgCl_2$
10.0	0.250 ml of 1.0 ppm $HgCl_2$
5.00	**0.250 ml of 1.0 ppm $HgCl_2$ in 50 ml total***
1.00	1.00 ml of 0.0250 ppm $HgCl_2$
0.500	0.50 ml of 0.0250 ppm $HgCl_2$
0.000	MQ water (40% acid)

Table 1: Dilution series for mercury calibration standards.

***Note: Make 50 ml of the 5.00 ppb standard as this is used as the CCV.**

6.4 Stock quality control check standard: purchased 1000 ppm from a different manufacturer than the calibration and spike standard solution.

6.5 Intermediate check standard: 1.0 ppm in 1.0% nitric acid. Partially fill a 100 ml volumetric flask with MQ water. Add 0.100 ml of 1000 ppm stock check standard $HgCl_2$. Bring to volume with MQ water. Expires 6 months.

6.6 Working check standard in 25 mL total volume (5.00 ppb in 40% nitric acid): Partially fill a standard tube with MQ water, add 10 ml concentrated nitric acid (40% acid) and let cool. Add 0.125 ml of the intermediate stock $HgCl_2$ standard volume with MQ water. Mix well. Prepare fresh daily.

7.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

7.1 All samples must have been collected using a sampling plan that addresses the considerations discussed in this manual.

7.2 All sample containers must be prewashed with detergents, acids, and MQ water. Plastic and glass containers are both suitable.

7.3 Nonaqueous samples shall be frozen, when possible, and analyzed as soon as possible.

8.0 SAMPLE PREPARATION

8.1 Before weighing samples, prepare an Excel spreadsheet

8.2 Balance calibration should be checked and recorded using more than one weight in order to bracket the sample weight and recorded in a log book including date and operator's initials.

8.3 With each set of analyses, prepare at least 1 method blank prepared from the rinse water from the Buchi Mixer-B400 Grinder or a routine method blank if the grinder is not used, 2 standard reference materials (~ 0.25 g dry tissue – Dorm 2 or NBS 1566a), a matrix spike and matrix spike duplicate, 1 laboratory control spike, and one duplicate for every lab batch (≤ 20 samples).

8.4 Samples with equal weight of water added: weigh 2.0 ± 0.05 g into a clean digestion tube.

8.5 Samples without added water: weigh 1.0 ± 0.05 g into a clean digestion tube.

8.6 Add 10 ml concentrated nitric acid, cap and let stand overnight.

8.7 The next morning digest the samples in a programmable hot block covering each with a ribbed watch glass. The parameters for heating are as follows:

Ramp: 5º C / min.
Set the temperature to 105ºC to achieve a temperature of 95 C (actual) in the hot block.
Hold: 2 ½ hours

8.8 After allowing the tubes to cool add MQ water to the 25 ml mark. Shake the samples 20 times to mix well. Filter the sample through a 20-25 um if necessary to remove particulate.

9.0 PERCENT MOISTURE DETERMINATION (if required)

9.1 Use only forceps to handle the aluminum weighing dish.

9.2 Number an aluminum weighing dish to correspond to the sample beaker number.

9.3 Weigh the aluminum weighing dish and record its weight.

9.4 Tare the aluminum weighing dish.

9.5 Weigh ~ 3g (minimum 1g) tissue and record the weight.

9.6 Place moisture samples in a 70°C oven for 24 hours (Nguyen, 2003).

9.7 After cooling samples, weigh and record the dry weights.

Percent Moisture Calculations

$$\% \text{ Moisture} = \frac{\text{Water Weight (g)}}{\text{Undiluted Sample Weight (g)}} \times 100$$

Where:

Water Weight = Undiluted Sample Weight (g) – Dry Sample Weight (g)

Undiluted Sample Weight (g) = Sample Weight in Planchette (g) x F

Where: F = Sample Dilution Factor = the water added at time of preparation

= 0.5 when added water equals the sample weight

= 1 when water was not added to the sample

Dry Sample Weight (g) = (Planchette Wt (g) & Dry Wt (g)) – Planchette Wt (g)

10.0 ANALYTICAL PROCEDURE

10.1 Prepare calibration standards and reagents (See section 5.0)

10.2 Transfer samples to 15 ml Rohre/Tubes numbered corresponding to sample numbers.

10.3 Switch on the fume ventilation system, then the carrier gas supply (argon), adjust the pressure to 52 psig and finally switch on FIMS 400.

10.4 Switch on the computer, printer and start Windows.

10.5 In the Program Manager, double-click on AA 2.50.

10.6 When ”AA WinLab” appears, proceed as follows:

10.6.1 From the Tools menu, choose Open Workspace, or on the Toolbar click on WkSpace.

10.6.2 Select **hgtissue.fms**, then click on OK.

10.6.3 Select **Tissue Hg Test** as the method. All of the desired parameters have been entered for:

Inst – Instrument parameters
Calib – Calibration parameters
FIAS – FIAS program instructions
Checks – Analytical checks for sample and calibration solutions
QC – Locations of quality control solutions and instructions for performing quality control procedures
Options – Remarks about the Method and options for saving and printing data

10.6.4 Click on SampInfo on the Toolbar to enter the pertinent information (e.g. description, batch ID, analyst)

- The first sample ID should be at auto sampler location # 9
- From the File menu, choose Save As Sample Info File to save the sample information file (also the autosampler loading list)
- Print the Autosampler Loading List from the File menu

10.6.5 Select the name of the Results Data Set where you will save the results. If the data set exists, new data will be added to it.

10.6.6 Select the Save Data check box if you want the results saved in the data set specified.

10.6.7 Select the Print Log check if you want the results to be printed.

10.6.8 On the Automated Analysis window, check “use Entire Sample Info File”

10.7 Place the liquid-gas separator membrane in the liquid-gas separator tower shiny side up.

10.8 Connect the gas sample line from the gas-liquid separator to the mercury lamp sample inlet.

10.9 The reagent flows must be checked before every run. An unused portion of the tubing should be used for a new run (never reuse the used portion of pump tubing). There are two runs available per pump tube.

10.9.1 Lower the sample probe into the acidified rinse water using the toggle switch in the Automated Analysis control window.

10.9.2 Place the inlets of the carrier pump tube (yellow/blue), and reductant pump tube (red/red) in 100 mL volumetric cylinders of deionized water.

(Analytical Procedure Cont.)

10.9.3 Swing the pump pressure levers over to press all (including waste tubing) pump tube magazines against the rollers.

10.9.4 Start both pumps and time the flows over a minute. The carrier pump tube (yellow/blue) should have a flow of 9-11 ml/min the reductant pump tube (red/red) should have a flow of 5-7 ml/min. Record reagent flows in the log book.

10.9.4.1 If the reagent pump flows are off the tension screws may be adjusted, or the tubing may need to be replaced. Adjust and recheck the reagent flows.

10.10 After setting the flows, position the inlets in the appropriate reagent.

10.11 Slight adjustments to the gas flow may improve sensitivity.

10.11.1 If the carrier gas flow is too high, the selenium vapor is dispersed too rapidly. If the flow is too low, selenium vapor flows into the cell too slowly. In both situations the signal and sensitivity are low. A flow in the range 60-100 ml/min is generally suitable.

10.11.2 If the peak maximum appears too early, slightly decrease the carrier gas flow. If the peak maximum appears too late, slightly increase the carrier gas flow.

10.12 On the Toolbar, click on Analyses, select Autozero signal to zero the instrument.

10.13 After the reagents have been prepared, the FIMS 400 flows have been set, the autosampler has been turn on, and the samples have been loaded click on the tab containing "Analyze" and click on "Analyze all" to establish a curve and run the samples.

10.14 PRECAUTIONS: Check that the drain tube is connected to the gas / liquid separator and freely drains into collection vessel. The end of the drain tube must not be submerged in liquid. The exhaust hood over the FIMS should be left on at all times.

11.0 INSTRUMENT SHUTDOWN

11.1 Rinsing procedure after automatic analyses

11.1.1 Place the inlets of the carrier and reagent (e.g. reductant, buffer) tubes in a container of MQ water.

11.1.2 Replace the probe acidified rinse water with MQ water.

11.1.3 In the Automated Analysis Control window, click on Move Probe Up/Down to lower the sampling probe.

11.1.4 On the Toolbar, click on FIAS. In the FIAS Control window:

11.1.4.1 Click on the Pump 1 and 2 to start the pump.

11.1.4.2 Click on Valve Fill/Inject a few times while the pumps are running. This ensures that sample channel and the inside of the FIAS-valve are rinsed effectively.

11.1.4.3 Rinse the tubing with the deionized water for approximately 5 minutes to remove all traces of the previous reagent.

11.1.4.4 Dry all tubing and the sample line prior to shut down.

12.0 QUALITY CONTROL

12.1 All calibration standards and the QC standard shall receive a unique ID.

12.2 A log book containing reagent flow, gas flow, project, calibration standard absorbances, r^2, calibration and QC standard IDs, project information, date and operators initials will be kept for every run.

12.3 The calibration curve must be composed of a minimum of three standards.

12.4 Run one QC standard for every batch.

12.5 A mid-curve standard from the curve must be analyzed at the beginning and end of every run and after every 10 samples.

12.6 Dilute samples if they are more concentrated than the highest standard.

12.7 Run a minimum of one blank per sample batch to determine if contamination or any carry over effects are occurring.

12.8 Verify the integrity of the sample run by analyzing two SRMs of a comparable matrix.

12.9 Run one duplicate sample for every twenty samples. A duplicate sample is a sample brought through the entire sample preparation and analytical process.

13.0 ACCEPTANCE CRITERIA

13.1 The correlation coefficient for the calibration curve must be 0.995 or greater.

13.2 The recovery of the standard reference material must be within 80% to 120% of the 95% confidence interval for the reference material.

13.3 Relative percent difference of sample duplicates must be within 15% for sample values greater than 30 times the MDL. An RPD between duplicates of 25% will be permitted if the sample value is less than 30 times the MDL.

13.4 Recovery values for sample spikes must fall within 80-120% if greater than 5 times the MDL. For spikes less than 5 times the MDL recoveries between 80-125% will be permitted.

14.0 CORRECTIVE ACTION

14.1 If the recovery for the standard reference material is unacceptable, the analysis must be terminated, the problem corrected and the samples reanalyzed.

14.2 If the precision falls outside of the acceptable range, the analysis must be terminated, the problem corrected and the previous samples reanalyzed.

14.3 If duplicate results are out side of the acceptance range, all pertinent data for samples associated with that duplicate sample will be flagged.

14.4 If the percent recovery for matrix spike is unacceptable, there might be interference due to the matrix. Dilute the sample to lower the interference and analyze the sample again. If it doesn't work, use standard addition.

14.5 If the blank value exceeds 3 times the MDL, the analysis must be terminated, the problem corrected and the previous samples associated with the blank reanalyzed.

14.6 If the correlation coefficient is <0.995 the analysis must be terminated and the standards reanalyzed. If the problem occurs after the second reanalysis, the standards will be remade and analyzed again.

15.0 REFERENCE

15.1 EPA Method 245.5, 1974. Mercury in Sediment (Manual Cold Vapor Technique), AA.

15.2 Evans, S.J., Johnson, M.S., Leah, R.T. 1986. Determination of mercury in Fish Tissue, a Rapid, Automated Technique for Routine Analysis. Varian Publication Number AA-60.

15.3 Perkin Elmer, Publication B3118.20. FIMS Flow Injection Mercury System. Setting Up and Performing Analyses by Atomic Spectroscopy.

15.4 Nguyen, L. 2003. Percent Moisture Analyzed for 24 Hours and 48 Hours. Department of Fish and Game, Water Pollution Control Lab.

Analyst: _____ Date:_____

Reviewed by: _____ Date:_____

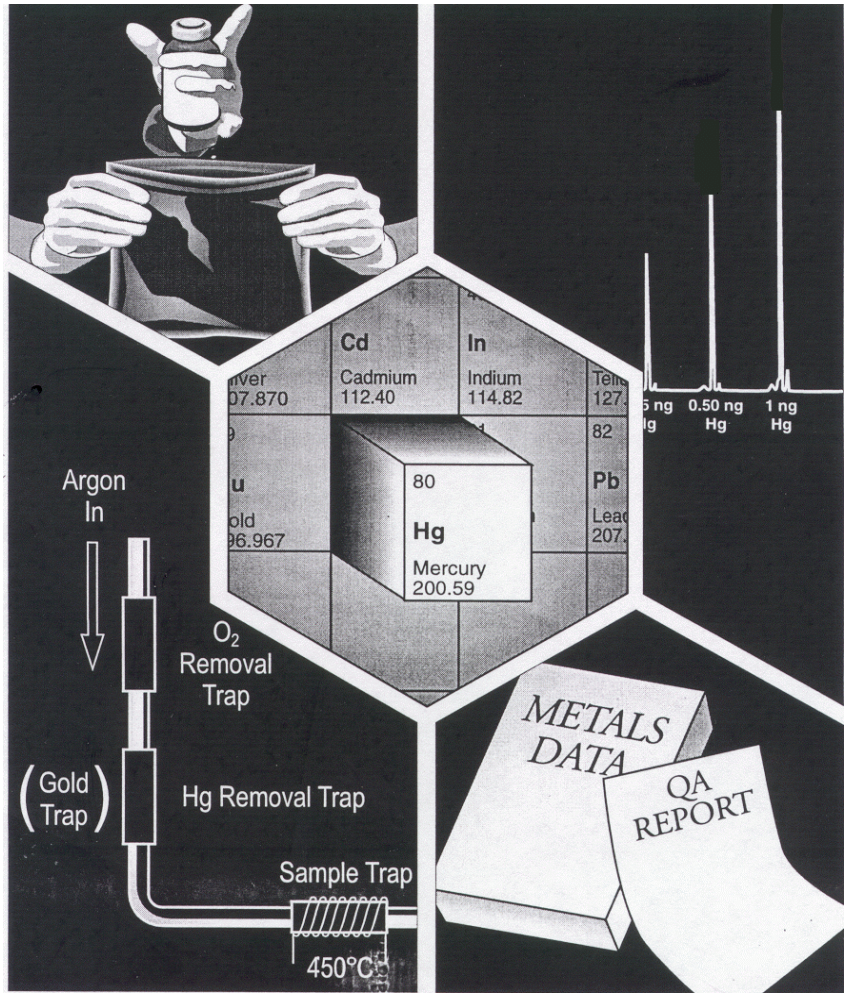
Lab Director: _____ Date:_____

QA Officer: _____ Date:_____

United States
Environmental Protection
Agency

Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry

August 2002



United States Environmental Protection Agency
Office of Water 4303
EPA-821-R-02-019
August 2002

Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry

Acknowledgments

This Method was developed under the direction of William A. Telliard and Maria Gomez-Taylor of the Engineering and Analysis Division (EAD) within the U.S. Environmental Protection Agency's (EPA's) Office of Science and Technology (OST). EPA acknowledges contributions to this method by Frontier Geosciences, Inc., Albion Environmental, Battelle Marine Sciences Laboratory, STL-Canton, and Tekran Inc. Additional assistance in preparing the Method was provided by DynCorp Environmental and Interface, Inc.

Disclaimer

This Method has been reviewed and approved for publication by the Statistics and Analytical Support Branch within EPA's Engineering and Analysis Division. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Questions concerning this Method or its application should be addressed to:

W.A. Telliard
Statistics and Analytical Support Branch (4303T)
U.S. Environmental Protection Agency
Ariel Rios Building
1200 Pennsylvania Avenue, N.W.
Washington, DC 20460
Phone: 202/566–1061
Fax: 202/566–1053

Table of Contents

Introduction . . . . . *iii*

1.0 Scope and Application 1

2.0 Summary of Method 2

3.0 Definitions 3

4.0 Contamination and Interferences 3

5.0 Safety 6

6.0 Apparatus and Materials 8

7.0 Reagents and Standards 11

8.0 Sample Collection, Preservation, and Storage 13

9.0 Quality Control 14

10.0 Calibration and Standardization 22

11.0 Procedure 25

12.0 Data Analysis and Calculations 27

13.0 Method Performance 29

14.0 Pollution Prevention 29

15.0 Waste Management 30

16.0 References 30

17.0 Glossary 31

18.0 Tables and Figures 34

Introduction

Method 1631 (the "Method") supports technology-based and water quality-based monitoring programs authorized under the Clean Water Act (CWA; the "Act").

CWA Sections 301 and 306 require EPA to publish effluent standards that restrict the direct discharge of pollutants to the nations waters, and CWA Sections 307(b) and (c) require EPA to promulgate nationally applicable pretreatement standards which restrict pollutant discharges into sewers flowing to publicly owned treatment works (POTWs). The effluent limitations guidelines are published at CFR parts 401-503.

CWA Section 303 requires each State to set a water quality standard for each body of water within its boundaries. A State water quality standard consists of a designated use or uses of a water body or a segment of a water body, the water quality criteria that are necessary to protect the designated use or uses, and an antidegradation policy. CWA Section 304(a) requires EPA to publish water quality criteria that reflect the latest scientific knowledge concerning the physical fate of pollutants, the effects of pollutants on ecological and human health, and the effect of pollutants on biological community diversity, productivity, and stability. These water quality standards serve two purposes: (1) they establish the water quality goals for a specific water body, and (2) they are the basis for establishing water quality-based treatment controls and strategies beyond the technology-based controls required by CWA Sections 301(b) and 306.

In 1987, amendments to the CWA required States to adopt numeric criteria for toxic pollutants (designated in Section 307(a) of the Act) based on EPA Section 304(a) criteria or other scientific data, when the discharge or presence of those toxic pollutants could reasonably be expected to interfere with designated uses. Method 1631 was specifically developed to provide reliable measurements of mercury at EPA WQC levels.

In developing methods for determination of trace metals, EPA found that one of the greatest difficulties was precluding sample contamination during collection, transport, and analysis. The degree of difficulty, however, is highly dependent on the metal and site-specific conditions. Method 1631 is designed to preclude contamination in nearly all situations. It also contains procedures necessary to produce reliable results at the lowest WQC levels published by EPA. In recognition of the variety of situations to which this Method may be applied, and in recognition of continuing technological advances, Method 1631 is performance based. Alternative procedures may be used so long as those procedures are demonstrated to yield reliable results.

Requests for additional copies of this draft Method should be directed to:

U.S. EPA Sample Control Center
6101 Stevenson Avenue
Alexandria, VA 22304-3540
703/461–2100

Note: This Method is performance based. The laboratory is permitted to omit steps or modify procedures provided that all performance requirements in this Method are met. The laboratory must not omit or modify any procedure defined by the term “shall” or “must” and must perform all quality control tests.

Method 1631, Revision E

Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry

1.0 Scope and Application

1.1 Method 1631, Revision E (the "Method") is for determination of mercury (Hg) in filtered and unfiltered water by oxidation, purge and trap, desorption, and cold-vapor atomic fluorescence spectrometry (CVAFS). This Method is for use in EPA's data gathering and monitoring programs associated with the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act. The Method is based on a contractor-developed procedure (Reference 16.1) and on peer-reviewed, published procedures for the determination of mercury in aqueous samples, ranging from sea water to sewage effluent (References 16.2–16.5).

1.2 This Method is accompanied by Method 1669: *Sampling Ambient Water for Determination of Trace Metals at EPA Water Quality Criteria Levels* (Sampling Method). The Sampling Method guidance document is recommended to preclude contamination during the sampling process.

1.3 This Method is for determination of Hg in the range of 0.5–100 ng/L. Application may be extended to higher levels by selection of a smaller sample size or by calibration of the analytical system across a higher range. For measurement of blank samples, the Method may be extended to a lower level by calibration to a lower calibration point. Section 10.4 gives requirements for extension of the calibration range.

1.4 The ease of contaminating ambient water samples with mercury and interfering substances cannot be overemphasized. This Method includes suggestions for improvements in facilities and analytical techniques that should minimize contamination and maximize the ability of the laboratory to make reliable trace metals determinations. Certain sections of this Method contain suggestions and other sections contain requirements to minimize contamination.

1.5 The detection limit and minimum level of quantitation in this Method usually are dependent on the level of interferences rather than instrument limitations. The method detection limit (MDL; 40 CFR 136, Appendix B) for Hg has been determined to be 0.2 ng/L when no interferences are present. The minimum level of quantitation (ML) has been established as 0.5 ng/L. An MDL as low as 0.05 ng/L can be achieved for low Hg samples by using a larger sample volume, a lower BrCl level (0.2%), and extra caution in sample handling.

1.6 Clean and ultraclean—The terms "clean" and "ultraclean" have been applied to the techniques needed to reduce or eliminate contamination in trace metals determinations. These terms are not used in this Method because they lack an exact definition. However, the information provided in this Method is consistent with the summary guidance on clean and ultraclean techniques (References 16.6-16.7).

1.7 This Method follows the EPA Environmental Methods Management Council's "Guidelines and Format for Methods to Be Proposed at 40 CFR, part 136 or part 141."

1.8 This Method is "performance based." The laboratory is permitted to modify the Method to overcome interferences or lower the cost of measurements if all performance criteria are met. Section 9.1.2.1 gives the requirements for establishing method equivalency.

1.9 Any modification of this Method, beyond those expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.10 This Method should be used only by analysts experienced in the use of CVAFS techniques and who are trained thoroughly in the sample handling and instrument techniques described in this Method. Each laboratory that uses this Method must demonstrate the ability to generate acceptable results using the procedures in Section 9.2.

1.11 This Method is accompanied by a data verification and validation guidance document, *Guidance on the Documentation and Evaluation of Trace Metals Data Collected for CWA Compliance Monitoring* (Reference 16.8), that can be used for verification and validation of the data obtained.

1.12 This Method uses either a bubbler or flow-injection system for determination of mercury in water. Separate calibration, analysis, and calculation procedures are provided for a bubbler system (Sections 10.2, 11.2.1, and 12.2) and for a flow-injection system (Sections 10.3, 11.2.2, and 12.3).

2.0 Summary of Method

2.1 A 100- to 2000-mL sample is collected directly into a cleaned, pretested, fluoropolymer or glass bottle using sample handling techniques designed for collection of mercury at trace levels (Reference 16.9).

2.2 For dissolved Hg, the sample is filtered through a 0.45-µm capsule filter prior to preservation.

2.3 The sample is preserved by adding either pretested 12N hydrochloric acid (HCl) or bromine monochloride (BrCl) solution. If a sample will also be used for the determination of methyl mercury, it should be preserved according to procedures in the method that will be used for determination of methylmercury.

2.4 Prior to analysis, all Hg in a 100-mL sample aliquot is oxidized to Hg(II) with BrCl.

2.5 After oxidation, the sample is sequentially reduced with $NH_2OH{\cdot}HCl$ to destroy the free halogens, then reduced with stannous chloride ($SnCl_2$) to convert Hg(II) to volatile Hg(0).

2.6 The Hg(0) is separated from solution either by purging with nitrogen, helium, or argon, or by vapor/liquid separation. The Hg(0) is collected onto a gold trap (Figures 1, 2, and 3).

2.7 The Hg is thermally desorbed from the gold trap into an inert gas stream that carries the released Hg(0) to a second gold (analytical) trap. The Hg is desorbed from the analytical trap into a gas stream that carries the Hg into the cell of a cold-vapor atomic fluorescence spectrometer (CVAFS) for detection (Figures 2 and 3).

2.8 Quality is assured through calibration and testing of the oxidation, purging, and detection systems.

3.0 Definitions

3.1 Total mercury—all BrCl-oxidizable mercury forms and species found in an unfiltered aqueous solution. This includes, but is not limited to, Hg(II), Hg(0), strongly organo-complexed Hg(II) compounds, adsorbed particulate Hg, and several tested covalently bound organo-mercurials (e.g., CH_3HgCl, $(CH_3)_2Hg$, and $C_6H_5HgOOCCH_3$). The recovery of Hg bound within microbial cells may require the additional step of UV photo-oxidation. In this Method, total mercury and total recoverable mercury are synonymous.

3.2 Dissolved mercury—all BrCl-oxidizable mercury forms and species found in the filtrate of an aqueous solution that has been filtered through a 0.45-µm filter.

3.3 Apparatus—Throughout this Method, the sample containers, sampling devices, instrumentation, and all other materials and devices used in sample collection, sample processing, and sample analysis that come in contact with the sample and therefore require careful cleaning will be referred to collectively as the Apparatus.

3.4 Definitions of other terms used in this Method are given in the glossary (Section 17.0).

4.0 Contamination and Interferences

4.1 Preventing samples from becoming contaminated during the sampling and analysis process constitutes one of the greatest difficulties encountered in trace metals determinations. Over the last two decades, marine chemists have come to recognize that much of the historical data on the concentrations of dissolved trace metals in seawater are erroneously high because the concentrations reflect contamination from sampling and analysis rather than ambient levels. Therefore, it is imperative that extreme care be taken to avoid contamination when collecting and analyzing samples for trace metals.

4.2 Samples may become contaminated by numerous routes. Potential sources of trace metals contamination during sampling include: metallic or metal-containing labware (e.g., talc gloves that contain high levels of zinc), containers, sampling equipment, reagents, and reagent water; improperly cleaned or stored equipment, labware, and reagents; and atmospheric inputs such as dirt and dust. Even human contact can be a source of trace metals contamination. For example, it has been demonstrated that dental work (e.g., mercury amalgam fillings) in the mouths of laboratory personnel can contaminate samples directly exposed to exhalation (Reference 16.9).

4.3 Contamination Control

4.3.1 Philosophy—The philosophy behind contamination control is to ensure that any object or substance that contacts the sample is metal free and free from any material that may contain mercury.

4.3.1.1 The integrity of the results produced cannot be compromised by contamination of samples. This Method and the Sampling Method give requirements and suggestions for control of sample contamination.

4.3.1.2 Substances in a sample cannot be allowed to contaminate the laboratory work area or instrumentation used for trace metals measurements. This Method gives requirements and suggestions for protecting the laboratory.

4.3.1.3 Although contamination control is essential, personnel health and safety remain the highest priority. The Sampling Method and Section 5 of this Method give suggestions and requirements for personnel safety.

4.3.2 Avoiding contamination—The best way to control contamination is to completely avoid exposure of the sample to contamination in the first place. Avoiding exposure means performing operations in an area known to be free from contamination. Two of the most important factors in avoiding/reducing sample contamination are (1) an awareness of potential sources of contamination and (2) strict attention to work being done. Therefore, it is imperative that the procedures described in this Method be carried out by well-trained, experienced personnel.

4.3.3 Use a clean environment—The ideal environment for processing samples is a class-100 clean room. If a clean room is not available, all sample preparation should be performed in a class-100 clean bench or a nonmetal glove box fed by mercury-and particle-free air or nitrogen. Digestion should be performed in a nonmetal fume hood equipped with HEPA filtration and ideally situated in a clean room.

4.3.4 Minimize exposure—The Apparatus that will contact samples, blanks, or standard solutions should be opened or exposed only in a clean room, clean bench, or glove box so that exposure to an uncontrolled atmosphere is minimized. When not being used, the Apparatus should be covered with clean plastic wrap, stored in the clean bench or in a plastic box or glove box, or bagged in clean zip-type bags. Minimizing the time between cleaning and use will also minimize contamination.

4.3.5 Clean work surfaces—Before a given batch of samples is processed, all work surfaces in the hood, clean bench, or glove box in which the samples will be processed should be cleaned by wiping with a lint-free cloth or wipe soaked with reagent water.

4.3.6 Wear gloves—Sampling personnel must wear clean, non-talc gloves during all operations involving handling of the Apparatus, samples, and blanks. Only clean gloves may touch the Apparatus. If another object or substance is touched, the glove(s) must be changed before again handling the Apparatus. If it is even suspected that gloves have become contaminated, work must be halted, the contaminated gloves removed, and a new pair of clean gloves put on. Wearing multiple layers of clean gloves will allow the old pair to be quickly stripped with minimal disruption to the work activity.

4.3.7 Use metal-free Apparatus—All Apparatus used for determination of mercury at ambient water quality criteria levels must be nonmetallic, free of material that may contain metals, or both.

4.3.7.1 Construction materials—Only fluoropolymer or glass containers must be used for collection of samples that will be analyzed for mercury because mercury vapors can diffuse in or out of other materials, leading to results that are biased low or high. Polyethylene and/or polypropylene labware may be used for digestion and other purposes because the time of sample exposure to these materials is relatively short. All materials, regardless of construction, that will directly or

indirectly contact the sample, must be known to be clean and free of Hg at the levels specified in this Method before proceeding.

4.3.7.2 Serialization—It is recommended that serial numbers be indelibly marked or etched on each piece of reusable Apparatus so that contamination can be traced, and logbooks should be maintained to track the sample from the container through the labware to introduction into the instrument. It may be useful to dedicate separate sets of labware to different sample types; e.g., receiving waters vs. effluents. However, the Apparatus used for processing blanks and standards must be mixed with the Apparatus used to process samples so that contamination of all labware can be detected.

4.3.7.3 The laboratory or cleaning facility is responsible for cleaning the Apparatus used by the sampling team. If there are any indications that the Apparatus is not clean when received by the sampling team (e.g., ripped storage bags), an assessment of the likelihood of contamination must be made. Sampling must not proceed if it is possible that the Apparatus is contaminated. If the Apparatus is contaminated, it must be returned to the laboratory or cleaning facility for proper cleaning before any sampling activity resumes.

4.3.8 Avoid sources of contamination—Avoid contamination by being aware of potential sources and routes of contamination.

4.3.8.1 Contamination by carryover—Contamination may occur when a sample containing a low concentration of mercury is processed immediately after a sample containing a relatively high concentration of mercury. The Hg concentration at which the analytical system (purge, traps, detector) will carry greater than 0.5 ng/L of Hg into a succeeding bubbler or system blank must be determined by analyzing calibration solutions containing successively larger concentrations of Hg. This test must be run prior to first use of the analytical system and whenever a change is made that would increase the amount of carryover. When a sample contains ½ or greater of this determined Hg concentration, a bubbler blank (bubbler system) or system blank (flow injection system) must be analyzed to demonstrate no carryover at the blank criteria level. For the bubbler system, the blank must be run using the same bubbler and sample trap used to run the high concentration sample. Samples analyzed following a sample that has been determined to result in carryover must be reanalyzed. Samples that are known or suspected to contain the lowest concentration of mercury should be analyzed first followed by samples containing higher levels.

4.3.8.2 Contamination by samples—Significant laboratory or instrument contamination may result when untreated effluents, in-process waters, landfill leachates, and other undiluted samples containing concentrations of mercury greater than 100 ng/L are processed and analyzed. Samples known or suspected to contain Hg concentrations greater than 100 ng/L should be diluted prior to bringing them into the clean room or laboratory dedicated for processing trace metals samples.

4.3.8.3 Contamination by indirect contact—Apparatus that may not directly come in contact with the samples may still be a source of contamination. For example, clean tubing placed in a dirty plastic bag may pick up contamination from the bag and subsequently transfer the contamination to the sample. It is imperative that every piece of the Apparatus that is directly or indirectly used in the collection, processing, and analysis of water samples be thoroughly cleaned (Section 6.1.2).

4.3.8.4 Contamination by airborne particulate matter—Less obvious substances capable of contaminating samples include airborne particles. Samples may be contaminated by airborne dust, dirt, particles, or vapors from unfiltered air supplies; nearby corroded or rusted pipes, wires, or other fixtures; or metal-containing paint. Whenever possible, sample processing and analysis should occur as far as possible from sources of airborne contamination.

4.3.8.5 Contamination from reagents— Contamination can be introduced into samples from method reagents used during processing and analysis. Reagent blanks must be analyzed for contamination prior to use (see Section 9.4.3). If reagent blanks are contaminated, a new batch of reagents must be prepared (see Section 9.4.3.2).

4.4 Interferences

4.4.1 At the time of promulgation of this Method, gold and iodide were known interferences. At a mercury concentration of 2.5 ng/L and at increasing iodide concentrations from 30 to 100 mg/L, test data have shown that mercury recovery will be reduced from 100 to 0 percent. At iodide concentrations greater than 3 mg/L, the sample should be pre-reduced with $SnCl_2$ (to remove the brown color) and additional or more concentrated $SnCl_2$ should be added. To preclude loss of Hg, the additional $SnCl_2$ should be added in a closed vessel or analysis should proceed immediately. If samples containing iodide concentrations greater than 30 mg/L are analyzed, it may be necessary to clean the analytical system with 4N HCl after the analysis (Reference 16.10).

4.4.2 The potential exists for destruction of the gold traps if free halogens are purged onto them, or if they are overheated (>500 °C). When the instructions in this Method are followed, neither of these outcomes is likely.

4.4.3 Water vapor may collect in the gold traps and subsequently condense in the fluorescence cell upon desorption, giving a false peak due to scattering of the excitation radiation. Condensation can be avoided by predrying the gold trap. Traps that tend to absorb large quantities of water vapor should not be used.

4.4.4 The fluorescent intensity is strongly dependent upon the presence of molecular species in the carrier gas that can cause "quenching" of the excited atoms. The dual amalgamation technique eliminates quenching due to trace gases, but it remains the laboratory's responsibility to ensure high purity inert carrier gas and a leak-free analytical train.

5.0 Safety

5.1 The toxicity or carcinogenicity of each chemical used in this Method has not been precisely determined; however, each compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level.

5.1.1 Chronic mercury exposure may cause kidney damage, muscle tremors, spasms, personality changes, depression, irritability and nervousness. Organo-mercurials may cause permanent brain damage. Because of the toxicological and physical properties of Hg, pure standards should be handled only by highly trained personnel thoroughly familiar with handling and cautionary procedures and the associated risks.

5.1.2 It is recommended that the laboratory purchase a dilute standard solution of the Hg in this Method. If primary solutions are prepared, they shall be prepared in a hood, and a NIOSH/MESA-approved toxic gas respirator shall be worn.

5.2 This Method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a current file of OSHA regulations for safe handling of the chemicals specified in this Method. OSHA rules require that a reference file of material safety data sheets (MSDSs) must be made available to all personnel involved in these analyses (29 CFR 1917.28, Appendix E). It also is suggested that the laboratory perform personal hygiene monitoring of each analyst who uses this Method and that the results of this monitoring be made available to the analyst. Personal hygiene monitoring should be performed using OSHA or NIOSH approved personal hygiene monitoring methods. Additional information on laboratory safety can be found in References 16.11-16.14. The references and bibliography included in Reference 16.14 are particularly comprehensive in dealing with the general subject of laboratory safety.

5.3 Samples suspected to contain concentrations of Hg at µg/L or higher levels are handled using essentially the same techniques employed in handling radioactive or infectious materials. Well-ventilated, controlled access laboratories are required. Assistance in evaluating the health hazards of particular laboratory conditions may be obtained from certain consulting laboratories and from State Departments of Health or Labor, many of which have an industrial health service. Each laboratory must develop a safety program for handling Hg.

5.3.1 Facility—When samples known or suspected of containing high concentrations of mercury are handled, all operations (including removal of samples from sample containers, weighing, transferring, and mixing) should be performed in a glove box demonstrated to be leak-tight or in a fume hood demonstrated to have adequate airflow. Gross losses to the laboratory ventilation system must not be allowed. Handling of the dilute solutions normally used in analytical and animal work presents no inhalation hazards except in an accident.

5.3.2 Protective equipment—Disposable plastic gloves, apron or lab coat, safety glasses or mask, and a glove box or fume hood adequate for radioactive work should be used. During analytical operations that may give rise to aerosols or dusts, personnel should wear respirators equipped with activated carbon filters.

5.3.3 Training—Workers must be trained in the proper method of removing contaminated gloves and clothing without contacting the exterior surfaces.

5.3.4 Personal hygiene—Hands and forearms should be washed thoroughly after each manipulation and before breaks (coffee, lunch, and shift).

5.3.5 Confinement—Isolated work areas posted with signs, segregated glassware and tools, and plastic absorbent paper on bench tops will aid in confining contamination.

5.3.6 Effluent vapors—The effluent from the CVAFS should pass through either a column of activated charcoal or a trap containing gold or sulfur to amalgamate or react mercury vapors.

5.3.7 Waste handling—Good technique includes minimizing contaminated waste. Plastic bag liners should be used in waste cans. Janitors and other personnel must be trained in the safe handling of waste.

5.3.8 Decontamination

5.3.8.1 Decontamination of personnel—Use any mild soap with plenty of scrubbing action.

5.3.8.2 Glassware, tools, and surfaces—Sulfur powder will react with Hg to produce mercuric sulfide, thereby eliminating the possible volatilization of Hg. Satisfactory cleaning may be accomplished by dusting a surface lightly with sulfur powder, then washing with any detergent and water.

5.3.9 Laundry—Clothing known to be contaminated should be collected in plastic bags. Persons that convey the bags and launder the clothing should be advised of the hazard and trained in proper handling. If the launderer knows of the potential problem, the clothing may be put into a washer without contact. The washer should be run through a cycle before being used again for other clothing.

5.3.10 Wipe tests—A useful method of determining cleanliness of work surfaces and tools is to wipe the surface with a piece of filter paper. Extraction and analysis by this Method can achieve a limit of detection of less than 1 ng per wipe. Less than 0.1 µg per wipe indicates acceptable cleanliness; anything higher warrants further cleaning. More than 10 µg on a wipe constitutes an acute hazard and requires prompt cleaning before further use of the equipment or work space, and indicates that unacceptable work practices have been employed.

6.0 Apparatus and Materials

Disclaimer: The mention of trade names or commercial products in this Method is for illustrative purposes only and does not constitute endorsement or recommendation for use by the Environmental Protection Agency. Equivalent performance may be achievable using apparatus, materials, or cleaning procedures other than those suggested here. The laboratory is responsible for demonstrating equivalent performance.

6.1 Sampling equipment

6.1.1 Sample collection bottles-fluoropolymer or glass, 125- to 1000-mL, with fluoropolymer or fluoropolymer-lined cap.

6.1.2 Cleaning

6.1.2.1 New bottles are cleaned by heating to 65–75 °C in 4 N HCl or concentrated HNO_3 for at least 48 h. The bottles are cooled, rinsed three times with reagent water, and filled with reagent water containing 1% HCl. These bottles are capped and placed in a clean oven at 60-70°C overnight. After cooling, they are rinsed three more times with reagent water, filled with reagent water containing 0.4% (v/v) HCl, and placed in a mercury-free Class-100 clean bench until the outside surfaces are dry. The bottles are tightly capped (with a wrench), double-bagged in new polyethylene zip-type bags until needed, and stored in wooden or plastic boxes until use. The bottles may be shipped to the sampling site containing dilute HCl solution (e.g., 0.04%), containing reagent water, or empty.

6.1.2.2 Used bottles known not to have contained mercury at high (>100 ng/L) levels are cleaned as above, except for only 6–12 h in hot 4 N HCl.

6.1.2.3 Bottle blanks must be analyzed as described in Section 9.4.7. To verify the effectiveness of the cleaning procedures, bottle blanks must be demonstrated to be free of mercury at the ML of this Method.

6.1.2.4 As an alternative to cleaning by the laboratory, bottles may be purchased from a commercial supplier and each lot certified to be clean. Bottles from the lot must be tested as bottle blanks (Section 9.4.7) and demonstrated to be free of mercury at the ML of this Method. If mercury is present above this level in any bottle, either the lot must be rejected or the bottles must be re-cleaned.

6.1.3 Filtration Apparatus

6.1.3.1 Filter—0.45-µm, 15-mm diameter capsule filter (Gelman Supor 12175, or equivalent)

6.1.3.2 Peristaltic pump—115-V a.c., 12-V d.c., internal battery, variable-speed, single-head (Cole-Parmer, portable, "Masterflex L/S," Catalog No. 07570-10 drive with Quick Load pump head, Catalog No. 07021-24, or equivalent).

6.1.3.3 Tubing—styrene/ethylene/butylene/silicone (SEBS) resin for use with peristaltic pump, approx 3/8-in ID by approximately 3 ft (Cole-Parmer size 18, Catalog No. 06424-18, or approximately 1/4-in OD, Cole-Parmer size 17, Catalog No. 06424-17, or equivalent). Tubing is cleaned by soaking in 5–10% HCl solution for 8–24 h, rinsing with reagent water in a clean bench in a clean room, and drying in the clean bench by purging with metal-free air or nitrogen. After drying, the tubing is double-bagged in clear polyethylene bags, serialized with a unique number, and stored until use.

6.2 Equipment for bottle and glassware cleaning

6.2.1 Vat, 100–200 L, high-density polyethylene (HDPE), half filled with 4 N HCl in reagent water.

6.2.2 Panel immersion heater, 500-W, all-fluoropolymer coated, 120 vac (Cole-Parmer H-03053-04, or equivalent)

WARNING: *Read instructions carefully!! The heater will maintain steady state, without temperature feedback control, of 60–75°C in a vat of the size described. However, the equilibrium temperature will be higher (up to boiling) in a smaller vat. Also, the heater plate MUST be maintained in a vertical position, completely submerged and away from the vat walls to avoid melting the vat or burning out!*

6.2.3 Laboratory sink—in Class-100 clean area, with high-flow reagent water (Section 7.1) for rinsing.

6.2.4 Clean bench—Class-100, for drying rinsed bottles.

6.2.5 Oven—stainless steel, in Class-100 clean area, capable of maintaining ± 5°C in the 60–70°C temperature range.

6.3 Cold vapor atomic fluorescence spectrometer (CVAFS): The CVAFS system used may either be purchased from a supplier, or built in the laboratory from commercially available components.

6.3.1 Commercially available CVAFS—Tekran (Toronto, ON) Series 2600 CVAFS, Brooks-Rand (Seattle, WA) Model III CVAFS, Leeman Labs Hydra AF Gold*plus* CVAFS, or equivalent

6.3.2 Custom-built CVAFS (Reference 16.15). Figure 2 shows the schematic diagram. The system consists of the following:

6.3.2.1 Low-pressure 4-W mercury vapor lamp

6.3.2.2 Far UV quartz flow-through fluorescence cell—12 mm x 12 mm x 45 mm, with a 10-mm path length (NSG Cells, or equivalent).

6.3.2.3 UV-visible photomultiplier (PMT)—sensitive to < 230 nm. This PMT is isolated from outside light with a 253.7-nm interference filter (Oriel Corp., Stamford, CT, or equivalent).

6.3.2.4 Photometer and PMT power supply (Oriel Corp. or equivalent), to convert PMT output (nanoamp) to millivolts

6.3.2.5 Black anodized aluminum optical block—holds fluorescence cell, PMT, and light source at perpendicular angles, and provides collimation of incident and fluorescent beams (Frontier Geosciences Inc., Seattle, WA, or equivalent).

6.3.2.6 Flowmeter—with needle valve capable of reproducibly keeping the carrier gas flow rate at 30 mL/min

6.4 Hg purging system—Figure 2 shows the schematic diagram for the purging system. The system consists of the following:

6.4.1 Flow meter/needle valve—capable of controlling and measuring gas flow rate to the purge vessel at 350 ± 50 mL/min.

6.4.2 Fluoropolymer fittings—connections between components and columns are made using 6.4-mm OD fluoropolymer tubing and fluoropolymer friction-fit or threaded tubing connectors. Connections between components requiring mobility are made with 3.2-mm OD fluoropolymer tubing because of its greater flexibility.

6.4.3 Acid fume pretrap—10-cm long x 0.9-cm ID fluoropolymer tube containing 2–3 g of reagent grade, nonindicating, 8–14 mesh soda lime chunks, packed between wads of silanized glass wool. This trap is cleaned of Hg by placing on the output of a clean cold vapor generator (bubbler) and purging for 1 h with N_2 at 350 mL/min.

6.4.4 Cold vapor generator (bubbler)—200-mL borosilicate glass (15 cm high x 5.0 cm diameter) with standard taper 24/40 neck, fitted with a sparging stopper having a coarse glass frit that extends to within 0.2 cm of the bubbler bottom (Frontier Geosciences, Inc. or equivalent).

6.5 The dual-trap Hg(0) preconcentrating system

6.5.1 Figures 2 and 3 show the dual-trap amalgamation system (Reference 16.5).

6.5.2 Gold-coated sand traps—10-cm long x 6.5-mm OD x 4-mm ID quartz tubing. The tube is filled with 3.4 cm of gold-coated 45/60 mesh quartz sand (Frontier Geosciences Inc., Seattle, WA, or equivalent). The ends are plugged with quartz wool.

6.5.2.1 Traps are fitted with 6.5-mm ID fluoropolymer friction-fit sleeves for making connection to the system. When traps are not in use, fluoropolymer end plugs are inserted in trap ends to eliminate contamination.

6.5.2.2 At least six traps are needed for efficient operation, one as the "analytical" trap, and the others to sequentially collect samples.

6.5.3 Heating of gold-coated sand traps—To desorb Hg collected on a trap, heat for 3.0 min to 450–500 °C (a barely visible red glow when the room is darkened) with a coil consisting of 75 cm of 24-gauge Nichrome wire at a potential of 10-14 vac. Potential is applied and finely adjusted with an autotransformer.

6.5.4 Timers—The heating interval is controlled by a timer-activated 120-V outlet (Gralab, or equivalent), into which the heating coil autotransformer is plugged. Two timers are required, one each for the "sample" trap and the "analytical" trap.

6.5.5 Air blowers—After heating, traps are cooled by blowing air from a small squirrel-cage blower positioned immediately above the trap. Two blowers are required, one each for the "sample" trap and the "analytical" trap.

6.6 Recorder—Any multi-range millivolt chart recorder or integrator with a range compatible with the CVAFS is acceptable. By using a two-pen recorder with pen sensitivity offset by a factor of 10, the dynamic range of the system is extended to 10^3.

6.7 Pipettors—All-plastic pneumatic fixed-volume and variable pipettors in the range of 10 µL to 5.0 mL.

6.8 Analytical balance capable of weighing to the nearest 0.01 g

7.0 Reagents and Standards

Note: The quantities of reagents and the preparation procedures in this section are for illustrative purposes. Equivalent performance may be achievable using quantities of reagents and procedures other than those suggested here. The laboratory is responsible for demonstrating equivalent performance.

7.1 Reagent water—18-MΩ minimum, ultrapure deionized water starting from a prepurified (distilled, reverse osmosis, etc.) source. Water should be monitored for Hg, especially after ion exchange beds are changed.

7.2 Air—It is very important that the laboratory air be low in both particulate and gaseous mercury. Ideally, mercury work should be conducted in a new laboratory with mercury-free paint on the walls. A source of air that is very low in Hg should be brought directly into the Class-100 clean bench air intake. If this is not possible, air coming into the clean bench can be cleaned for mercury by placing a gold-coated cloth prefilter over the intake. Gold-coated cloth filter: Soak 2 m^2 of cotton gauze in 500 mL of 2% gold chloride solution at pH 7. In a hood, add 100 mL of 30% $NH_2OH{\cdot}HCl$ solution, and homogenize into the cloth with gloved hands. The material will turn black as colloidal gold is precipitated. Allow the mixture to set for several hours, then rinse

with copious amounts of deionized water. Squeeze-dry the rinsed cloth, and spread flat on newspapers to air-dry. When dry, fold and place over the intake prefilter of the laminar flow hood.

CAUTION: Great care should be taken to avoid contaminating the laboratory with gold dust. This could cause interferences with the analysis if gold becomes incorporated into the samples or equipment. The gilding procedure should be done in a remote laboratory if at all possible.

7.3 Hydrochloric acid—trace-metal purified reagent-grade HCl containing less than 5 pg/mL Hg. The HCl should be analyzed for Hg before use.

7.4 Hydroxylamine hydrochloride—Dissolve 300 g of $NH_2OH{\cdot}HCl$ in reagent water and bring to 1.0 L. This solution may be purified by the addition of 1.0 mL of $SnCl_2$ solution and purging overnight at 500 mL/min with Hg-free N_2. Flow injection systems may require the use of less $SnCl_2$ for purification of this solution.

7.5 Stannous chloride—Bring 200 g of $SnCl_2{\cdot}2H_2O$ and 100 mL concentrated HCl to 1.0 L with reagent water. Purge overnight with mercury-free N_2 at 500 mL/min to remove all traces of Hg. Store tightly capped.

7.6 Bromine monochloride (BrCl)—In a fume hood, dissolve 27 g of reagent grade KBr in 2.5 L of low-Hg HCl. Place a clean magnetic stir bar in the bottle and stir for approximately 1 h in the fume hood. Slowly add 38 g reagent grade $KBrO_3$ to the acid while stirring. When all of the $KBrO_3$ has been added, the solution color should change from yellow to red to orange. Loosely cap the bottle, and allow to stir another hour before tightening the lid.

WARNING: *This process generates copious quantities of free halogens (Cl_2, Br_2, BrCl), which are released from the bottle. Add the $KBrO_3$ slowly in a fume hood!*

7.7 Stock mercury standard—NIST-certified 10,000-ppm aqueous Hg solution (NIST-3133). This solution is stable at least until the NIST expiration date.

7.8 Secondary Hg standard—Add approx 0.5 L of reagent water and 5 mL of BrCl solution (Section 7.6) to a 1.00-L Class A volumetric flask. Add 0.100 mL of the stock mercury standard (Section 7.7) to the flask and dilute to 1.00 L with reagent water. This solution contains 1.00 µg/mL (1.00 ppm) Hg. Transfer the solution to a fluoropolymer bottle and cap tightly. This solution is considered stable until the NIST expiration date.

7.9 Working Hg Standard A—Dilute 1.00 mL of the secondary Hg standard (Section 7.8) to 100 mL in a Class A volumetric flask with reagent water containing 0.5% by volume BrCl solution (Section 7.6). This solution contains 10.0 ng/mL and should be replaced monthly, or longer if extended stability is demonstrated.

7.10 Working Hg Standard B—Dilute 0.10 mL of the secondary Hg standard (Section 7.8) to 1000 mL in a Class A volumetric flask with reagent water containing 0.5% by volume BrCl solution (Section 7.6). This solution contains 0.10 ng/mL and should be replaced monthly, or longer if extended stability is demonstrated.

7.11 Initial Precision and Recovery (IPR) and Ongoing Precision and Recovery (OPR) solutions—Using the working Hg standard A (Section 7.9), prepare IPR and OPR solutions at a

concentration of 5 ng/L Hg in reagent water. IPR/OPR solutions are prepared using the same amounts of reagents used for preparation of the calibration standards.

7.12 Nitrogen—Grade 4.5 (standard laboratory grade) nitrogen that has been further purified by the removal of Hg using a gold-coated sand trap.

7.13 Argon—Grade 5.0 (ultra high-purity, GC grade) argon that has been further purified by the removal of Hg using a gold-coated sand trap.

8.0 Sample Collection, Preservation, and Storage

8.1 Before samples are collected, consideration should be given to the type of data required (i.e., dissolved or total), so that appropriate preservation and pretreatment steps can be taken. An excess of BrCl should be confirmed either visually (presence of a yellow color) or with starch iodide indicating paper, using a separate sample aliquot, prior to sample processing or direct analysis to ensure the sample has been properly preserved.

8.2 Samples are collected into rigorously cleaned fluoropolymer bottles with fluoropolymer or fluoropolymer-lined caps. Glass bottles may be used if Hg is the only target analyte. It is critical that the bottles have tightly sealing caps to avoid diffusion of atmospheric Hg through the threads (Reference 16.4). Polyethylene sample bottles must not be used (Reference 16.15).

8.3 Collect samples using guidance provided in the Sampling Method (Reference 16.9). Procedures in the Sampling Method are based on rigorous protocols for collection of samples for mercury (References 16.4 and 16.15).

NOTE: *Discrete samplers have been found to contaminate samples with Hg at the ng/L level. Therefore, great care should be exercised if this type of sampler is used. It may be necessary for the sampling team to use other means of sample collection if samples are found to be contaminated using the discrete sampler.*

8.4 Sample filtration—For dissolved Hg, a sample is filtered through a 0.45-µm capsule filter (Section 6.1.3.1) in a mercury-free clean area prior to preservation. If the sample is filtered, it must be accompanied by a blank that has been filtered under the same conditions. The Sampling Method describes sample filtration procedures.

8.5 Preservation—Samples are preserved by adding either 5 mL/L of pretested 12N HCl or 5 mL/L BrCl solution to the sample bottle. If a sample will be used also for the determination of methyl mercury, it should be collected and preserved according to procedures in the method that will be used for determination of methyl mercury (e.g., HCl or H_2SO_4 solution). Preserved samples are stable for up to 90 days of the date of collection.

8.5.1 Samples to be analyzed for total or dissolved Hg only may be shipped to the laboratory unpreserved and unrefrigerated if they are collected in fluoropolymer or glass bottles and capped tightly. Samples must be either preserved or analyzed within 48 hours of collection. If a sample is oxidized in the sample bottle, the time to preservation can be extended to 28 days.

8.5.2 Samples that are acid-preserved may lose Hg to coagulated organic materials in the water or condensed on the walls (Reference 16.16). The best approach is to add BrCl directly to the sample bottle at least 24 hours before analysis. If other Hg species are to be analyzed, these aliquots must be removed prior to the addition of BrCl. If BrCl

cannot be added directly to the sample bottle, the bottle must be shaken vigorously prior to sub-sampling.

8.5.3 Handling of the samples in the laboratory should be undertaken in a mercury-free clean bench, after rinsing the outside of the bottles with reagent water and drying in the clean air hood.

NOTE: *Because of the potential for contamination, it is recommended that filtration and preservation of samples be performed in the clean room in the laboratory. However, if circumstances prevent overnight shipment of samples, samples should be filtered and preserved in a designated clean area in the field in accordance with the procedures given in Method 1669 (Reference 16.9). If filtered in the field, samples ideally should be filtered into the sample bottle.*

8.6 Storage—Sample bottles should be stored in clean (new) polyethylene bags until sample analysis.

8.7 Sample preservation, storage, and holding time requirements also are given at 40 CFR part 136.3(e) Table II.

9.0 Quality Control

9.1 Each laboratory that uses this Method is required to operate a formal quality assurance program (Reference 16.17). The minimum requirements of this program consist of an initial demonstration of laboratory capability, ongoing analysis of standards and blanks as a test of continued performance, and the analysis of matrix spikes (MS) and matrix spike duplicates (MSD) to assess precision and recovery. Laboratory performance is compared to established performance criteria to determine that the results of analyses meet the performance characteristics of the Method.

9.1.1 The laboratory shall make an initial demonstration of the ability to generate acceptable accuracy and precision. This ability is established as described in Section 9.2.

9.1.2 In recognition of advances that are occurring in analytical technology, the laboratory is permitted certain options to improve results or lower the cost of measurements. These options include automation of the dual-amalgamation system, single-trap amalgamation (Reference 16.18), direct electronic data acquisition, calibration using gas-phase elemental Hg standards, use of the bubbler or flow-injection systems, or changes in the detector (i.e., CVAAS) when less sensitivity is acceptable or desired. Changes in the determinative technique, such as the use of colorimetry, are not allowed. If an analytical technique other than the CVAFS technique specified in this Method is used, that technique must have a specificity for mercury equal to or better than the specificity of the technique in this Method.

9.1.2.1 Each time this Method is modified, the laboratory is required to repeat the procedure in Section 9.2 to demonstrate that an MDL (40 CFR part 136, Appendix B) less than or equal to one-third the regulatory compliance limit or less than or equal to the MDL of this Method (Table 1), whichever is greater, can be achieved. If the change will affect calibration, the instrument must be recalibrated according to Section 10.

Note: If the compliance limit is greater than the concentration of Hg in the OPR/OPR (5 ng/L), the acceptance criteria for blanks and the concentrations of mercury spiked into quality control samples may be increased to support measurements at the compliance limit. For example, if the compliance limit is 12

ng/L (National Toxics Rule, 40 CFR 131.36), the MDL must be less than or equal to 4 ng/L; concentrations of the calibration standards may be 5, 10, 20, 50 , and 100 ng/L; concentrations of the IPR/OPR samples may be 10 ng/L; spike concentrations and acceptance criteria for MS/MSD samples would remain as specified in Section 9.3; and an appropriate blank acceptance criterion would be 5 ng/L.

9.1.2.2 The laboratory is required to maintain records of modifications made to this Method. These records include the following, at a minimum:

9.1.2.2.1 The names, titles, addresses, and telephone numbers of the analyst(s) who performed the analyses and modification, and the quality control officer who witnessed and will verify the analyses and modification

9.1.2.2.2 A narrative stating the reason(s) for the modification(s)

9.1.2.2.3 Results from all quality control (QC) tests demonstrating the performance of the modified method, including the following:

(a) Calibration (Section 10)
(b) Initial precision and recovery (Section 9.2.2)
(c) Analysis of blanks (Section 9.4)
(d) Matrix spike/matrix spike duplicate analysis (Section 9.3)
(e) Ongoing precision and recovery (Section 9.5)
(f) Quality control sample (Section 9.6)
(g) Method detection limit (Section 9.2.1)

9.1.2.2.4 Data that will allow an independent reviewer to validate each determination by tracking the instrument output to the final result. These data are to include the following:

(a) Sample numbers and other identifiers
(b) Processing dates
(c) Analysis dates
(d) Analysis sequence/run chronology
(e) Sample weight or volume
(f) Copies of logbooks, chart recorder, or other raw data output
(g) Calculations linking raw data to the results reported

9.1.3 Analyses of MS and MSD samples are required to demonstrate the accuracy and precision and to monitor matrix interferences. Section 9.3 describes the procedure and QC criteria for spiking.

9.1.4 Analyses of blanks are required to demonstrate acceptable levels of contamination. Section 9.4 describes the procedures and criteria for analyzing blanks.

9.1.5 The laboratory shall, on an ongoing basis, demonstrate through analysis of the ongoing precision and recovery (OPR) sample and the quality control sample (QCS) that the system is in control. Sections 9.5 and 9.6 describe these procedures, respectively.

9.1.6 The laboratory shall maintain records to define the quality of the data that are generated. Sections 9.3.7 and 9.5.3 describe the development of accuracy statements.

9.1.7 Quality of the analyses is controlled by an analytical batch. An analytical batch is a set of samples oxidized with the same batch of reagents, and analyzed during the same 12-hour shift. A batch may be from 1 to as many as 20 samples. Each batch must be accompanied by 3 system blanks (Section 9.4.2 for the flow-injection system), a

minimum of 3 bubbler blanks (Section 9.4.1 for the bubbler system), 1 OPR sample at the beginning and end of the batch (Section 9.5), a QCS (Section 9.6), and at least 3 method blanks (Section 9.4.4). In addition, there must be 1 MS and 1 MSD sample for every 10 samples (a frequency of 10%). A typical analytical sequence would be:

(a) Three system blanks (Section 9.4.2) or a minimum of 3 bubbler blanks (Section 9.4.1)
(b) A minimum of five, non-zero calibration standards (Section 10.2.2.1)
(c) On-going precision and recovery (Section 9.5)
(d) Quality control sample (Section 9.6)
(e) Method blank (Section 9.4.4)
(f) Seven samples
(g) Method blank (Section 9.4.4)
(h) Three samples
(i) Matrix spike (Section 9.3)
(j) Matrix spike duplicate (Section 9.3)
(k) Four samples
(l) Method blank (Section 9.4.4)
(m) Six samples
(n) Matrix spike (Section 9.3)
(o) Matrix spike duplicate (Section 9.3)
(p) Ongoing precision and recovery (Section 9.5)

The above sequence includes calibration. If system performance is verified at the end of the sequence using the OPR, analysis of samples and blanks may proceed without recalibration (i.e., the analytical sequence would be entered at Step (d) above), unless more than 12 hours has elapsed since verification of system performance. If more than 12 hours has elapsed, the sequence would be initiated at Step (c) above.

9.2 Initial demonstration of laboratory capability

9.2.1 Method detection limit—To establish the ability to detect Hg, the laboratory shall achieve an MDL that is less than or equal to the MDL listed in Section 1.5 or one-third the regulatory compliance limit, whichever is greater. The MDL shall be determined according to the procedure at 40 CFR 136, Appendix B using the apparatus, reagents, and standards that will be used in the practice of this Method. This MDL shall be used for determination of laboratory capability only, and should be determined when a new operator begins work or whenever, in the judgment of the laboratory, a change in instrument hardware or operating conditions would dictate reevaluation of capability.

9.2.2 Initial precision and recovery (IPR)—To establish the ability to generate acceptable precision and recovery, the laboratory shall perform the following operations:

9.2.2.1 Analyze four replicates of the IPR solution (5 ng/L, Section 7.11) according to the procedure beginning in Section 11.

9.2.2.2 Using the results of the set of four analyses, compute the average percent recovery (X), and the standard deviation of the percent recovery (s) for Hg.

9.2.2.3 Compare s and X with the corresponding limits for initial precision and recovery in Table 2. If s and X meet the acceptance criteria, system performance is acceptable and analysis of samples may begin. If, however, s exceeds the

precision limit or X falls outside the acceptance range, system performance is unacceptable. Correct the problem and repeat the test (Section 9.2.2.1).

9.3 Matrix spike (MS) and matrix spike duplicate (MSD)—To assess the performance of the Method on a given sample matrix, the laboratory must spike, in duplicate, a minimum of 10% (1 sample in 10) from a given sampling site or, if for compliance monitoring, from a given discharge. Therefore, an analytical batch of 20 samples would require two pairs of MS/MSD samples (four spiked samples total).

9.3.1 The concentration of the spike in the sample shall be determined as follows:

9.3.1.1 If, as in compliance monitoring, the concentration of Hg in the sample is being checked against a regulatory compliance limit, the spiking level shall be at that limit or at 1–5 times the background concentration of the sample (as determined in Section 9.3.2), whichever is greater.

9.3.1.2 If the concentration of Hg in a sample is not being checked against a limit, the spike shall be at 1–5 times the background concentration or at 1-5 times the ML in Table 1, whichever is greater.

9.3.2 To determine the background concentration (B), analyze one sample aliquot from each set of 10 samples from each site or discharge according to the procedure in Section 11. If the expected background concentration is known from previous experience or other knowledge, the spiking level may be established *a priori*.

9.3.2.1 If necessary, prepare a standard solution to produce an appropriate level in the sample (Section 9.3.1).

9.3.2.2 Spike two additional sample aliquots with identical amounts of the spiking solution and analyze these aliquots as described in Section 11.1.2 to determine the concentration after spiking (A).

9.3.3 Calculate the percent recovery (R) in each aliquot using the following equation:

$$\% R = 100 \frac{(A-B)}{T}$$

where:

A = *Measured concentration of analyte after spiking*
B = *Measured concentration of analyte before spiking*
T = *True concentration of the spike*

9.3.4 Compare percent recovery (R) with the QC acceptance criteria in Table 2.

9.3.4.1 If results of the MS/MSD are similar and fail the acceptance criteria, and recovery for the OPR standard (Section 9.5) for the analytical batch is within the acceptance criteria in Table 2, an interference is present and the results may not be reported or otherwise used for permitting or regulatory compliance purposes. If the interference can be attributed to sampling, the site or discharge should be resampled. If the interference can be attributed to a method deficiency, the laboratory must modify the method, repeat the test required in Section 9.1.2, and repeat analysis of the sample and MS/MSD. However, during the development

of Method 1631, very few interferences have been noted in the determination of Hg using this Method. (See Section 4.4 for information on interferences.)

9.3.4.2 If the results of both the spike and the OPR test fall outside the acceptance criteria, the analytical system is judged to be not in control, and the results may not be reported or used for permitting or regulatory compliance purposes. The laboratory must identify and correct the problem and reanalyze all samples in the sample batch.

9.3.5 Relative percent difference (RPD)—Compute the RPD between the MS and MSD results according to the following equation using the concentrations found in the MS and MSD. Do not use the recoveries calculated in Section 9.3.3 for this calculation because the RPD is inflated when the background concentration is near the spike concentration.

$$RPD = 200 \times \frac{(|D1-D2|)}{(D1+D2)}$$

Where:
D1 = concentration of Hg in the MS sample
D2 = concentration of Hg in the MSD sample

9.3.6 The RPD for the MS/MSD pair must not exceed the acceptance criterion in Table 2. If the criterion is not met, the system is judged to be out of control. The problem must be identified and corrected, and the MS/MSD and corresponding samples reanalyzed.

9.3.7 As part of the QC program for the laboratory, method precision and recovery for samples should be assessed and records maintained. After analyzing five samples in which the recovery passes the test in Section 9.3.4, compute the average percent recovery (R_a) and the standard deviation of the percent recovery (s_r). Express the accuracy assessment as a percent recovery interval from R_a - $2s_r$ to R_a + $2s_r$. For example, if R_a = 90% and s_r = 10% for five analyses, the accuracy interval is expressed as 70–110%. Update the accuracy assessment regularly (e.g., after every five to ten new accuracy measurements).

9.4 Blanks—Blanks are critical to the reliable determination of Hg at low levels. The sections below give the minimum requirements for analysis of blanks. Analysis of additional blanks is recommended as necessary to pinpoint sources of contamination in, and external to, the laboratory.

9.4.1 Bubbler blanks—Bubbler blanks are analyzed to demonstrate that bubbler systems are free from contamination at levels that could affect data quality. At least three bubbler blanks must be run during calibration and with each analytical batch.

9.4.1.1 To analyze a bubbler blank, place a clean gold trap on the bubbler. Purge and analyze previously purged water using the procedure in Section 11, and determine the amount of Hg remaining in the system.

9.4.1.2 If the bubbler blank is found to contain more than 50 pg Hg, the system is out of control. The problem must be investigated and remedied, and the samples run on that bubbler must be reanalyzed. If the blanks from other bubblers contain less than 50 pg Hg, the data associated with those bubblers remain valid, provided that all other criteria in Section 9 also are met.

9.4.1.3 The mean result for all bubbler blanks (from bubblers passing the specification in Section 9.4.1.2) must be < 25 pg (0.25 ng/L) Hg with a standard deviation (n-1) of <10 pg (0.10 ng/L). If the mean is < 25 pg, the average peak area or height is subtracted from all raw data before results are calculated (Section 12.2).

9.4.1.4 If Hg in the bubbler blank exceeds the acceptance criteria in Section 9.4.1.3, the system is out of control. The problem must be resolved and the system recalibrated. Usually, the bubbler blank is too high for one of the following reasons:

(a) Bubblers need rigorous cleaning;
(b) Soda-lime is contaminated; or
(c) Carrier gas is contaminated.

9.4.2 System blanks— System blanks are analyzed to demonstrate that flow injection systems are free from contamination at levels that could affect data quality. Three system blanks must be run during calibration and with each analytical batch.

9.4.2.1 To analyze a system blank, analyze reagent water containing the same amount of reagents used to prepare the calibration standards.

9.4.2.2 If a system blank is found to contain ≥ 0.50 ng/L Hg, the system is out of control. The problem must be investigated and remedied, and the system recalibrated. If the blanks contain < 0.50 ng/L Hg, the data associated with the blanks remain valid, provided that all other criteria in Section 9 also are met.

9.4.2.3 The mean result for the three system blanks must be <0.5 ng/L Hg with a standard deviation (n-1) <0.1 ng/L. If the mean exceeds these criteria, the system is out of control, and the problem must be resolved and the system recalibrated. If the mean is <0.5 ng/L, the average peak height or area is subtracted from all raw data before results are calculated (Section 12.3).

9.4.3 Reagent blanks—Reagent blanks are used to demonstrate that the reagents used to prepare samples for Hg analyses are free from contamination. The Hg concentration in reagent blanks is determined by analyzing the reagent solutions using either the bubbler or flow-injection system. For the bubbler system, reagent may be added directly to previously purged water in the bubbler.

9.4.3.1 Reagent blanks are required when the batch of reagents (bromine monochloride plus hydroxylamine hydrochloride) are prepared. The amount of Hg in a reagent blank containing 0.5% (v/v) BrCl solution (Section 7.6) and 0.2% (v/v) hydroxylamine hydrochloride solution (Section 7.4) must be < 20 pg (0.2 ng/L).

9.4.3.2 The presence of more than 20 pg (0.2 ng/L) of Hg indicates a problem with the reagent solution. The purging of certain reagent solutions, such as $SnCl_2$ or NH_2OH, with mercury-free nitrogen or argon can reduce Hg to acceptable levels. Because BrCl cannot be purified, a new batch must be prepared and tested if the BrCl is contaminated.

9.4.4 Method blanks— Method blanks are used to demonstrate that the analytical system is free from contamination that could otherwise compromise sample results. Method blanks are prepared and analyzed using sample containers, labware, reagents, and analytical procedures identical to those used to prepare and analyze the samples.

9.4.4.1 A minimum of three method blanks per analytical batch are required for both the bubbler and flow-injection systems.

9.4.4.2 If the result for any method blank containing the nominal amount of reagent used to prepare a sample (Section 11.1.1) is found to contain ≥ 0.50 ng/L (50 pg) Hg, the system is out of control. Mercury in the analytical system must be reduced until a method blank is free from contamination at the 0.50 ng/L level. Samples associated with a contaminated method blank must be reanalyzed.

9.4.4.3 Because method blanks are analyzed using procedures identical to those used to analyze samples, any sample requiring an increased amount of reagent must be accompanied by at least one method blank that includes an identical amount of reagent.

9.4.5 Field blanks–Field blanks are used to demonstrate that samples have not been contaminated by the sample collection and transport activities.

9.4.5.1 Analyze the field blank(s) shipped with each set of samples (samples collected from the same site at the same time, to a maximum of 10 samples). Analyze the blank immediately before analyzing the samples in the batch.

9.4.5.2 If Hg or any potentially interfering substance is found in the field blank at a concentration equal to or greater than the ML (Table 1), or greater than one-fifth the level in the associated sample, whichever is greater, results for associated samples may be the result of contamination and may not be reported or otherwise used for regulatory compliance purposes.

9.4.5.3 Alternatively, if sufficient multiple field blanks (a minimum of three) are collected, and the average concentration (of the multiple field blanks) plus two standard deviations is equal to or greater than the regulatory compliance limit or equal to or greater than one-half of the level in the associated sample, results for associated samples may be the result of contamination and may not be reported or otherwise used for regulatory compliance purposes.

9.4.5.4 If contamination of the field blanks and associated samples is known or suspected, the laboratory should communicate this to the sampling team so that the source of contamination can be identified and corrective measures taken before the next sampling event.

9.4.6 Equipment blanks—Before any sampling equipment is used at a given site, the laboratory or cleaning facility is required to generate equipment blanks on all sampling equipment that will be used to demonstrate that the sampling equipment is free from contamination.

9.4.6.1 Equipment blanks are generated in the laboratory or at the equipment cleaning facility by processing reagent water through the sampling devices using the same procedures that are used in the field (see Sampling Method). Therefore, the "clean hands/dirty hands" technique used during field sampling should be followed when preparing equipment blanks at the laboratory or cleaning facility for low level mercury measurements. If grab samples are to be collected using any ancillary equipment, e.g., an extension pole or a dipper, an equipment blank

is generated by submersing this equipment into the reagent water and analyzing the resulting reagent water collected.

9.4.6.2 The equipment blank must be analyzed using the procedures in this Method. If mercury or any potentially interfering substance is detected in the blank at or above the level specified for the field blank (Section 9.4.5), the source of contamination or interference must be identified, and the problem corrected. The equipment must be demonstrated to be free from mercury and interferences before the equipment may be used in the field.

9.4.7 Bottle blanks— Bottles must be subjected to conditions of use to verify the effectiveness of the cleaning procedures. A representative set of sample bottles (Section 6.1.2) should be filled with reagent water acidified to pH <2 and allowed to stand for a minimum of 24 h. At least 5% of the bottles from a given lot should be tested, and the time that the bottles are allowed to stand should be as close as possible to the actual time that the sample will be in contact with the bottle. After standing, the water must be analyzed for any signs of contamination. If a bottle shows contamination at or above the level specified for the field blank (Section 9.4.5), the problem must be identified, the cleaning procedures corrected or cleaning solutions changed, and all affected bottles re-cleaned.

9.5 Ongoing precision and recovery (OPR)—To demonstrate that the analytical system is within the performance criteria of this Method and that acceptable precision and recovery is being maintained within each analytical batch, the laboratory shall perform the following operations:

9.5.1 Analyze the OPR solution (5 ng/L, Section 7.11) prior to the analysis of each analytical batch according to the procedure beginning in Section 11. An OPR also must be analyzed at the end of an analytical sequence or at the end of each 12-hour shift.

9.5.2 Compare the recovery with the limits for ongoing precision and recovery in Table 2. If the recovery is in the range specified, the analytical system is in control and analysis of samples and blanks may proceed. If, however, the concentration is not in the specified range, the analytical process is not in control. Correct the problem and repeat the ongoing precision and recovery test. All reported results must be associated with an OPR that meets the Table 2 performance criteria at the beginning and end of each batch.

9.5.3 The laboratory should add results that pass the specification in Section 9.5.2 to IPR and previous OPR data and update QC charts to form a graphic representation of continued laboratory performance. The laboratory also should develop a statement of laboratory data quality by calculating the average percent recovery (R_a) and the standard deviation of the percent recovery (s_r). Express the accuracy as a recovery interval from $R_a - 2s_r$ to $R_a + 2s_r$. For example, if $R_a = 95\%$ and $s_r = 5\%$, the accuracy is 85–105%.

9.6 Quality control sample (QCS) – The laboratory must obtain a QCS from a source different from the Hg used to produce the standards used routinely in this Method (Sections 7.7–7.10). The QCS should be analyzed as an independent check of system performance.

9.7 Depending on specific program requirements, the laboratory may be required to analyze field duplicates and field spikes collected to assess the precision and accuracy of the sampling, sample transportation, and storage techniques. The relative percent difference (RPD) between field duplicates should be less than 20%. If the RPD of the field duplicates exceeds 20%, the laboratory should communicate this to the sampling team so that the source of error can be identified and corrective measures taken before the next sampling event.

10.0 Calibration and Standardization

10.1 Calibration and standardization— Separate calibration procedures are provided for a bubbler system (Section 10.2) and flow-injection system (Section 10.3). Both systems are calibrated using standards traceable to NIST Standard Reference Materials. If system performance is verified at the end of an analytical batch using the OPR, analysis of samples and blanks may proceed without recalibration, unless more than 12 hours has elapsed since verification of system performance.

10.2 Bubbler system calibration

10.2.1 Establish the operating conditions necessary to purge Hg from the bubbler and to desorb Hg from the traps in a sharp peak. Further details for operation of the purge-and-trap, desorption, and analysis systems are given in Sections 11.2.1 and 11.2.2.

10.2.2 The calibration must contain a minimum of five non-zero points and the results of analysis of three bubbler blanks. The lowest calibration point must be at the Minimum Level (ML).

NOTE: *The purge efficiency of the bubbler system is 100% and is independent of volume at the volumes used in this Method. Calibration of this system is typically performed using units of mass. For purposes of working in concentration, the volume is assumed to be 100 mL.*

10.2.2.1 Standards are analyzed by the addition of aliquots of Hg working standard A (Section 7.9) and Hg working standard B (Section 7.10) directly into the bubblers. Add 0.50 mL of working standard B and 0.5 mL $SnCl_2$ to the bubbler. Swirl to produce a standard containing 50 pg of Hg (0.5 ng/L). Purge under the optimum operating conditions (Section 10.2.1). Sequentially follow with the addition of aliquots of 0.05, 0.25, 0.50 and 1.0 mL of working standard A to produce standards of 500, 2500, 5000, and 10,000 pg Hg (5.0, 25.0, 50.0 and 100.0 ng/L).

NOTE: *If calibration to the higher levels results in carryover (Section 4.3.8.1), calibrate the system across a narrower range (Section 10.4)*

10.2.2.2 Analyze the standards beginning with the lowest concentration and proceeding to the highest. Tabulate the height or area for each peak.

10.2.2.3 Prepare and analyze a minimum of 3 bubbler blanks. If multiple bubblers are used, there must be 1 bubbler blank per bubbler (to a maximum of 4 bubblers). Calculate the mean peak area or height for the bubbler blanks.

10.2.2.4 For each calibration point, subtract the mean peak height or area of the bubbler blanks from the peak height or area for each standard. Calculate the calibration factor (CF_x) for Hg in each of the five standards using the mean bubbler-blank-subtracted peak height or area and the following equation:

$$CF_x = \frac{(A_x) - (\overline{A}_{BB})}{(C_x)}$$

Where:

A_x = *peak height or area for Hg in standard*
$\overline{A}_{BB}$ = *mean peak height or area for Hg in bubbler blank*
C_x = *mass in standard analyzed (ng)*

10.2.2.5 Calculate the mean calibration factor (CF_m), the standard deviation of the calibration factor (SD; n-1), and the relative standard deviation (RSD) of the calibration factor, where RSD = 100 x SD/CF_m.

10.2.2.6 If RSD ≤ 15%, calculate the recovery for the lowest standard using CF_m. If the RSD ≤ 15% and the recovery of the lowest standard is in the range of 75-125%, the calibration is acceptable and CF_m may be used to calculate the concentration of Hg in samples. If RSD > 15% or if the recovery of the lowest standard is not in the range of 75-125%, recalibrate the analytical system and repeat the test.

10.2.2.7 Calculate the concentration of Hg in the bubbler blanks (Section 10.2.2.1) using CF_m. The bubbler blanks must meet the criteria in Section 9.4.1; otherwise, mercury in the system must be reduced and the calibration repeated until the bubbler blanks meet the criteria.

10.3 Flow-injection system calibration

10.3.1 Establish the operating conditions necessary to purge Hg from the gas-liquid separator and dryer tube and desorb Hg from the traps in a sharp peak. Further details for operating the analytical system are given in Section 11.2.1.

10.3.2 The calibration must contain a minimum of 5 non-zero points and the results of analysis of 3 system blanks. The lowest calibration point must be at the minimum level (ML).

10.3.2.1 Place 25-30 mL of reagent water and 250 µL of concentrated BrCl solution (Section 7.6) in each of 5 calibrated 50-mL autosampler vials. Prepare the 0.5 ng/L calibration standard by adding 250 µL of working standard B (Section 7.10) to the vial. Dilute to the mark with reagent water. Sequentially follow with the addition of aliquots of 25, 125, 250 and 500 µL of working standard A (Section 7.9) to produce standards of 5.0, 25.0, 50.0 and 100.0 ng/L, respectively. Cap the vials and invert once to mix.

10.3.2.2 Immediately prior to analysis, remove the caps and add 125 µL of NH_2OH solution (Section 7.4). Re-cap, invert once to mix, and allow to stand until the yellow color disappears. Remove all caps and place vials into the analysis rack.

10.3.2.3 Analyze the standards beginning with the lowest concentration and proceeding to the highest. Tabulate the height or area for the Hg peak.

10.3.2.4 Prepare and analyze a minimum of 3 system blanks and tabulate the peak heights or areas. Calculate the mean peak area or height for the system blanks.

10.3.2.5 For each calibration point, subtract the mean peak height or area of the system blanks (Section 9.4.2) from the peak height or area for each standard. Calculate

the calibration factor (CF_x) for Hg in each of the five standards using the mean reagent-blank-subtracted peak height or area and the following equation:

$$CF_x = \frac{(A_x) - (\overline{A}_{SB})}{(C_x)}$$

Where:

A_x = *peak height or area for Hg in standard*
$\overline{A}_{SB}$ = *mean peak height or area for Hg in calibration blanks*
C_x = *concentration of standard analyzed (ng/L)*

10.3.2.6 Calculate the mean calibration factor (CF_m), the standard deviation of the calibration factor (SD; n-1), and the relative standard deviation (RSD) of the calibration factor, where RSD = 100 x SD/CF_m.

10.3.2.7 If RSD ≤ 15%, calculate the recovery for the lowest standard (0.5 ng/L) using CF_m. If the RSD ≤ 15% and the recovery of the lowest standard is in the range of 75-125%, the calibration is acceptable and CF_m may be used to calculate the concentration of Hg in samples, blanks, and OPRs. If RSD > 15% or if the recovery of the lowest standard is not in the range of 75-125%, recalibrate the analytical system and repeat the test.

10.3.2.8 Calculate the concentration of Hg in the system blanks (Section 9.4.2) using CF_m. The system blanks must meet the criteria in Section 9.4.2; otherwise, mercury in the system must be reduced and the calibration repeated until the system blanks meet the criteria.

10.4 Calibration to a range other than 0.5 to 100 ng/L—This Method may be calibrated to a range other than 0.5 to 100 ng/L, provided that the following requirements are met:

(a) There must be a minimum of five non-zero calibration points.
(b) The difference between successive calibration points must be no greater than a factor of 10 and no less than a factor of 2 and should be approximately evenly spaced on a logarithmic scale over the calibration range.
(c) The relative standard deviation (RSD) of the calibration factors for all calibration points must be less than 15%.
(d) The calibration factor for any calibration point at a concentration greater than 100 ng/L must be within ±15% of the average calibration factor for the points at or below 100 ng/L.
(e) The calibration factor for any point <0.5 ng/L must be within 25% of the average calibration factor for all points.
(f) If calibration is to a higher range and this Method is used for regulatory compliance, the ML must be less than one-third the regulatory compliance limit

11.0 Procedure

NOTE: *The following procedures for analysis of samples are provided as guidelines. Laboratories may find it necessary to optimize the procedures, such as drying time or potential applied to the Nichrome wires, for the laboratory's specific instrument set-up.*

11.1 Sample Preparation

11.1.1 Pour a 100-mL aliquot from a thoroughly shaken, acidified sample, into a 125-mL fluoropolymer bottle. If BrCl was not added as a preservative (Section 8.5), add the amount of BrCl solution (Section 7.6) given below, cap the bottle, and digest at room temperature for a 12 h minimum.

11.1.1.1 For clear water and filtered samples, add 0.5 mL of BrCl; for brown water and turbid samples, add 1.0 mL of BrCl. If the yellow color disappears because of consumption by organic matter or sulfides, more BrCl should be added until a permanent (12-h) yellow color is obtained.

11.1.1.2 Some highly organic matrices, such as sewage effluent, will require high levels of BrCl (e.g., 5 mL/100 mL of sample) and longer oxidation times, or elevated temperatures (e.g., place sealed bottles in oven at 50 °C for 6 h). The oxidation must be continued until it is complete. Complete oxidation can be determined by either observation of a permanent yellow color remaining in the sample or the use of starch iodide indicating paper to test for residual free oxidizer. The sample also may be diluted to reduce the amount of BrCl required, provided that the resulting level of mercury is sufficient for reliable determination.

11.1.2 Matrix spikes and matrix spike duplicates—For every 10 or fewer samples, pour 2 additional 100-mL aliquots from a selected sample (see Section 9.3), spike at the level specified in Section 9.3, and process in the same manner as the samples. There must be a minimum of 2 MS/MSD pairs for each analytical batch of 20 samples.

11.2 Hg reduction and purging—Separate procedures are provided for the bubbler system (Section 11.2.1) and flow-injection (Section 11.2.2).

11.2.1 Hg reduction and purging for the bubbler system

11.2.1.1 Add 0.2-0.25 mL of NH_2OH solution to the BrCl-oxidized sample in the 125-mL sample bottle. Cap the bottle and swirl the sample. The yellow color will disappear, indicating the destruction of the BrCl. Allow the sample to react for 5 min with periodic swirling to be sure that no traces of halogens remain.

NOTE: *Purging of free halogens onto the gold trap will result in damage to the trap and low or irreproducible results.*

11.2.1.2 Connect a fresh trap to the bubbler, pour the reduced sample into the bubbler, add 0.5 mL of $SnCl_2$ solution, and purge the sample onto a gold trap with N_2 at 350 ± 50 mL/min for 20 min.

11.2.1.3 When analyzing Hg samples, the recovery is quantitative, and organic interferents are destroyed. Thus, standards, bubbler blanks, and small amounts of high-level samples may be run directly in previously purged water. After very high samples (Section 4.3.8.1), a small degree of carryover (<0.01%) may occur. Bubblers that contain such samples must be demonstrated to be clean prior to proceeding with low level samples. Samples run immediately following a sample that has been determined to result in carryover must be reanalyzed using a bubbler that is demonstrated to be clean as per Section 4.3.8.1.

11.2.2 Hg reduction and purging for the flow-injection system

11.2.2.1 Add 0.2-0.25 mL of NH_2OH solution (Section 7.4) to the BrCl-oxidized sample in the 125-mL sample bottle or in the autosampler tube (the amount of NH_2OH required will be approximately 30 percent of the BrCl volume). Cap the bottle and swirl the sample. The yellow color will disappear, indicating the destruction of the BrCl. Allow the sample to react for 5 minutes with periodic swirling to be sure that no traces of halogens remain.

NOTE: *Purging of free halogens onto the gold trap will result in damage to the trap and low or irreproducible results.*

11.2.2.2 Pour the sample solution into an autosampler vial and place the vial in the rack.

11.2.2.3 Carryover may occur after analysis of a sample containing a high level of mercury. Samples run immediately following a sample that has been determined to result in carryover (Section 4.3.8.1) must be reanalyzed using a system demonstrated to be clean as per Section 4.3.8.1.

11.3 Desorption of Hg from the gold trap

11.3.1 Remove the sample trap from the bubbler, place the Nichrome wire coil around the trap and connect the trap into the analyzer train between the incoming Hg-free argon and the second gold-coated (analytical) sand trap (Figure 2).

11.3.2 Pass argon through the sample and analytical traps at a flow rate of approximately 30 mL/min for approximately 2 min to drive off condensed water vapor.

11.3.3 Apply power to the coil around the sample trap for 3 minutes to thermally desorb the Hg (as Hg(0)) from the sample trap onto the analytical trap.

11.3.4 After the 3-min desorption time, turn off the power to the Nichrome coil, and cool the sample trap using the cooling fan.

11.3.5 Turn on the chart recorder or other data acquisition device to start data collection, and apply power to the Nichrome wire coil around the analytical trap. Heat the analytical trap for 3 min (1 min beyond the point at which the peak returns to baseline).

11.3.6 Stop data collection, turn off the power to the Nichrome coil, and cool the analytical trap to room temperature using the cooling fan.

11.3.7 Place the next sample trap in line and proceed with analysis of the next sample.

NOTE: *Do not heat a sample trap while the analytical trap is still warm; otherwise, the analyte may be lost by passing through the analytical trap.*

11.4 Peaks generated using this technique should be very sharp and almost symmetrical. Mercury elutes at approximately 1 minute and has a width at half-height of about 5 seconds.

11.4.1 Broad or asymmetrical peaks indicate a problem with the desorption train, such as improper gas flow rate, water vapor on the trap(s), or an analytical trap damaged by chemical fumes or overheating.

11.4.2 Damage to an analytical trap is also indicated by a sharp peak, followed by a small, broad peak.

11.4.3 If the analytical trap has been damaged, the trap and the fluoropolymer tubing downstream from it should be discarded because of the possibility of gold migration onto downstream surfaces.

11.4.4 Gold-coated sand traps should be tracked by unique identifiers so that any trap producing poor results can be quickly recognized and discarded.

12.0 Data Analysis and Calculations

12.1 Separate procedures are provided for calculation of sample results using the bubbler system (Section 12.2) and the flow-injection system (Section 12.3), and for method blanks (Section 12.4).

12.2 Calculations for the bubbler system

12.2.1 Calculate the mean peak height or area for Hg in the bubbler blanks measured during system calibration or with the analytical batch (A_{BB}; n = 3 minimum).

12.2.2 Calculate the concentration of Hg in ng/L (parts-per-trillion; ppt) in each sample according to the following equation:

$$[Hg]\ (ng/L) = \frac{A_s - \overline{A}_{BB}}{CF_m \times V}$$

where:
A_s = *peak height (or area) for Hg in sample*
$\overline{A}_{BB}$ = *peak height (or area) for Hg in bubbler blank*
CF_m = *mean calibration factor (Section* 10.2.2.5)
V = *Volume of sample (L)*

12.3 Calculations for the flow-injection system

12.3.1 Calculate the mean peak height or area for Hg in the system blanks measured during system calibration or with each analytical batch (A_{SB}; n = 3)

12.3.2 Calculate the concentration of Hg in ng/L in each sample according to the following equation:

$$[Hg]\ (ng/L) = \frac{(A_s - \overline{A}_{SB})}{CF_m} \times \frac{V_{std}}{V_{sample}}$$

where:
A_s = *peak height (or area) for Hg in sample*
$\overline{A}_{SB}$ = *mean peak height (or area) for Hg in system blanks*
CF_m = *mean calibration factor (Section* 10.3.2.6)
V_{std} = *volume (mL) used for standards – volume (mL) reagent used in standards*
V_{sample} = *volume (mL) of sample – volume (mL) reagent used in sample*

12.4 Calculations for concentration of Hg in method blanks, field blanks, and reagent blanks.

12.4.1 Calculate the concentration of Hg in the method blanks (C_{MB}), field blanks (C_{FB}), or reagent blanks (C_{RB}) in ng/L, using the equation in Section 12.2.2 (if bubbler system is used) or Section 12.3.2 (if flow injection system is used) and substituting the peak height or area resulting from the method blank, field blank, or reagent blank for A_s.

12.4.2 Determine the mean concentration of Hg in the method blanks associated with the analytical batch (a minimum of three). If a sample requires additional reagent(s) (e.g., BrCl), a corresponding method blank containing an identical amount of reagent must be analyzed (Section 9.4.4.3). The concentration of Hg in the corresponding method blank may be subtracted from the concentration of Hg in the sample per Section 12.5.2.

12.5 Reporting

12.5.1 Report results for Hg at or above the ML, in ng/L, to three significant figures. Report results for Hg in samples below the ML as <0.5 ng/L, or as required by the regulatory authority or in the permit. Report results for Hg in reagent blanks and field blanks at or above the ML, in ng/L, to three significant figures. Report results for Hg in reagent blanks, method blanks, or field blanks below the ML but at or above the MDL to two significant figures. Report results for Hg not detected in reagent blanks, method blanks, or field blanks as <0.2 ng/L, or as required by the regulatory authority or in the permit.

12.5.2 Report results for Hg in samples, method blanks and field blanks separately. In addition to reporting results for the samples and blank(s) separately, the concentration of Hg in the method blanks or field blanks associated with the sample may be subtracted from the results for that sample, or must be subtracted if requested or required by a regulatory authority or in a permit.

12.5.3 Results from tests performed with an analytical system that is not in control must not be reported or otherwise used for permitting or regulatory compliance purposes, but do not relieve a discharger or permittee of reporting timely results.

13.0 Method Performance

13.1 This Method was tested in 12 laboratories using reagent water, freshwater, marine water and effluent (Reference 16.19). The quality control acceptance criteria listed in Table 2 were verified by data gathered in the interlaboratory study, and the method detection limit (MDL) given in Section 1.5 was verified in all 12 laboratories. In addition, the techniques in this Method have been compared with other techniques for low-level mercury determination in water in a variety of studies, including ICES-5 (Reference 16.20) and the International Mercury Speciation Intercomparison Exercise (Reference 16.21).

13.2 Precision and recovery data for reagent water, freshwater, marine water, and secondary effluent are given in Table 3.

14.0 Pollution Prevention

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Many opportunities for pollution prevention exist in laboratory operation. EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address waste generation. When it is not feasible to reduce wastes at the source, the Agency recommends recycling as the next best option. The acids used in this Method should be reused as practicable by purifying by electrochemical techniques. The only other chemicals used in this Method are the neat materials used in preparing standards. These standards are used in extremely small amounts and pose little threat to the environment when managed properly. Standards should be prepared in volumes consistent with laboratory use to minimize the disposal of excess volumes of expired standards.

14.2 For information about pollution prevention that may be applied to laboratories and research institutions, consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's Department of Governmental Relations and Science Policy, 1155 16th Street NW, Washington DC 20036, 202/872–4477.

15.0 Waste Management

15.1 The laboratory is responsible for complying with all Federal, State, and local regulations governing waste management, particularly hazardous waste identification rules and land disposal restrictions, and for protecting the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required. An overview of requirements can be found in *Environmental Management Guide for Small Laboratories* (EPA 233-B-98-001).

15.2 Acids, samples at pH <2, and BrCl solutions must be neutralized before being disposed of, or must be handled as hazardous waste.

15.3 For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* and *Less is Better: Laboratory Chemical Management for Waste Reduction*, both available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington, DC 20036.

16.0 References

16.1 Bloom, Nicolas, Draft “Total Mercury in Aqueous Media,” Frontier Geosciences, Inc., September 7, 1994.

16.2 Fitzgerald, W.F.; Gill, G.A. "Sub-Nanogram Determination of Mercury by Two-Stage Gold Amalgamation and Gas Phase Detection Applied to Atmospheric Analysis," *Anal. Chem.* 1979, *15*, 1714.

16.3 Bloom, N.S; Crecelius, E.A. "Determination of Mercury in Sea water at Subnanogram per Liter Levels," *Mar. Chem.* 1983, *14*, 49.

16.4 Gill, G.A.; Fitzgerald, W.F. "Mercury Sampling of Open Ocean Waters at the Picogram Level," *Deep Sea Res* 1985, *32*, 287.

16.5 Bloom, N.S.; Fitzgerald, W.F. "Determination of Volatile Mercury Species at the Picogram Level by Low-Temperature Gas Chromatography with Cold-Vapor Atomic Fluorescence Detection," *Anal. Chim. Acta.* 1988, *208*, 151.

16.6 Guidance on Establishing Trace Metal Clean Rooms in Existing Facilities, U.S. EPA, Office of Water, Office of Science and Technology, Engineering and Analysis Division (4303), 401 M Street SW, Washington, DC 20460, January 1996, EPA 821-B-96-001.

16.7 Trace Metal Cleanroom, prepared by Research Triangle Institute for U.S. Environmental Protection Agency, 26 W. Martin Luther King Dr., Cincinnati, OH 45268, RTI/6302/04-02 F.

16.8 Guidance on the Documentation and Evaluation of Trace Metals Data Collected for Clean Water Act Compliance Monitoring, U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, Engineering and Analysis Division (4303), 401 M Street SW, Washington, DC 20460, July 1996, EPA 821-B-96-004.

16.9 Method 1669, "Method for Sampling Ambient Water for Determination of Metals at EPA Ambient Criteria Levels," U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, Engineering and Analysis Division (4303), 401 M Street SW, Washington, DC 20460, April 1995 with January 1996 revisions.

16.10 Correspondence from Nicolas Bloom, Frontier Geosciences, Inc. to Dale Rushneck, Interface, Inc., December 31, 1998.

16.11 "Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service. Centers for Disease Control. NIOSH Publication 77-206, Aug. 1977, NTIS PB-277256.

16.12 "OSHA Safety and Health Standards, General Industry," OSHA 2206, 29 CFR 1910.

16.13 "Safety in Academic Chemistry Laboratories," ACS Committee on Chemical Safety, 1979.

16.14 "Standard Methods for the Examination of Water and Wastewater," 18th ed. and later revisions, American Public Health Association, 1015 15th Street NW, Washington, DC 20005. 1-35: Section 1090 (Safety), 1992.

16.15 Bloom, N.S. "Trace Metals & Ultra-Clean Sample Handling," *Environ. Lab.* 1995, *7*, 20.

16.16 Bloom, N.S. "Influence of Analytical Conditions on the Observed 'Reactive Mercury,' Concentrations in Natural Fresh Waters," In *Mercury as a Global Pollutant*; Huckabee, J. and Watras, C.J., Eds.; Lewis Publishers, Ann Arbor, MI: 1994.

16.17 "Handbook of Analytical Quality Control in Water and Wastewater Laboratories," U.S. Environmental Protection Agency. Environmental Monitoring Systems Laboratory, Cincinnati, OH 45268, EPA-600/4-79-019, March 1979.

16.18 Liang, L.; Bloom, N.S. "Determination of Total Mercury by Single-Stage Gold Amalgamation with Cold Vapor Atom Spectrometric Detection," *J. Anal. Atomic Spectrom.* 1993, *8*, 591.

16.19 "Results of the EPA Method 1631 Validation Study," February, 1998. Available from the EPA Sample Control Center, 6101 Stevenson Avenue, Alexandria, VA, 22304; 703/461-2100.

16.20 Cossa, D.; Couran, P. "An International Intercomparison Exercise for Total Mercury in Sea Water," *App. Organomet. Chem.* 1990, *4*, 49.

16.21 Bloom, N.S.; Horvat, M.; Watras, C.J. "Results of the International Mercury Speciation Intercomparison Exercise," *Wat. Air. Soil Pollut.*, 1995, 80, 1257.

17.0 Glossary

The definitions and purposes below are specific to this Method, but have been conformed to common usage as much as possible.

17.1 **Ambient Water**—Waters in the natural environment (e.g., rivers, lakes, streams, and other receiving waters), as opposed to effluent discharges.

17.2 **Analytical Batch**—A batch of up to 20 samples that are oxidized with the same batch of reagents and analyzed during the same 12-hour shift. Each analytical batch must also include at least three bubbler blanks, an OPR, and a QCS. In addition, MS/MSD samples must be prepared at a frequency of 10% per analytical batch (one MS/MSD for every 10 samples).

17.3 **Bottle Blank**—The bottle blank is used to demonstrate that the bottle is free from contamination prior to use. Reagent water known to be free of mercury at the MDL of this Method is added to a bottle, acidified to pH <2 with BrCl or HCl, and allowed to stand for a minimum of 24 hours. The time that the bottle is allowed to stand should be as close as possible to the actual time that the sample will be in contact with the bottle. After standing, the water is analyzed.

17.4 **Bubbler Blank**—For this Method, the bubbler blank is specific to the bubbler system and is used to determine that the analytical system is free from contamination. After analysis of a standard, blank, or sample, the solution in the bubbler is purged and analyzed. A minimum of three bubbler blanks is required for system calibration.

17.5 **Equipment Blank**—Reagent water that has been processed through the sampling device at a laboratory or other equipment cleaning facility prior to shipment of the sampling equipment to the sampling site. The equipment blank is used to demonstrate that the sampling equipment is free from contamination prior to use. Where appropriate, the "clean hands/dirty hands" technique used during field sampling should be followed when preparing equipment blanks at the laboratory or cleaning facility.

17.6 **Field Blank**—Reagent water that has been transported to the sampling site and exposed to the same equipment and operations as a sample at the sampling site. The field blank is used to demonstrate that the sample has not been contaminated by the sampling and sample transport systems.

17.7 **Intercomparison Study**—An exercise in which samples are prepared and split by a reference laboratory, then analyzed by one or more testing laboratories and the reference laboratory. The intercomparison, with a reputable laboratory as the reference laboratory, serves as the best test of the precision and accuracy of the analyses at natural environmental levels.

17.8 **Matrix Spike (MS) and Matrix Spike Duplicate (MSD)**—Aliquots of an environmental sample to which known quantities of the analyte(s) of interest is added in the laboratory. The MS and MSD are analyzed exactly like a sample. Their purpose is to quantify the bias and precision caused by the sample matrix. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the MS and MSD corrected for these background concentrations.

17.9 **May**—This action, activity, or procedural step is allowed but not required.

17.10 **May not**—This action, activity, or procedural step is prohibited.

17.11 **Method blank**— Method blanks are used to determine the concentration of mercury in the analytical system during sample preparation and analysis, and consist of a volume of reagent water that is carried through the entire sample preparation and analysis. Method blanks are prepared by placing reagent water in a sample bottle and analyzing the water using reagents and procedures identical to those used to prepare and analyze the corresponding samples. A minimum of three method blanks is required with each analytical batch.

17.12 **Minimum Level (ML)**—The lowest level at which the entire analytical system must give a recognizable signal and acceptable calibration point for the analyte. It is equivalent to the concentration of the lowest calibration standard, assuming that all method-specified sample weights, volumes, and cleanup procedures have been employed. The ML is calculated by multiplying the MDL by 3.18 and rounding the result to the number nearest to (1, 2, or 5) x 10^n, where n is an integer (See Section 1.5).

17.13 **Must**—This action, activity, or procedural step is required.

17.14 **Quality Control Sample (QCS)**—A sample containing Hg at known concentrations. The QCS is obtained from a source external to the laboratory, or is prepared from a source of standards different from the source of calibration standards. It is used as an independent check of instrument calibration.

17.15 **Reagent blank**—Reagent blanks are used to determine the concentration of mercury in the reagents (BrCl, $NH_2OH{\cdot}HCl$, and $SnCl_2$) that are used to prepare and analyze the samples. In this Method, reagent blanks are required when each new batch of reagents is prepared.

17.16 **Reagent Water**—Water demonstrated to be free of mercury at the MDL of this Method. It is prepared from 18 MΩ ultrapure deionized water starting from a prepurified source. Reagent water is used to wash bottles, as trip and field blanks, and in the preparation of standards and reagents.

17.17 **Regulatory Compliance Limit**—A limit on the concentration or amount of a pollutant or contaminant specified in a nationwide standard, in a permit, or otherwise established by a regulatory authority.

17.18 **Shall**—This action, activity, or procedure is required.

17.19 **Should**—This action, activity, or procedure is suggested, but not required.

17.20 **Stock Solution**— A solution containing an analyte that is prepared from a reference material traceable to NIST, or a source that will attest to the purity and authenticity of the reference material.

17.21 **System Blank**— For this Method, the system blank is specific for the flow-injection system and is used to determine contamination in the analytical system and in the reagents used to prepare the calibration standards. A minimum of three system blanks is required during system calibration.

17.22 **Ultraclean Handling**— A series of established procedures designed to ensure that samples are not contaminated during sample collection, storage, or analysis.

18.0 Tables and Figures

Table 1

Lowest Ambient Water Quality Criterion for Mercury and the Method Detection Limit and Minimum Level of Quantitation for EPA Method 1631

Metal	Lowest Ambient Water Quality Criterion[1]	Method Detection Limit (MDL) and Minimum Level (ML)	
		MDL[2]	ML[3]
Mercury (Hg)	1.3 ng/L	0.2 ng/L	0.5 ng/L

1. Lowest water quality criterion for the Great Lakes System (Table 4, 40 CFR 132.6). The lowest Nationwide criterion is 12 ng/L (40 CFR 131.36).
2. Method detection limit (40 CFR 136, Appendix B)
3. Minimum level of quantitation (see Glossary)

Table 2

Quality Control Acceptance Criteria for Performance Tests in EPA Method 1631

Acceptance Criteria	Section	Limit (%)
Initial Precision and Recovery (IPR)	9.2.2	
Precision (RSD)	9.2.2.3	21
Recovery (X)	9.2.2.3	79-121
Ongoing Precision and Recovery (OPR)	9.5.2	77-123
Matrix Spike/Matrix Spike Duplicate (MS/MSD)	9.3	
Recovery	9.3.4	71-125
Relative Percent Difference (RPD)	9.3.5	24

Table 3

Precision and Recovery for Reagent Water, Fresh Water, Marine Water, and Effluent Water Using Method 1631

Matrix	*Mean Recovery (%)	*Precision (% RSD)
Reagent Water	98.0	5.6
Fresh Water (Filtered)	90.4	8.3
Marine Water (Filtered)	92.3	4.7
Marine Water (Unfiltered)	88.9	5.0
Secondary Effluent (Filtered)	90.7	3.0
Secondary Effluent (Unfiltered)	92.8	4.5

*Mean percent recoveries and RSDs are based on expected Hg concentrations.

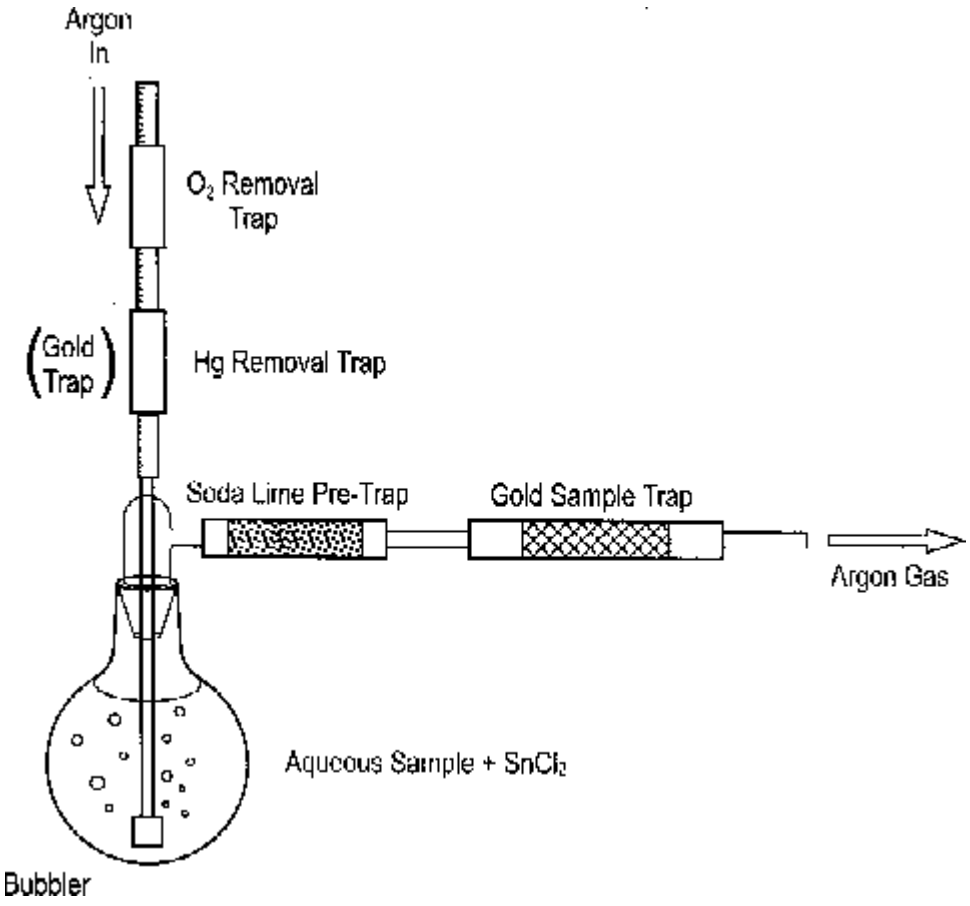


Figure 1. Schematic Diagram of Bubbler Setup

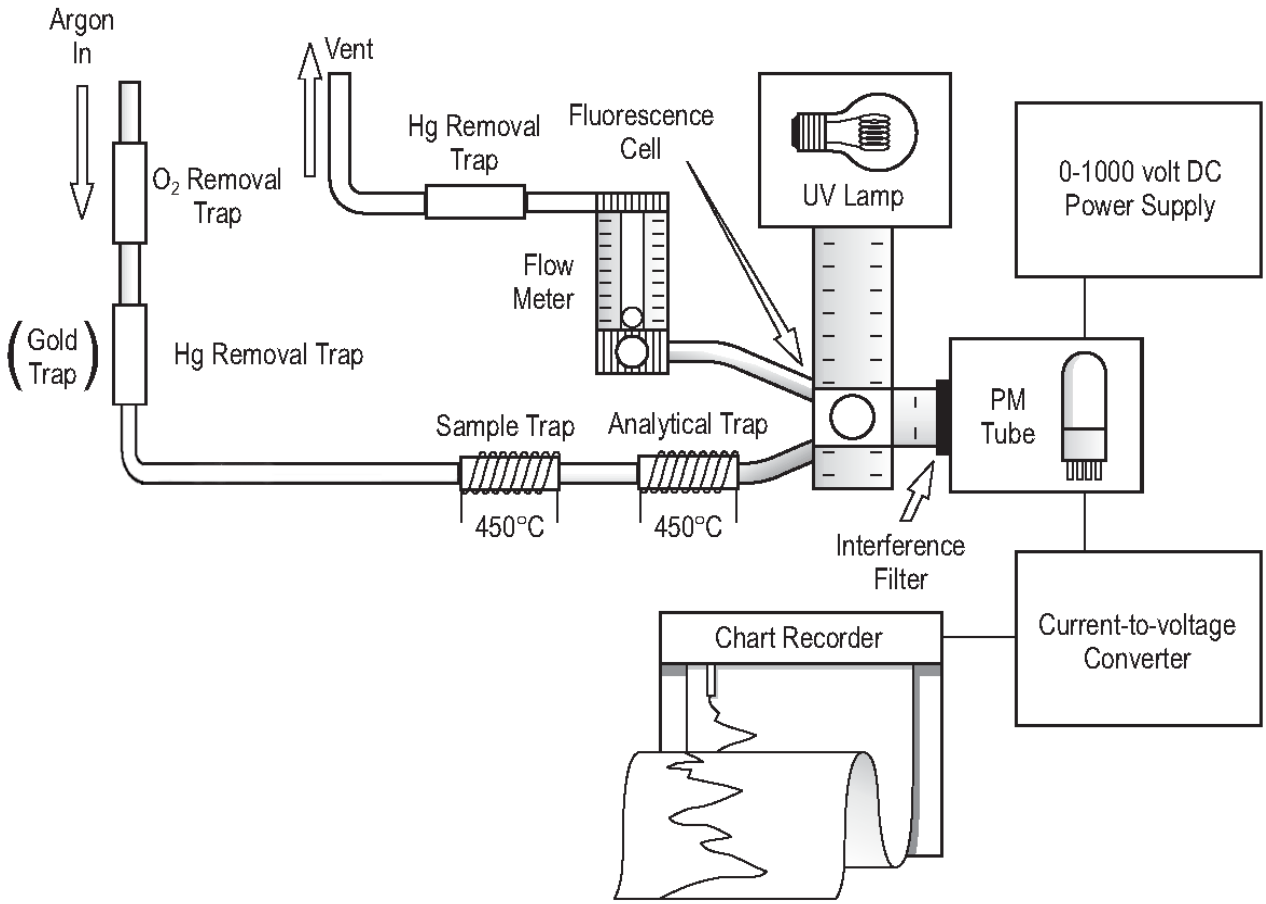


Figure 2. Schematic Diagram of the Bubbler, Purge and Trap, Cold Vapor Atomic Fluorescence Spectrometer (CVAFS) System

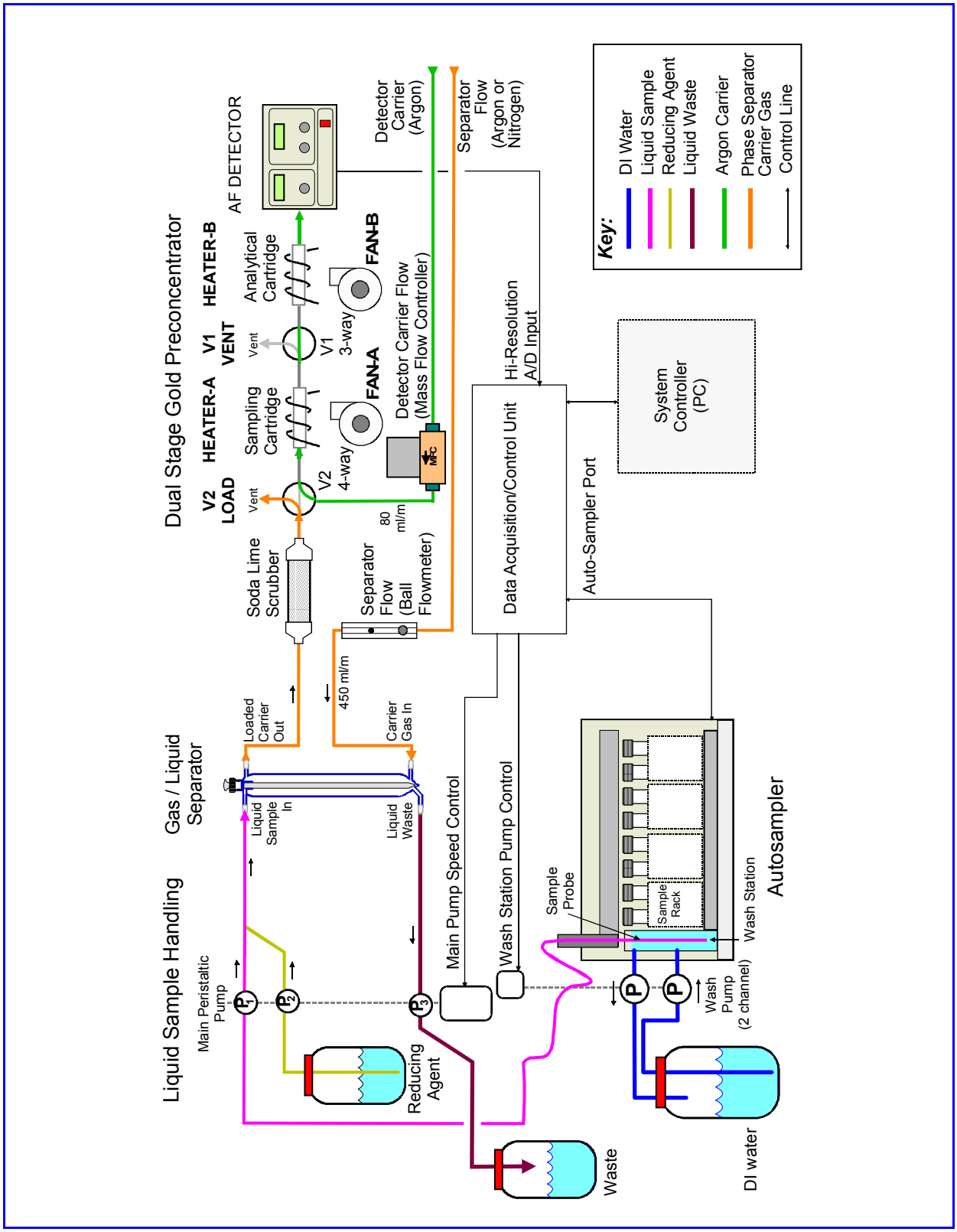


Figure 3. Schematic Diagram of the Flow-Injection, Cold Vapor Atomic Fluorescence Spectrometer (CVAFS) System

Modification of EPA Method 1631e

Amy Byington, Analyst
Marine Pollution Studies Lab
7544 Sandholdt Road
Moss Landing CA 95039
831-771-4165

Tamara Voss, Analyst
Marine Pollution Studies Lab
7544 Sandholdt Road
Moss Landing CA 95039
831-771-4167

Mark Stephenson, Director
Marine Pollution Studies Lab
7544 Sandholdt Road
Moss Landing CA 95039
831-771-4177

Sample bottle cleaning methods were modified from that described in EPA 1631e in order to shorten cleaning time and reduce hazards to staff. It was felt that heating concentrated acid for a period of 48 hours would result in unsafe working conditions and would unnecessarily increase the wear and tear on equipment.

It was determined through R&D that bottles cleaned under the following conditions resulted in mercury levels below the detection limit set forth in EPA 1631e (modifications are listed according to section number):

6.1.2.1 New environmentally clean bottles are cleaned by heating to 65-70°C in 7.5N HNO_3 for 8 hours or soaking for 3 days unheated in the same acid. The bottles are rinsed 5 times with reagent water and filled with reagent water containing 0.5% HCl. The bottles are capped tightly and placed on a mercury free clean bench until the outside surfaces are dry. The bottles are double-bagged in new polyethylene zip-type bags until needed, and stored in the original cardboard container.

6.1.2.2 Used sample bottles are not re-cleaned and/or re-used.

8.5 Preservation- samples are preserved by adding 0.5% v/v BrCl to the sample bottle. Preserved samples are stable for up to 90 days of the date of collection. (This is a clarification, rather than a modification.)

Determination of Microcystins and Microcystin Metabolites in Water and Tissue by Enhanced Liquid Chromatography Tandem Mass Spectrometry

1.0 Scope and Application

A liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) method has been developed and thoroughly validated to identify and quantify trace levels of cyanotoxins or microcystins (MC) in water, bivalves and fish tissue with enhanced sensitivity and specificity. The method enables confirmation and quantification of six MCs (MC-LA, LF, LR, LW, RR and YR) with a single chromatographic run. The applied chromatography also allows determination of certain MC metabolites (Desmethyl-LR and -RR). By using LC-ESI-MS/MS in Multiple Reaction Monitoring (MRM) mode, the limit of detection and quantitation for the microcystins studied, were determined to be between 0.2 pg and 1 pg on column (5:1 S/N ratio).

2.0 Summary of Method

An aliquot of water sample is mixed with 10% acidified methanol and extracted using sonication techniques. Each batch of samples (20 or less) contains a blank, laboratory control sample (LCS), matrix spike and duplicate (MS/MSD) and field sample duplicate, when provided (100 mL total). Identification and quantification of MCs is performed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in MRM mode. All quantitation is performed using certified standards, except the demethylated (dm) congeners which are quantified as the parent non-methylated analog since no certified standard is commercially available. All extracts are analyzed using a five level calibration curve and second source standards are obtained when available. The microcystins currently analyzed are MC-RR, -dmRR, -LR, -dmLR, -YR, -LA, -LF, and -LW. Nodularin is used for internal standard. The reporting limit for all microcystins is 1 µg/L (ppb). The average method recovery range for MCs in water is 65-120%.

3.0 Interferences

3.1 Solvents, reagents, glassware, and other sample processing hardware may cause LC artifacts and/or elevated baselines, resulting in the misinterpretation of chromatograms. All materials should be demonstrated to be free from interferences under the conditions of the analysis by running method blanks initially and with each sample lot. Specific selection of reagents and purification of solvents by distillation in all-glass systems are required. High-purity distilled-in-glass solvents are commercially available.

An effective way of cleaning laboratory glassware is by rinsing with polar and non-polar solvents before use. The cleaning procedure used must be tested by analyzing procedural blanks prior to analyzing samples.

3.2 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source. Solid phase extraction (SPE) can be used to overcome many of these interferences.

3.3 SPE Clean Up Procedure

Pre-filtered water samples (100 mL) were extracted with J.T.Baker™ C18, 6 mL, 500 mg solid phase cartridges (Milford, MA) mounted on a Resprep™ vacuum manifold, (Restek Corp., Bellefonte, PA). The cartridges were first pre-conditioned with 10 mL methanol followed by 10 mL water. The samples were loaded through the cartridges at a rate of 5 mL/min, not to exceed 20 psi. The cartridges were then dried for 5 minutes with vacuum and finally, eluded with 2 mL methanol, vortexed and filtered through 0.45 µm filters.

4.0 Material and methods

4.1 Chemicals and reagents

Certified MC standards (LR, RR, LF, LW and NOD-R) were purchased from Calbiochem (EMD Chemicals, La Jolla, CA) and LR, RR, YR, LA were purchased from Sigma-Aldrich (Allentown, PA). Burdick and Jackson HPLC grade solvents (acetonitrile, methanol, water), glass fiber filters (Type A/E, 90mm, 1 µm) and Gelman Acrodisc® CR PTFE syringe filters (13 mm, 0.45 µm) were obtained from Pall Corp., Ann Arbor MI, USA. Mobile phase additives, ACS grade formic acid (98%) and trifluoroacetic acid (99%) were purchased from Sigma Aldrich, Milwaukee, WI, USA). For method validation purposes, Sacramento River water and Rainbow Trout tissue and livers were obtained from the Nimbus Fish hatchery, Rancho Cordova, CA. Mussels were purchased from a local fish market. A combined intermediate working solution of MCs was made in methanol from the purchased standards.

4.2 Sample storage

Tissue samples are kept frozen until time of extraction. Water samples for cyanotoxin analysis should be refrigerated in the dark to prevent toxin degradation but it is essential that storage be kept to a minimum (preferably less than 72 hours). Where prolonged storage is required, samples can be frozen, although this will release toxins from the cells and only the total amount of toxin in the sample can then be determined.

4.3 Sample preparation

4.3.1. Liquid sample extraction

MCs in water bodies at the time of a bloom will be present in both the water (free, dissolved or extra cellular toxins) and the cyanobacterial cells (intracellular toxins). In order to determine total MC in the water the cell wall must be ruptured or lysed by repeated freeze-thawing and sonication. An aliquot of sample (100 mL) was filtered under vacuum through a glass fiber filter (1 µm). The water and filters were extracted separately, as follows: 1) Pre-filtered water samples were acidified with 0.1 % FA and 0.05 % TFA to obtain pH~2 and extracted by SPE using JT BakerBond C18, 6 cc, 500 mg solid phase cartridges (Mallinckrodt Baker, Phillipsburg, NJ) mounted on a Resprep™ vacuum manifold (Restek Corp., Bellefonte, PA). The cartridges were first pre-conditioned with 10 mL methanol followed by 10 mL acidified water. The samples were loaded through the cartridges at a rate of 5 mL/min, not to exceed 20 psi. The cartridges were then dried for 5 minutes with vacuum and finally, eluded with 2 x 1 mL mixture methanol:water (90:10) acidified with 0.1% TFA, vortexed and filtered through 0.45 µm filters. Extracts are now ready for analysis. 2) Filters with planktonic material or lyophilized biomass shellfish were extracted twice with 15 mL of methanol:acidified water (90:10, v/v) by homogenizing for 1-2 minutes using a Polytron, followed by 10 minute sonication in ultrasonic bath. The extracts were centrifuged and the supernatant was evaporated at 35°C to 5 mL with rotary-evaporator. The concentrated extract was diluted to 100 mL in order to decrease the methanol concentration, acidified and followed by the SPE procedure.

4.3.2 Bivalve sample extraction

Tissue (mussel, liver, fish tissue) samples were homogenized using a Bucchi B-400 mixer equipped with a titanium knife assembly. A 2-5 g sample was transferred to conical centrifuge tubes with 10 mL methanol:acidified water (90:10, v/v) and finely-ground with an Arrow 850 tissue grinder (Arrow Engineering Co., Inc., Hillside, NJ) equipped with a glass pestle for five minutes, followed by sonication with a Branson® 3510 Ultrasonic for one hour. The extracts were then centrifuged at 3500 rpm for 30 minutes using a HN-S centrifuge (Damon-IEC Division, Needham Heights, MA). The extract is reduced to minimum volume and diluted with water (not to exceed 5 % methanol), acidified and finally, cleaned-up using SPE, as described above. For extremely dirty samples, an extra step is recommended by washing the HLB cartridge with 10-20% methanol:water solution before eluting the target analytes.

4.4 Analysis parameters and set up

The LC-MSD single quadrupole used was an Agilent 1100 liquid chromatograph connected to Hewlett Packard MSD-SL and the LC-MS/MS used was an Agilent 1200 liquid chromatograph connected to a 6410 triplequadrupole (QqQ). Both LCs were equipped with a vacuum degasser, a binary pump, an autosampler and a thermostatted column compartment kept at 40°C. These instruments were purchased from Agilent Technologies, Santa Clara, CA. Agilent Chemstation

software was used to collect and process data from LC-MS, while Agilent Mass Hunter software was used for LC triplequadrupole.

4.4.1. Chromatography

The mobile phase consisted of HPLC water (A) and acetonitrile (B) (both contained 0.1 % formic acid (v/v)). The gradient elution program started with 5 % B and held for 2 min. The first linear gradient from 5 % B to 50 % B over 8 min, a second linear gradient from 50 % B to 75 % B over 3 min and held at this gradient for 5 min before returning to initial mobile phase ratio at 19 min and held for 1 min. The run time was 20 minutes. The flow rate was set at 0.25 mL/min. The injection volume was 10 µL. After each run, the column was equilibrated for 5 min at the initial conditions before the next injection. A Guard column C18, 3.5 µm, 2.1 x 30 mm was used to protect the analytical column; dC18, 3 µm and 2.1 X 100mm (Waters Atlantis). The effluent from the LC column was directed from the waste to the mass spectrometer source after the first 5 minutes of the run.

4.4.2. MS-SIM parameters

Nitrogen (less than 1 ppm oxygen, Praxair, Rancho Cordova, CA) was used as the nebulizing and drying gas. The MSD was run using electrospray ionization (ESI) interface operated in positive mode as follows: 350°C drying gas temp, 13.0 L/min drying gas flow, 40 psi nebulizer pressure, 110 fragment voltage and 4.0 kV electrospray capillary voltage. MS detection was performed in Selected Ion Monitoring (SIM). The following MC ions (m/z) were monitored: 519.8 RR and 512.8 desmethyl-RR are both $[M+2H]^{2+}$; 1045.6 YR, 995.7 LR, 981.7 demethyl-LR, 910.6 LA, 1026.6 LW, 987.6 LF and 825.5 NOD-R were monitored using $[M+H]^{+}$. Full scan was also collected over the range 100-1100 Da. The UV-Diode Array Detector (DAD) was set at 238 nm , Agilent ChemStation was used to collect data.

First, experiments were carried out by direct injection of high concentrations of individual toxin standards into the mass spectrometer using the Flow Injection Automated (FIA) program. The obtained full scan spectra showed the exclusive presence of protonated molecular ions $[M+H]^{+}$ for all microcystins (MC) except MC-RR, which had a doubly charged $[M+2H]^{2+}$ ion. This correlates well with the presence of two arginine residues in MC-RR whose side chains are capable of retaining external protons and producing stabilized $[M+2H]^{2+}$. Those ions were then chosen for SIM mode. Table 1 shows the toxin fragments monitored and their respective m/z values. Nodularin could be used as surrogate (Sur) or internal standard (IS) since it is a pentacyclicpeptide and found primarily in marine water.

Table 1. MC toxin fragments monitored by LC-MS.

MC toxin fragment	m/z $[M+H]^{+}$	m/z $[M+2H]^{2+}$
Adda fragment	135.1	
MC-RR		519.8

dm-MC-RR		512.8
MC-LR	995.5	
dm-MC-LR	981.5	
MC-YR	1045.5	
MC-LA	910.6	
MC-LF	987.5	
MC-LW	1026.5	
NOD-(IS)	825.5	

The fragment voltage was optimized by re-analyzing the individual microcystin standards in SIM mode using FIA programming and changing the fragment voltage over the range from 10-130 volts. The fragment voltage corresponding to the most intense peak was chosen (110V). Instrument default settings were used for drying gas, capillary voltage and the remaining MS parameters. Finally, the LC system was attached to the MS and MCs standard mixture was analyzed. All analytes were well separated under the LC conditions listed above. It was possible to increase analyte response in the SIM mode by using multiple acquisitions and time programming modes, where five segments were used.

4.4.3. MS/MS-MRM parameters

The triplequadrupole was operated using the same conditions as the single quadrupole, except the detection was in multiple reaction monitoring (MRM) mode. The parameters for running MRM are as follows: ultra-pure nitrogen gas was used for collision induced dissociation (CID). The protonated fragment ions used for SIM mode served as the precursor ions for MRM mode, therefore, only the transition from the precursor to the product ion needed to be optimized by varying the voltage of collision induced dissociation (CID) gas from 0 to 50 eV. As a result, the most intense product ions obtained by these voltage settings were selected. The collision energy (CE) was set at 50V for MC-RR/dm-RR and 70V for the remaining MCs. The MRM windows were established for MCs using the daughter ions, which are the Adda fragments of m/z 135.2 and m/z 213 produced by the transition of the protonated precursor ions (SIM). Fragment at m/z 135 corresponding to the O-methylphenylacetaldehyde $[Ph\text{-}CH2\text{-}CHOMe]^+$ structure from the Adda moiety and the fragment at m/z 213 corresponding to $[Glu\text{-}Mdha+H]^+$ resulted from the MRM transition were the predominate product ions for all MC analytes. The fragment ions, m/z 135.2 and m/z 213 were chosen as quantifier and qualifier ions, respectively. Fragment of Adda at m/z 135.2 and fragment ion at m/z 227.1 correspond to $[Glu\text{-}Mdhb+H]^+$ were obtained for the internal standard, NDLN. Table 2 shows the optimum CE setting for all MCs and time segments of the MRM method.

Table 2. Time segments and MRM parameters.

Time Segment #	Time (min)	Target Compound	Scan Type	Precursor Ion	Product Ions	Fragment (V)	Collision Energy (V)
1	0	na*	MS2 Scan (to waste)				
2	5	MC-RR	MRM	520	213, 135.2	110	50
		dm-MC-RR	MRM	512.8	213, 135.2	110	50
3	12.9	MC-LR	MRM	995.7	213, 135.2	110	70
		dm-MC-LR	MRM	981.7	213, 135.2	110	70
		MC-YR	MRM	1045.6	213, 135.2	110	70
4	14.5	MC-LA	MRM	910.6	213, 135.2	110	70
5	15.5	MC-LF	MRM	987.6	213, 135.2	110	70
		MC-LW	MRM	1026.6	213, 135.2	110	70
* na: not applicable							

Under these LC-MS/MS conditions a 0.2 µg/L microcystins standard mixture was analyzed and easily identified with S/N greater than 5.0 for most toxins. Typical MRM and reconstructed ion chromatograms are shown in Fig. 1.

Fig. 1. MRM constructed ion chromatogram for microcystin standard.

4.5 Calibration curve

To evaluate the linearity of the system various concentrations of certified MC mixture standard solution were prepared in methanol-water (90:10) (v/v) to obtain a seven level calibration curve ranging from 0.2 µg/L to 200 µg/L. An individual calibration curve was drawn for all the toxins except the demethylated variants. A linear response was found between concentration and area for MCs. As shown in Fig. 2, the linearity was very good for all MCs with correlation coefficient (r^2) greater than 0.998. The limit of detection in MRM mode was calculated using USEPA procedures found in Title 40 Code of Federal Regulations Part 136 (40CFR 136, Appendix B, revision 1.11) and were below 0.1 µg/L or lower for all the MCs.

Using the above listed MRM parameters, 1 µg/L microcystin standard mixture was easily detected and separated (Fig. 3). The constructed ion chromatogram (1 µg/L) showing the transition from the individual microcystin precursor ion to its corresponding product ions chromatogram and spectra are shown in Fig. 4. By using LC triplequadrupole MS with ESI in Multiple Reaction Monitoring (MRM) mode, the limit of detection and quantitation for all microcystins were determine to be between 0.2 and 1 pg on column, with 5:1 S/N ratio.

Fig. 2. Calibration curves of individual MCs ranging from 1-200 µg/L.

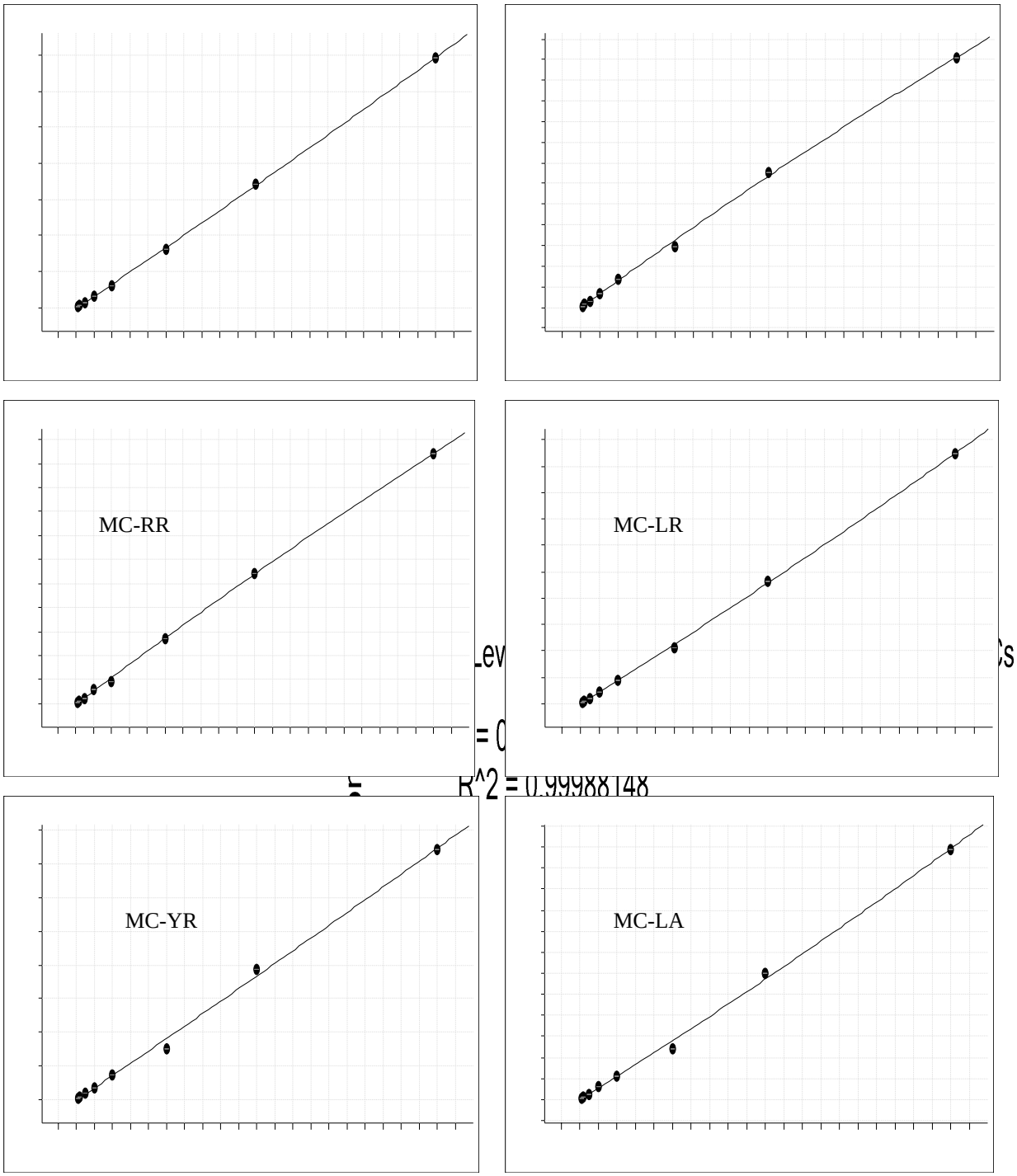


Fig. 3. Total ion chromatogram of a microcystin standard at 1 pg on column.

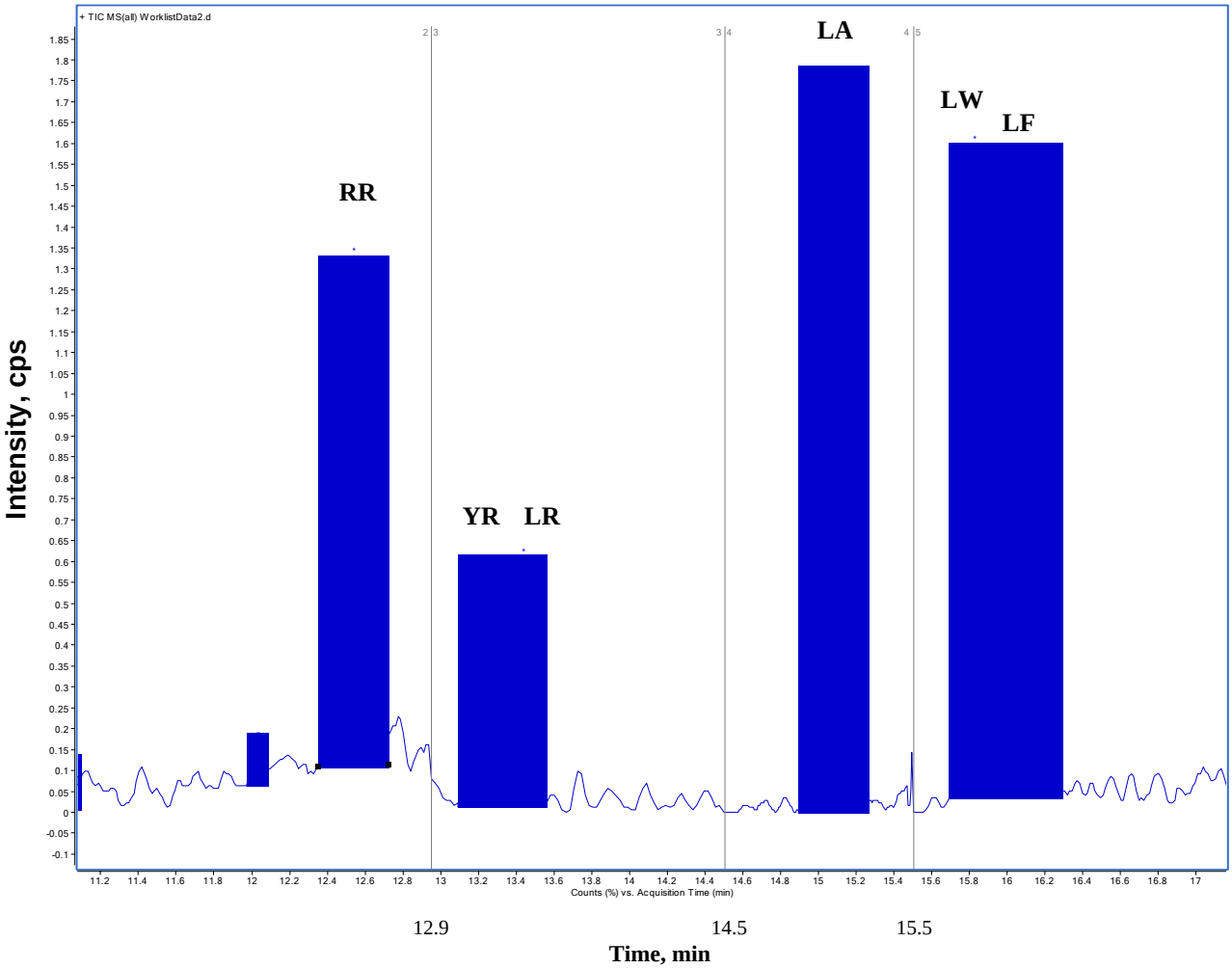
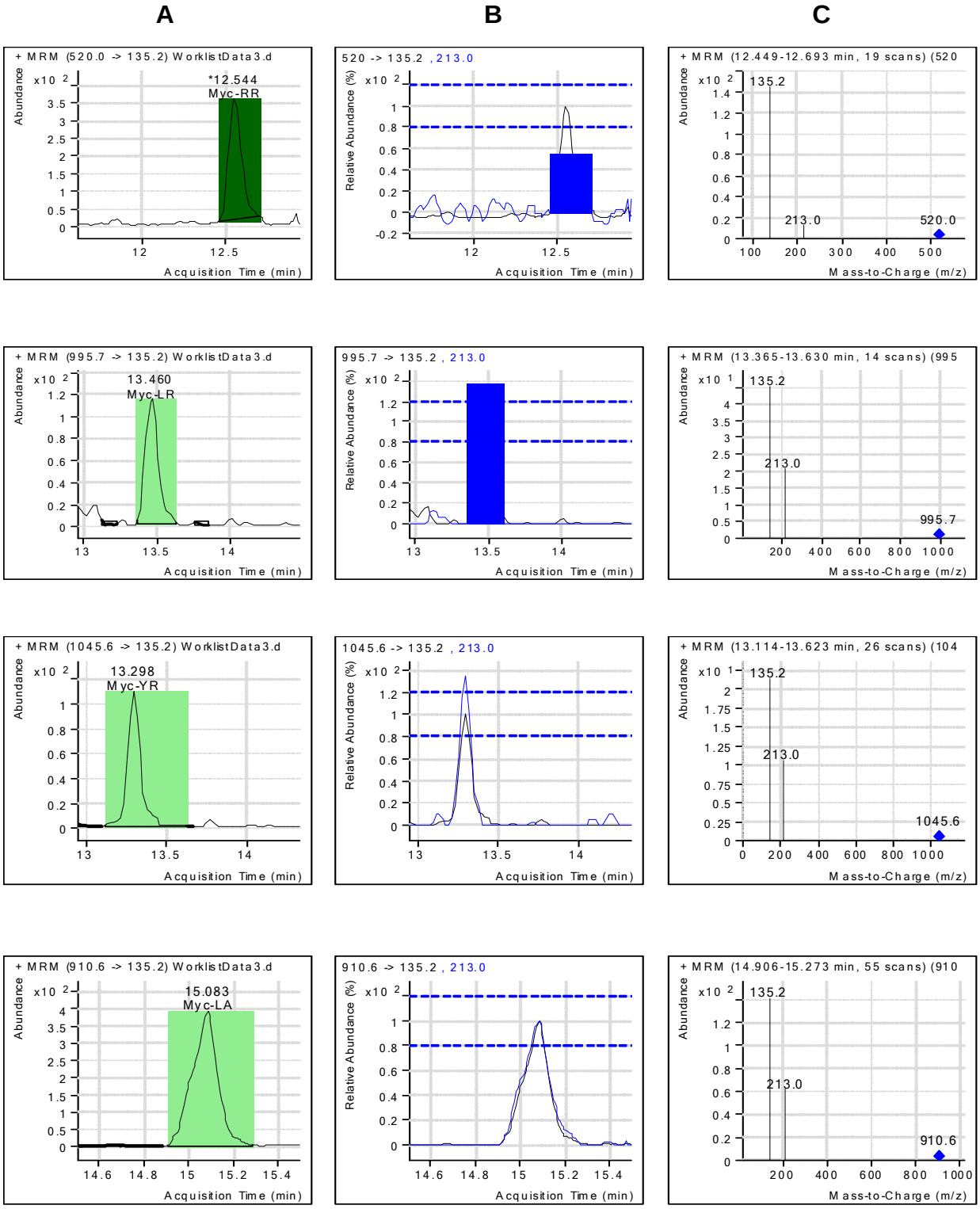
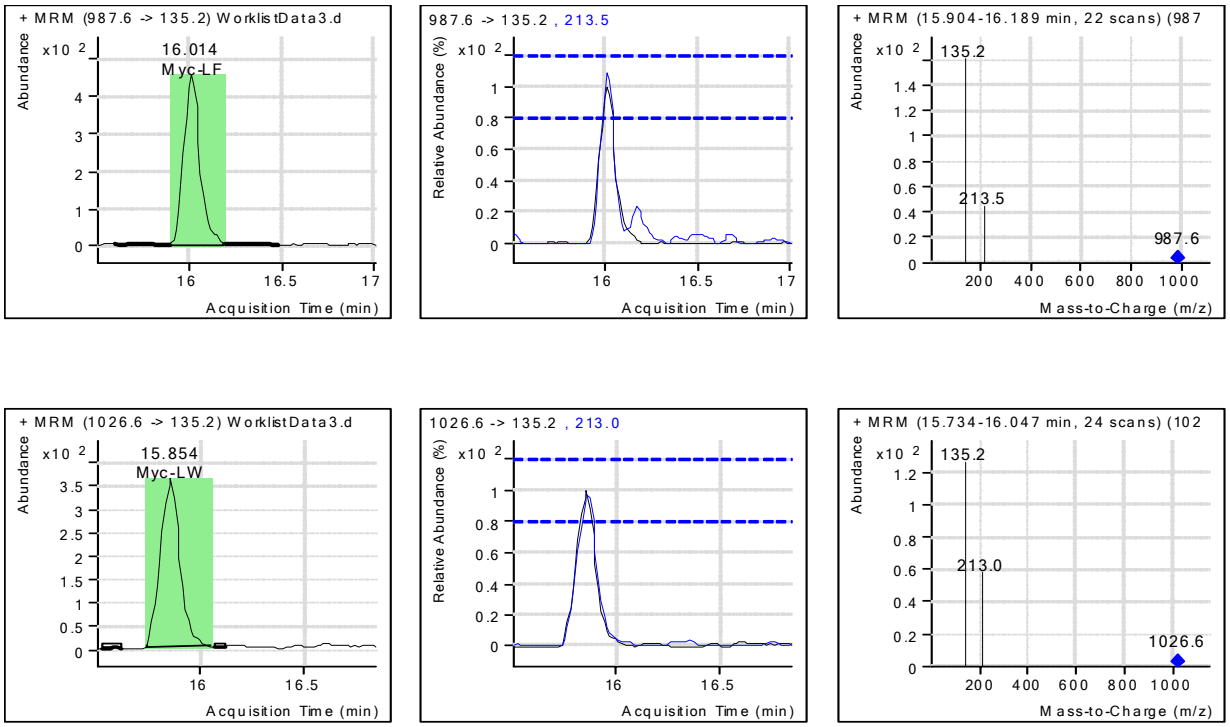


Fig. 4. Microcystin chromatograms in MRM mode: precursor ion (A), product ion (B) and spectra (C).





5.0 Method validation

5.1 Liquid samples method validation

5.1.1. Fresh water solid phase extraction (SPE)

The method was first tested with several types of SPE cartridges (Supelco C8, Oasis HLB, C18 J.T Baker Strata X). Acidified water samples (100 mL), fortified with MCs mixture at 5 µg/L, went thru the SPE procedure detailed earlier in Section 3.3.3. The result from this study shows that J.T Baker C18 cartridges extract all the tested microcystins and Nodularin from the water with acceptable recovery as shown in Table 3.

Table 3. Microcystin Solid Phase Extraction Results.

Biotoxin Compounds	C8-Supelco	C18-JTBaker	C18-HLB Oasis	Strata X
	% Recovery	% Recovery	% Recovery	% Recovery
Myc-YR	114	118	60.5	60.0
Noduralin	78.8	82.8	56.3	50.0
Myc-LW	74.1	139	10.8	8.08
Myc-LF	86.1	157	13.2	ND
Myc-LR	60.8	109	58.8	60.3
Myc-LA	4.99	48.1	18.6	13.2
Myc-RR	151	143	95.4	114

The selected SPE C18 cartridge (J.T.Baker) was used to validate the method. Triplicate river water samples fortified with MCs and NDLN at 5µg/L level (LCS) and triplicate glass fiber filters spiked (FS) with MCs and NDLN at 0.2u/g were extracted following the procedure listed in Section 4.3. Recoveries obtained for all tested MCs were ranging from 74.0-125 % and from 73.8-110% for water and filter extracts respectively. NDLN recoveries were ranging from 89.7-113 for both. MC-LW showed lower recoveries in the filter extracts. This loss could be contributed to the sorption of this MC to the wall of the C18 cartridges. Recoveries for all individual MC and the estimated method detection limit (MDL) for MCs calculated from students *t* times standard deviation for water samples (200mL) using this procedure are listed in Table 4.

Table 4. C18 SPE water (LCS) and filters (FS) method validation.

Microcystin Congeners	LCS1	LCS2	LCS3	Ave% Rec	StDev	%RSD	MDL=t*stdev µg/L
MCY-RR	102	99.6	102	101.3	1.45	1.43	0.009
MCY-LR	104	103	111	106.0	4.51	4.25	0.005
MCY-YR	114	115	125	118	6.08	5.15	0.015
MCY-LA	92.0	89.9	101	94.3	5.90	6.25	0.013
MCY-LF	85.6	74.0	84.8	81.5	6.48	7.95	0.020
MCY-LW	66.7	72.3	81.9	73.6	7.69	10.4	0.024
Nodularin	100	101	113	104.7	7.51	7.18	0.009

Microcystin Congeners	FS1	FS2	FS3	Ave% Rec	StDev	%RSD	MDL=t*stdev µg/L
MCY-RR	73.8	79.2	76.0	76.3	2.72	3.56	0.005
MCY-LR	87.2	89.6	86.8	87.9	1.51	1.72	0.016
MCY-YR	103	111	110	108	4.36	4.04	0.021
MCY-LA	82.0	77.0	84.0	81.0	3.61	4.45	0.021
MCY-LF	92.0	84.8	80.8	85.9	5.68	6.61	0.023
MCY-LW	38.8	44.8	52.7	45.4	6.97	15.3	0.027
Nodularin	94.7	89.7	92.0	92.1	2.50	2.72	0.026

5.2 Fresh water direct injection

LC-MS/MS triplequadrupole operated in MRM has shown the ability to achieve extremely low detection of MCs (2 pg on column). For this reason a direct injection method was validated on the most common microcystins (MC-RR, MC-LR and MC-YR). A set of nine fortified river water samples (0.5 µg/L) were diluted with methanol to obtain (9:1) water-methanol (v/v). A portion of the sample was filtered through 0.45 µm Gelman filters then directly injected into LC-MS/MS (QqQ).The MDL for water using direct injection was determined to be 0.1 µg/L based on signal-to-noise equivalent to 7:2. The MRM results obtained in Table 5 shows the mean recoveries were 104, 97.0 and 95.4 % for MC RR, -LR and -YR, respectively, with RSD< 11%.

Table 5. Direct injection results from 0.2 µg/L fortified water samples.

Toxins	LCS1	LCS2	LCS3	LCS4	LCS5	LCS6	LCS7	LCS8	LCS9	Avg	StDev
MC-RR	106	100	102	98.8	104	110	110	110	96.8	104	5.09
MC-LR	88.0	85.1	90.2	87.2	93.9	107	104	101	116	97.0	10.6
MC-YR	100	88.4	96.5	94.0	103	106	95.0	83.5	92.4	95.4	7.00

This validated method was tested by analyzing split contaminated water samples received as part of a Round Robin study organized by Florida Department of Environmental Protection (EPA). Twelve laboratories throughout the United States participated in this exercise. Three types of water samples were received:

1) water from natural bloom (2007) occurred in Lake Munson (M) which was caused by cyanobacteria, *Microcystis aeruginosa*, 2) water sample containing toxin produced by cultures from University of Texas laboratory (T) and 3) a microcystin standard (S) diluted in water. Each laboratory was provided with 10 blind water samples. Either three or four replicates were provided to each laboratory for each sample type. Laboratories were required to hold the samples in the dark at 4°C for no more than one week before analysis. Two different extractions were performed on the water samples for comparison:

a) An aliquot of round robin water sample (200mL) was first filtered thru 0.45 µm glass fibers filters then extracted with SPE cartridge. The filters were sonicated and both SPE and filters were extracted according to the procedure outlined in Section 4.3.
b) The second extraction consisted of 1 mL of methanol added to 9 mL of the round robin water sample sonicated for 45 min, centrifuged for 30 min and a portion of the methalonic solution was filtered thru Gelman filters and directly injected into LC-MS/MS. Results from both type of extraction are listed in Tables 6-8.

Table 6. Comparison of direct injection (sonication) vs. SPE and filters from Round Robin standard (S).

Sonication	S-1	S-2	S-3	Avg (ppb)	StDev (ppb)	RSD (%)
Myc-RR						
Myc-LR	2.80	2.96	2.96	2.91	0.09	3.18
Myc-YR						
Total MCs	2.80	2.96	2.96	2.91	0.09	3.18

SPE	S-1	S-2	S-3	Avg (ppb)	StDev (ppb)	RSD (%)
Myc-RR						
Myc-LR	0.37	0.49	0.55	0.47	0.09	19.4
Myc-YR						
Total MCs	0.37	0.49	0.55	0.47	0.09	19.4

Filters	S-1	S-2	S-3	Avg (ppb)	StDev (ppb)	RSD (%)
Myc-RR						
Myc-LR	1.28	1.27	1.17	1.24	0.06	5.12
Myc-YR						
Total MCs	1.28	1.27	1.17	1.24	0.06	5.12

Total MC	S-1	S-2	S-3
Sonication	2.80	2.96	2.96
SPE+filters	1.65	1.76	1.72

Table 6 contains the results obtained from the analysis of round robin sample (S) showing the presence of low levels of MC-LR (~2 ppb). The combined results obtained from SPE and filters correlate well with the direct injection results. The average MC-LR value obtained from sonication was 2.90 ppb compared to 1.71 ppb obtained with SPE and filters, the difference could be contributed to losses during sample preparation.

Table 7. Comparison of direct injection vs. SPE and filters from University of Texas culture (T).

Sonication	T-1	T-2	T-3	Avg (ppb)	StDev (ppb)	RSD (%)
Myc-RR						
Myc-LR	65.0	60.6	63.1	62.9	2.21	3.51
Myc-YR						
Total MCs	65.0	60.6	63.1	62.9	2.21	3.51

SPE	T-1	T-2	T-3	Avg (ppb)	StDev (ppb)	RSD (%)
Myc-RR	0.51					
Myc-LR	37.5	40.0	39.6	39.0	1.37	3.51
Myc-YR						
Total MCs	38.0	40.0	39.6	39.2	1.1	2.7

Filters	T-1	T-2	T-3	Avg (ppb)	StDev (ppb)	RSD (%)
Myc-RR						
Myc-LR	11.69	7.54	6.27	8.50	2.84	33.4
Myc-YR						
Total MCs	11.69	7.54	6.27	8.5	2.84	33.4

Total MC	T-1	T-2	T-3
Sonication	65.0	60.6	63.1
SPE+filters	49.7	47.5	45.9

Microcystins	T-1	T-2	T-3
Demethyl-RR	6.20	5.65	7.61
Demethyl-LR	16.9	16.5	18.0

Table 7 shows the presence of mainly MC-LR in the Texas culture samples. With an average of 62.9 ppb for the direct injection compare to 48.1 ppb obtained by SPE extraction. The RSD values were below 5% for all replicates. Desmethylated

microcystin (demethyl-MC) RR and LR were also found in these samples with an average value of 5.82 and 17.13 ppb, respectively.

Table 8. Comparison of direct injection vs. SPE and filters from Lake Munson (M) natural bloom.

Sonication	M-1	M-2	M-3	M-4	Avg (ppb)	StDev (ppb)	RSD (%)
Myc-RR	57.3	71.9	72.7	70.7	68.2	7.28	10.7
Myc-LR	63.6	84.2	79.4	78.8	76.5	8.93	11.7
Myc-YR	1.80	1.50	1.50	1.90	1.68	0.21	12.3
Total MCs	123	158	154	151	147	15.9	10.9

SPE	M-1	M-2	M-3	M-4	Avg (ppb)	StDev (ppb)	RSD (%)
Myc-RR	60.3	67.3	60.3	72.1	65.0	5.77	8.88
Myc-LR	58.5	81.5	70.0	80.4	72.6	10.8	14.8
Myc-YR	0.19	0.79	1.25	0.65	0.72	0.44	60.6
Total MCs	119	150	132	153	138	16.0	11.6

Filters	M-1	M-2	M-3	M-4	Avg (ppb)	StDev (ppb)	RSD (%)
Myc-RR	9.88	11.4	16.7	10.1	12.0	3.21	26.7
Myc-LR	8.73	7.08	8.96	9.08	8.46	0.93	11.0
Myc-YR	0.35	0.54	0.74	0.54	0.54	0.16	29.4
Total MCs	19.0	19.0	26.4	19.7	21.0	3.62	17.2

Total MC	M-1	M-2	M-3	M-4
Sonication	123	158	154	151
SPE+filters	138	169	158	173

Microcystins	M-1	M-2	M-3	M-4
Demethyl-RR	60.0	74.2	74.2	72.6
Demethyl-LR	56.5	72.5	68.1	68.8

MC-RR, MC-LR and MC YR were found in Munson Lake samples with an average of 68.2, 76.5 and 1.68 ppb, respectively. The total microcystins obtained from direct

injection of the four replicates were practically the same as the one obtained from SPE and filters combined and were 151 and 173 ppb, respectively. Desmethyl-MC-RR and desmethyl-MC-LR were also found in the samples with an average of 70.2 and 66.5 ppb, respectively (Table 8).

In summary, the total concentration of microcystins obtained from direct injection was slightly better compared to the combined MCs obtained from SPE and filters extractions. The method showed excellent precision by comparing replicate results. Only this LC-MS/MS technique was able to detect and report the presence of desmethylated variants compared to other participating laboratory methods. Since desmethylated-MC standards were not available at the time, the desmethyl-MCs values were estimated using the methylated congeners response factor.

5.3 Biota samples (fish and mussels)

California coastal mussels (M), oysters (O), Rainbow Trout fillets and livers were used for method validation. Samples (2-5 g fresh weight) were polytronned, homogenized, fortified with 5 ng/g microcystins mixture standard and extracted with methanol-water (90:10) using the sonication procedure listed in Section 4.3. Recovery experiments were performed using replicate samples. The results showed that all tested MCs were extracted with high degree of efficiency using sonication technique (Table 9). Recoveries obtained from mussels ranged from 79.9-104 % with percent RSD<15 (n=8). The average microcystin recovery for oysters was 102 % with average standard deviation of ±14.9. The mean recoveries were 106 % for fish fillet (n=4) and 85.7 % for fish liver (n=3). The % RSD was below 11 % for both.

Table 9. Recoveries of microcystins by sonication in various matrices: mussel (M), oyster (O), fish fillet (FF) and fish liver (FL).

	M-1	M-2	M-3	M-4	M-5	M-6	M-7	M-8
MC-RR	112	115	125	118	85.4	92.5	94.0	89.2
MC-LR	82.9	77.5	81.8	114	114	107	115	114
MC-YR	72.0	87.3	97.9	116	109	117	121	115
MC-LA	73.5	72.9	73.6	80.3	82.5	74.5	75.6	106
MC-LW	75.4	74.3	83.8	92.0	81.9	74.3	79.0	85.6
MC-LF	82.8	80.7	89.1	96.1	85.9	71.5	78.1	89.2

The method detection limit (MDL) calculated from student's *t* times standard deviation for mussels (n=8) determine to be ≤ 1 ng/g using MRM (Table 10).

Table 10. Determination of microcystin MDL in tissue (n=8).

Microcystins	Avg (%Rec)	StDev	RSD (%)	MDL=t* StDev
MCY-RR	104	15.2	14.6	0.91
MCY-LR	101	16.8	16.7	1.01
MCY-YR	104	17.3	16.5	1.04
MCY-LA	79.9	11.1	13.9	0.67
MCY-LF	80.8	6.27	7.77	0.38
MCY-LW	84.2	7.62	9.05	0.46

	O-1	O-2	O-3	O-4	FF-1	FF-2	FF-3	FF-4	FL-1	FL-2	FL-3
MC-RR	106	103	105	93.3	114	106	99.1	101	81.6	77.0	83.8
MC-LR	79.1	74.8	107	117	120	122	108	103	78.4	84.4	82.4
MC-YR	80.1	79.0	115	118	123	119	109	108	89.0	83.2	87.0
MC-LA	102	101	103	110	108	107	110	107	88.6	76.4	84.8
MC-LW	118	116	101	88.8	125	111	112	103	79.8	68.8	85.4
MC-LF	120	118	103	90.3	110	106	105	104	78.4	84.4	82.4

6. QUALITY CONTROL

Quality control checks are routinely performed in the WPCL operations. These checks may be increased or modified to meet the needs of a particular analysis or project.

6.1 QA Samples

Internal quality assurance samples (fortified samples and duplicates, appropriate reference materials, duplicate samples, and method or procedural blanks) will be analyzed with each set or every twenty analyses being performed. These internal quality assurance analyses are conducted for the parameters being monitored by that analytical procedure. In addition, the compounds contained in the quality assurance sample will be representative of those compounds being monitored.

Accuracy is measured by calculating percent recovery for laboratory control spikes (fortified reagent sample), matrix spikes (fortified samples) and when available, certified reference materials (CRMs or SRMs). Accuracy is also determined for CRMs by comparing the analysis results with the certified (consensus) or reference (non-certified) values. CRM results are acceptable if they are within 65-135% of the 95th percentile confidence interval of the consensus values for certified materials.

Precision is measured by calculating the relative percent difference (RPD) for analytes from duplicate analysis of samples, fortified samples and fortified blanks.

The results of all QA analyses and the percent recoveries for fortified samples and reference materials will be calculated and documented.

6.2 Duplicate Samples

One duplicate sample and/or a matrix spike duplicate or laboratory control spike duplicate will be analyzed for each set of twenty samples analyzed. The relative percent difference for each constituent is calculated as follows:

$$RPD = \{(D_1-D_2)/[(D_1+D_2)/2]\}x100$$

Where, RPD = Relative Percent Difference
D_1 = First Sample Value
D_2 = Second Sample Value (duplicate)

The results of all duplicate determinations and the calculated relative percent difference will be reported with the data sets. For RPD, use a control limit of 25 percent unless otherwise specified by a project specific QAPP.

If either sample value is less than the MDL, the notation of "ND" (not detected) will be reported. If the precision falls outside the control limits, the analysis results will be reported with the appropriate data qualifier.

6.3 Fortified Matrix (MS/MSD) Sample Analyses

When required, matrix spike and matrix spike duplicate analyses will be conducted at a rate of five percent. The spike will be added prior to any digestion, extraction, or distillation steps as a check on the sample preparation and analysis. An amount of analyte will be added to the sample that is five to ten times the reporting limit for the analyte of interest. Recovery values are calculated as follows:

$$\text{Recovery} = [(D_a - D)/D_s] \times 100$$

Where, Recovery = Percent Recovery

D_a = Analysis value of fortified sample
D = Analysis value of sample without spike
D_s = Amount of spike added

Recovery values for fortified samples must be greater than 50 percent except where a specific method (SOP) or project specific QAPP require a different acceptable range. Exceptions shall be noted in the project specific data quality objectives. When a specific method and analyte require a different acceptable recovery range, as determined by actual spike recovery runs, the acceptable range shall be noted in the Standard Operating Procedure for that method. If the recovery falls outside of the acceptable recovery range, the analysis results will be qualified or rejected. If the results are rejected, the batch of samples associated with the rejected results may need to be re-analyzed. When sample concentrations are less than the MDL, the value of "0" will be used as the sample result concentration for purposes of calculating spike recoveries. All fortified sample results will be reported with the data package.

If the percent recovery for matrix spike is unacceptable, there might be an interference due to the matrix. The sample will be diluted to lower the interference and re-analyzed. If matrix interference is determined to be the cause of unacceptable recoveries, the data will be qualified.

6.4 Method Blanks

Method blanks will be analyzed at a minimum of once for every batch of samples. Blank concentrations should not exceed the reporting limit for the analyte. If blank values exceed the reporting limit, the source of the contamination should be investigated and corrected, and the results associated with the contaminated blank re-analyzed or qualified. All blank analysis results will be reported with the data package.

6.5 Laboratory Control Samples

While reference materials are not available for all analytes, a way of assessing the accuracy of an analytical method is still required. Laboratory control samples (LCSs) provide an alternate method of assessing accuracy. An LCS is a specimen of known composition prepared using contaminant-free reagent water or an inert solid spiked with the target analyte at the midpoint of the calibration curve or at the level of concern. The LCS must be analyzed using the same preparation, reagents, and analytical methods employed for regular samples.

SOP Section Approval: _____ Date: _____

SOP Final Approval: _____ Date: _____

SOP QA Officer Approval: _____ Date: _____

STANDARD OPERATING PROCEDURE
TITLE: Determination of Organochlorine Pesticides in Water

REVISION HISTORY		
Revision #	**Summary of Changes**	**Date**
1	Separated organochlorine pesticides and PCBs analyses into separate SOPs. Original source SOP was "Method #31, revision 1."	11/12/2009
0	Initial release as Method 31.	06/20/2005

Author:	Gloria Blondina		Date: 11/12/09
Approved:	Laboratory Director *David B. Crane*		Date:
Approved:	Section Lead *Abdu Mekebri*		Date:
Approved:	Quality Assurance *Gail Cho*		Date:
Approved:	Health and Safety *Thomas Lew*		Date:

Determination of Organochlorine Pesticides in Water Samples

1.0 Scope and Application

1.1 This is a modified EPA Method 608/8081B and describes the sample preparation and quantitative analysis of trace level organochlorine (OCH) pesticides in surface, municipal and wastewater using liquid-liquid extraction and high resolution gas chromatography with electron capture detectors (GC/ECD) and gas chromatography with mass spectrometer and ion trap detector (GC/MS-ITD) for confirmation. The following target analytes can be determined by this method:

Target Analyte	CAS Registry No.
Aldrin	309-00-2
Chlordane, cis	5103-71-9
Chlordane, trans	5103-74-2
Chlordene	3734-48-3
Dacthal	1861-32-1
DDD(o,p')	53-19-0
DDD(p,p')	72-54-8
DDE(o,p')	3424-82-6
DDE(p,p')	72-55-9
DDMU(p,p')	1022-22-6
DDT(o,p')	789-02-6
DDT(p,p')	50-29-3
Dieldrin	60-57-1
Endosulfan I	959-98-8
Endosulfan II	33213-65-9
Endosulfan sulfate	1031-07-8
Endrin	72-20-8
Endrin Aldehyde	7421-93-4
Endrin Ketone	53494-70-5
HCH, alpha	319-84-6
HCH, beta	319-85-7
HCH, gamma	58-89-9
HCH, delta	319-86-8
Heptachlor	76-44-8

Heptachlor epoxide	1024-57-3
Hexachlorobenzene	118-74-1
Methoxychlor	72-43-5
Mirex	2385-85-5
Nonachlor, cis	5103-73-1
Nonachlor, trans	39765-80-5
Oxadiazon	19666-30-9
Oxychlordane	27304-13-8
Tedion	116-29-0
PCB 207 (Surrogate)	52663-79-3
Dibromooctafluorobiphenyl (Surrogate)	10386-84-2

1.2 The estimated detection limit for each analyte is listed in Table 1. The actual MDL may differ from those listed, depending upon the nature of interferences in the sample matrix. Validation of the target analytes produced recoveries greater than 75 percent, except for Aldrin (62.5%). The mean percent recoveries for each analyte are also included in Table 1.

1.3 Upon request, unknowns of sufficient signal in the sample may be qualitatively confirmed for compound identification by gas chromatography equipped with an ion trap mass spectrometer detector (GC/MS-ITD).

2.0 Summary of Method

2.1 A measured volume of sample (1000 ml) is extracted with methylene chloride (DCM) using a separatory funnel. The DCM extract is dried with sodium sulfate, evaporated using Kuderna-Danish (K-D) and solvent exchanged into petroleum ether. The extract is concentrated with micro-snyder (micro K-D) apparatus to approximately 1 ml and adjusted to 2.0 ml with iso-octane. The extracts are analyzed by gas chromatography using conditions which permit the separation and measurement of the target analytes in the extracts by GC/ECD.

2.2 Interferences in analyses may be encountered in very dirty samples and cleanup may be needed to aid in the elimination or reduction of these interferences. Florisil column cleanup or Gel Permeation Chromatography (GPC) procedures will be followed.

Table 1. Organochlorine pesticides analyzed by GC/ECD, their Method Detection Limits (MDL), Reporting Limits (RL) and Mean Percent Recovery in water.

Target Analytes	MDL (µg/l)	RL (µg/l)	Mean % Recovery
Aldrin	0.001	0.002	62.5
Chlordane, cis	0.001	0.002	86.2
Chlordane, trans	0.001	0.002	93.5
Chlordene	0.001	0.002	95.7
Dacthal	0.001	0.002	97.4
DDD(o,p')	0.001	0.002	83.2
DDD(p,p')	0.001	0.002	88.1
DDE(o,p')	0.001	0.002	91.4
DDE(p,p')	0.001	0.002	104
DDMU(p,p')	0.001	0.002	84.8
DDT(o,p')	0.001	0.002	83.5
DDT(p,p')	0.002	0.005	88.5
Dieldrin	0.001	0.002	79.3
Endosulfan I	0.001	0.002	91.4
Endosulfan II	0.001	0.002	90.3
Endosulfan sulfate	0.001	0.002	101
Endrin	0.001	0.002	101
Endrin Aldehyde	0.002	0.005	90.8
Endrin Ketone	0.002	0.005	95.2
HCH, alpha	0.001	0.002	96.0
HCH, beta	0.001	0.002	107
HCH, gamma	0.001	0.002	94.2
HCH, delta	0.001	0.002	95.4
Heptachlor	0.001	0.002	104
Heptachlor epoxide	0.001	0.002	94.1
Hexachlorobenzene	0.0005	0.001	79.1
Methoxychlor	0.001	0.002	94.6
Mirex	0.001	0.002	89.5
Nonachlor, cis	0.001	0.002	78.6
Nonachlor, trans	0.001	0.002	86.3
Oxadiazon	0.001	0.002	99.6
Oxychlordane	0.001	0.002	93.5
Tedion	0.001	0.002	101
PCB 207 (Surrogate)	0.001	0.002	109

Dibromooctafluorobiphenyl (Surrogate)	0.001	0.002	100

3.0 Interferences

3.1 Solvents, reagents, glassware, and other sample processing hardware may cause GC artifacts and/or elevated baselines, resulting in the misinterpretation of chromatograms. All materials should be demonstrated to be free from interferences under the conditions of the analysis by running method blanks initially and with each sample lot. Specific selection of reagents and purification of solvents by distillation in all-glass systems are required. High-purity distilled-in-glass solvents are commercially available.

An effective way of cleaning laboratory glassware is by rinsing with polar and non-polar solvents before use. The cleaning procedure used must be tested by analyzing procedural blanks prior to analyzing samples.

3.2 Phthalates are common laboratory contaminants that are used widely as plasticizers. Sources of phthalate contamination include plastic lab-ware, plastic tubing, plastic gloves, plastic coated glassware clamps, and have been found as a contaminant in Na_2SO_4.

Polytetrafluoroethylene (PTFE) can be used instead of polypropylene or polyethylene to minimize this potential source of contamination. However, use of PTFE lab-ware will not necessarily preclude all phthalate contamination. Na_2SO_4 can be solvent rinsed to eliminate contaminants.

3.3 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source. A Florisil or GPC cleanup procedure can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4.0 Apparatus and Laboratory Supplies

4.1 Separatory funnel. 2000-ml, with TFE-fluorocarbon stopcock, ground glass or TEF stopper.

4.2 Automatic shaker designed to fit 2 liter separatory funnels with rpm and timer controls.

4.3 Beakers. Borosilicate glass, 400 mL

4.4 Glass wool. Pyrex - solvent washed prior to use.

4.5 Kuderna-Danish (K-D) Apparatus.

4.5.1 Concentrator tube. 15 mL, graduate (Kontes K0570012-0500, or equivalent). A ground stopper, 19/22 joint, is used to prevent evaporation of extracts.

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

4.5.3 Snyder column. Three ball (Kontes K-503000-0121, or equivalent).

4.5.4 Micro-Snyder column. Alltech 9058 or equivalent.

4.5.5 Boiling chips. Hengar granules, high purity amphoteric alundum - extracted with acetone and petroleum ether. Note that boiling chips can be a significant source of contamination if not properly cleaned.

4.6 Water bath. Blue M, 115 V, thermostatically controlled with stainless steel cover to fit K-D apparatus, installed in a fume hood.

4.7 GC vials. GC autosampler vials, borosilicate glass, 2 mL with PTFE-lined screw cap.

4.8 Analytical balance. Capable of weighing 0.1 mg.

4.9 Drying oven.

4.10 Disposable Pasteur Pipettes. 2 mL, rinsed with solvents before use.

4.11 Glass filter funnel. Fluted, 75 mm or larger.

4.12 Graduated cylinder. 1000 ml, 250 mL and 100 mL.

4.13 Culture tubes. 13 x 100 mm with PTFE lined screw cap.

4.14 Analytical systems

4.14.1 Gas chromatograph. Agilent 6890 equipped with dual ^{63}Ni micro electron capture detectors (ECD) with EPC, split-splitless injector, a 7683 autosampler and dual capillary columns (J&W Scientific) connected to a single injection port using a 5 meter pre-column with a "Y" press fit connector. Section 9 describes the acquisition and analysis procedures while Table 2 lists the operating parameters.

4.14.2 Gas chromatograph. Varian 3400, equipped with a Varian Saturn model 2000 Ion Trap Mass Spectrometer, split-splitless injector, LEAP Model CTC A200SE autosampler and a 30 meter capillary column (J&W Scientific).

4.14.3 Data System. Hewlett-Packard, to collect and record GC/ECD data, generates reports, computes and records response factors for multi-level calibrations. Data system should be capable of calibrating a method using a minimum of 5 concentrations of analytical standards and calculating in external standard mode.

Table 2 Operating parameters for Agilent 6890 GC/ECD

Gases
Carrier: Helium, 1 mL/min
Makeup: Nitrogen, 1 mL/min

Columns
DB-5, 60 m x 0.25 mm I.D. x 0.25 µm film thickness
DB-17-MS, 60 m x 0.25 mm I.D. x 0.25 µm film thickness

Mode: Constant flow
Initial flow: 1.9 mL/min

Inlet
Isocratic temp: 240 ºC

Oven
Initial temperature: 80 ºC, initial time: 1.00 min
Ramp 1: 15.0 deg/min, final temp 210 ºC, hold time 10.00 min
Ramp 2: 2.0 deg/min, final temp 290 ºC, hold time 14.00 min

Detectors (^{63}Ni µECD)
Temperature: 310 ºC
Combined Flow: 31.0 mL/min (column + make-up flow)

Injection Volume: 3 µL

5.0 Reagents, materials, gases and standards

5.1 Reagent water is defined as water in which an interferent is not observed at method detection limit of each parameter of interest. Deionized (DI) water was used for method validation and as method blank.

5.2 Petroleum ether (PE), acetone, methylene chloride (DCM), diethyl ether, isooctane. Pesticide residue quality or equivalent.

5.3 Sodium sulfate. Anhydrous granular reagent grade, rinsed with PE prior to use.

5.4 Nitrogen. Ultra-pure (99.99999%) for GC/ECD

5.5 Helium. Ultra-pure (99.99999%) for GC/ECD

5.6 Stock standards. Individual stock standards (100 µg/ml) are purchased as certified solutions from AccuStandard (New Haven, CT).

6.0 Sample Collection, Preservation, and Storage

6.1 Samples are collected in one liter amber glass bottles and iced or refrigerated at 4 °C from time of collection until extraction.

6.2 All samples must be extracted within 7 days and completely analyzed within 40 days of extraction.

7.0 Sample Extraction

7.1 Remove water samples from refrigerator and allow samples to reach room temperature prior to extraction. Transfer contents to a pre-cleaned 2-liter separatory funnel. Immediately add 1.0 ml of the 20 ppb OCH pesticide surrogate solution to every sample. For laboratory control spike (LCS) and matrix spikes (MS/MSD) also add 1.0 ml of 20 ppb OCH pesticide spiking solution.

7.2 Add 60 ml of methylene chloride (DCM) to the empty bottle, replace the cap and rinse the bottle. Pour the DCM into the separatory funnel and repeat with another 60 mL aliquot of DCM. Extract the sample by shaking the funnel for 5 minutes on the auto-shaker with periodic venting to

release excess pressure. Allow organic layer to separate from the water phase for a minimum of 10 minutes. Collect the methylene chloride extract in a 400 ml beaker.

7.3 Add a second 120 ml volume of methylene chloride to the separatory funnel and repeat the extraction procedure a second time, combining the extracts in the beaker.

7.4 Set up and label pre-cleaned K-D flasks with concentrator tubes and attached with a blue clamp on ring stands in the fume hood. Add 0.5 ml iso-octane as “keeper” and a solvent rinsed micro-boiling chip to each K-D concentrator tube. Place a filter funnel containing a plug of pre-cleaned glass wool in the bottom of the funnel and place the funnel in the top of the K-D flask. Add about two inches of solvent rinsed sodium sulfate to the funnel.

7.5 Pour the combined extracts from the beaker through sodium sulfate into the K-D flask. Rinse the beaker with about 10 mL of DCM and add this rinse to the sodium sulfate. Repeat with another 10 mL DCM rinse. Rinse the sodium sulfate with an additional portion of DCM (~10-20 mL).

7.6 Place a Snyder column on the K-D flask, clamp with a green clamp and place the flask on the hot water bath set at 78-82 ^{o}C. Evaporate solvent on the hot water bath. When the apparent volume of solvent in the concentrator tube is 5-10 mL, add 20-30 mL of petroleum ether through the top of the Snyder column. Repeat this procedure when the apparent volume is again at 5-10 mL. When the reflux line falls below the top of the Snyder column, the K-D apparatus should be removed from the hot water bath. Dry the outer KD apparatus with a Kimwipe to prevent condensation water from entering the concentrator tube. Upon cooling, remove the concentrator tube from the K-D apparatus.

7.7 Place a clean micro-Snyder column on the concentrator tube with a blue clamp, add a new micro boiling chip and place in a 400 mL beaker containing water heated to approximately 78 ^{o}C on a hot plate. If the solvent does not begin to boil, remove the tube from the bath immediately, allow it to cool slightly, add a new micro boiling stone to prevent it from bumping and place it back in the bath.

7.8 When the solvent has been evaporated to 0.5-1 mL remove the tube from the bath and allow it to cool in a test tube rack. Dry the outer KD apparatus with a Kimwipe to prevent condensation water from entering the concentrator tube. Remove the micro-Snyder column and add iso-octane to the concentrator tube to reach a final volume of 2.0 mL. Mix the tube contents by tapping the bottom of the tube causing a vortex which will

rinse the sides of the tube. A Vortex Genie mixer may be used for this step.

7.9 Transfer the solution from the concentrator tube to a culture tube and cap with a Teflon™ faced cap. Place extracts in a refrigerator for storage until analysis or cleanup, if necessary.

7.10 When ready for analysis, transfer extract to labeled GC vials and cap.

8.0 Cleanup Procedure

8.1 Cleanup of dirty samples may be necessary due to interferences in the analysis of baseline or co-elution with target analytes of the sample extract. Follow the in-house SOP for Florisil® column or GPC method, as needed.

9.0 Analytical Procedure

9.1 The final extract will be analyzed on an Agilent 6890 GC/ECD. Chromatographic conditions for operating the Agilent 6890 GC/ECD are found in Table 2.

9.2 GC acquisition

9.2.1 Analyze a Pesticide Check Solution at the beginning and end of each run to ensure GC performance.

9.2.2 Pour several isooctanes into GC vials using the same lot as used for samples with each GC run.

9.2.3 Pour standard curves into GC vials using 0.5, 1.0, 2.0, 5.0, 10, 20 and 50 ppb OCH Std in isooctane. Pour extra vials of a mid-level concentration for use as CCV (to be analyzed every 20 samples or less).

9.2.4 Create sequence file and sequence table on computer. Use the WPCL login number for “Data Subdirectory” and “Save As” sequence name.

9.2.5 Acquire data and recap each vial daily to preserve sample integrity.

9.3 Analysis

9.3.1 Recalibrate OCH curves and analyze samples in external

standard mode. Add a printed chromatogram and report for each standard and sample to folder.

9.3.2 Certain analytes will co-elute on a given column. However, using two columns with different polarities will allow for confirmation of target analytes.

10.0 References

U.S. Environmental Protection Agency, Office of Water, EPA 821-R-92-002, April 1992, Methods For The Determination of Nonconventional Pesticides In Municipal And Industrial Wastewater, p. 227.
Method 622, *The Determination of Organochlorine Pesticides in Municipal and Industrial Wastewater*.

APPENDIX I: Validation of organochlorine compounds in water.

1.0 Scope

A method validation for organochlorine compounds in water was performed using a modified EPA 8081A method, as detailed in this OCH-Water SOP. Recoveries shall be 75-125% with RPE of less than 20 percent. Method detection and reporting limits were determined during the method development phase.

2.0 Setup

Perform nine replicate spiked extractions and analysis following the SOP for water extraction of OCHs. Using 1 liter of DI water, add 1 mL of 10 ppb OCH spike mix and 1 mL of 10 ppb PCB 207 and DBOB (surrogate) to each replicate. Also, prepare a method blank using 1 liter of DI water and 1 mL of 10 ppb OCH surrogates.

Repeat this procedure at the reporting limit (1 ppb Standard) using 5 replicates.

3.0 Results

All recoveries fell within the allowable range.

Determination of Select Organophosphorous Pesticides in Sediment Samples

1.0 Scope and Application

1.1 This is a modified EPA Method 8141B and describes the sample preparation and quantitative analysis of trace level organophosphorous pesticides using an automated extraction system for the determination of trace residue levels of a selected list of organophosphorous pesticides in sediments using high resolution gas chromatography with Flame Photometric Detector (FPD) in phosphorous mode. Table 1 lists the target pesticide compounds currently analyzed with their CAS registry numbers, reporting limits and average percent recoveries for sediments.

Table 1. Organophosphorous pesticides analyzed, their CAS registry number, Reporting Limit (RL) and Average Percent Recovery in sediment, based on 50% moisture.

Target Analytes	CAS Registry No.	RL, ppb ng/g, dry wt.	Ave. Rec (%)*
Chlorpyrifos	2921-88-2	10	72.8
Chlorpyrifos methyl	5598-13-0	50	79.4
Diazinon	333-41-5	10	100
Dichlofenthion	97-17-6	50	92.4
Dioxathion	78-34-2	50	92.4
Ethion	563-12-2	50	99.0
Ethoprop (Prophos)	13194-48-4	50	98.2
Fenchlorphos (Ronnel)	299-84-3	50	79.8
Fenitrothion	122-14-5	50	89.4
Fonofos (Dyfonate)	944-22-9	50	84.5
Malathion	121-75-5	50	82.6
Merphos	150-50-5	50	71.1
Parathion, Ethyl	56-38-2	20	86.7
Parathion, Methyl	298-00-0	20	78.6
Phosphamidon	13171-21-6	50	86.7
Sulfotep	3689-24-5	50	72.6
Thionazin	297-97-2	50	84.5
Tokuthion	34643-46-4	50	85.5
Trichloronate	327-98-0	20	85.1
Triphenyl phosphate(Surrogate)	115-86-6	NA	105

* Recoveries fall within 60-130% accept as discussed in Section 10.3.3.

1.2 These procedures are applicable when low parts per billion analyses are required to monitor differences between burdens in soils and sediment from relatively uncontaminated reference areas and contaminated areas.

1.3 The actual MDL may differ from those listed, depending upon the nature of interferences in the sample matrix. Validation of the target analytes produced recoveries greater than 60 percent for all analytes. Some target compounds are widely accepted as having lower recoveries, as listed in Section 10.3.3.

1.4 If possible, unknowns in the sample will be qualitatively confirmed for compound identification by Gas Chromatography with a Mass Spectrometer – Ion Trap Detector (GC/MS-ITD).

2.0 Summary of Method

2.1 Sets of 12-16 homogenized sediment samples are scheduled for extraction by the project lead chemist. Extraction methods employed were developed and validated by the Water Pollution Control Laboratory (WPCL). Extract cleanup and partitioning methods are modifications of the multi-residue methods for fatty and non-fatty foods described in the U.S. Food and Drug Administration, Pesticide Analytical Manual, Vol. 1, 3rd Edition 1994, Chapter 3, Multi-residue Methods, Section 303-C1.

2.2 Homogenized sediment samples are removed from the freezer and allowed to thaw. A 1-5 g (sediment homogenate) sample is weighed into a pre-weighed aluminum planchet and placed in a 70oC oven for 48 hours to determine moisture content. A 10 g sample is mixed using a clean glass stirring rod with approximately 7 g of pre-extracted Hydromatrix® (Varian Corp) in a 250 mL Trace Clean Wide Mouth Jar until the mixture is free flowing. The mixture is then poured into a 33 mL stainless steel Dionex Accelerated Solvent Extractor (ASE 200) extractor cell and packed by tamping the mixture. A solution containing TPP surrogate is added to the cell and the cap is screwed onto the cell. The extractor cells (maximum of 24) are placed on the ASE 200 autosampler rack and the samples are extracted twice with a 50/50 mixture of acetone/ dichloromethane (DCM) using heat and pressure. The extracts are automatically collected in 60 mL VOA vials.

2.3 The combined extracts are dried using sodium sulfate, evaporated to approximately 0.5-1.0 mL using Kuderna-Danish (K-D) glassware equipped with 3-ball Snyder columns and micro-Snyder apparatus and diluted to 10 mL using DCM. The extracts are then filtered through a 0.45 µm syringe filter into J_2 Scientific AccuPrep 170 (GPC) autosampler tubes.

2.4 The GPC autosampler tubes are then placed on the GPC autosampler for initial sample cleanup.

2.5 The cleaned-up extracts are evaporated using K-D apparatus and solvent exchanged into petroleum ether. The extracts are further cleaned up using Florisil® in a 22 mm x 300 mm column. The solvent rinsed columns are prepared by placing a glass wool plug at the bottom of the tube followed by one half inch of pre-cleaned sodium sulfate, four inches of Florisil® and one inch of sodium sulfate. OP extracts are eluted in one fraction with 50 % diethyl ether/petroleum ether. The collected fraction is concentrated to an appropriate volume using K-D/micro K-D apparatus prior to analysis by dual column high resolution gas chromatography.

3.0 Interferences

3.1 Solvents, reagents, glassware, and other sample processing hardware may cause GC artifacts and/or elevated baselines, resulting in the misinterpretation of chromatograms. All materials should be demonstrated to be free from interferences under the conditions of the analysis by running method blanks initially and with each sample lot. Specific selection of reagents and purification of solvents by distillation in all-glass systems are required. High-purity distilled-in-glass solvents are commercially available.

An effective way of cleaning laboratory glassware is by rinsing with polar and non-polar solvents before use. The cleaning procedure used must be tested by analyzing procedural blanks prior to analyzing samples.

3.2 Phthalates are common laboratory contaminants that are used widely as plasticizers. Sources of phthalate contamination include plastic lab-ware, plastic tubing, plastic gloves, plastic coated glassware clamps, and have been found as a contaminant in Na_2SO_4.

Polytetrafluoroethylene (PTFE) can be used instead of polypropylene or polyethylene to minimize this potential source of contamination. However, use of PTFE lab-ware will not necessarily preclude all phthalate contamination. Na_2SO_4 can be solvent rinsed to eliminate contaminants.

3.3 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source. A Florisil® or GPC cleanup procedure can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL.

4.0 Apparatus and Laboratory Supplies

4.1 Wide mouth, borosilicate glass, pre-cleaned and certified, 250 mL, Qorpak or equivalent.

4.2 Chromatographic Column - 22 mm x 300 mm, borosilicate glass chromatography column with a Teflon stopcock.

4.3 Glass wool, Pyrex - solvent washed prior to use.

4.4 Kuderna Danish (K D) Apparatus

4.4.1 Concentrator tube - 10 mL, graduate (Kontes K0570050 1025, or equivalent). A ground stopper, 19/22 joint, is used to prevent evaporation of extracts.

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

4.4.3 Snyder column - three ball (Kontes K 503000 0121, or equivalent).

4.4.4 Micro-Snyder column - (Kontes VWR KT569261-0319 or equivalent).

4.4.5 Boiling stones - Chemware® Ultra-Pure PTFE, extracted with acetone and petroleum ether. Note that boiling chips can be a significant source of contamination if not properly cleaned.

4.5 Water bath, Organomation Assoc. Inc.(OA-SYS/S-EVAP-KD), 115 thermostatically controlled with stainless steel cover to fit 5 K-D apparatus, Installed in a fume hood.

4.6 Extractor, automated, Dionex Accelerated Solvent Extractor (ASE 200), Dionex P/N 047046.

4.6.1 Extraction Cells, 33 mL, Dionex P/N 049562

4.6.2 Filters, cellulose for ASE extraction cells, Dionex P/N 049458.

4.6.3 VOA Vials, 60 mL, pre-cleaned and certified.

4.7 Sample vials, glass, 2.5 mL with PTFE lined screw cap.

4.8 Analytical balance capable of weighing 0.1 mg.

4.9 Drying oven.

4.10 Balance capable of 100 g to the nearest 0.01 g.

4.11 Disposable Pasteur Pipettes (rinsed with solvents before use).

4.12 Aluminum dishes for moisture and lipid determination.

4.13 Desiccator with indicating desiccant.

4.14 Glass funnel, 75 mm.

4.15 Graduated cylinder, 250 mL and 100 mL.

4.16 Culture tubes, 13 x 100mm and 16 x 100 mm, with PTFE lined cap.

4.17 Analytical systems

4.17.1 Gas chromatograph. Agilent 6890*plus* equipped with dual FPD detectors with phosphorous filters, split-splitless injector in pulsed splitless mode with EPC, a 7683 autosampler and dual capillary columns (J&W Scientific) connected to a single injection port using a "Y" press fit connector. Section 10 describes the acquisition and analysis procedures while Table 2 lists the operating parameters.

4.17.2 Data System. Agilent, to collect and record GC data, generates reports, computes and records response factors for multi-level calibrations. Data system should be capable of calibrating a method using a minimum of 5 concentrations of analytical standards and calculating in external standard mode.

4.18 Homogenizer, Brinkman Polytron or equivalent equipped Teflon and titanium generator assembly (for homogenization of small sample amounts).

4.19 Homogenizer, Bucchi Model B-400 (Brinkman P/N 16-07-200-1) or equivalent equipped with titanium knife assembly (Brinkman P/N 16-07-222-2) and glass sample vessel (Brinkman P/N 16-07-245-1).

4.20 Gel Permeation (size exclusion) Chromatograph, automated, J_2 Scientific AccuPrep 170, equipped with 70 g S-X3 BioBeads J_2 Scientific P/N C0070G (100% DCM).

Table 2. Operating parameters for Agilent 6890 GC/FPD

Gases
- Carrier: Helium, 1 mL/min
- Makeup: Nitrogen, 1 mL/min
- Flame: Air and Hydrogen

Columns
- DB5, 30 m x 0.25 mm I.D. x 0.25 µm film thickness
- DB17MS, 30 m x 0.25 mm I.D. x 0.25 µm film thickness

Inlet Isocratic temp: 200 ºC

Oven
- Initial temperature: 90 ºC Initial time: 1.00 min
- Ramp 1: 8.0 deg/min, final temp 220 ºC, hold time 5.00 min
- Ramp 2: 20.0 deg/min, final temp 250 ºC, hold time 13.00 min

Detectors (FPD)
- Temperature: 225 ºC

Injection Volume: 3 µL

5.0 Reagents, materials, gases and standards

5.1 Petroleum ether (PE), Burdick and Jackson, distilled in glass and pesticide residue or HRGC grade or equivalent.

5.2 Acetone. (Same as above).

5.3 Iso-octane. (Same as above).

5.4 Diethyl ether. (Same as above).

5.5 Dichloromethane (DCM). (Same as above).

5.6 Chem Elut-Hydromatrix®, Varian P/N 0019-8003. Pre-extracted on ASE-200 with acetone/DCM prior to use.

5.7 Sodium sulfate. Anhydrous granular reagent grade, rinsed with PE prior to use.

5.8 Florisil® , 60/100 mesh, PR grade, U.S. Silica.

5.9 Nitrogen, pre-purified grade (99.9999%) or better (used for ASE and GPC.)

5.10 Air, compressed, breathing quality, for ASE pneumatics and GC/FPD

5.11 Nitrogen. Ultra-pure (99.99999%) for GC/FPD
5.12 Helium. Ultra-pure (99.99999%) for GC/FPD

5.13 Hydrogen. Ultra high purity for GC/FPD

5.14 Organophosphorous Surrogate Solution containing 800 ppb of triphenyl phosphate (TPP) obtained from AccuStandard and Chem Service.

5.15 Stock standards. Individual stock standards (100 µg/mL) are purchased as certified solutions from ChemService as well as premixed solutions of 8140 and 8141A. Additional compounds analyzed are prepared as WPCL solution "OP Mix C".

5.16 Organophosphorous Instrument Calibration Standards: Individual compounds obtained from AccuStandard and Chem Service (see 5.14) are mixed in iso-octane with concentrations ranging from 10.0 ppb to 500 ppb.

5.16.1 Second Source Standards: Organophosphorous analytes were obtained from AccuStandard, New Haven, CT for verification of calibration standards.

CAUTION

The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of data handling Material Safety Data Sheets should also be made available to all personnel involved in these analyses.

6.0 Sample Collection and Storage

6.1 In the field, sources of contamination include sampling gear, grease from ship winches or cables, ship and/or motor vehicle engine exhaust, dust, and ice used for cooling. Efforts should be made to minimize handling and to avoid sources of contamination.

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

6.3 Sediment samples may be refrigerated at 4°C for up to 14-days maximum or must be stored frozen at minus (-) 20°C for up to 12 months maximum.

7.0 Sample Extraction

7.1 Frozen homogenized sediment samples are removed from the freezer and allowed to thaw. Prior to extraction, sediment samples are thoroughly mixed by hand using a clean glass rod or may be homogenized using a Polytron homogenizer equipped with stainless steel generator equipped with Teflon bearings. Sample sets of 12-16 should be extracted when possible. The ASE-200 extractor will extract 24 cells. Be sure to reserve enough cells for method blanks, matrix spikes, and laboratory control spikes.

7.2 Prepare a glass rod or Teflon spatula for each sample to be weighed by rinsing 3 times with petroleum ether using a Teflon wash bottle.

7.3 Label 60 mL VOA vials for the collection of the sample extract. The labels must be placed between 1.5" and 3" from the top of the VOA cap; if they are placed outside of this area, they will interfere with the ASE optical sensor. Use two VOA vials for each sample. Label the first VOA vial with the ASE position number, bench sheet number and the sample name. Label the second VOA vial the same but add “RE” to distinguish between the two vials. Label and weigh aluminum planchets for moisture determinations (samples ID can be made on the bottom of planchets using a ball point pen.

7.4 Tare a 250 mL glass jar. Using a clean (solvent rinsed) glass rod, stir the sediment so that the mixture is homogeneous. Weigh 10 g of sample into the jar, record the weight on the bench sheet, and add the twice-extracted Hydromatrix® from one ASE cell. Stir the mixture thoroughly and go on to the next sample. After approximately 15 minutes stir the sample again. Repeat this at 15 minute intervals two more times or until the sample mixture is free flowing.

7.5 Weigh 1-5 g of additional sample into a pre-weighed and tared aluminum planchet for % moisture analysis. Place planchets in 70°C oven for 48

hours and re-weigh dry weight.

7.6 Place a pre-rinsed powder funnel on top of a 33 mL ASE cell containing a pre-extracted cellulose filter (the filter is the one that was used to pre-extract the Hydromatrix®.

7.7 Pour the sediment/Hydromatrix® mixture through the powder funnel back into the extraction cell that the Hydromatrix® was poured from. Tap the cell against the counter top to settle the contents. The mixture will fill the cell and it may be necessary to pack it slightly using the glass rod and the end of the powder funnel. The cells used for the method blank and laboratory control spike and its duplicate (if used) will contain only Hydromatrix®.

7.8 All of the extraction cells are spiked with the pesticide surrogate standard. Spike each cell with exactly 1.0 mL of the OP surrogate solution (800 µg/L). Surrogate spikes must be witnessed, recorded and dated on the extraction bench sheet.

7.9 The extraction cells used for the matrix spike (MS) and duplicate matrix spike (MSD) and laboratory control spike (LCD) and its duplicate (LCSD) (if used) are spiked with exactly 1.0 mL of the OP matrix spike solution (20 ng/mL). Matrix spikes must be witnessed, recorded and dated on the extraction bench sheet.

7.10 The extraction cells are capped (firmly tightened but do not over tighten) and placed on the ASE 200 carrousel. The first set of labeled VOA collection vials are placed on the ASE 200 collection carrousel with the position numbers corresponding to the position numbers of the extraction cells. Make sure that the solvent reservoir contains enough solvent for the extraction.

7.11 Samples are extracted with acetone/methylene chloride (DCM) 50:50 using the following conditions:

Pre-heat	0 min.
Heat	5 min.
Static	5 min.
Flush	60%
Purge	300 sec.
Cycles	1
Pressure	1500 psi
Temp	100 ^{o}C
Sol A Other	100%

7.12 After the initial extraction is complete, remove full VOA vials and place in a Wheaton rack. Place the second set of collection VOA vials labeled “RE”

on the ASE carrousel. Check each of the extraction cells to make sure that the caps are (firmly tightened) as they tend to loosen with the first extraction. Make sure that the replacement vials are in the correct order. Make sure that the solvent reservoir contains enough solvent for the re-extraction. Re-start the ASE-200.

7.13 When extraction is completed, place VOA vials in a Wheaton rack with the "RE" vials next to the vials from the first extraction. The extracts should be re-capped with solid green caps (Qorpak) and placed in a refrigerator for storage until they are removed for the GPC cleanup procedure.

8.0 Gel Permeation Chromatography

IMPORTANT: ***All glassware, glass wool, and sodium sulfate must be triple-rinsed with petroleum ether before they are used for this procedure.***

8.1 Remove VOA vials containing the sample extracts from the refrigerator. Make sure the vials are capped with the green Qorpak caps. Allow them to sit out until they are at room temperature.

8.2 Set up and label pre-cleaned K-D flasks (4-6) with concentrator tubes attached on ring stands in the fume hood. Place a funnel containing a plug of pre-cleaned glass wool in the bottom of the funnel and place the funnel in the top of the K-D flask. Add about two inches of pre-rinsed sodium sulfate to the funnel. Make sure that the level of the sodium sulfate is uniform across the funnel to prevent any possible splashing out.

8.3 Pour sample extracts from the VOA vials through sodium sulfate into the K-D flask. Add about 10 mL of DCM to the VOA vial, cap and shake and add this rinse to the sodium sulfate. Repeat with another 10 mL DCM rinse. Rinse the sodium sulfate with an additional portion of DCM (~50 mL) by pouring from a clean and rinsed 400 mL beaker. After the solvent has completely drained through the sodium sulfate add one more additional rinse of DCM (~50 mL) from the beaker of clean DCM. Allow the DCM to completely drain through the sodium sulfate (~3-5 minutes).

8.4 Add 0.5 mL Iso-Octane using a macro-pipetter and a solvent rinsed boiling chip to each K-D flask. Place a Snyder column on the K-D flask and place the flask on the hot water bath set at 80-82°C. Evaporate the solvent until the reflux line falls below the top of the Snyder column. At this point there should be between 1-5 mL visible in the concentrator tube while the K-D apparatus is still on the hot water bath and 10 mL or less of the solvent remaining after the K-D flask is removed from the hot water bath and the solvent drains from the Snyder column. Dry off the water using a WyPall X60 towel to remove any water from around the ground glass union of the concentrator tube and the K-

D flask to prevent any of it from entering the concentrator tube upon removal.

8.5 After the K-D apparatus has cooled and all of the solvent has drained from the Snyder column, remove the Snyder column, label the concentrator tube and then remove the concentrator tube from the flask and place the tube in a test tube rack and cover with pre-rinsed aluminum foil. Rinse the Snyder column with petroleum ether and place back in the column rack for storage. After all of the flasks have been removed from the hot water bath, repeat steps 2-5 for the remaining samples extracted with this set.

8.6 Add a new micro-boiling stone and place a clean micro-Snyder column on the concentrator tube with a blue clamp and place in a 400 mL beaker containing hot water heated to approximately 75°C on a hot plate. If the solvent does not begin to boil, remove the tube from the bath immediately, allow it to cool slightly, add a new micro boiling stone to prevent it from bumping and place it back in the bath. Evaporate the solvent until only 0.5 mL remains in the concentrator tube. Four or five tubes can be evaporated at one time.

8.7 When the solvent has been evaporated to 0.5-1.0 mL remove the tube from the bath and allow it to cool in a test tube rack. Remove the micro-Snyder column and add DCM to the concentrator tube to reach a final volume of 10.0 mL.

8.8 Draw the sample up into a clean 10 mL syringe with a 0.45 µm filter attached. Filter the sample into a 12 mL culture tube. Cap the culture tube with the Teflon-insert style caps. Mark the bottom of the meniscus with a pen in case of evaporation before clean-up on GPC.

8.9 All samples are cleaned using a J_2 Scientific GPC (Autoinject 110, AccuPrep 170, DFW-20 Fixed Wavelength Detector, 1” ID glass column with 70g Bio-Beads SX-3 in 100% DCM)

8.9.1 From the desktop double click on the AccuPrep.exe shortcut to open the program. Click on the Use Injector button and allow the instrument time to initialize. Activate the pump by using the top left hand button. A solvent Control Pump window will open up. Click on the Apply Defaults button and then OK on the Selected Pressure Limit 30 psi. The pump should audibly be heard coming on and the green light should show that the system is on line and status flowing. Make sure that the bottle of clean DCM is full and the waste bottle is empty. Allow the system to pump for about 5 minutes before switching the column in-line (gray button next to Column that has ‘Put in line’ on it). The pressure will be observed to normally go up to the 12-16 psi range. Turn the power on to the detector to allow it at least 30 minutes of time to warm up before use. Because the scale is auto-adjusted in the software now it is no longer necessary to manually adjust the range on the unit itself.

8.9.2 While the system is equilibrating, the sequence can be entered. Click on the Seq button next to the Pump button. An 'Editing new sequence' window will pop up. This gives a view of the instrument which clearly shows the sample tray locations and the corresponding sample collection locations. By clicking on the sample tray position, a new window 'Adding sample at tray position #' will pop up. This allows information to be included about each specific sample. Sample position 1 will always be a calibration standard (CLP-340) which is run prior to any sequence of runs to verify instrument integrity. In the Sample ID field just type in 'CLP-340'. In the Descrip (optional), information pertaining to the project, laboratory control number, bench sheet number and date are typically added. The Method File needs to be changed to 'GPC-Sed' for only this sample and in the Sample Type field the 'Calibration' type can be chosen. After this information is completed click on the OK to continue. This returns you back to the main sequence window but now the first position will be highlighted in green. Continue by adding the next sample information to tray position 2, again following the same steps as before. The Method File name to be used is 'Sed-Pest'. Also by default, the Sample Type field will already be set at 'Sample'. This will not need to be changed until a duplicate sample (Duplicate), matrix spike (Matrix Spike), matrix spike duplicate (Spike Duplicate) and laboratory control spike (Spiked Blank) are encountered. After all the samples have been added to the sequence, save it as the WPCL log-in number (L-###-##). From the Editing sequence window print out the sample list. Compare the information to your original bench sheet to insure there are no mistakes. Make sure the GPC-Sed method is being used for the calibration standard and 'Sed-Pest' method is being used for the samples. Next verify that the samples are still at the marked line on the culture tubes (add DCM to the marked line if they are not). Place a tube with the GPC Calibration Standard Solution (CLP-340) in sample tray position 1 and then follow as the sequence was made in the remaining positions.

8.9.3 Get two boxes of the 125 mL Trace Clean amber bottles for sample collection. A bottle does not need to be placed in collection position #1 because that is the GPC Calibration Std (all goes to waste). Remove the white caps from the bottles and place them on top of the detector (so that Teflon side is not exposed to possible contamination). Label the boxes with laboratory control numbers and keep them for the post-GPC samples to be stored in. Now that the pump has had plenty of time to equilibrate the system and the detector has had plenty of time to warm up, in the Signal field click to adjust the setting to 'Absorbance Units' and click on the 'Zero Signal' button to set the baseline.

8.9.4 If the pressure seems to be pretty stable between the 12-16 psi range and all the samples positions and collection positions have been loaded, then click on the large button with the stop watch to begin the

program. A window will pop up asking if the correct column method is loaded (100%DCM). Click on 'yes' to engage the syringe pump to begin priming. The sample probe will move over to sample position #1 and aspirate the sample. After the samples have all been processed (~1 hour per sample), remove the label from the sample position and place it on the bottle in corresponding collection position. Cap the bottle and place it back in the box that was retained for their storage. At the end of the sequence there will be a window that pops up saying that the 'Sequence has been successfully completed'. The column will switch offline and the pump will automatically shut down. The only thing that has to manually be turned off is the power to the detector.

8.10 Pour the GPC eluate into a rinsed K-D flask. Rinse the bottle with some DCM and add that to the K-D flask. Add 0.5 mL Iso-Octane and a micro boiling chip to each K-D flask. Attach a Snyder column to the flask and place in the hot water bath. When the volume of the solvent in the concentrator tube is level with the base of the K-D flask, lift the K-D apparatus up enough to be able to angle it slightly and add 40-50 mL Petroleum Ether through the top of the Snyder column. By holding the K-D apparatus at an angle, it allows the solvent to more easily drain back into the flask. Return to the K-D apparatus back into the hot water bath. Repeat this step 2 more times to successfully solvent exchange the sample from DCM to Petroleum Ether. When the apparent volume in the concentrator tube is 5-10 mL remove it from the hot water bath. Wipe down the K-D apparatus with a WyPall X60 towel especially around the ground glass junction. Remove the Snyder column from the K-D apparatus and allow to completely drain into the concentrator tube. After it has finished cooling, remove the concentrator tube and place a micro-Snyder column on it. Add a new micro boiling chip and place it in a 400 mL beaker containing water heated to approximately 75°C on a hot plate (4-5 tubes can be evaporated at one time). Evaporate the solvent down to 1-2 mL. Remove it from the water bath and allow it to cool.

8.11 Transfer the solution to a 13 x 100 culture tube with a Pasteur pipette; rinse the concentrator tube with 0.5 ml of Petroleum Ether, vortex, and transfer the rinse to the culture tube. Repeat the rinse step two more times, and add each rinse to the culture tube. Cap the culture tube with a Teflon faced cap. Place extracts in a refrigerator for storage until the final Florisil® column cleanup is done.

9.0 Florisil® Column Fractionation

IMPORTANT: ***All glassware, glass wool, and sodium sulfate must be triple-rinsed with petroleum ether (PE) before they are used for this procedure. Florisil® must be activated in an oven at 130°C for at least 24 hours prior to use.***

9.1 This procedure is performed after the GPC cleanup procedure for all sediment samples analyzed for organophosphorous pesticides.

9.2 Prepare the reagent to be used for Florisil® cleanup: 50% ethyl ether in petroleum ether. Make an amount slightly in excess of what is actually needed to allow for any loss which may occur during solvent transfer. The required volume is 280 mL per sample.

9.3 Prepare the chromatography columns. Place a small piece of PE rinsed glass wool in the bottom of the 22 x 300 mm column and tap into place with a PE rinsed glass rod. Cover with a small portion (0.5 inch) of sodium sulfate. Fill the column with four inches of Florisil®. Tap column with rubber "mallet" to firmly settle the Florisil®. Top the column with 3/4-1 inch of sodium sulfate. This will prevent the column from being disrupted when solvent is added and will remove any residual water.

9.4 Place a 600 mL beaker under the column and pre-wet the column with about 25 mL of petroleum ether.

IMPORTANT: From this point and through the elution process, the solvent level should never be allowed to go below the top of the sodium sulfate layer.

9.5 When approximately 1 inch of PE remains above the surface of the column, place a K-D flask under column making sure that the stopcock is in the full open position. This will allow for a flow rate of about 2 to 3 mL/min. When the meniscus of the PE rinse reaches the column bed surface, decant the sample onto the column. Immediately add approximately 0.5 mL of PE to the tube, vortex, and add the rinse to the sample extract on the column. Add another 0.5 ml of PE to the tube, vortex, and add this final rinse to the sample extract on the column. Start the columns in a sequential fashion, and the lag time will be adequate to perform the necessary tasks for up to six columns.

9.6 When the combined sample and rinses reach the sodium sulfate layer, add 280 mL of 50% ethyl ether/PE that has been carefully measured out using a graduated cylinder to the column. Make sure that the stopcock is fully open in order to achieve the desired flow rate of 5 mL per minute.

9.7 After all of the solvent drains through the column, add a micro boiling stone and attach a Snyder column with a green clamp to the K-D flask and place vessel in the hot water bath with the temperature set at 80-82°C and reduce volume to an apparent volume of 1 mL. Tap the Snyder column to make sure solvent is not trapped between the balls then remove the vessel from the bath and place in the vessel stand to cool.

9.8 When the vessels are cool, remove the concentrator tube from the K-D flask

add a new micro boiling stone and attach a clean micro-Snyder column to the concentrator tube with a blue clamp and place in a 400 mL beaker containing hot water heated to approximately 75°C on a hot plate. Evaporate the solvent until only 0.5-1 mL remains in the concentrator tube. Four or five tubes can be evaporated at one time.

9.9 When the solvent has been evaporated to 0.5-1 mL remove the tube from the bath and allow it to cool in a test tube rack. Remove the micro-Snyder column and add iso-octane to the concentrator tube to reach a final volume of 2.0 mL. Mix the tube contents by tapping the bottom of the tube causing a vortex which will rinse the sides of the tube. A Vortex Genie mixer may be used for this step. Transfer the extract to a labeled GC vial. The extracts are ready for analysis by GC-FPD.

10.0 Analytical Procedure

10.1 The final extract will be analyzed on an Agilent 6890*plus* GC/FPD. The operating parameters are listed in Table 3.

10.2 GC acquisition

10.2.1 Pour several iso-octanes into GC vials using the same lot as used for samples with each GC run.

10.2.2 Pour standard curves into GC vials using 10, 20, 50, 100, 200 and 500 ppb in isooctane. Pour extra vials of a midlevel concentration for use as CCV (to be analyzed every 20 samples or less).

10.2.3 Create sequence file and sequence table on computer. Use the WPCL login number for “Data Subdirectory” and “Save As” sequence name.

10.2.4 Acquire data and recap each vial daily to preserve sample integrity.

10.3 Analysis

10.3.1 Recalibrate OP curves and analyze samples in external standard mode. Add a printed chromatogram and report for each standard and sample to folder.

10.3.2 Certain analytes will co-elute on a given column. However, using two columns with different polarities will allow for confirmation of target analytes.

10.3.3 EPA Method 8141B cites the following common analytical difficulties encountered for target analytes:

10.3.3.1 Dioxathion is a single-component pesticide. However, several extra peaks are observed in the chromatograms of standards. These peaks appear to be the result of spontaneous oxygen-sulfur isomerization.

10.3.3.2 Merphos (tributyl phosphorotrithioite) is a single-component pesticide that is readily oxidized to its phosphorotrithioate (merphos oxone). Chromatographic analysis of Merphos almost always results in two peaks (unoxidized merphos elutes first). As the relative amounts of oxidation of the sample and the standard are probably different, quantitation based on the sum of both peaks may be most appropriate.

10.3.3.3 Many analytes will degrade on reactive sites in the chromatographic system. Analysts must ensure that injectors and splitters are free from contamination and are silanized. Columns should be installed and maintained properly.

10.3.3.4 Performance of chromatographic systems will degrade with time. Column resolution, analyte breakdown and baselines may be improved by column washing. Oxidation of columns is not reversible.

11.0 References

U.S. Environmental Protection Agency, Office of Solid Waste, SW-846 On-Line:
Method 3545A, *Pressurized Fluid Extraction*, Revision 1, February 2007, http://www.epa.gov/epawaste/hazard/testmethods/sw846/pdfs/3545a.pdf [11/10/08]
Method 3620C, *Florisil Cleanup*, Revision 3, February 2007, http://www.epa.gov/epawaste/hazard/testmethods/sw846/pdfs/3620c.pdf [11/10/08]
Method 3640A, *Gel Permeation Cleanup*, Revision 1, September 1994, http://www.epa.gov/epaoswer/hazwaste/test/pdfs/3640a.pdf [03/29/07]
Method 8141B, *Organophosphorous Compounds by Gas Chromatography*, Revision 2, February 2007, http://www.epa.gov/epawaste/hazard/testmethods/sw846/pdfs/8141b.pdf [11/10/08]

SOP Section Approval: _____ Date: _____

SOP Final Approval: _____ Date: _____

SOP QA Officer Approval: _____ Date: _____

Determination of Organophosphorous Pesticides in Water Samples

1.0 Scope and Application

1.1 This is a modified EPA Method 8141A and describes the sample preparation and quantitative analysis of trace level organophosphorous pesticides in surface, municipal and wastewater using liquid-liquid extraction and high resolution gas chromatography with Flame Photometric Detector (FPD) in phosphorous mode and Thermionic Bead Specific Detector (TSD). The following target analytes can be determined by this method:

Target Analyte	CAS Registry No.
Aspon	3244-90-4
Azinphos ethyl	2642-71-9
Azinphos methyl	86-50-0
Bolstar (Sulprofos)	35400-43-2
Carbophenothion	786-19-6
Chlorfenvinphos	470-90-6
Chlorpyrifos	2921-88-2
Chlorpyrifos methyl	5598-13-0
Ciodrin (Crotoxyphos)	7700-17-6
Coumaphos	56-72-4
Demeton-s	126-75-0
Diazinon	333-41-5
Dichlofenthion	97-17-6
Dichlorvos	62-73-7
Dicrotophos	141-66-2
Dimethoate	60-51-5
Dioxathion	78-34-2
Disulfoton	298-04-4
Ethion	563-12-2
Ethoprop	13194-48-4
Famphur	52-85-7
Fenchlorphos (Ronnel)	299-84-3
Fenitrothion	122-14-5
Fensulfothion	115-90-2

Fenthion	55-38-9
Fonofos (Dyfonate)	944-22-9
Leptophos	21609-90-5
Malathion	121-75-5
Merphos	150-50-5
Methidathion	950-37-8
Mevinphos (Phosdrin)	7786-34-7
Molinate	2212-67-1
Naled (Dibrom)	300-76-5
Parathion, Ethyl	56-38-2
Parathion, Methyl	298-00-0
Phorate	298-02-2
Phosmet	732-11-6
Phosphamidon	13171-21-6
Sulfotep	3689-24-5
Terbufos	13071-79-9
Tetrachlorvinphos	22248-79-9
Thiobencarb	28249-77-6
Thionazin	297-97-2
Tokuthion	34643-46-4
Trichlorfon	52-68-6
Trichloronate	327-98-0
Triphenyl phosphate (surrogate)	115-86-6

1.2 The estimated detection limit for each analyte is listed in Table 1. The actual MDL may differ from those listed, depending upon the nature of interferences in the sample matrix. Validation of the target analytes produced recoveries greater than 70 percent for most analytes. Some target compounds are widely accepted as having lower recoveries, as listed in Section 9.3.3. The range of percent recoveries for each analyte is also included in Table 1.

1.3 If possible, unknowns in the sample will be qualitatively confirmed for compound identification by Gas Chromatography with a Mass Spectrometer – Ion Trap Detector (GC/MS-ITD).

Table 1. Organophosphorous pesticides analyzed, their Minimum Detection Limits (MDL), Reporting Limits (RL) and Range of Percent Recovery in water.

Target Analytes	MDL (µg/l)	RL (µg/l)	Recovery Range (%)*
Aspon	0.030	0.050	85 – 105
Azinphos ethyl	0.030	0.050	95 – 110
Azinphos methyl (Guthion)	0.030	0.050	50 – 90
Bolstar (Sulprofos)	0.030	0.050	80 – 95
Carbophenothion	0.030	0.050	90 – 100
Chlorfenvinphos	0.030	0.050	80 – 100
Chlorpyrifos	**0.010**	**0.020**	**80 – 100**
Chlorpyrifos methyl	0.020	0.050	95 – 110
Ciodrin (Crotoxyphos)	0.030	0.050	90 – 110
Coumaphos	0.040	0.050	50 – 90
Demeton-s	0.040	0.050	30 – 80
Diazinon	**0.005**	**0.020**	**95 – 110**
Dichlofenthion	0.030	0.050	95 – 105
Dichlorvos	0.030	0.050	85 – 105
Dicrotophos	0.030	0.050	20 – 70
Dimethoate	0.030	0.050	90 – 100
Dioxathion	0.030	0.050	50 – 90
Disulfoton	0.010	0.050	80 – 95
Ethion	0.020	0.050	80 – 105
Ethoprop	0.030	0.050	80 – 100
Famphur	0.030	0.050	90 – 105
Fenchlorphos (Ronnel)	0.030	0.050	90 – 105
Fenitrothion	0.030	0.050	90 – 110
Fensulfothion	0.030	0.050	40 – 80
Fenthion	0.030	0.050	80 – 100
Fonofos (Dyfonate)	0.020	0.050	85 – 110
Leptophos	0.030	0.050	80 – 100
Malathion	0.030	0.050	95 – 105
Merphos	0.030	0.050	85 – 110
Methidathion	0.030	0.050	95 – 105
Mevinphos (Phosdrin)	0.030	0.050	80 – 90
Molinate	0.100	0.200	65 – 100
Naled (Dibrom)	0.030	0.050	40 – 80
Parathion, Ethyl	0.030	0.050	85 – 110

Parathion, Methyl	0.010	0.050	90 – 105
Phorate	0.030	0.050	80 – 95
Phosmet	0.030	0.050	80 – 100
Phosphamidon	0.030	0.050	85 – 100
Sulfotep	0.030	0.050	95 – 110
Terbufos	0.030	0.050	85 – 100
Tetrachlorvinphos	0.030	0.050	80 – 105
Thiobencarb	0.100	0.200	90 – 110
Thionazin	0.040	0.050	95 – 110
Tokuthion	0.030	0.050	85 – 105
Trichlorfon	0.030	0.050	90 – 115
Trichloronate	0.030	0.050	80 – 105
Triphenyl phosphate (surrogate)	0.030	0.050	90 – 105

* Recoveries fall within 75-125% accept as discussed in Section 9.3.3.

2.0 Summary of Method

2.1 A measured volume of sample (1000 ml) is extracted with methylene chloride (DCM) using a separatory funnel. The DCM extract is dried with sodium sulfate, evaporated using Kuderna-Danish (K-D) and solvent exchanged into petroleum ether. The extract is concentrated with micro-snyder (micro K-D) apparatus to approximately 1 ml and adjusted to 2.0 ml with iso-octane. The extracts are analyzed by gas chromatography using conditions which permit the separation and measurement of the target analytes in the extracts by FPD and TSD detection.

2.2 Interferences in analyses may be encountered in very dirty samples and cleanup may be needed to aid in the elimination or reduction of these interferences. Florisil column cleanup or Gel Permeation Chromatography (GPC) procedures will be followed.

3.0 Interferences

3.1 Solvents, reagents, glassware, and other sample processing hardware may cause GC artifacts and/or elevated baselines, resulting in the misinterpretation of chromatograms. All materials should be demonstrated to be free from interferences under the conditions of the analysis by running method blanks initially and with each sample lot. Specific selection of reagents and purification of solvents by distillation in all-glass systems are required. High-purity distilled-in-glass solvents are

commercially available.

An effective way of cleaning laboratory glassware is by rinsing with polar and non-polar solvents before use. The cleaning procedure used must be tested by analyzing procedural blanks prior to analyzing samples.

3.2 Phthalates are common laboratory contaminants that are used widely as plasticizers. Sources of phthalate contamination include plastic lab-ware, plastic tubing, plastic gloves, plastic coated glassware clamps, and have been found as a contaminant in Na_2SO_4.

Polytetrafluoroethylene (PTFE) can be used instead of polypropylene or polyethylene to minimize this potential source of contamination. However, use of PTFE lab-ware will not necessarily preclude all phthalate contamination. Na_2SO_4 can be solvent rinsed to eliminate contaminants.

3.3 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source. A Florisil or GPC cleanup procedure can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4.0 Apparatus and Laboratory Supplies

4.1 Separatory funnel. 2000-ml, with TFE-fluorocarbon stopcock, ground glass or TEF stopper.

4.2 Automatic shaker designed to fit 2 liter separatory funnels with rpm and timer controls.

4.3 Beakers. Borosilicate glass, 400 mL

4.4 Chromatographic Column. 300 cm x 22 cm borosilicate glass chromatography column with Teflon stopcock.

4.5 Glass wool. Pyrex - solvent washed prior to use.

4.6 Kuderna-Danish (K-D) Apparatus.

4.6.1 Concentrator tube. 15 mL, graduate (Kontes K0570012-0500, or equivalent). A ground stopper, 19/22 joint, is used to prevent evaporation of extracts.

4.6.2 Evaporation flask. 500 mL (Kontes K-570050-0500, or equivalent),

attached to concentrator tube with blue clamp (Kontes K-662750-0012).

4.6.3 Snyder column. Three ball (Kontes K-503000-0121, or equivalent).

4.6.4 Micro-Snyder column. Alltech 9058 or equivalent.

4.6.5 Boiling chips. Hengar granules, high purity amphoteric alundum - extracted with acetone and petroleum ether. Note that boiling chips can be a significant source of contamination if not properly cleaned.

4.7 Water bath. Blue M, 115 V, thermostatically controlled with stainless steel cover to fit K-D apparatus, installed in a fume hood.

4.8 GC vials. GC autosampler vials, borosilicate glass, 2 mL with PTFE-lined screw cap.

4.9 Analytical balance. Capable of weighing 0.1 mg.

4.10 Drying oven.

4.11 Disposable Pasteur Pipettes. 2 mL, rinsed with solvents before use.

4.12 Glass filter funnel. Fluted, 75 mm or larger.

4.13 Graduated cylinder. 1000 ml, 250 mL and 100 mL.

4.14 Culture tubes. 13 x 100 mm with PTFE lined screw cap.

4.15 Analytical systems

4.15.1 Gas chromatograph. **Agilent 6890** equipped with dual FPD detectors with phosphorous filters, split-splitless injector in pulsed splitless mode with EPC, a **7683** autosampler and dual capillary columns (J&W Scientific) connected to a single injection port using a "Y" press fit connector. Section 9 describes the acquisition and analysis procedures while Table 2 lists the operating parameters.

4.15.2 Gas chromatograph. **Varian 3600**, equipped with dual Thermionic Specific Detectors (TSD), direct and Septum Programmable Injector (SPI), an **8200** autosampler and dual megabore columns (J&W Scientific). Section 9 describes the acquisition and analysis procedures while Table 3 lists the operating parameters.

4.15.3 Data System. Hewlett-Packard, to collect and record GC data, generates reports, computes and records response factors for multi-level

calibrations. Data system should be capable of calibrating a method using a minimum of 5 concentrations of analytical standards and calculating in external standard mode.

Table 2 Operating parameters for Agilent 6890 GC/FPD

Gases
- Carrier: Helium, 1 mL/min
- Makeup: Nitrogen, 1 mL/min
- Flame: Air and Hydrogen

Columns
- DB5, 30 m x 0.32 mm I.D. x 0.25 µm film thickness
- DB17, 30 m x 0.32 mm I.D. x 0.25 µm film thickness

Inlet
- Isocratic temp: 200 ºC

Oven
- Initial temperature: 90 ºC Initial time: 1.00 min
- Ramp 1: 8.0 deg/min, final temp 220 ºC, hold time 5.00 min
- Ramp 2: 20.0 deg/min, final temp 250 ºC, hold time 13.00 min

Detectors (FPD)
- Temperature: 225 ºC

Injection Volume: 3 µL

Table 3 Operating parameters for Varian 3600 GC/TSD

Gases
- Carrier: Helium
- Makeup: Nitrogen
- Flame: Air and Hydrogen

Columns
- DB5, 15 m x 0.53 mm I.D. x 1.5 µm film thickness
- DB17, 15 m x 0.53 mm I.D. x 1.5 µm film thickness

Inlet
- Isocratic temp: 190 ºC

Oven
Initial temperature: 190 ºC Initial time: 3.00 min
Ramp 1: 5.0 deg/min, final temp 250 ºC, hold time 10.00 min

Detectors (TSD)
Temperature: 225 ºC

Injection Volume: 3 µL

5.0 Reagents, materials, gases and standards

5.1 Reagent water is defined as water in which an interferent is not observed at method detection limit of each parameter of interest. Deionized (DI) water was used for method validation and as method blank.

5.2 Petroleum ether (PE), acetone, methylene chloride (DCM), diethyl ether, isooctane. Pesticide residue quality or equivalent.

5.3 Sodium sulfate. Anhydrous granular reagent grade, rinsed with PE prior to use.

5.4 Nitrogen. Ultra-pure (99.99999%) for GC/FPD/TSD

5.5 Helium. Ultra-pure (99.99999%) for GC/FPD/TSD

5.6 Air. Compressed, breathing quality for GC/FPD/TSD

5.7 Hydrogen. Ultra high purity for GC/FPD/TSD

5.8 Stock standards. Individual stock standards (100 µg/ml) are purchased as certified solutions from ChemService as well as premixed solutions of 8140 and 8141A, as shown in Table 4. Additional compounds analyzed are prepared as WPCL solution “OP Mix C”

Table 4 Organophosphorous analyte spiking solutions and standard curves.

EPA 8140 Analytes	EPA 8141A Analytes	OP Mix C Analytes
Azinphos methyl(Guthion)	Aspon	Dimethoate
Bolstar (Sulprofos)	Azinphos ethyl	Malathion
Chlorpyrifos	Carbophenothion	Methidathion
Coumaphos	Chlorfenvinphos	Molinate

Demeton-s
Diazinon
Dichlorvos
Disulfoton
Ethoprop (Prophos)
Fenchlorphos (Ronnel)
Fensulfothion
Fenthion
Merphos
Mevinphos (Phosdrin)
Naled (Dibrom)
Parathion, Methyl
Phorate
Tetrachlorvinphos
Tokuthion
Trichloronate
Chlorpyrifos methyl
Ciodrin (Crotoxyphos)
Dichlofenthion
Dicrotophos
Dioxathion
Ethion
Famphur
Fenitrothion
Fonofos (Dyfonate)
Leptophos
Phosmet (Imidan)
Phosphamidon
Terbufos
Thionazin (Zinophos)
Trichlorfon (Dylox)
Parathion,Ethyl
Sulfotep
Thiobencarb

6.0 Sample Collection, Preservation, and Storage

6.1 Samples are collected in one liter amber glass bottles and iced or refrigerated at 4 °C from time of collection until extraction.

6.2 All samples must be extracted within 7 days and completely analyzed within 40 days of extraction.

7.0 Sample Extraction

7.1 Remove water samples from refrigerator and transfer contents to a pre-cleaned 2-liter separatory funnel. Immediately add 1.0 ml of the 200 ppb OP pesticide surrogate solution to every sample. For Method Blank, add 1.0 ml of the 200 ppb OP pesticide surrogate solution (TPP) to 1 liter DI water. For laboratory control spike (LCS) and matrix spikes (MS/MSD) also add 1.0 ml of 200 ppb OP pesticide spiking solution for each mix (8140, 8141A and Mix C)

7.2 Add 60 ml of methylene chloride (DCM) to the empty bottle, replace the cap and rinse the bottle. Pour the DCM into the separatory funnel and repeat with another 60 mL aliquot of DCM. Extract the sample by shaking the funnel for 5 minutes on the auto-shaker with periodic venting to release excess pressure. Allow organic layer to separate from the water phase for a minimum of 10 minutes. Collect the methylene chloride extract in a 400 ml beaker.

7.3 Add a second 120 ml volume of methylene chloride to the separatory funnel and repeat the extraction procedure a second time, combining the extracts in the beaker.

7.4 Set up and label pre-cleaned K-D flasks with concentrator tubes and attached with a blue clamp on ring stands in the fume hood. Add 0.5 ml iso-octane as “keeper” and a solvent rinsed micro-boiling chip to each K-D concentrator tube. Place a filter funnel containing a plug of pre-cleaned glass wool in the bottom of the funnel and place the funnel in the top of the K-D flask. Add about two inches of solvent rinsed sodium sulfate to the funnel.

7.5 Pour the combined extracts from the beaker through sodium sulfate into the K-D flask. Rinse the beaker with about 10 mL of DCM and add this rinse to the sodium sulfate. Repeat with another 10 mL DCM rinse. Rinse the sodium sulfate with an additional portion of DCM (~10-20 mL).

7.6 Place a Snyder column on the K-D flask, clamp with a green clamp and place the flask on the hot water bath set at 78-82 ^{o}C. Evaporate solvent on the hot water bath. When the apparent volume of solvent in the concentrator tube is 5-10 mL, add 20-30 mL of petroleum ether through the top of the Snyder column. Repeat this procedure when the apparent volume is again at 5-10 mL. When the reflux line falls below the top of the Snyder column, the K-D apparatus should be removed from the hot water bath. Dry outer KD apparatus with a Kimwipe to prevent condensation water from entering the concentrator tube. Upon cooling, remove the concentrator tube from the K-D apparatus.

7.7 Place a clean micro-Snyder column on the concentrator tube with a blue clamp, add a new micro boiling chip and place in a 400 mL beaker containing water heated to approximately 78 ^{o}C on a hot plate. If the solvent does not begin to boil, remove the tube from the bath immediately, allow it to cool slightly, add a new micro boiling stone to prevent it from bumping and place it back in the bath.

7.8 When the solvent has been evaporated to 0.5-1 mL remove the tube from the bath and allow it to cool in a test tube rack. Dry outer KD apparatus with a Kimwipe to prevent condensation water from entering the concentrator tube. Remove the micro-Snyder column and add iso-octane to the concentrator tube to reach a final volume of 2.0 mL. Mix the tube contents by tapping the bottom of the tube causing a vortex which will rinse the sides of the tube. A Vortex Genie mixer may be used for this step.

7.9 Transfer the solution from the concentrator tube to a culture tube and cap with a Teflon faced cap. Place extracts in a refrigerator for storage until analysis or cleanup, if necessary.

7.10 When ready for analysis, transfer extract to labeled GC vials and cap.

8.0 Cleanup Procedure

8.1 Cleanup of dirty samples may be necessary due to interferences in the analysis of baseline or co-elution with target analytes of the sample extract. Follow the in-house SOP for Florisil® column or GPC method, as needed.

9.0 Analytical Procedure

9.1 The final extract will be analyzed on an Agilent 6890 GC/FPD and a Varian 3600 GC/TSD.

9.1.1 Chromatographic conditions for operating the Agilent 6890 GC/FPD are found in Table 2.

9.1.2 Chromatographic conditions for operating the Varian 3600 GC/TSD are found in Table 3.

9.2 GC acquisition

9.2.1 Pour several isooctanes into GC vials using the same lot as used for samples with each GC run.

9.2.2 Pour standard curves into GC vials using 20, 50, 100, 200 and 500 ppb Std 8140 and 8141A and 50, 100, 200 and 500 ppb OP Mix C in isooctane. Pour extra vials of a midlevel concentration for use as CCV (to be analyzed every 20 samples or less).

9.2.3 Create sequence file and sequence table on computer. Use the WPCL login number for “Data Subdirectory” and “Save As” sequence name.

9.2.4 Acquire data and recap each vial daily to preserve sample integrity.

9.3 Analysis

9.3.1 Recalibrate OP curves and analyze samples in external standard mode. Add a printed chromatogram and report for each standard and sample to folder.

9.3.2 Certain analytes will coelute on a given column.

However, using two columns with different polarities will allow for confirmation of target analytes.

9.3.3 EPA Method 8141A cites the following common analytical difficulties encountered for target analytes:

9.3.3.1 The water solubility of Dichlorvos (DDVP) is 10 g/L at 20EC, and recovery is poor from aqueous solution.

9.3.3.2 Naled is converted to Dichlorvos (DDVP) on column by debromination. This reaction may also occur during sample workup. The extent of debromination will depend on the nature of the matrix being analyzed. The analyst must consider the potential for debromination when Naled is to be determined.

9.3.3.3 Trichlorfon rearranges and is dehydrochlorinated in acidic, neutral, or basic media to form Dichlorvos (DDVP) and hydrochloric acid. If this method is to be used for the determination of organophosphates in the presence of Trichlorfon, the analyst should be aware of the possibility of rearrangement to Dichlorvos to prevent misidentification.

9.3.3.4 Demeton (Systox) is a mixture of two compounds; O,O-diethyl O-[2-(ethylthio)ethyl]phosphorothioate (Demeton-O) and O,O-diethyl S-[2-(ethylthio)ethyl]phosphorothioate (Demeton-S). Two peaks are observed in all the chromatograms corresponding to these two isomers. It is recommended that the early eluting compound (Demeton-S) be used for quantitation.

9.3.3.5 Dioxathion is a single-component pesticide. However, several extra peaks are observed in the chromatograms of standards. These peaks appear to be the result of spontaneous oxygen-sulfur isomerization. Because of this, Dioxathion is not included in composite standard mixtures.

9.3.3.6 Merphos (tributyl phosphorotrithioite) is a single-component pesticide that is readily oxidized to its phosphorotrithioate (Merphos oxone). Chromatographic analysis of Merphos almost always results two peaks (unoxidized Merphos elutes first). As the relative amounts of oxidation of

the sample and the standard are probably different, quantitation based on the sum of both peaks may be most appropriate.

9.3.3.7 Many analytes will degrade on reactive sites in the chromatographic system. Analysts must ensure that injectors and splitters are free from contamination and are silanized. Columns should be installed and maintained properly.

9.3.3.8 Performance of chromatographic systems will degrade with time. Column resolution, analyte breakdown and baselines may be improved by column washing. Oxidation of columns is not reversible.

10.0 References

U.S. Environmental Protection Agency, Office of Water, EPA 821-R-92-002, April 1992, Methods For The Determination of Nonconventional Pesticides In Municipal And Industrial Wastewater, p. 227.
Method 622, *The Determination of Organophosphorous Pesticides in Municipal and Industrial Wastewater*.
Method 8141A, *Organophosphorous Compounds by Gas Chromatography: Capillary Column Technique.*

SOP Section Approval: _____ Date: _____

SOP Final Approval: _____ Date: _____

SOP QA Officer Approval: _____ Date: _____

WPCL Operating Procedures for Analysis of Extractable PAH Compounds in Tissue and Sediments

1.0 Scope and Application

1.1 This method describes the sample preparation using an automated extraction system for the determination of trace residue levels of a selected list of polynuclear aromatic hydrocarbons (PAH) in tissue and sediment samples by gas chromatography-mass spectrometry. Table 1 lists the target PAH compounds currently analyzed with their method detection limits and reporting limits for tissues and sediments. Table 2 lists the deuterated PAH compounds used as surrogates for this procedure. Table 3 lists the deuterated PAH compounds used as internal standards for this procedure.

Table 1. Polynuclear aromatic hydrocarbons (PAH) Compounds Analyzed and Their Approximate Dry Weight Reporting Limits (RL) in Tissue and Sediment. These values are based on individual sample size and moisture content and can vary between samples.

	MDL/RL ng/g dry wt.
Naphthalene	5
2-Methylnaphathalene	5
1-Methylnaphthalene	5
2,6-Dimethylnaphthalene	5
2,3,5-Trimethylnaphthalene	5
C1 – Naphthalenes	5
C2 – Naphthalenes	5
C3 – Naphthalenes	5
C4 – Naphthalenes	5
Biphenyl	5
Acenaphthylene	5
Acenaphthene	5
Fluorene	5
1-Methylfluorene	5
C1 – Fluorenes	5
C2 – Fluorenes	5
C3 – Fluorenes	5
Dibenzothiophene	5
4-Methyldibenzothiophene	5
Phenanthrene	5
1-Methylphenanthrene	5
3,5-Dimethylphenanthrene	5
Anthracene	5
C1 - Phenanthrene/Anthracenes	5
C2 - Phenanthrene/Anthracenes	5
C3 - Phenanthrene/Anthracenes	5
C4 - Phenanthrene/Anthracenes	5
Fluoranthene	5

2-Methylfluoranthene	5
Pyrene	5
C1 - Fluoranthene/Pyrenes	5
Benz[a]anthracene	5
Chrysene	5
C1 – Chrysenes	5
C2 – Chrysenes	5
C3 – Chrysenes	5
Benzo[b]fluoranthene	5
Benzo[k]fluoranthene	5
Benzo[e]pyrene	5
Benzo[a]pyrene	5
Perylene	5
Indeno[1,2,3-cd]pyrene	5
Dibenz[a,h]anthracene	5
Benzo[ghi]perylene	5

Table 2. Deuterated polynuclear aromatic hydrocarbons (PAH) compounds used as surrogates for target correction.

Naphthalene-d8
Biphenyl-d10
Acenaphthene-d10
Phenanthrene-d10
Pyrene-d10
Benz[a]anthracene-d12
Perylene-d12
Benzo[ghi]perylene-d12

Table 3. Deuterated polynuclear aromatic hydrocarbons (PAH) compounds used as internal standards for this method.

1-Methylnaphthalene-d10
Acenaphthylene-d8
Fluorene-d10
Dibenzothiophene-d8
Chrysene-d12
Benzo[a]pyrene-d12

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

2.0 Summary of Method

2.1 Sets of 12-16 homogenized tissue samples are scheduled for extraction by the project lead chemist. Extraction methods employed were developed and validated

by the Water Pollution Control Laboratory. Extract cleanup and partitioning methods are modifications of the multi-residue methods for solids described in EPA Method 3500B-3545 from EPA Test Methods for Evaluating Solid Waste Vol. 1B.

2.2 Homogenized tissue and sediment samples are removed from the freezer and allowed to thaw. A separate extraction bench sheet is initiated for each project, sample matrix type, and analysis type.

2.3 A 1-5 g (tissue or sediment homogenate) sample is mixed using a clean glass stirring rod and weighed into a pre-weighed aluminum planchet and placed in a 70°C oven for 48 hours to determine moisture content. A 10 g sample is weighed using a clean glass stirring rod and mixed with approximately 7 g of twice pre-extracted Hydromatrix (Varian Part NO: 0019-8003) in a 250 mL Trace Clean Wide Mouth Jar until the mixture is free flowing. The mixture is then poured into a 33 mL stainless steel Dionex Accelerated Solvent Extractor (ASE 200) extractor cell and packed by tamping the mixture. A solution containing PAH surrogate compounds [Table 2.] is added to the cell and the cap is screwed onto the cell. The extractor cells (maximum of 24) are placed on the ASE 200 autosampler rack and the samples are extracted twice with a 50/50 mixture of acetone-dichloromethane (DCM) using heat and pressure. The extracts are automatically collected in 60 mL VOA vials.

2.4 The extracts are combined and dried using sodium sulfate, 1 mL of iso-octane is added and then they are evaporated to approximately 5 mL using Kuderna-Danish (K-D) glassware equipped with 3-ball Snyder column. The extracts are then evaporated to approximately 1 mL using nitrogen. The extracts are then diluted to 10 mL using DCM. The extracts are filtered through a 0.45 µm syringe filter into J2 Scientific AccuPrep 170 (GPC) autosampler tubes equipped with teflon septum lined caps.

2.5 The GPC autosampler tubes are then placed on the GPC autosampler for initial sample cleanup. All samples are cleaned up using the large (1 inch i.d.) GPC column.

2.6 Two mL of iso-octane “keeper” are added to the cleaned-up extracts. The extracts are evaporated using a K-D apparatus to 5 mL. The extracts are then fractionated using a standard 10 mm x 300 mm small column packed with 1 mL sodium sulfate (drying agent), 2 mL alumina, 4 mL silica and another 1 mL sodium sulfate. The alumina/silica columns are eluted with 1:1 dichloromethane: pentane. The fractions are concentrated to 1 mL using K-D/nitrogen blow down apparatus prior to analysis by gas chromatography/mass spectrometry.

3.0 Interferences

3.1 Solvents, reagents, glassware, and other sample processing hardware may cause GC artifacts and/or elevated baselines, resulting in the misinterpretation of chromatograms. All materials should be demonstrated to be free from interferences under the conditions of the analysis by running method blanks initially and with each sample lot. Specific selection of reagents and purification of solvents by distillation in all-glass systems are required. High-purity, distilled-in-glass solvents are commercially available. An effective way of cleaning laboratory glassware is by rinsing with polar and non-polar solvents before use. The cleaning procedure used must be tested by analyzing procedural blanks prior to analyzing samples.

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

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

4.0 Apparatus and Materials

4.1 Wide mouth, borosilicate glass, pre-cleaned and certified, 250 mL, Qorpak or equivalent.

4.2 Chromatographic Column - 300 mm x 11 mm (o.d.) borosilicate glass chromatography column with reservoir and Teflon stopcock.

4.3 Glass wool, Pyrex - solvent washed prior to use.

4.4 Kuderna-Danish (K-D) Apparatus

4.4.1 Concentrator tube - 10 mL, graduate (Kontes K0570050-1025, or equivalent). A ground stopper, 19/22 joint, is used to prevent evaporation of extracts.

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

4.4.3 Snyder column - three ball (Kontes K-503000-0121, or equivalent).

4.4.4 Micro-Snyder column - (Kontes VWR KT569261-0319 or equivalent).

4.4.5 Boiling chips, Hengar granules, high purity amphoteric alundum – crushed to uniform size [~ 1-2 mm diameter] and muffled in a ceramic dish at 400 °C for 24 hours. Note that boiling chips can be a significant source of contamination if not properly cleaned. Solvent rinsing alone does not eliminate trace level PAH contamination.

4.5 Water bath, Organomation Assoc. Inc.(OA-SYS/S-EVAP-KD), 115 V, thermostatically controlled with stainless steel cover to fit 5 K-D apparatus, installed in a fume hood.

4.6 Nitrogen evaporator/water bath, Organomation Assoc. Inc.(N-EVAP 112), 115 V, thermostatically controlled with stainless steel cover for culture tubes, installed in a fume hood.

4.7 Extractor, automated, Dionex Accelerated Solvent Extractor (ASE 200), Dionex P/N 047046.

4.7.1 Extraction Cells, 33 mL, Dionex P/N 049562

4.7.2 Filters, cellulose for ASE extraction cells, Dionex P/N 049458.

4.7.3 VOA Vials, 60 mL, pre-cleaned and certified.

4.8 Autosampler vials – amber glass, 1.5 mL [12 X 32] with PTFE-lined screw cap.

4.9 Analytical balance - capable of weighing 0.1 mg.

4.10 Drying oven.

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

4.12 Disposable Pasteur Pipets - (rinsed with solvents before use).

4.13 Aluminum dishes for moisture determination.

4.14 Desiccator with indicating desiccant.

4.15 Glass funnel, 75 mm.

4.16 Graduated cylinder, 250 mL and 100 mL.

4.17 Culture tubes, 16 x 100 mm, with PTFE lined cap.

4.18 Gas chromatographs (2), Hewlett-Packard HP 6890 plus, equipped with HP 5973 "Inert Source" Quadrapole mass selective detector, split-splitless injector with EPC (electronic pneumatic control), and autosampler.

4.19 Capillary columns, 60 meter DB5 (J&W Scientific) (0.25 mm I.D. and 25 µm film thickness) connected to a single injection port.

4.20 Data System, Enviroquant Chemstation, to collect and record GC/MS data, generate reports, and compute and record response factors for multi-level calibrations. Data system should be capable of calibrating a method using a minimum of 5 concentrations of analytical standards.

4.21 Homogenizer, Bucchi Model B-400 (Brinkman P/N 16-07-200-1) or equivalent equipped with titanium knife assembly (Brinkman P/N 16-07-222-2) and glass sample vessel (Brinkman P/N 16-07-245-1).

4.22 Homogenizer, Brinkman Polytron or equivalent equipped Teflon and titanium generator assembly (for homogenization of small sample amounts).

4.23 Gel Permeation (size exclusion) Chromatograph, automated, J2 Scientific AccuPrep 170, equipped with a 25 mm (1") i.d. glass column packed with 70 g S-X3 BioBeads. J2 Scientific P/N C0100 (100% DCM).

5.0 Reagents

5.1 Acetone, Burdick and Jackson, distilled in glass and pesticide residue or HRGC grade or equivalent.

5.2 Iso-Octane Burdick and Jackson, distilled in glass and pesticide residue or HRGC grade or equivalent.

5.3 Dichloromethane (DCM). Burdick and Jackson, distilled in glass and pesticide residue or HRGC grade or equivalent..

5.4 Chem Elut-Hydromatrix, Varian P/N 0019-8003. Pre-extracted on ASE-200 with acetone/DCM prior to use.

5.5 Sodium sulfate. Anhydrous granular reagent grade, rinsed with PE prior to use.

EMD, GR ACS.

5.6 Nitrogen, pre-purified grade (99.9999%) or better (used for ASE, GPC and solvent evaporation).

5.7 Helium, ultra-pure (99.9999%) for GC carrier gas.

5.8 Air, compressed, breathing quality, for ASE pneumatics.

5.9 PAH Surrogate Mix, Custom Surrogate mix, Ultra Scientific.

5.10 PAH Standard Mix, Custom Standard mix, Ultra Scientific.

5.11 PAH Internal Standard Mix, Custom Internal Standard Mix, Ultra Scientific.

5.12 Silca Gel, Mallincrodt Silicar, 100-200 mesh for column chromatography.

5.13 Alumina Gel, Sigma for Thin Layer Chromatography, type F-20. 80-200 mesh – muffled in a ceramic dish at 400 °C for 24 hours prior to use. Note that untreated alumnia can be a significant source of trace level PAH contamination.

CAUTION

The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of data handling Material Safety Data Sheets should also be made available to all personnel involved in these analyses.

6.0 Sample Collection, Preparation, and Storage

6.1 In the field, sources of contamination include sampling gear, grease from ship winches or cables, ship and/or motor vehicle engine exhaust, dust, and ice used for cooling. Efforts should be made to minimize handling and to avoid sources of contamination. In the case of tissues, this will usually require that resection (i.e.,

surgical removal) of tissue be performed in a controlled environment (e.g., a laboratory). Tissue samples should be double wrapped in aluminum foil and immediately frozen with dry ice in a covered ice chest. Sediment samples should be placed in wide mouth straight sided glass jars and may be frozen or refrigerated. If frozen, sufficient space should be left in the jar to allow for expansion or the glass will break on freezing. Ice should be in water tight plastic bags for transporting live shellfish.

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

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

6.4 The resected tissue sample should be placed in a clean glass or PTFE container which has been washed with detergent, rinsed twice with tap water, rinsed once with distilled water, rinsed with acetone, and, finally, rinsed with high-purity petroleum ether.

The U.S. EPA has published a guidance document containing specific recommendations regarding holding times and temperatures for tissue samples to be analyzed for semi-volatile organic compounds. The following holding conditions should be observed. Tissue samples should be maintained at $\leq$-20° C and analyzed as soon as possible, but within 12 months of sample receipt.

6.5 Sediment samples (unfrozen) must be extracted within 14 days. Frozen sediment samples should be extracted within 12 months of sample collection.

7.0 Sample Extraction

7.1 A separate extraction bench sheet is started for each project, sample matrix type, and analysis type. Several bench sheets may be used for a single project.

7.2 Prepare one glass rod or Teflon spatula for each sample to be weighed by rinsing 3 times with petroleum ether using a Teflon wash bottle.

7.3 Label 60 mL VOA vials used for the collection of the sample extract. The labels must be placed between 1.5" and 3" from the top of the VOA cap, if they are placed outside of this area they will interfere with the ASE optical sensor. Label two VOA vials for each sample to be extracted. Label vials with the Bench Sheet Number (BS#), the Sample I.D #, and Project Number (L#) or project name. Label the second vial with RE-EXTRACT followed by all of the information listed above. Label and weigh aluminum planchets for moisture determinations (Sample I.D. can be made on the bottom of planchets using a ball point pen.)

7.4 Tare 250 mL glass jar. Using a clean glass rod, stir the tissue or sediment and make sure that water has not separated from the tissue (if water has separated sample must be re-homogenized). Weigh 10 g of tissue or sediment into the jar, record the weight on the bench sheet, and add the Hydromatrix from one ASE cell. Stir the mixture thoroughly and go on to the next sample. After approximately 15 minutes, stir the sample again. Repeat this at 15 minute intervals two more times or until the sample mixture is free flowing.

7.5 Weigh 1-5 g of additional sample into a pre-weighed and tared aluminum planchet for % moisture analysis. Place planchets in 70°C oven for 48 hours and re-weigh dry weight.

7.6 Place a pre-rinsed powder funnel on top of a 33 mL ASE cell containing a pre-extracted cellulose filter *(the filter is the one that was used to pre-extract the Hydromatrix twice)*.

7.8 Pour the tissue or sediment/Hydromatrix mixture through the powder funnel back into the extraction cell that the Hydromatrix was poured from. Tap the cell against the counter top to settle the contents. The mixture will fill the cell and it may be necessary to pack it slightly using the glass rod and the end of the powder funnel. The cells used for the **method blank** and **laboratory control spike** (LCS) and LCS duplicate *(if used)* will contain only Hydromatrix.

7.9 **All** of the extraction cells are spiked with the PAH surrogate standard

[compounds listed in Table 2]. Spike each cell with exactly **1.0 mL** of the PAH surrogate solution (100 ng/mL). Add **1.0 mL Acetone** to **method blank** to match acetone volumes in **laboratory control spikes**. Surrogate spikes must be witnessed, recorded and dated on the extraction bench sheet.

7.10 The extraction cells used for the matrix spike (MS) and duplicate matrix spike (MSD) and laboratory control spike (LCD) and its duplicate (LCSD) (if used) are spiked with exactly **1.0 mL** of the PAH matrix spike solution (100 ng/mL) [compounds listed in Table 1]. Matrix spikes must be witnessed, recorded and dated on the extraction bench sheet.

7.11 The extraction cells are capped (Firmly tightened but do not over-tighten) and placed on the ASE 200 carrousel. The first set of labeled VOA collection vials are placed on the ASE 200 collection carrousel with the position numbers corresponding to the numbers of the extraction cells. Make sure that the solvent reservoir contains enough solvent for the extraction. Also verify that the air and nitrogen gas pressures are above 500 lbs.

7.12 Samples are extracted with acetone/methylene chloride (DCM) 50:50 using the following conditions:

Pre-heat	0 min.
Heat	5 min.
Static	5 min.
Flush	60%
Purge	300 sec.
Cycles	1
Pressure	1500 psi
Temp	100 ^{o}C
Sol A	Other 100%

7.13 After the initial extraction is complete, remove full VOA vials and place in a Nalgene rack and replace collection VOA vials with the vials labeled RE-EXTRACT. Check each of the extraction cells to make sure that the caps are firmly tightened as they tend to loosen with the first extraction. Make sure that the replacement vials are in the correct order. Make sure that the solvent reservoir contains enough solvent for the re-extraction. Re-start the ASE-200.

7.14 When extraction is completed place VOA vials in a Nalgene rack with the RE-EXTRACT vials next to the vials from the first extraction. The extracts should be re-capped with solid green caps (Qorpak) and placed in a refrigerator for storage until they are removed for the GPC cleanup procedure.

8.0 Gel Permeation Chromatography

IMPORTANT: All glassware, glass wool, and sodium sulfate must be triple-rinsed with petroleum ether before they are used for this procedure.

8.1 Remove VOA vials containing the sample extracts from the refrigerator. Make sure the vials are capped with the green Qorpak caps. Allow them to sit out until they are at room temperature.

8.2 Set up and label pre-cleaned K-D flasks (4-6) with concentrator tubes attached on ring stands in the fume hood. Place a funnel containing a plug of pre-cleaned glass wool in the bottom of the funnel and place the funnel in the top of the K-D flask. Add about two inches of pre-rinsed sodium sulfate to the funnel. Make sure that the level of the sodium sulfate is uniform across the funnel to prevent any possible splashing out.

8.3 Pour sample extracts from the VOA vials through sodium sulfate into the K-D flask. Add about 10 mL of DCM to the VOA vial, cap and shake, and add this rinse to the sodium sulfate. Repeat with another 10 mL DCM rinse. Rinse the sodium sulfate with an additional portion of DCM (~50 mL) by poring from a clean and rinsed 400 mL beaker. After the solvent has completely drained through the sodium sulfate add one more additional rinse of DCM (~50 mL) from the beaker of clean DCM. Allow the DCM to completely drain through the sodium sulfate (~3-5 minutes).

8.4 Add 1.0 mL Iso-Octane using a macropipetter and a solvent rinsed boiling chip to each K-D flask. Place a Snyder column on the K-D flask, clamp with a green clamp and place the flask on the hot water bath set at 80-82°C. Drop down the inverted Hopkins condenser from the solvent recovery system and attach it to the top of the Snyder column. Turn the water supply on to the solvent recovery system until the water flow is between 1500-2000 cc/min. Evaporate the solvent until the reflux line falls below the top of the Snyder column. Remove the inverted Hopkins condenser and secure using the set clamps so that it is out of the way. At this point there should be between 1-5 mL visible in the concentrator tube while the K-D apparatus is still on the hot water bath and about 5 mL of the solvent remaining after the K-D flask is removed from the hot water bath and the solvent drains from the Snyder column. Dry off the water using a WyPall X60 towel to remove any water from around the ground glass union of the concentrator tube and the K-D flask to prevent any of it from entering the concentrator tube upon removal.

8.5 After the K-D apparatus has cooled and all of the solvent has drained from the Snyder column, remove the Snyder column, label the concentrator tube and then

remove the concentrator tube from the flask and place the tube in a test tube rack and cover with pre-rinsed glass stoppers. Rinse the Snyder column with petroleum ether and place back in the column rack for storage. After all of the flasks have been removed from the hot water bath, repeat steps 8.2-8.5 for the remaining samples that were extracted with this set.

8.6 Using the N-Evap 112 set to 35 º C with clean disposable pipettes installed, reduce sample volumes in the concentrator tubes to approximately 1 mL. Set the nitrogen pressure at the cylinder at 30 p.s.i. and adjust the regulator on the side of the water bath to 4 L/min.

8.7 When the solvent has been evaporated to 1 mL remove the tube from the bath and allow it to cool in a test tube rack. Add DCM to the concentrator tube to reach a final volume of 10.0 mL.

8.8 Draw the sample up into a clean 10 mL syringe with a 0.45 µm filter attached. Filter the sample into a 12 mL culture tube. Cap the culture tube with the Teflon-insert style caps. Mark the bottom of the meniscus with a pen in case of evaporation before clean-up on GPC.

8.9 All samples are cleaned using a J_2 Scientific GPC (Autoinject 110, AccuPrep 170, DFW-20 Fixed Wavength Detector, 1” ID glass column with 70g Bio-Beads SX-3 in 100% DCM)

8.9.1 From the desktop double click on the AccuPrep.exe shortcut to open the program. Click on the Use Injector button and allow the instrument time to initialize. Activate the pump by using the top left hand button. A solvent Control Pump window will open up. Click on the Apply Defaults button and then OK on the Selected Pressure Limit 30 psi. The pump should audibly be heard coming on and the green light should show that the system is on line and status flowing. Make sure that the bottle of clean DCM is full or at least has more than 5L in it and, the waste bottle is empty. Allow the system to pump for about 5 minutes before switching the column in-line (gray button next to Column that has ‘Put in line’ on it). The pressure will be observed to normally go up to the 12-16 psi range. Turn the power on to the detector to allow it at least 30 minutes of time to warm up before use. Because the scale is auto-adjusted in the software now it is no longer necessary to manually adjust the range on the unit itself.

8.9.2 While the system is equilibrating, the sequence can be entered. Click on the Sequence button next to the Pump button. An ‘Editing new sequence’ window will pop up. This gives a view of the instrument which clearly shows the sample tray locations and the corresponding sample collection

locations. By clicking on the sample tray position, a new window 'Adding sample at tray position #' will pop up. This allows information to be included about each specific sample. Sample position 1 will always be a calibration standard (CLP-340) which is run prior to any sequence of runs to verify instrument integrity. In the Sample ID field just type in 'CLP-340'. In the Descrip (optional), information pertaining to the project, laboratory control number, bench sheet number and date are typically added. The Method File needs to be changed to 'ZGPC Calib' for only this sample and in the Sample Type field the 'Calibration' type can be chosen. After this information is completed click on the OK to continue. This returns you back to the main sequence window but now the first position will be highlighted in green. Continue by adding the next sample information to tray position 2, again following the same steps as before. By default the Method File will be on the program SOPAH which is used for both pesticide (SO) and petroleum (PAH) clean-up. Also by default, the Sample Type field will already be set at 'Sample'. This will not need to be changed until a duplicate sample (Duplicate), matrix spike (Matrix Spike), matrix spike duplicate (Spike Duplicate), laboratory control spike (Spiked Blank), and the SRM (Lab Control Std) are encountered. After all the samples have been added to the sequence, save it as the bench sheet number (BS###). From the Editing sequence window print out the sample list. Compare the information to your original bench sheet to insure there are no mistakes. Make sure the ZGPC method is being used for the calibration standard and the SOPAH method is being used for the samples. Next verify that the samples are still at the marked line on the culture tubes (add DCM to the marked line if they are not). Place a tube with the GPC Calibration Standard Solution (CLP-340) in sample tray position 1 and then follow as the sequence was made in the remaining positions.

8.9.3 Get two boxes of the 125 mL Trace-Clean amber bottles for sample collection. A bottle does not need to be placed in collection position #1 because that is the GPC Calibration Std (all goes to waste). Remove the white caps from the bottles and place them on top of the detector (with the Teflon side not being left exposed to possible contamination). Label the boxes with bench sheet and laboratory control numbers and keep them for the post-GPC samples to be stored in. Now that the pump as had plenty of time to equilibrate the system and the detector has had plenty of time to warm up, in the Signal field click to adjust the setting to 'Absorbance Units' and click on the 'Zero Signal' button to set the baseline.

8.9.4 If the pressure seems to be stable between the 12-16 p.s.i. range and all the samples positions and collection positions have been loaded, then click on the large button with the stop watch to begin the program. A window will pop up asking if the correct column method is loaded (100%DCM). Click

on 'yes' to engage the syringe pump to begin priming. The sample probe will move over to sample position #1 and aspirate the sample. After the samples have all been processed (~1 hour per sample), remove the label from the sample position and place it on the bottle in the corresponding collection position. Cap the bottle and place it back in the box that was retained for their storage. At the end of the sequence there will be a window that pops up saying that the 'Sequence has been successfully completed'. The column will switch offline and the pump will automatically shut down. The only thing that has to manually be turned off is the power to the detector. Empty the waste container into a 4L waste bottle labeled with a hazardous waste label.

8.10 Pour the GPC eluate into a rinsed K-D flask. Rinse the bottle twice with DCM and add that to the K-D flask. Add 2.0 mL Iso-Octane and a micro boiling chip to each K-D flask. Attach a Snyder column to the flask and place in the hot water bath. When the apparent volume in the concentrator tube is 1-5 mL remove it from the hot water bath. Wipe down the K-D apparatus with a WyPall X60 towel especially around the ground glass junction. Remove the Snyder column from the K-D apparatus after allowing it to completely drain into the concentrator tube. After it has finished cooling, adjust the volume with DCM to 5 mL. The sample is ready for columns.

9.0 Silica Gel/Alumina cleanup procedure for all tissue and sediment samples analyzed for Poly Aromatic Hydrocarbons (PAH's).

9.1 **Note: When using the small columns, all rinses, washes and elutions should be preformed with the stopcock wide open [3 mL/min].**

9.2 All glassware, glass wool, and sodium sulfate must be triple-rinsed with dichloromethane (DCM) before they are used for this procedure. Silica gel and alumina must be activated in an oven at 130°C for at least 2 hours prior to use.

IMPORTANT: The solvent level should <u>never</u> be allowed to go below the top of the packing layer.

9.3 Place a glass wool plug in the bottom of the small columns and add 1 cc of sodium sulfate.

9.4 With the stopcock closed add 50 mL **1:1 DCM/pentane (eluent)**. Pour 2 cc of dry alumina into the bulb and rotate to make a slurry. Open the stopcock and allow the slurry to pack onto the column. Rinse with small amounts of eluent until all of the alumina is on the column. Close the stopcock and gently tap the column to insure uniform packing.

9.5 With the stopcock closed add 30 mL **1:1 DCM/pentane (eluent)**. Pour 4 cc of dry silica gel into the bulb and rotate to make a slurry. Open the stopcock and allow the slurry to pack onto the column. Rinse with small amounts of eluent until all of the silica is on the column. Close the stopcock and gently tap the column to insure uniform packing.

9.6 Top off the column with another 1 cc of sodium sulfate.

Note: Prior to adding the GPC sample to the column, the sample must be KD'd to an approximate volume of 5 mL (8.10).

9.7 Place a 200 mL beaker under the column and rinse the column with 20 mL of the 1:1 DCM/pentane eluent. When approximately 1 cm of the rinse solvent remains above the surface of the column, turn the stopcock off.
Discard the 1:1 DCM/pentane wash.

9.8 Place a K-D flask under the column. When the meniscus of the eluent reaches the column bed surface, transfer the sample extract onto the column using a long stemmed pipette. Immediately add about 1 mL of 1:1 DCM/pentane to the concentrator tube, shake vigorously, and set aside.

9.9 When the sample extract reaches the sodium sulfate layer, add the 1:1 DCM/pentane rinse from the concentrator tube and open the stopcock so that the flow is approximately 1 – 2 mL/min to load the sample rinse. Add another 1 mL to the sample tube, shake and immediately add this rinse to the top of the column. Repeat rinse one more time.

9.10 When approximately 1 cm of the 1:1 DCM/pentane rinse solvent remains above the surface of the column, add 30 mL of 1:1 DCM/pentane to the column and begin eluting the sample with the stopcock wide open [flow of 5 mL/min].

9.11 When the column stops dripping into the K-D flask, add a boiling chip to the flask and attach a Snyder column using a green clamp to the K-D flask and place the vessel in the hot water bath with the temperature set at 80-85°C and reduce volume to an apparent volume of 1-5 mL. Tap the Snyder column to make sure solvent is not trapped.

9.12 When the vessels are cool, remove the concentrator tube from the K-D flask being careful to remove any residual moisture from the ground glass joint between the concentrator tube and the K-D flask. Pour the solvent into a 15 mL culture tube that has been pre-calibrated to 1000 µL (1.0 mL). Rinse the concentrator tube into the culture tube twice with DCM.

9.13 Place the culture tube onto the N-EVAP 112 with the water bath set at 35°C and with a new disposable Pasteur pipette above it. Set the nitrogen pressure at the cylinder to 30 p.s.i. and adjust the regulator on the side of the water bath to 4 L/min. Lower the secured pipette to ~1 inch above the level of the solvent in the culture tube and adjust the nitrogen flow at the individual valve above the tube so that the surface of the solvent is slightly "dimpled", but not splashing. Checking frequently and adjusting the height of the pipette to maintain ~1 inch of clearance above the surface of the solvent, allow the level of the solvent to reach **exactly 1.0 mL.** Quantitatively transfer the solvent to a pre-rinsed amber autosampler vial with a small glass disposable pipette. Label and cap the vial. Mark the level of the solvent on the outside of the vial with a permanent marker.

9.14 Prior to analysis, add 10 µL of PAH internal standard mix [compounds listed in Table 3] (10,000 µg/mL) to the sample extract vials.

10.0 Analytical Procedure

10.1 PAH analysis:

10.1.1 Instrumentation:
Agilent 6890 gas chromatograph equipped with a 5973 *Network* "Inert Source" Quadrapole Mass Selective Detector, a split-splitless injector with EPC (electronic pneumatic control), and a 7683 autosampler. One 60 meter DB5-MS (J&W Scientific) (0.25 mm I.D. and 25 µm film thickness) fused silica column is used.

10.1.2 Chromatograph conditions:
The injector is operated isothermal at 275°C in the "Pulsed Splitless" mode with a pressure of 16.9 psi and a total flow of 52.4 mL/min. The injection pulse pressure is set to 30 psi until 0.80 min. The purge flow to split vent is set for 48.5 mL/min @ 1 min. The "Gas Saver" option is used with a setting of 20 mL/min @ 3 min. The oven has an initial temperature of 80°C which is held for 1 minute and then temperature programmed to 200°C at a rate of 10°C/min and held for 0 min. It is then programmed to 210°C at a rate of 3°C/min and is held for 0 min. It is then programmed to 290°C at a rate of 10°C/min and is held for 5 min. It is then programmed to 325°C at a rate of 20°C/min and is held for 11 min. Ultra High Purity Grade Helium is used as the carrier gas at a linear velocity of 24 cm/sec and a flow rate of 0.9 mL/min. All methods are retention time locked.

10.1.3 Sample volume:
Two microliters of both samples and standards are injected via the pulsed splitless method.

10.1.4 Mass Spectrometer Conditions:

The transfer line from the GC to the MS detector is held at 300 ^{o}C. The MS ion source is held at 300 ^{o}C. The MS Quad is held at 180 ^{o}C. The MS detector is operated in the Selected Ion Monitoring [SIM] mode. Table 4 lists the compounds, ions and groups used.

Compounds	Ion	Q Ion	RT	
1-Methylnaphthalene-d10	152		**12.58**	
Naphthalene-d8	136		**10.82**	**Group 1: 7.50 - 13.00**
Naphthalene	128	102	**10.87**	Cycles/sec = 1.23
2-Methylnaphathalene	141	115	**12.44**	Dwell = 100
C1 - Naphthalenes	141	115	**12.44**	
1-Methylnaphthalene	141	115	**12.68**	
Biphenyl-d10	164	80	**13.49**	**Group 2: 13.00 -15.00**
Biphenyl	154	76	**13.55**	Cycles/sec = 1.08
2,6-Dimethylnaphthalene	156	141	**13.97**	Dwell = 100
C2 - Naphthalenes	156	141	**14.17**	
Acenaphthylene-d8	160		**14.66**	
Acenaphthylene	152	76	**14.70**	
Acenaphthene-d10	162	80	**15.09**	**Group 3: 15.00 - 19.00**
Acenaphthene	153		**15.18**	Cycles/sec = 0.98
C3 - Naphthalenes	170	155	**16.10**	Dwell = 100
2,3,5-Trimethylnaphthalene	170	155	**16.30**	
Fluorene-d10	176		**16.63**	
Fluorene	166	82	**16.74**	
C4 - Naphthalenes	170	155	**17.90**	
1-Methylfluorene	180	165	**18.56**	
C1 - Fluorenes	180	165	**18.56**	
Dibenzothiophene-d8	192	160	**19.30**	**Group 4: 19.00 - 20.36**
Dibenzothiophene	184	139	**19.38**	Cycles/sec = 1.08
Phenanthrene-d10	188		**19.70**	
Phenanthrene	178	152	**19.77**	Dwell = 100
Anthracene	178	152	**19.93**	
C2 - Fluorenes	194		**20.26**	
4-Methyldibenzothiophene	198	175	**20.73**	**Group 5: 20.36 - 22.25**
C1 - Dibenzothiophenes	198	175	**20.73**	
C1 - Phenanthrene/Anthracene	192	165	**21.25**	Cycles/sec = 0.96
1-Methylphenanthrene	192	165	**21.65**	Dwell = 100
C3 - Fluorenes	208		**22.20**	Carry over ions: 194,178,212
C2 - Dibenzothiophenes	212		**21.95**	**Group 6: 22.25 - 23.65**
3,6-Dimethylphenanthrene	206	191	**22.57**	Cycles/sec = 1.08
C2 - Phenanthrene/Anthracene	206	191	**22.88**	Dwell = 100
Fluoranthene	202	101	**23.34**	carry over ions: 208,226,220
Pyrene-d10	212	106	**23.92**	**Group 7: 23.65 - 26.50**

Pyrene	202	101	**23.99**	Cycles/sec = 1.53
C3 - Dibenzothiophenes	226		**23.22**	Dwell = 50
C3 - Phenanthrene/Anthracene	220		**24.16**	
2-Methylfluoranthene	216	189	**24.61**	
C1 - Fluoranthene/Pyrenes	216	189	**25.22**	
C4 - Phenanthrene/Anthracene	234		**25.72**	
Benz[a]anthracene-d12	240	120	**27.62**	**Group 8: 26.50 - 31.31**
Benz[a]anthracene	228	226	**27.71**	Cycles/sec = 1.15
Chrysene-d12	240		**27.75**	
Chrysene	228	226	**27.85**	Dwell = Mixed 50/100
C1 - Chrysenes	242		**29.35**	
C2 - Chrysenes	256		**30.99**	Carry over ions: 234
Benzo[b]fluoranthene	252	126	**31.66**	**Group 9: 31.31 - 32.30**
Benzo[k]fluoranthene	252	126	**31.74**	Cycles/sec = 2.15
				Dwell = 100
				Carry over ions: 270,256
Benzo[a]pyrene-d12	264	132	**32.63**	**Group 10: 32.30 - 33.39**
C3 - Chrysenes	270		**32.31**	Cycles/sec =1.72
Benzo[e]pyrene	252	126	**32.54**	Dwell = 100
Benzo[a]pyrene	252	126	**32.72**	
Perylene-d12	264	132	**32.90**	
Perylene	252		**32.98**	
Indeno[1,2,3-cd]pyrene	276	138	**36.70**	**Group 11: 33.39 - 37.26**
Dibenz[a,h]anthracene-d14	292		**36.64**	Cycles/sec = 1.23
Dibenz[a,h]anthracene	278	139	**36.76**	Dwell = 100
				Carry over ions: 270
				Group 12: 37.26 to end of run
Benzo[ghi]perylene-d12	288	144	**37.66**	
Benzo[ghi]perylene	276	138	**37.78**	Cycles/sec = 1.08
				Dwell =100
				Carry over ions: 278

Table 4.

10.1.4 Data processing:
Enviroquant Chemstation, to collect and record GC/MS data, generate reports, and compute and record response factors for multi-level calibrations. Data system should be capable of calibrating a method using a minimum of 5 concentrations of analytical standards.

11.0 References

U.S. Environmental Protection Agency. 1996. EPA Test Methods for Evaluating Solid Waste. Vol 1B. EPA Method 3500B and 3545.

U.S. Environmental Protection Agency. 1993. Guidance For Assessing Chemical Contaminant Data For Use In Fish Advisories, Volume I, Fish Sampling and Analysis. EPA 823-R-93-002. U.S. EPA, Office of Water, Washington D.C.

Section Approval: _____

Final Approval: _____

QA Officer Approval_____

WPCL Operating Procedures for Analysis of Extractable PAH Compounds in Water

1.0 Scope and Application

1.1 This method describes the sample preparation using an automated extraction system for the determination of trace residue levels of a selected list of polynuclear aromatic hydrocarbons (PAH) in water samples by gas chromatography-mass spectrometry. Table 1 lists the target PAH compounds currently analyzed with their method detection limits and reporting limits for water samples. Table 2 lists the deuterated PAH compounds used as surrogates for this procedure. Table 3 lists the deuterated PAH compounds used as internal standards for this procedure.

Table 1. Polynuclear aromatic hydrocarbons (PAH) Compounds Analyzed and Their Approximate Reporting Limits (RL) in Water.

	MDL/RL ng/mL
Naphthalene	0.005
2-Methylnaphathalene	0.005
1-Methylnaphthalene	0.005
2,6-Dimethylnaphthalene	0.005
2,3,5-Trimethylnaphthalene	0.005
C1 – Naphthalenes	0.005
C2 – Naphthalenes	0.005
C3 – Naphthalenes	0.005
C4 – Naphthalenes	0.005
Biphenyl	0.005
Acenaphthylene	0.005
Acenaphthene	0.005
Fluorene	0.005
1-Methylfluorene	0.005
C1 – Fluorenes	0.005
C2 – Fluorenes	0.005
C3 – Fluorenes	0.005
Dibenzothiophene	0.005
4-Methyldibenzothiophene	0.005
Phenanthrene	0.005
1-Methylphenanthrene	0.005
3,5-Dimethylphenanthrene	0.005
Anthracene	0.005
C1 - Phenanthrene/Anthracenes	0.005
C2 - Phenanthrene/Anthracenes	0.005
C3 - Phenanthrene/Anthracenes	0.005
C4 - Phenanthrene/Anthracenes	0.005
Fluoranthene	0.005
2-Methylfluoranthene	0.005
Pyrene	0.005

C1 - Fluoranthene/Pyrenes	0.005
Benz[a]anthracene	0.005
Chrysene	0.005
C1 – Chrysenes	0.005
C2 – Chrysenes	0.005
C3 – Chrysenes	0.005
Benzo[b]fluoranthene	0.005
Benzo[k]fluoranthene	0.005
Benzo[e]pyrene	0.005
Benzo[a]pyrene	0.005
Perylene	0.005
Indeno[1,2,3-cd]pyrene	0.005
Dibenz[a,h]anthracene	0.005
Benzo[ghi]perylene	0.005

Table 2. Deuterated polynuclear aromatic hydrocarbons (PAH) compounds used as surrogates for target correction.

Naphthalene-d8
Biphenyl-d10
Acenaphthene-d10
Phenanthrene-d10
Pyrene-d10
Benz[a]anthracene-d12
Perylene-d12
Benzo[ghi]perylene-d12

Table 3. Deuterated polynuclear aromatic hydrocarbons (PAH) compounds used as internal standards for this method.

1-Methylnaphthalene-d10
Acenaphthylene-d8
Fluorene-d10
Dibenzothiophene-d8
Chrysene-d12
Benzo[a]pyrene-d12

1.2 These procedures are applicable when low parts per billion analyses are required to monitor differences between relatively uncontaminated reference areas and contaminated areas.

2.0 Summary of Method

2.1 Sets of up to 19 1 liter water samples are scheduled for extraction by the project lead chemist. Extraction methods employed were developed and validated by the Water Pollution Control Laboratory and are based on modifications of methods described in EPA Methods 3500B and 3510c from the EPA SW-846.

2.2 A measured volume of sample, usually 1 liter, is serially extracted with 60 mL

aliquots of methylene chloride [3 times] using a separatory funnel and an automated water extraction apparatus.

2.3 The extract is dried, concentrated to ~5 mL using a Kuderna-Danish (K-D) apparatus with an isooctane keeper, further concentrated to a final volume of 1 mL [in isooctane] using a nitrogen blow down apparatus and analyzed by gas chromatography-mass spectrometry.

2.4 A separate extraction bench sheet is initiated for each project. Each bench sheet prepared has up to 19 samples as well as a method blank, sample/sample duplicate set and a sample/matrix spike, matrix spike duplicate set for quality control/quality assurance.

3.0 Interferences

3.1 Solvents, reagents, glassware, and other sample processing hardware may cause GC artifacts and/or elevated baselines, resulting in the misinterpretation of chromatograms. All materials should be demonstrated to be free from interferences under the conditions of the analysis by running method blanks initially and with each sample lot. Specific selection of reagents and purification of solvents by distillation in all-glass systems are required. High-purity, distilled-in-glass solvents are commercially available. An effective way of cleaning laboratory glassware is by rinsing with polar and non-polar solvents before use. The cleaning procedure used must be tested by analyzing procedural blanks prior to analyzing samples.

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

3.3 Interferences co-extracted from water samples limit the method detection and quantitation limits.

4.0 Apparatus and Materials

4.1 Separatory funnel – 2 liter, with polytetrafluoroethylene (PTFE) stopcock and ground glass stopper.

4.2 250 mL round bottom flask and ground glass stopper.

4.3 Glass wool, Pyrex - solvent washed prior to use.

4.4 Kuderna-Danish (K-D) Apparatus

4.4.1 Concentrator tube - 10 mL, graduate (Kontes K0570050-1025, or equivalent). A ground stopper, 19/22 joint, is used to prevent evaporation of extracts.

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

4.4.3 Snyder column - three ball (Kontes K-503000-0121, or equivalent).

4.4.4 Boiling chips, Hengar granules, high purity amphoteric alundum – crushed to uniform size [~ 1-2 mm diameter] and muffled in a ceramic dish at 400 °C for 24 hours. Note that boiling chips can be a significant source of contamination if not properly cleaned. Solvent rinsing alone does not eliminate trace level PAH contamination.

4.5 Water bath, Organomation Assoc. Inc.(OA-SYS/S-EVAP-KD), 115 V, thermostatically controlled with stainless steel cover to fit 5 K-D apparatus equipped with solvent recovery system, installed in a fume hood.

4.6 Nitrogen evaporator/water bath, Organomation Assoc. Inc.(N-EVAP 112), 115 V, thermostatically controlled with stainless steel cover for culture tubes, installed in a fume hood.

4.7 Culture tube calibrated to 1 mL .

4.8 Autosampler vials – amber glass, 1.5 mL [12 X 32] with PTFE-lined screw cap.

4.9 1 mL glass volumetric pipette and rubber bulb.

4.10 100 mL glass beaker.

4.11 Disposable Pasteur Pipets - (rinsed with solvents before use).

4.12 Glass funnel – wide mouth, 75 mm

4.13 Graduated cylinder, 250 mL and 100 mL.

4.14 3 or 7 position liquid/liquid motorized water extractor fitted for 2 L separatory funnels.

4.15 Gas chromatographs, Hewlett-Packard HP 6890 plus, equipped with HP 5973 "Inert Source" Quadrapole mass selective detector, split-splitless injector with EPC (electronic pneumatic control), and autosampler.

4.16 Capillary columns, 60 meter DB5_MS (J&W Scientific) (0.25 mm I.D. and 25 µm film thickness) connected to a single injection port.

4.17 Data System, Enviroquant Chemstation, to collect and record GC/MS data, generate reports, and compute and record response factors for multi-level calibrations. Data system should be capable of calibrating a method using a minimum of 5 concentrations of analytical standards.

5.0 Reagents

5.1 Acetone, Burdick and Jackson, distilled in glass and pesticide residue or HRGC grade or equivalent.

5.2 Isooctane, Burdick and Jackson, distilled in glass and pesticide residue or HRGC grade or equivalent.

5.3 Dichloromethane (DCM). Burdick and Jackson, distilled in glass and pesticide residue or HRGC grade or equivalent..

5.4 Sodium sulfate. Anhydrous granular reagent grade, rinsed with PE prior to use. EMD, GR ACS.

5.5 Nitrogen, pre-purified grade (99.9999%) or better
(used for ASE, GPC and solvent evaporation).

5.6 Helium, ultra-pure (99.9999%) for GC carrier gas.

5.7 PAH Surrogate Mix, Custom Surrogate mix, Ultra Scientific.

5.8 PAH Standard Mix, Custom Standard mix, Ultra Scientific.

5.9 PAH Internal Standard Mix, Custom Internal Standard Mix, Ultra Scientific.

5.10 PAH Working Spike Mix, Custom Spiking Mix, Ultra Scientific.

CAUTION

The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of data handling Material Safety Data Sheets should also be made available to all personnel involved in these analyses.

6.0 Sample Collection, Preparation, and Storage

6.1 In the field, sources of contamination include sampling gear, grease from ship winches or cables, ship and/or motor vehicle engine exhaust, dust, and ice used for cooling. Efforts should be made to minimize handling and to avoid sources of contamination. Water samples should be collected in amber 1 L bottles with PTFE or Teflon lined caps and stored on ice until they can be transferred to the Lab.

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

6.3 Water samples must be extracted within 7 days of collection.

6.4 Water samples must be stored at ~ 10 °C prior to extraction.

7.0 Sample Extraction

IMPORTANT: All glassware must be triple-rinsed with dichloromethane before being used for this procedure.

7.1 A separate extraction bench sheet is started for each project. Several bench sheets may be used for a single project.

7.2 Mark the sample's water level on the outside of the sample container using a

permanent marker for a volume determination.

7.3 Using a clean solvent rinsed wide mouthed glass funnel, transfer the entire contents of the sample container into a 2 L separatory funnel.

7.4 Using a volumetric pipette, add 1 mL of PAH Surrogate Mix to the separatory funnel and mix well by swirling.

7.5 Add 60 mL of dichloromethane to the sample container, replace the cap and extract the walls of the sample container by gently shaking or rolling the solvent over the inner surface.

7.6 Transfer the dichloromethane from the sample container into the separatory funnel using the wide mouthed glass funnel. Set the sample container aside in a fume hood to completely vent prior to determining the sample volume.

7.7 Rinse the wide mouthed funnel into the separatory funnel with dichloromethane and set the funnel aside.

7.8 Replace the ground glass stopper onto the separatory funnel and secure the stopper in place with the strap provided on the sample turner. The strap should be tight when correctly engaged.

7.9 Turn the samples (3 or 7 depending on the sample turner) 2 complete rotations at normal speed (a setting of "6" on the dial). Stop the samples with the stopcocks pointing upward and vent the separatory funnels.

NOTE: Methylene chloride creates excessive pressure very rapidly; therefore, initial venting should be done immediately after the separatory funnel has been sealed and turned no more than two times.

7.10 Repeat step 7.9 until little or no pressure builds up when the samples are turned.

7.11 Turn the samples at normal speed for 5 minutes.

7.12 Vent the samples to remove any excess pressure that may have built up during the extraction.

7.13 Turn the separatory funnels into the upright position and remove the straps and ground glass stoppers.

7.14 Collect the dichloromethane layer into a 250 mL round bottom flask.

7.15 Repeat steps 7.5 through 7.14 two times for a total of three 60 mL extractions.

Combine the extract in the round bottom flask.

7.16 With each set of samples, a Method Blank and a Laboratory Control Spike (LCS) must be extracted and analyzed. Prepare the Method Blank and LCS by adding 1000 mL of deionized water to cleaned and solvent rinsed separatory funnels. Add 1 mL of PAH Surrogate Mix to each separatory funnel. Add 1 mL of PAH Working Spike Mix to the LCS. Extract, dry, concentrate and analyze the Method Blank and LCS as if they were real samples.

7.17 With each set of samples, a sample duplicate must also be extracted and analyzed. The field sampling crew will supply enough of one sample to perform a duplicate extraction and analysis. This duplicate sample should be extracted, concentrated, dried and analyzed as if it was a real sample.

7.18 With each set of samples, a matrix spike and a matrix spike duplicate must be extracted and analyzed. The field sampling crew will supply enough of one sample to perform these duplicate extractions and analyses. Add 1 mL of PAH Working Spike Mix to each matrix spike sample after adding 1 mL of the PAH Surrogate Mix. These matrix spikes should be extracted, concentrated, dried and analyzed as if they were real samples.

8.0 Drying and Concentrating the Extract

IMPORTANT: All glassware, glass wool, and sodium sulfate must be triple-rinsed with dichloromethane before they are used for this procedure.

8.1 Set up and label pre-cleaned K-D flasks (1-6) with concentrator tubes attached on ring stands in the fume hood. Place a funnel containing a plug of pre-cleaned glass wool in the bottom of the funnel and place the funnel in the top of the K-D flask. Add about two inches of pre-rinsed sodium sulfate to the funnel. Make sure that the level of the sodium sulfate is uniform across the funnel to prevent any possible splashing out.

8.2 Pour the sample extract from the round bottom flask through sodium sulfate into the K-D flask. Add about 10 mL of DCM to the round bottom flask, swirl to rinse the sides of the flask, and add this rinse to the sodium sulfate. Repeat with another 10 mL DCM rinse. Rinse the sodium sulfate with an additional portion of DCM (~25 mL) by pouring from a clean and rinsed 100 mL beaker. After the solvent has completely drained through the sodium sulfate add one more additional rinse of DCM (~25 mL) from the beaker of clean DCM. Allow the DCM to completely drain through the sodium sulfate (~3-5 minutes).

8.4 Add 1.0 mL isooctane using a macropipetter and a solvent rinsed boiling chip to each K-D flask. Place a Snyder column on the K-D flask, clamp with a green

clamp and place the flask on the hot water bath set at 80-82°C. Drop down the inverted Hopkins condenser from the solvent recovery system and attach it to the top of the Snyder column. Turn the water supply on to the solvent recovery system until the water flow is between 1500-2000 cc/min. Evaporate the solvent until the reflux line falls below the top of the Snyder column. Remove the inverted Hopkins condenser and secure using the set clamps so that it is out of the way. At this point there should be between 1-5 mL visible in the concentrator tube while the K-D apparatus is still on the hot water bath and about 5 mL of the solvent remaining after the K-D flask is removed from the hot water bath and the solvent drains from the Snyder column. Dry off the water using a WyPall X60 towel to remove any water from around the ground glass union of the concentrator tube and the K-D flask to prevent any of it from entering the concentrator tube upon removal.

8.5 When the vessels are cool, remove the concentrator tube from the K-D flask after removing, with a Wypall towel, any residual moisture from the ground glass joint between the concentrator tube and the K-D flask. Pour the solvent into a 15 mL culture tube that has been pre-calibrated to 1000 µL (1.0 mL). Rinse the concentrator tube into the culture tube twice with DCM. Rinse the Snyder column with petroleum ether and place back in the column rack for storage.

8.6 Place the culture tube onto the N-EVAP 112 with the water bath set at 35°C and with a new disposable Pasteur pipette above it. Set the nitrogen pressure at the cylinder to 30 p.s.i. and adjust the regulator on the side of the water bath to 4 L/min. Lower the secured pipette to ~1 inch above the level of the solvent in the culture tube and adjust the nitrogen flow at the individual valve above the tube so that the surface of the solvent is slightly "dimpled", but not splashing. Checking frequently and adjusting the height of the pipette to maintain ~1 inch of clearance above the surface of the solvent, allow the level of the solvent to reach **exactly 1.0 mL.** Quantitatively transfer the solvent to a pre-rinsed amber autosampler vial with a small glass disposable pipette. Label and cap the vial. Mark the level of the solvent on the outside of the vial with a permanent marker.

8.7 Prior to analysis, add 10 µL of PAH internal standard mix [compounds listed in Table 3] (10,000 µg/mL) to the sample extract vials.

8.8 After the original sample container has been vented completely in the fume hood, add water back to the mark made prior to transferring the sample to the separatory funnel. Using a graduated cylinder, measure and record the sample volume on the project bench sheet for each sample.

10.0 Analytical Procedure

10.1 PAH analysis:

10.1.1 Instrumentation:
Agilent 6890 gas chromatograph equipped with a 5973 *Network* "Inert Source" Quadrapole Mass Selective Detector, a split-splitless injector with EPC (electronic pneumatic control), and a 7683 autosampler. One 60 meter DB5-MS (J&W Scientific) (0.25 mm I.D. and 25 µm film thickness) fused silica column is used.

10.1.2 Chromatograph conditions:
The injector is operated isothermal at 275°C in the "Pulsed Splitless" mode with a pressure of 16.9 psi and a total flow of 52.4 mL/min. The injection pulse pressure is set to 30 psi until 0.80 min. The purge flow to split vent is set for 48.5 mL/min @ 1 min. The "Gas Saver" option is used with a setting of 20 mL/min @ 3 min. The oven has an initial temperature of 80°C which is held for 1 minute and then temperature programmed to 200°C at a rate of 10°C/min and held for 0 min. It is then programmed to 210°C at a rate of 3°C/min and is held for 0 min. It is then programmed to 290°C at a rate of 10°C/min and is held for 5 min. It is then programmed to 325°C at a rate of 20°C/min and is held for 11 min. Ultra High Purity Grade Helium is used as the carrier gas at a linear velocity of 24 cm/sec and a flow rate of 0.9 mL/min. All methods are retention time locked.

10.1.3 Sample volume:
Two microliters of both samples and standards are injected via the pulsed splitless method.

10.1.4 Mass Spectrometer Conditions:
The transfer line from the GC to the MS detector is held at 300 ^{o}C. The MS ion source is held at 300 ^{o}C. The MS Quad is held at 180 ^{o}C. The MS detector is operated in the Selected Ion Monitoring [SIM] mode. Table 4 lists the compounds, ions and groups used.

Compounds	Ion	Q Ion	RT	
1-Methylnaphthalene-d10	152		**12.58**	
Naphthalene-d8	136		**10.82**	**Group 1: 7.50 - 13.00**
Naphthalene	128	102	**10.87**	Cycles/sec = 1.23
2-Methylnaphathalene	141	115	**12.44**	Dwell = 100
C1 - Naphthalenes	141	115	**12.44**	
1-Methylnaphthalene	141	115	**12.68**	
Biphenyl-d10	164	80	**13.49**	**Group 2: 13.00 -15.00**
Biphenyl	154	76	**13.55**	Cycles/sec = 1.08
2,6-Dimethylnaphthalene	156	141	**13.97**	Dwell = 100
C2 - Naphthalenes	156	141	**14.17**	
Acenaphthylene-d8	160		**14.66**	
Acenaphthylene	152	76	**14.70**	
Acenaphthene-d10	162	80	**15.09**	**Group 3: 15.00 - 19.00**
Acenaphthene	153		**15.18**	Cycles/sec = 0.98
C3 - Naphthalenes	170	155	**16.10**	Dwell = 100
2,3,5-Trimethylnaphthalene	170	155	**16.30**	
Fluorene-d10	176		**16.63**	
Fluorene	166	82	**16.74**	
C4 - Naphthalenes	170	155	**17.90**	
1-Methylfluorene	180	165	**18.56**	
C1 - Fluorenes	180	165	**18.56**	
Dibenzothiophene-d8	192	160	**19.30**	**Group 4: 19.00 - 20.36**
Dibenzothiophene	184	139	**19.38**	Cycles/sec = 1.08
Phenanthrene-d10	188		**19.70**	
Phenanthrene	178	152	**19.77**	Dwell = 100
Anthracene	178	152	**19.93**	
C2 - Fluorenes	194		**20.26**	
4-Methyldibenzothiophene	198	175	**20.73**	**Group 5: 20.36 - 22.25**
C1 - Dibenzothiophenes	198	175	**20.73**	
C1 - Phenanthrene/Anthracene	192	165	**21.25**	Cycles/sec = 0.96
1-Methylphenanthrene	192	165	**21.65**	Dwell = 100
C3 - Fluorenes	208		**22.20**	Carry over ions: 194,178,212
C2 - Dibenzothiophenes	212		**21.95**	**Group 6: 22.25 - 23.65**
3,6-Dimethylphenanthrene	206	191	**22.57**	Cycles/sec = 1.08
C2 - Phenanthrene/Anthracene	206	191	**22.88**	Dwell = 100
Fluoranthene	202	101	**23.34**	carry over ions: 208,226,220
Pyrene-d10	212	106	**23.92**	**Group 7: 23.65 - 26.50**

Pyrene	202	101	**23.99**	Cycles/sec = 1.53
C3 - Dibenzothiophenes	226		**23.22**	Dwell = 50
C3 - Phenanthrene/Anthracene	220		**24.16**	
2-Methylfluoranthene	216	189	**24.61**	
C1 - Fluoranthene/Pyrenes	216	189	**25.22**	
C4 - Phenanthrene/Anthracene	234		**25.72**	
Benz[a]anthracene-d12	240	120	**27.62**	**Group 8: 26.50 - 31.31**
Benz[a]anthracene	228	226	**27.71**	Cycles/sec = 1.15
Chrysene-d12	240		**27.75**	
Chrysene	228	226	**27.85**	Dwell = Mixed 50/100
C1 - Chrysenes	242		**29.35**	
C2 - Chrysenes	256		**30.99**	Carry over ions: 234
Benzo[b]fluoranthene	252	126	**31.66**	**Group 9: 31.31 - 32.30**
Benzo[k]fluoranthene	252	126	**31.74**	Cycles/sec = 2.15
				Dwell = 100
				Carry over ions: 270,256
Benzo[a]pyrene-d12	264	132	**32.63**	**Group 10: 32.30 - 33.39**
C3 - Chrysenes	270		**32.31**	Cycles/sec =1.72
Benzo[e]pyrene	252	126	**32.54**	Dwell = 100
Benzo[a]pyrene	252	126	**32.72**	
Perylene-d12	264	132	**32.90**	
Perylene	252		**32.98**	
Indeno[1,2,3-cd]pyrene	276	138	**36.70**	**Group 11: 33.39 - 37.26**
Dibenz[a,h]anthracene-d14	292		**36.64**	Cycles/sec = 1.23
Dibenz[a,h]anthracene	278	139	**36.76**	Dwell = 100
				Carry over ions: 270
				Group 12: 37.26 to end of run
Benzo[ghi]perylene-d12	288	144	**37.66**	
Benzo[ghi]perylene	276	138	**37.78**	Cycles/sec = 1.08
				Dwell =100
				Carry over ions: 278

Table 4.

10.1.4 Data processing:
Enviroquant Chemstation, to collect and record GC/MS data, generate reports, and compute and record response factors for multi-level calibrations. Data system should be capable of calibrating a method using a minimum of 5 concentrations of analytical standards.

11.0 References

U.S. Environmental Protection Agency. 1996. EPA Test Methods for Evaluating Solid Waste. Vol 1B. 3500B and 3510c.

Section Approval: _____

Final Approval: _____

QA Officer Approval_____

STANDARD OPERATING PROCEDURE
TITLE: Determination of Polychlorinated biphenyls (PCBs) in Water

REVISION HISTORY		
Revision #	**Summary of Changes**	**Date**
1	Separated PCB and organochlorine pesticides analyses into separate SOPs. Original source SOP was "Method #31, revision 1."	11/12/2009
0	Initial release.	06/20/2005

Author:	Gloria Blondina		Date: 11/12/09
Approved:	Laboratory Director *David B. Crane*		Date:
Approved:	Section Lead *Abdu Mekebri*		Date:
Approved:	Quality Assurance *Gail Cho*		Date:
Approved:	Health and Safety *Thomas Lew*		Date:

Determination of Polychlorinated-biphenyls in Water Samples

1.0 Scope and Application

1.1 This is a modified EPA Method 608/8082A and describes the sample preparation and quantitative analysis of trace level polychlorinated biphenyls (PCB) in surface, municipal and wastewater using liquid-liquid extraction and high resolution gas chromatography with electron capture detector (GC/ECD) and gas chromatography with optional mass spectrometer and ion trap detector (GC/MS-ITD) for confirmation. This method provides extraction and analysis of Arochlor mixtures as well as individual PCB congeners. The following target analytes can be determined by this method:

Arochlor Mixtures

Arochlor 1016	**Arochlor 1242**
Arochlor 1221	**Arochlor 1248**
Arochlor 1232	**Arochlor 1254**
Arochlor 1260	

PCB Congeners

PCB 005	**PCB 087**	**PCB 157**
PCB 008	**PCB 095**	**PCB 158**
PCB 015	**PCB 097**	**PCB 170**
PCB 018	**PCB 099**	**PCB 174**
PCB 027	**PCB 101**	**PCB 177**
PCB 028	**PCB 105**	**PCB 180**
PCB 029	**PCB 110**	**PCB 183**
PCB 031	**PCB 114**	**PCB 187**
PCB 033	**PCB 118**	**PCB 189**
PCB 044	**PCB 128**	**PCB 194**

PCB 049	**PCB 137**	**PCB 195**
PCB 052	**PCB 138**	**PCB 200**
PCB 056	**PCB 141**	**PCB 201**
PCB 060	**PCB 149**	**PCB 203**
PCB 066	**PCB 151**	**PCB 206**
PCB 070	**PCB 153**	**PCB 209**
PCB 074	**PCB 156**	**PCB 207 (Surrogate)**

1.2 The estimated detection limit for each analyte is listed in Table 1. The actual MDL may differ from those listed, depending upon the nature of interferences in the sample matrix. Validation of the target analytes produced recoveries greater than 65 percent. The mean percent recoveries for each analyte are also included in Table 1.

1.3 Upon request, unknowns with sufficient signal in the sample may be qualitatively confirmed for compound identification by gas chromatography equipped with an ion trap mass spectrometer detector (GC/MS-ITD).

2.0 Summary of Method

2.1 A measured volume of sample (1000 mL) is extracted with methylene chloride (DCM) using a separatory funnel. The DCM extract is dried with sodium sulfate, evaporated using Kuderna-Danish (K-D) and solvent exchanged into petroleum ether. The extract is concentrated with micro-snyder (micro K-D) apparatus to approximately 1 mL and adjusted to 2.0 mL with iso-octane. The extracts are analyzed by gas chromatography using conditions which permit the separation and measurement of the target analytes in the extracts by GC/ECD.

2.2 Interferences in analyses may be encountered in very dirty samples and cleanup may be needed to aid in the elimination or reduction of these interferences. Florisil column cleanup or Gel Permeation Chromatography (GPC) procedures will be followed.

Table 1. Polychlorinated biphenyls (PCB) analyzed by GC/ECD, their Method Detection Limits (MDL), Reporting Limits (RL) and Mean Percent Recovery in water.

Target Analytes	MDL (µg/L)	RL (µg/L)	Mean % Recovery
PCB 005	0.001	0.002	102
PCB 008	0.001	0.002	96.1
PCB 015	0.001	0.002	95.4
PCB 018	0.001	0.002	102
PCB 027	0.001	0.002	109
PCB 028	0.001	0.002	102
PCB 029	0.001	0.002	104
PCB 031	0.001	0.002	103
PCB 033	0.001	0.002	106
PCB 044	0.001	0.002	105
PCB 049	0.001	0.002	103
PCB 052	0.001	0.002	101
PCB 056	0.001	0.002	110
PCB 060	0.001	0.002	108
PCB 066	0.001	0.002	107
PCB 070	0.001	0.002	107
PCB 074	0.001	0.002	105
PCB 087	0.001	0.002	109
PCB 095	0.001	0.002	100
PCB 097	0.001	0.002	108
PCB 099	0.001	0.002	111
PCB 101	0.001	0.002	105
PCB 105	0.001	0.002	113
PCB 110	0.001	0.002	113
PCB 114	0.001	0.002	111
PCB 118	0.001	0.002	115
PCB 128	0.001	0.002	114
PCB 137	0.001	0.002	112
PCB 138	0.001	0.002	118
PCB 141	0.001	0.002	112
PCB 149	0.001	0.002	114
PCB 151	0.001	0.002	111

PCB 153	**0.001**	**0.002**	**111**
PCB 156	**0.001**	**0.002**	**113**
PCB 157	**0.001**	**0.002**	**114**
PCB 158	**0.001**	**0.002**	**115**
PCB 170	**0.001**	**0.002**	**115**
PCB 174	**0.001**	**0.002**	**114**
PCB 177	**0.001**	**0.002**	**115**
PCB 180	**0.001**	**0.002**	**114**
PCB 183	**0.001**	**0.002**	**113**
PCB 187	**0.001**	**0.002**	**113**
PCB 189	**0.001**	**0.002**	**113**
PCB 194	**0.001**	**0.002**	**113**
PCB 195	**0.001**	**0.002**	**113**
PCB 200	**0.001**	**0.002**	**110**
PCB 201	**0.001**	**0.002**	**115**
PCB 203	**0.001**	**0.002**	**114**
PCB 206	**0.001**	**0.002**	**113**
PCB 209	**0.001**	**0.002**	**108**
PCB 207 (Surrogate)	**0.001**	**0.002**	**110**

3.0 Interferences

3.1 Solvents, reagents, glassware, and other sample processing hardware may cause GC artifacts and/or elevated baselines, resulting in the misinterpretation of chromatograms. All materials should be demonstrated to be free from interferences under the conditions of the analysis by running method blanks initially and with each sample lot. Specific selection of reagents and purification of solvents by distillation in all-glass systems are required. High-purity distilled-in-glass solvents are commercially available.

An effective way of cleaning laboratory glassware is by rinsing with polar and non-polar solvents before use. The cleaning procedure used must be tested by analyzing procedural blanks prior to analyzing samples.

3.2 Phthalates are common laboratory contaminants that are used widely as plasticizers. Sources of phthalate contamination include plastic lab-ware, plastic tubing, plastic gloves, plastic coated glassware clamps, and have been found as a contaminant in Na_2SO_4.

Polytetrafluoroethylene (PTFE) can be used instead of polypropylene or polyethylene to minimize this potential source of contamination. However, use of PTFE lab-ware will not necessarily preclude all phthalate contamination. Na_2SO_4 can be solvent rinsed to eliminate contaminants.

3.3 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source. A Florisil or GPC cleanup procedure can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4.0 Apparatus and Laboratory Supplies

4.1 Separatory funnel. 2000-mL, with TFE-fluorocarbon stopcock, ground glass or TEF stopper.

4.2 Automatic shaker designed to fit 2 liter separatory funnels with rpm and timer controls.

4.3 Beakers. Borosilicate glass, 400 mL

4.4 Glass wool. Pyrex - solvent washed prior to use.

4.5 Kuderna-Danish (K-D) Apparatus.

4.5.1 Concentrator tube. 15 mL, graduate (Kontes K0570012-0500, or equivalent). A ground stopper, 19/22 joint, is used to prevent evaporation of extracts.

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

4.5.3 Snyder column. Three ball (Kontes K-503000-0121, or equivalent).

4.5.4 Micro-Snyder column. Alltech 9058 or equivalent.

4.5.5 Boiling chips. Hengar granules, high purity amphoteric alundum - extracted with acetone and petroleum ether. Note that boiling chips can be a significant source of contamination if not properly cleaned.

4.6 Water bath. Blue M, 115 V, thermostatically controlled with stainless steel cover to fit K-D apparatus, installed in a fume hood.

4.7 GC vials. GC autosampler vials, borosilicate glass, 2 mL with PTFE-lined screw cap.

4.8 Analytical balance. Capable of weighing 0.1 mg.

4.9 Drying oven.

4.10 Disposable Pasteur Pipettes. 2 mL, rinsed with solvents before use.

4.11 Glass filter funnel. Fluted, 75 mm or larger.

4.12 Graduated cylinder. 1000 mL, 250 mL and 100 mL.

4.13 Culture tubes. 13 x 100 mm with PTFE lined screw cap.

4.14 Analytical systems

4.14.1 Gas chromatograph. Agilent 6890 equipped with dual ^{63}Ni micro electron capture detectors (ECD) with EPC, split-splitless injector, a 7683 autosampler and dual capillary columns (J&W Scientific) connected to a single injection port using a 5 meter pre-column with a "Y" press fit connector. Section 9 describes the acquisition and analysis procedures while Table 2 lists the operating parameters.

4.14.2 Gas chromatograph. Varian 3400, equipped with a Varian Saturn model 2000 Ion Trap Mass Spectrometer, split-splitless injector, LEAP Model CTC A200SE autosampler and a 30 meter capillary column (J&W Scientific).

4.14.3 Data System. Hewlett-Packard, to collect and record GC/ECD data, generates reports, computes and records response factors for multi-level calibrations. Data system should be capable of calibrating a method using a minimum of 5 concentrations of analytical standards and calculating in external standard mode.

Table 2 Operating parameters for Agilent 6890 GC/ECD

Gases
Carrier: Helium, 1 mL/min
Makeup: Nitrogen, 1 mL/min

Columns
DB-5, 60 m x 0.25 mm I.D. x 0.25 µm film thickness
DB-17-MS, 60 m x 0.25 mm I.D. x 0.25 µm film thickness

Mode: Constant flow
Initial flow: 1.9 mL/min

Inlet
Isocratic temp: 240 ºC

Oven
Initial temperature: 80 ºC, initial time: 1.00 min
Ramp 1: 15.0 deg/min, final temp 210 ºC, hold time 10.00 min
Ramp 2: 2.0 deg/min, final temp 290 ºC, hold time 14.00 min

Detectors (^{63}Ni µECD)
Temperature: 310 ºC
Combined Flow: 31.0 mL/min (column + make-up flow)

Injection Volume: 3 µL

5.0 Reagents, materials, gases and standards

5.1 Reagent water is defined as water in which an interferent is not observed at method detection limit of each parameter of interest. Deionized (DI) water was used for method validation and as method blank.

5.2 Petroleum ether (PE), acetone, methylene chloride (DCM), diethyl ether, isooctane. Pesticide residue quality or equivalent.

5.3 Sodium sulfate. Anhydrous granular reagent grade, rinsed with PE prior to use.

5.4 Nitrogen. Ultra-pure (99.99999%) for GC/ECD

5.5 Helium. Ultra-pure (99.99999%) for GC/ECD

5.6 Stock standards. Individual stock standards (100 µg/mL) are purchased as certified solutions from AccuStandard (New Haven, CT).

6.0 Sample Collection, Preservation, and Storage

6.1 Samples are collected in one liter amber glass bottles and iced or refrigerated at 4 °C from time of collection until extraction.

6.2 All samples must be extracted within 7 days and completely analyzed within 40 days of extraction.

7.0 Sample Extraction

7.1 Remove water samples from refrigerator and allow samples to reach room temperature prior to extraction. Transfer contents to a pre-cleaned 2-liter separatory funnel. Immediately add 1.0 mL of the 20 ppb PCB pesticide surrogate solution to every sample. For laboratory control spike (LCS) and matrix spikes (MS/MSD) also add 1.0 mL of 20 ppb PCB pesticide spiking solution.

7.2 Add 60 mL of methylene chloride (DCM) to the empty bottle, replace the cap and rinse the bottle. Pour the DCM into the separatory funnel and repeat with another 60 mL aliquot of DCM. Extract the sample by shaking the funnel for 5 minutes on the auto-shaker with periodic venting to release excess pressure. Allow organic layer to separate from the water phase for a minimum of 10 minutes. Collect the methylene chloride extract in a 400 mL beaker.

7.3 Add a second 120 mL volume of methylene chloride to the separatory funnel and repeat the extraction procedure a second time, combining the extracts in the beaker.

7.4 Set up and label pre-cleaned K-D flasks with concentrator tubes and attached with a blue clamp on ring stands in the fume hood. Add 0.5 mL iso-octane as “keeper” and a solvent rinsed micro-boiling chip to each K-D concentrator tube. Place a filter funnel containing a plug of pre-cleaned glass wool in the bottom of the funnel and place the funnel in the top of the K-D flask. Add about two inches of solvent rinsed sodium sulfate to the funnel.

7.5 Pour the combined extracts from the beaker through sodium sulfate into the K-D flask. Rinse the beaker with about 10 mL of DCM and add this rinse to the sodium sulfate. Repeat with another 10 mL DCM rinse. Rinse the sodium sulfate with an additional portion of DCM (~10-20 mL).

7.6 Place a Snyder column on the K-D flask, clamp with a green clamp and place the flask on the hot water bath set at 78-82 ^{o}C. Evaporate solvent on the hot water bath. When the apparent volume of solvent in the

concentrator tube is 5-10 mL, add 20-30 mL of petroleum ether through the top of the Snyder column. Repeat this procedure when the apparent volume is again at 5-10 mL. When the reflux line falls below the top of the Snyder column, the K-D apparatus should be removed from the hot water bath. Dry the outer KD apparatus with a Kimwipe to prevent condensation water from entering the concentrator tube. Upon cooling, remove the concentrator tube from the K-D apparatus.

7.7 Place a clean micro-Snyder column on the concentrator tube with a blue clamp, add a new micro boiling chip and place in a 400 mL beaker containing water heated to approximately 78 ^{o}C on a hot plate. If the solvent does not begin to boil, remove the tube from the bath immediately, allow it to cool slightly, add a new micro boiling stone to prevent it from bumping and place it back in the bath.

7.8 When the solvent has been evaporated to 0.5-1 mL remove the tube from the bath and allow it to cool in a test tube rack. Dry the outer KD apparatus with a Kimwipe to prevent condensation water from entering the concentrator tube. Remove the micro-Snyder column and add iso-octane to the concentrator tube to reach a final volume of 2.0 mL. Mix the tube contents by tapping the bottom of the tube causing a vortex which will rinse the sides of the tube. A Vortex Genie mixer may be used for this step.

7.9 Transfer the solution from the concentrator tube to a culture tube and cap with a Teflon™ faced cap. Place extracts in a refrigerator for storage until analysis or cleanup, if necessary.

7.10 When ready for analysis, transfer extract to labeled GC vials and cap.

8.0 Cleanup Procedure

8.1 Cleanup of dirty samples may be necessary due to interferences in the analysis of baseline or co-elution with target analytes of the sample extract. Follow the in-house SOP for Florisil® column or GPC method, as needed.

9.0 Analytical Procedure

9.1 The final extract will be analyzed on an Agilent 6890 GC/ECD. Chromatographic conditions for operating the Agilent 6890 GC/ECD are found in Table 2.

9.2 GC acquisition

9.2.1 Analyze a Pesticide Check Solution at the beginning and end of each run to ensure GC performance.

9.2.2 Pour several isooctanes into GC vials using the same lot as used for samples with each GC run.

9.2.3 Pour standard curves into GC vials using 0.5, 1.0, 2.0, 5.0, 10, 20 and 50 ppb PCB Std in isooctane. Pour extra vials of a mid-level concentration for use as CCV (to be analyzed every 20 samples or less).

9.2.4 Create sequence file and sequence table on computer. Use the WPCL login number for “Data Subdirectory” and “Save As” sequence name.

9.2.5 Acquire data and recap each vial daily to preserve sample integrity.

9.3 Analysis

9.3.1 Recalibrate PCB curves and analyze samples in external standard mode. Add a printed chromatogram and report for each standard and sample to folder.

9.3.2 Certain analytes will co-elute on a given column. However, using two columns with different polarities will allow for confirmation of target analytes.

10.0 References

U.S. Environmental Protection Agency, Office of Water, EPA 821-R-92-002, April 1992, Methods For The Determination of Nonconventional Pesticides In Municipal And Industrial Wastewater, p. 21.
Method 608, *The Determination of Organochlorine Pesticides in Municipal and Industrial Wastewater*.

U.S. Environmental Protection Agency, Office of Solid Waste, SW-846 On-Line, Method 3510C, *Separatory Funnel Liquid-Liquid Extraction*, Revision 3, December 1996, http://www.epa.gov/epaoswer/hazwaste/test/pdfs/3510c.pdf, [06-27-06]

U.S. Environmental Protection Agency, Office of Solid Waste, SW-846 On-Line, Draft Update IVB, Method 8082A, *Organochlorine Pesticides by Gas Chromatography,* Revision 2, November 2000. http://www.epa.gov/epaoswer/hazwaste/test/pdfs/8081b_ivb.pdf[08-14-07]

APPENDIX I: Validation of method detection limits of Arochlors in water.

1.0 Scope

A method validation for method detection limits of Arochlors in water was performed using a modified EPA 8082A method, as detailed in this PCB-Water SOP. Method detection and reporting limits were determined during the method development phase and will be checked for compliance with these results using MDL=t*StdDev, where t = the student's t value for n-1 degrees of freedom and StdDev is the standard deviation calculated from the replicate recoveries of this study. Recoveries shall be 50-150% with RPE of less than 20 percent.

2.0 Setup

Perform nine replicate spiked extractions and analysis following the SOP for water extraction of PCBs. Using 1 liter of DI water, add 1 mL of 10 ppb PCB spike mix and 1 mL of 10 ppb PCB 207 (surrogate) to each replicate. Also, prepare a method blank using 1 liter of DI water and 1 mL of 10 ppb PCB 207 surrogate.

Repeat this procedure at the reporting limit (1 ppb Standard) using 5 replicates.

3.0 Results

All recoveries fell within the allowable range.

ANALYSIS OF EXTRACTABLE SYNTHETIC ORGANIC COMPOUNDS IN TISSUE AND SEDIMENT (Organochlorine Pesticides, Polychlorinated Biphenyls and Polybrominated Diphenyl Ethers)

1.0 Scope and Application

1.1 This method describes the sample preparation using an automated extraction system for the determination of trace residue levels of a selected list of organochlorine (OCs) pesticides, polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in fish and shellfish tissues and sediments. Dual column gas chromatography with dual electron capture detectors (GC-ECD) and/or gas chromatography with triple quadrupole mass spectrometry (GC-MSMS) are used to analyze OC pesticides, PCBs and PBDEs. Table 1 lists the target OC pesticide compounds currently analyzed with their method detection limits and reporting limits. Table 2 lists the PCB congeners and Aroclor mixtures analyzed with their reporting limits. Table 3 lists the PBDE congeners analyzed with their method detection limits and reporting limits.

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

Table 1. Organochlorine Compounds Analyzed and their Minimum Detection Limits (MDL) and Reporting Limits (RL) in Tissue (ng/g, wet wt.) and Sediment (ng/g dry weight), based on 50 % moisture.

	Tissue		Sediment	
	MDL, ng/g wet wt.	RL, ng/g wet wt.	MDL, ng/g dry wt.	RL, ng/g dry wt.
aldrin	0.414	1.00	0.800	2.00
chlordane, cis	0.400	1.00	0.800	2.00
chlordane, trans	0.450	1.00	0.900	2.00
chlorpyrifos	0.204	1.00	0.400	2.00
dacthal	0.096	1.00	0.200	2.00
DDD, o,p'	0.096	1.00	0.200	2.00
DDD, p,p'	0.124	1.00	0.250	2.00
DDE, o,p'	0.178	2.00	0.400	4.00
DDE, p,p'	0.480	2.00	1.00	4.00
DDMU, p,p'	0.108	3.00	0.200	6.00
DDT, o,p'	0.216	3.00	0.400	6.00
DDT, p,p'	0.156	5.00	0.300	10.0
diazinon	4.80	20.0	10.0	40.0
dieldrin	0.432	0.500	1.00	1.00
endosulfan I	0.560	2.00	1.00	4.00
endosulfan II	0.682	5.00	1.40	10.0
endosulfan sulfate	0.546	5.00	1.00	10.0
endrin	0.180	2.00	0.400	4.00
HCH, alpha	0.262	0.500	0.500	1.00
HCH, beta	0.210	1.00	0.400	2.00
HCH, gamma	0.144	0.500	0.300	1.00
heptachlor	0.356	1.00	0.700	2.00
heptachlor epoxide	0.246	1.00	0.500	2.00
hexachlorobenzene	0.346	0.692	0.700	1.40
methoxychlor	0.146	3.00	0.300	6.00
mirex	0.300	1.50	0.600	3.00
nonachlor, cis	0.308	1.00	0.600	2.00
nonachlor, trans	0.194	1.00	0.400	2.00
oxadiazon	0.544	1.00	1.00	2.00
oxychlordane	0.474	1.00	1.00	2.00
parathion, ethyl	0.524	2.00	1.00	4.00
parathion, methyl	0.756	4.00	1.50	8.00
tedion	1.07	2.00	2.00	4.00
DBOB(surrogate)	NA	NA	NA	NA
DBCE(surrogate)	NA	NA	NA	NA
DDD*deuterated (surrogate)	NA	NA	NA	NA

Table 2. PCB Congeners and Aroclor mixtures Analyzed and their Reporting Limits (RL) in Tissue (ng/g, wet weight) and Sediment (ng/g, dry weight).

NIST PCB Congeners:

PCB Congener 8	PCB Congener 128
PCB Congener 18	PCB Congener 138
PCB Congener 28	PCB Congener 153
PCB Congener 44	PCB Congener 170
PCB Congener 52	PCB Congener 180
PCB Congener 66	PCB Congener 187
PCB Congener 87	PCB Congener 195
PCB Congener 101	PCB Congener 206
PCB Congener 105	PCB Congener 209
PCB Congener 118	PCB Congener 209 C^{13}(surrogate)

Additional PCB Congeners:

PCB Congener 27	PCB Congener 141
PCB Congener 29	PCB Congener 146
PCB Congener 31	PCB Congener 149
PCB Congener 33	PCB Congener 151
PCB Congener 49	PCB Congener 156
PCB Congener 56	PCB Congener 157
PCB Congener 60	PCB Congener 158
PCB Congener 64	PCB Congener 169
PCB Congener 70	PCB Congener 174
PCB Congener 74	PCB Congener 177
PCB Congener 77	PCB Congener 183
PCB Congener 95	PCB Congener 189
PCB Congener 97	PCB Congener 194
PCB Congener 99	PCB Congener 198_199
PCB Congener 110	PCB Congener 200
PCB Congener 114	PCB Congener 201
PCB Congener 126	PCB Congener 203
	PCB Congener 137

All individual PCB Congener reporting limits (RL) are 0.2 ng/g (wet weight) or 0.4 ng/g (dry weight, based on 50 % moisture). Estimated Aroclor concentrations calculated from the congener concentrations have the following RLs:

Aroclors:	RL ng/g (wet wt.)	RL ng/g (dry wt.)
Aroclor 1248	25	50
Aroclor 1254	10	20
Aroclor 1260	10	20

Table 3. Polybrominated Diphenyl Ethers (PBDEs) and their Minimum Detection Limits (MDL) and Reporting Limits (RL) in Tissue (ng/g, wet wt.) and Sediment (ng/g, dry wt., based on 50 % moisture.)

	Tissue		Sediment	
	MDL, ng/g wet wt.	RL, ng/g wet wt.	MDL, ng/g dry wt.	RL, ng/g dry wt.
BDE 17	0.139	0.600	0.278	1.20
BDE 28	0.148	0.600	0.296	1.20
BDE 47	0.196	0.800	0.391	1.60
BDE 66	0.135	0.600	0.269	1.20
BDE 100	0.157	0.600	0.314	1.20
BDE 99	0.197	0.800	0.394	1.60
BDE 85	0.177	0.800	0.354	1.60
BDE 154	0.165	0.600	0.329	1.20
BDE 153	0.185	0.800	0.370	1.60
BDE 138	0.200	0.800	0.400	1.60
BDE 183	0.297	1.20	0.594	2.40
BDE 190	0.437	1.80	0.874	3.60
BDE 209	1.00	10.0	2.00	20.0

2.0 Summary of Method

2.1 Sets of 10-18 homogenized tissue or sediment samples are scheduled for extraction by the project lead chemist. Extraction method employed was developed and validated by the Water Pollution Control Laboratory (WPCL) and is a modification of EPA Method 3545A Pressurized Fluid Extraction (PFE). Extract cleanup and partitioning methods are modifications of EPA Methods 3640A Gel Permeation Cleanup and 3620C Florisil Cleanup and the multi-residue methods for fatty and non-fatty foods described in the U.S. Food and Drug Administration, Pesticide Analytical Manual, Vol. 1, 3rd Edition 1994, Chapter 3, Multi-residue Methods, Section 303-C1.

Homogenized tissue or sediment samples are removed from the freezer and allowed to thaw. A separate extraction bench sheet is initiated for each set of samples which are distinguished by project, sample matrix type and analysis type.

2.2 A 1-5 g (tissue or sediment homogenate) sample is weighed into a pre-weighed aluminum planchet and placed in a 70°C oven for 48 hours to determine moisture content. A 10 g sample is mixed using a clean glass stirring rod with approximately
7 g of pre-extracted Hydromatrix® in a 250 mL Trace Clean Wide Mouth Jar until the mixture is free flowing. The mixture is then poured into a 33 mL stainless steel Dionex Accelerated Solvent Extractor (ASE 200) extractor cell and packed by tamping the mixture. A solution containing pesticide, PCB and

PBDE surrogate compounds is added to the cell and the cap is screwed onto the cell. The extractor cells (maximum of 24) are placed on the ASE 200 autosampler rack and the samples are extracted twice with a 50/50 mixture of acetone/dichloromethane (DCM) using heat and pressure. The extracts are automatically collected in two 60 mL VOA vials.

2.3 The combined extracts (~100 mL) are dried using sodium sulfate, evaporated to approximately 1.0 mL using Kuderna-Danish (K-D) glassware equipped with 3-ball Snyder columns and micro-Snyder apparatus and diluted to 10 mL using DCM. The extracts are then filtered through a 0.45 µm syringe filter into J_2 Scientific AccuPrep 170 (GPC) autosampler tubes. If the lipid content needs to be determined, two milliliters each of the filtered extracts are removed and placed in a pre-weighed aluminum planchet.

2.4 The GPC autosampler tubes are then placed on the GPC autosampler for initial sample cleanup by gel permeation (size exclusion) chromatography.

2.5 The cleaned-up extracts are evaporated using K-D apparatus and solvent exchanged into petroleum ether. The extracts are then fractionated using 5 grams of Florisil® in a 11 mm x 300 mm column with a 250 mL reservoir. The Florisil® columns prepared for tissue samples are eluted with 6% diethyl ether/PE (Fraction 1), 15% diethyl ether/PE (Fraction 2), and 50% diethyl ether/PE (Fraction 3). Florisil® columns prepared for sediment samples are eluted with 6% diethyl ether/PE (Fraction 1) and 50% diethyl ether/PE (Fraction 2).The fractions are concentrated to an appropriate volume using K-D/micro K-D apparatus prior to analysis by dual column high resolution gas chromatography and/or GC-MSMS. The distribution of synthetic organic compounds in the fractions is listed in Table 4.

Table 4. Distribution of Synthetic Organic Compounds Among the Three Fractions of a Standard Florisil® Column.

6% Fraction 1/	**15% Fraction 2/**	**50% Fraction 3/**
aldrin	dacthal	endosulfan II 5/
chlordane (cis-)	DBCE*	endosulfan sulfate
chlordane (trans-)	dieldrin	
DBOB*	endosulfan I 4/	
DDE, o,p'	endosulfan II 5/	
DDE, p,p'	endrin	
DDD, o,p'	oxadiazon	
DDD, p,p'/DDD-d10*,p,p'	tetradifon	
DDMU, p,p'		
DDT, o,p'		
DDT, p,p'		
endosulfan I 4/		
heptachlor		
heptachlor epoxide		
hexachlorobenzene		
HCH-alpha		
HCH-beta		
HCH-gamma		
methoxychlor		
nonachlor (cis-)		
nonachlor (trans-)		
oxychlordane		
polybrominated diphenyl ethers (PBDEs)		
polychlorinated biphenyls (PCBs)/PCB 209*(C^{13})		
toxaphene		

* surrogate
1/ 6% ethyl ether in petroleum ether (analysis by GC-MSMS)
2/ 15% ethyl ether in petroleum ether (analysis by GC-ECD)
3/ 50% ethyl ether in petroleum ether (analysis by GC-ECD).
4/ In both 6% and 15% fractions.
5/ In both 15% and 50% fractions.

3.0 Interferences

3.1 Solvents, reagents, glassware, and other sample processing hardware may cause GC artifacts and/or elevated baselines, resulting in the misinterpretation of chromatograms. All materials should be demonstrated to be free from interferences under the conditions of the analysis by running method blanks initially and with each sample lot. Specific selection of reagents and purification of solvents by distillation in all-glass systems are required. High-purity, distilled-in-glass solvents are commercially available.

An effective way of cleaning laboratory glassware is by rinsing with polar and non-polar solvents before use. The cleaning procedure used must be tested by analyzing procedural blanks prior to analyzing samples.

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

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

4.0 Apparatus and Materials

4.1 Wide mouth, borosilicate glass, pre-cleaned and certified, 250 mL, Qorpak or equivalent.

4.2 Chromatographic Column - (300 mm x 11 mm) borosilicate glass chromatography column with 250 mL reservoir and Teflon stopcock.

4.3 Glass wool, Pyrex - solvent washed prior to use.

4.4 Kuderna-Danish (K-D) Apparatus

4.4.1 Concentrator tube - 10 mL, graduate (Kontes K0570050-1025, or equivalent). A ground stopper, 19/22 joint, is used to prevent evaporation of extracts.

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

4.4.3 Snyder column - three ball (Kontes K-503000-0121, or equivalent).

4.4.4 Micro-Snyder column - (Kontes VWR KT569261-0319 or equivalent).

4.4.5 Boiling stones, Chemware® Ultra-Pure PTFE, extracted with acetone and petroleum ether. Note that boiling chips can be a significant source of contamination if not properly cleaned.

4.5 Water bath, Organomation Assoc. Inc.(OA-SYS/S-EVAP-KD), 115 V, thermostatically controlled with stainless steel cover to fit 5 K-D apparatus, installed in a fume hood. Water bath is equipped with solvent recovery system.

4.6 Extractor, automated, Dionex Accelerated Solvent Extractor (ASE 200), Dionex P/N 047046.

4.6.1 Extraction Cells, 33 mL, Dionex P/N 049562

4.6.2 Filters, cellulose for ASE extraction cells, Dionex P/N 049458.

4.6.3 VOA Vials, 60 mL, pre-cleaned and certified.

4.7 Sample vials - glass, 2.5 mL with PTFE-lined screw cap.

4.8 Analytical balance - capable of weighing 0.1 mg.

4.9 Drying oven.

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

4.11 Disposable Pasteur Pipettes - (rinsed with solvents before use).

4.12 Aluminum dishes for moisture and lipid determination.

4.13 Desiccator with indicating desiccant.

4.14 Glass funnel, 75 mm.

4.15 Graduated cylinder, 250 mL and 100 mL.

4.17 Culture tubes, 13 x 100mm and 16 x 100 mm, with PTFE lined cap.

4.18 Centrifuge tubes, 15 mL, graduated to 0.1 mL and calibrated to 1.0 mL.

4.19 Gas chromatographs (GC) (3): Hewlett-Packard HP 6890 plus, equipped with dual micro-ECD. All are equipped with split-splitless injector with EPC and autosampler.

4.20 GC Capillary columns, 60 meter DB5 and 60 meter DB17MS (J&W Scientific) (0.25 mm I.D. and 25 µm film thickness) connected to a single injection port using a "Y" press fit connector.

4.21 GC Data System, Hewlett-Packard, to collect and record GC data, generate reports, and compute and record response factors for multi-level calibrations.

Data system should be capable of calibrating a method using a minimum of 5 concentrations of analytical standards.

4.22 Gas chromatograph-mass spectrometer (triple quadrupole), Varian Model 1200L with Varian Model 3800 gas chromatograph, split-splitless injector with EPC and Combi-Pal autosampler.

4.23 Homogenizer, Bucchi Model B-400 (Brinkman P/N 16-07-200-1) or equivalent equipped with titanium knife assembly (Brinkman P/N 16-07-222-2) and glass sample vessel (Brinkman P/N 16-07-245-1).

4.24 Homogenizer, Brinkman Polytron or equivalent equipped Teflon and titanium generator assembly (for homogenization of small sample amounts).

4.25 Gel Permeation (size exclusion) Chromatograph, automated, J2 Scientific AccuPrep 170, equipped with 70 g S-X3 BioBeads J_2 Scientific P/N C0070G (100% DCM).

5.0 Reagents

5.1 Petroleum ether (PE), Burdick and Jackson, distilled in glass and pesticide residue or HRGC grade or equivalent.

5.2 Acetone. (Same as above).

5.3 Iso-Octane. (Same as above).

5.4 Diethyl ether preserved with 2% ethanol.(Same as above).

5.5 Dichloromethane (DCM). (Same as above).

5.6 Chem Elut-Hydromatrix®, Varian P/N 0019-8003. Pre-extracted on ASE-200 with acetone/DCM prior to use.

5.7 Sodium sulfate. Anhydrous granular reagent grade, rinsed with PE prior to use.

5.8 Florisil®, 60/100 mesh, PR grade, U.S. Silica.

5.9 Nitrogen, pre-purified grade (99.9999%) or better (used for ASE and GPC).

5.10 Nitrogen, ultra-pure (99.99999%) for ECD makeup.

5.11 Helium, ultra-pure (99.99999%) for GC carrier gas.

5.12 Air, compressed, breathing quality, for ASE pneumatics.

5.13 OC/PCB/PBDE Surrogate Mix containing: 40 ppb of deuterated p,p'-DDD-d10, PCB 209(C^{13}), and dibutylchlorendate (DBCE).

5.14 Standard Reference Material (SRM), National Institute of Standards and Technology (NIST): SRM 1588b (Organics in Cod Liver Oil) and SRM 1944 (New York/New Jersey Waterway sediment).

CAUTION

The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of data handling Material Safety Data Sheets should also be made available to all personnel involved in these analyses.

6.0 Sample Collection, Preparation, and Storage

6.1 In the field, sources of contamination include sampling gear, grease from ship winches or cables, ship and/or motor vehicle engine exhaust, dust, and ice used for cooling. Efforts should be made to minimize handling and to avoid sources of contamination. This will usually require that resection (i.e., surgical removal) of tissue be performed in a controlled environment (e.g., a laboratory). The samples should be double wrapped in aluminum foil and immediately frozen with dry ice in a covered ice chest. Ice should be in water tight plastic bags for transporting live shellfish.

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

6.3 Resection should be carried out by or under the supervision of a competent biologist. Each organism should be handled with clean high carbon steel, titanium, quartz, or Teflon instruments (except for external surfaces). The specimens should come into contact with pre-cleaned glass surfaces only. Polypropylene and polyethylene surfaces are a potential source of contamination and should not be used. To control contamination when resecting tissue, separate sets of utensils should be used for removing outer

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

6.4 The resected tissue sample should be placed in a clean glass or PTFE container which has been washed with detergent, rinsed twice with tap water, rinsed once with distilled water, rinsed with acetone, and, finally, rinsed with high-purity petroleum ether.

6.5 The U.S. EPA has published a guidance document containing specific recommendations regarding holding times and temperatures for tissue samples to be analyzed for semi-volatile organic compounds. The following holding conditions should be observed. Tissue samples should be maintained at $\leq$-20° C and analyzed as soon as possible, but within 12 months of sample receipt.

6.6 Sediment samples may be refrigerated at 4°C for up to 14-days maximum or <u>must</u> be stored frozen at minus (-) 20°C for up to 12 months maximum.

7.0 Sample Extraction

7.1 Remove homogenized tissue or sediment samples from freezer and allow to thaw. Prior to extraction, the tissue samples are homogenized using a Bucchi B-400 mixer equipped with a titanium knife assembly or for small samples a Brinkman Polytron® equipped with a titanium and Teflon generator. Decant any excess water from the sediment samples prior to thoroughly mixing by hand using a clean glass rod or may be homogenized using a Polytron homogenizer equipped with stainless steel generator equipped with Teflon bearings. Sample sets of 10-18 should be extracted when possible. The ASE-200 extractor will extract 24 cells. Be sure to reserve enough cells for method blanks, matrix spikes, and laboratory control spikes.

7.2 A separate extraction bench sheet is initiated for each project, sample matrix type, and analysis type. Several bench sheets may be used for an extraction set.

7.3 Prepare a glass rod or Teflon spatula for each sample to be weighed by rinsing 3 times with petroleum ether using a Teflon wash bottle.

7.4 Label 60 mL VOA vials for the collection of the sample extract. The labels must be placed between 1.5" and 3" from the top of the VOA cap; if they are placed outside of this area, they will interfere with the ASE optical sensor. Use two VOA vials for each sample. Label the first VOA vial with the ASE position number, bench sheet number and the sample name. Label the second VOA vial the same but add “RE” to distinguish between the two vials. Label and weigh aluminum planchets for lipid and moisture determinations (write sample ID on the bottom of planchets using a ball point pen).

7.5 Tare a 250 mL glass jar. Using a clean (solvent rinsed) glass rod, stir the tissue or sediment so that the mixture is homogeneous. Weigh 10 g of sample into the jar, record the weight on the bench sheet, and add the twice-extracted Hydromatrix® from one ASE cell. Stir the mixture thoroughly and go on to the next sample. After approximately 15 minutes stir the sample again. Repeat this at 15 minute intervals two more times or until the sample mixture is free flowing.

7.6 Weigh 1-5 g of additional sample into a pre-weighed and tared aluminum planchet for % moisture analysis. Place planchets in 70°C oven for 48 hours and re-weigh dry weight.

7.7 Place a pre-rinsed powder funnel on top of a 33 mL ASE cell containing a pre-extracted cellulose filter *(the filter is the one that was used to pre-extract the Hydromatrix®)*.

7.8 Pour the tissue or sediment/Hydromatrix® mixture through the powder funnel back into the extraction cell that the Hydromatrix® was poured from. Tap the cell against the counter top to settle the contents. The mixture will fill the cell and it may be necessary to pack it slightly using the glass rod and the end of the powder funnel. The cells used for the method blank and laboratory control spike and its duplicate *(if used)* will contain only Hydromatrix®.

7.9 <u>All</u> of the extraction cells are spiked with the OC/PCB/PBDE pesticide surrogate standard. Spike each cell with exactly 0.5 mL of the appropriate surrogate solution. Surrogate spikes must be witnessed, recorded and dated on the extraction bench sheet.

7.10 The extraction cells used for the matrix spike (MS) and duplicate matrix spike (MSD) and laboratory control spike (LCS) and its duplicate (LCSD) (if used) are spiked with exactly 0.5 mL of the OC/PCB/PBDE matrix spike solution (40 ng/mL). A separate MS/MSD and LCS/LCSD (if used) is required for each class of compounds being analyzed. Matrix spikes must be witnessed, recorded and dated on the extraction bench sheet.

7.11 The extraction cells are capped *(Firmly tightened but do not overtighten)* and placed on the ASE 200 carrousel. The first set of labeled VOA collection vials

are placed on the ASE 200 collection carrousel with the position numbers corresponding to the position numbers of the extraction cells. Make sure that the solvent reservoir contains enough solvent for the extraction.

7.12 Samples are extracted with acetone/methylene chloride (DCM) 50:50 using the following conditions:

Pre-heat	0 min.
Heat	5 min.
Static	5 min.
Flush	60%
Purge	300 sec.
Cycles	1
Pressure	1500 psi
Temp	100 ^{o}C
Sol A Other	100%

7.13 After the initial extraction is complete, remove full VOA vials and place in a Wheaton rack. Place the second set of collection VOA vials labeled “RE” on the ASE carrousel. Check each of the extraction cells to make sure that the caps are *(firmly tightened)* as they tend to loosen with the first extraction. Make sure that the replacement vials are in the correct order. Make sure that the solvent reservoir contains enough solvent for the re-extraction. Re-start the ASE-200.

7.14 When extraction is completed, place VOA vials in a Wheaton rack with the “RE” vials next to the vials from the first extraction. The extracts should be re-capped with solid green caps (Qorpak) and placed in a refrigerator for storage until they are removed for the GPC cleanup procedure.

8.0 Gel Permeation Chromatography

IMPORTANT: ***All glassware, glass wool, and sodium sulfate must be triple-rinsed with petroleum ether before they are used for this procedure.***

8.1 Remove VOA vials containing the sample extracts from the refrigerator. Make sure the vials are capped with the green Qorpak caps. Allow them to sit out until they are at room temperature.

8.2 Set up and label pre-cleaned K-D flasks (4-6) with concentrator tubes attached on ring stands in the fume hood. Place a funnel containing a plug of pre-cleaned glass wool in the bottom of the funnel and place the funnel in the top of the K-D flask. Add about two inches of pre-rinsed sodium sulfate to the funnel. Make sure that the level of the sodium sulfate is uniform across the

funnel to prevent any possible splashing out.

8.3 Pour sample extracts from the VOA vials through sodium sulfate into the K-D flask. Add about 10 mL of DCM to the VOA vial, cap and shake and add this rinse to the sodium sulfate. Repeat with another 10 mL DCM rinse. Rinse the sodium sulfate with an additional portion of DCM (~50 mL) by pouring from a clean and rinsed 400 mL beaker. After the solvent has completely drained through the sodium sulfate add one more additional rinse of DCM (~50 mL) from the beaker of clean DCM. Allow the DCM to completely drain through the sodium sulfate (~3-5 minutes).

8.4 Add 0.5 mL Iso-Octane using a macro-pipetter and a solvent rinsed boiling chip to each K-D flask. Place a Snyder column on the K-D flask, clamp with a green clamp and place the flask on the hot water bath set at 80-82°C. Drop down the inverted Hopkins condenser from the solvent recovery system and attach it to the top of the Snyder column. Turn the water supply on to the solvent recovery system until the water flow is between 1500-2000 cc/min. Evaporate the solvent until the apparent volume is 2-5 mL. Remove the inverted Hopkins condenser and secure using the set clamps so that it is out of the way. At this point there should be between 2-5 mL visible in the concentrator tube while the K-D apparatus is still on the hot water bath and 10 mL or less of the solvent remaining after the K-D flask is removed from the hot water bath and the solvent drains from the Snyder column. Dry off the water using a WyPall X60 towel to remove any water from around the ground glass union of the concentrator tube and the K-D flask to prevent any of it from entering the concentrator tube upon removal.

8.5 After the K-D apparatus has cooled and all of the solvent has drained from the Snyder column, remove the Snyder column, label the concentrator tube and then remove the concentrator tube from the flask and place the tube in a test tube rack and cover with pre-rinsed aluminum foil. Rinse the Snyder column with petroleum ether and place back in the column rack for storage. After all of the flasks have been removed from the hot water bath, repeat steps 2-5 for the remaining samples extracted with this set.

8.6 Add a new micro-boiling stone and place a clean micro-Snyder column on the concentrator tube with a blue clamp and place in a 400 mL beaker containing hot water heated to approximately 75°C on a hot plate. If the solvent does not begin to boil, remove the tube from the bath immediately, allow it to cool slightly, add a new micro boiling stone to prevent it from bumping and place it back in the bath. Evaporate the solvent until only 1.0 mL remains in the concentrator tube. Four or five tubes can be evaporated at one time.

8.7 When the solvent has been evaporated to 1.0 mL remove the tube from the bath and allow it to cool in a test tube rack. Remove the micro-Snyder column and add DCM to the concentrator tube to reach a final volume of 10.0 mL.

8.8 Gelman filter (0.45 µm) the sample into a 12 mL culture tube. Using a volumetric pipette remove 2.0 mL of the filtered sample and place it in a pre-weighed aluminum planchet if lipid determination is needed. Cap the culture tube with the Teflon-insert style caps. Mark the bottom of the meniscus with a pen in case of evaporation before clean-up on GPC.

8.9 All samples are cleaned using a J_2 Scientific GPC (Autoinject 110, AccuPrep 170, DFW-20 Fixed Wavelength Detector, 1" ID glass column with 70g Bio-Beads SX-3 in 100% DCM)

8.9.1 From the desktop double click on the AccuPrep.exe shortcut to open the program. Click on the Use Injector button and allow the instrument time to initialize. Activate the pump by using the top left hand button. A solvent Control Pump window will open up. Click on the Apply Defaults button and then OK on the Selected Pressure Limit 30 psi. The pump should audibly be heard coming on and the green light should show that the system is on line and status flowing. Make sure that the bottle of clean DCM is full and the waste bottle is empty. Allow the system to pump for about 5 minutes before switching the column in-line (gray button next to Column that has 'Put in line' on it). The pressure will be observed to normally go up to the 12-16 psi range. Turn the power on to the detector to allow it at least 30 minutes of time to warm up before use. Because the scale is auto-adjusted in the software now it is no longer necessary to manually adjust the range on the unit itself.

8.9.2 While the system is equilibrating, the sequence can be entered. Click on the Seq button next to the Pump button. An 'Editing new sequence' window will pop up. This gives a view of the instrument which clearly shows the sample tray locations and the corresponding sample collection locations. By clicking on the sample tray position, a new window 'Adding sample at tray position #' will pop up. This allows information to be included about each specific sample. Sample position 1 will always be a calibration standard (CLP-340) which is run prior to any sequence of runs to verify instrument integrity. In the Sample ID field just type in 'CLP-340'. In the Descrip (optional), information pertaining to the project, laboratory control number, bench sheet number and date are typically added. The Method File needs to be changed to 'ZGPC Calib' for only this sample and in the Sample Type field the 'Calibration' type can be chosen. After this information is completed click on the OK to continue. This returns you back to the main sequence window but now the first position will be highlighted in green. Continue by adding the next sample information to tray position 2, again following the same steps as before. By default the Method File will be on the program SOPAH which is used for both pesticides (SO) and petroleum (PAH) clean-up. Also by default, the Sample Type field will already be set at 'Sample'. This will not need to be changed until a duplicate sample (Duplicate), matrix spike (Matrix Spike), matrix spike duplicate (Spike Duplicate), laboratory control spike (Spiked

Blank), and the SRM (Lab Control Std) are encountered. After all the samples have been added to the sequence, save it as the bench sheet number (BS###). From the Editing sequence window print out the sample list. Compare the information to your original bench sheet to insure there are no mistakes. Make sure the ZGPC method is being used for the calibration standard and the SOPAH method is being used for the samples. Next verify that the samples are still at the marked line on the culture tubes (add DCM to the marked line if they are not). Place a tube with the GPC Calibration Standard Solution (CLP-340) in sample tray position 1 and then follow as the sequence was made in the remaining positions.

8.9.3 Get two boxes of the 125 mL Trace Clean amber bottles for sample collection. A bottle does not need to be placed in collection position #1 because that is the GPC Calibration Std (all goes to waste). Remove the white caps from the bottles and place them on top of the detector (so that Teflon side is not exposed to possible contamination). Label the boxes with bench sheet and laboratory control numbers and keep them for the post-GPC samples to be stored in. Now that the pump as had plenty of time to equilibrate the system and the detector has had plenty of time to warm up, in the Signal field click to adjust the setting to 'Absorbance Units' and click on the 'Zero Signal' button to set the baseline.

8.9.4 If the pressure seems to be pretty stable between the 12-16 psi range and all the sample positions and collection positions have been loaded, then click on the large button with the stop watch to begin the program. A window will pop up asking if the correct column method is loaded (100%DCM). Click on 'yes' to engage the syringe pump to begin priming. The sample probe will move over to sample position #1 and aspirate the sample. After the samples have all been processed (~1 hour per sample), remove the label from the sample position and place it on the bottle in corresponding collection position. Cap the bottle and place it back in the box that was retained for their storage. At the end of the sequence there will be a window that pops up saying that the 'Sequence has been successfully completed'. The column will switch offline and the pump will automatically shut down. The only thing that has to manually be turned off is the power to the detector. Empty the waste container into a 4L waste bottle labeled with a hazardous waste label.

8.10 Pour the GPC eluate into a rinsed K-D flask. Rinse the bottle with some DCM and add that to the K-D flask. Add 0.5 mL Iso-Octane and a micro boiling chip to each K-D flask. Attach a Snyder column to the flask and place in the hot water bath. Attach the inverted Hopkins condenser to the top of the Snyder column and turn to water on to the solvent recovery system (~1500-2000 cc/min). When the volume of the solvent in the concentrator tube is level with the base of the K-D flask, remove the inverted Hopkins condenser and secure out of the way. Lift the K-D apparatus up enough to be able to angle it slightly and add 40-50 mL Petroleum Ether through the top of the Snyder column. By

holding the K-D apparatus at an angle, it allows the solvent to more easily drain back into the flask. Return to the K-D apparatus back into the hot water bath. Repeat this step 2 more times to successfully solvent exchange the sample from DCM to Petroleum Ether. When the apparent volume in the concentrator tube is 5-10 mL remove it from the hot water bath. Wipe down the K-D apparatus with a WyPall X60 towel especially around the ground glass junction. Remove the Snyder column from the K-D apparatus and allow to completely drain into the concentrator tube. After it has finished cooling, remove the concentrator tube and bring to a final volume of 10 mL in DCM. Split the sample using a 5 mL volumetric pipette. One aliquot is transferred to a labeled 13 x 100 mm test tube. Add a new micro boiling chip to the remaining aliquot and place it in a 400 mL beaker containing water heated to approximately 75°C on a hot plate (4-5 tubes can be evaporated at one time). Evaporate the solvent down to 1-2 mL. Remove it from the water bath and allow it to cool. Exactly one-half of the extract is removed and placed in a GC autosampler vial for PAH silica/alumina column cleanup or for archive if PAHS are not requested.

8.11 Transfer the solution to a 13 x 100 culture tube with a Pasteur pipette, rinse the concentrator tube with 0.5 ml of Petroleum Ether, vortex, and transfer the rinse to the culture tube. Repeat the rinse step two more times, and add each rinse to the culture tube. Cap the culture tube with a Teflon faced cap.

8.12 SEDIMENT SAMPLES ONLY: Check the GPC chromatogram for a sulfur peak. If a sulfur peak is present, add acid rinsed copper to the culture tubes to remove any residual sulfur from the extract.

9.0 Florisil® Column Fractionation

IMPORTANT: ***All glassware, glass wool, and sodium sulfate must be triple-rinsed with petroleum ether (PE) before they are used for this procedure.*** **Florisil®** ***must be activated in an oven at 130°C for at least 24 hours prior to use.***

9.1 This procedure is performed after the GPC cleanup procedure for all tissue and sediment samples analyzed for pesticides and PCBs.

9.2 **PCB ONLY:** When the samples are to be analyzed for only PCBs prepare only the 6% ethyl ether in petroleum ether Florisil column eluant. Make an amount slightly in excess of what is actually needed to allow for any loss which may occur during solvent transfer. The required volume is 40 mL per sample for the 6% eluant.

9.3 **TISSUE:** Prepare the reagents to be used for Florisil® cleanup <u>for tissue</u>: 6% ethyl ether in petroleum ether, 15% ethyl ether in PE, and 50% ethyl ether in

PE. Make an amount slightly in excess of what is actually needed to allow for any loss which may occur during solvent transfer. The required volume is 40 mL per sample for the 6%, 50 ml per sample for the 15% (F2), and 40 ml per sample for the 50% (F3) fractions.

9.4 **SEDIMENT:** Prepare the reagents to be used for Florisil® cleanup for sediment: 6% ethyl ether in petroleum ether and 50% ethyl ether in PE. Make an amount slightly in excess of what is actually needed to allow for any loss which may occur during solvent transfer. The required volume is 40 mL per sample for the 6% and 40 ml per sample for the 50% fraction.

9.5 Prepare the chromatography columns. Place a small piece of PE rinsed glass wool in the bottom of the column and tap into place with a PE rinsed glass rod. Cover with a small portion (0.5 inch) of sodium sulfate. Fill the column with 5 grams of Florisil® that has been measured using a dedicated pre-calibrated culture tube. Tap column with rubber "mallet" to firmly settle the Florisil®. Top the column with 3/4-1 inch of sodium sulfate. This will prevent the column from being disrupted when solvent is added and will remove any residual water.

9.6 Place a 600 mL beaker under the column and pre-wet the column with about 25 mL of petroleum ether.

IMPORTANT: ***From this point and through the elution process, the solvent level should never be allowed to go below the top of the sodium sulfate layer.***

9.7 When approximately 1 inch of PE remains above the surface of the column, add 0.5 mL of iso-octane to a K-D flask and place it under the column making sure that the stopcock is in the full open position. This will allow for a flow rate of about 2 to 3 mL/min. When the meniscus of the PE rinse reaches the column bed surface, decant the sample onto the column. Immediately add approximately 0.5 mL of PE to the tube, vortex, and add the rinse to the sample extract on the column. Add another 0.5 ml of PE to the tube, vortex, and add this final rinse to the sample extract on the column. Start the columns in a sequential fashion, and the lag time will be adequate to perform the necessary tasks for up to six columns.

9.8 When the combined sample and rinses reach the sodium sulfate layer, add 40 mL of 6% diethyl ether/petroleum ether that has been carefully measured out using a graduated cylinder to the column reservoir. Make sure that the stopcock is fully open in order to achieve the desired flow rate of 2 to 3 mL per minute. Place a 50 mL clean, dry, petroleum ether rinsed beaker over the top of the reservoir to prevent evaporation during the elution process. If only **PCB** analyses are requested, allow the column to completely drain and stop here.

TISSUE SAMPLES

9.9 Just as the last of the 6% diethyl ether/PE solvent reaches the top of the sodium sulfate layer, add 0.5 mL of iso-octane to a new K-D flask and exchange it for the K-D containing the 6% elution, add 50 mL of the 15% diethyl ether/PE mixture to the column reservoir, replace the 50mL beaker, and elute as before. Add a micro boiling stone and attach a Snyder column with a green clamp to the K-D flask containing the 6% diethyl ether/PE fraction and place vessel in the hot water bath with the temperature set at 80-82 ^{o}C and reduce volume to an apparent volume of 1 mL. Tap the Snyder column to make sure solvent is not trapped between the balls then remove the vessel from the bath and place in the vessel stand to cool.

9.10 Repeat the above adding 0.5 mL of iso-octane to a new K-D flask and exchange it for the K-D flask containing the 15% eluant. Add 40 mL of 50% diethyl ether/PE mixture to the solvent reservoir. Allow all of the eluant to drain into the K-D flask.

SEDIMENT SAMPLES

9.11 Just as the last of the 6% diethyl ether/PE solvent reaches the top of the sodium sulfate layer, add 0.5 mL of iso-octane to a new K-D flask and exchange it for the K-D flask containing the 6% eluant, add 40 mL of the 50% diethyl ether/PE mixture to the column reservoir, replace the 50mL beaker, and elute as before. Add a micro boiling stone and attach a Snyder column with a green clamp to the K-D flask containing the 6% diethyl ether/PE fraction and place vessel in the hot water bath with the temperature set at 80-82 ^{o}C and reduce volume to an apparent volume of 1 mL. Tap the Snyder column to make sure solvent is not trapped between the balls then remove the vessel from the bath and place in the vessel stand to cool.

9.12 When the vessels are cool, remove the concentrator tube from the K-D flask add a new micro boiling stone and attach a clean micro-Snyder column to the concentrator tube with a blue clamp and place in a 400 mL beaker containing hot water heated to approximately 75°C on a hot plate. Evaporate the solvent until only 0.5-1 mL remains in the concentrator tube. Four or five tubes can be evaporated at one time.

9.13 When the solvent has been evaporated to 0.5-1 mL remove the tube from the bath and allow it to cool in a test tube rack. Remove the micro-Snyder column and transfer the contents to a calibrated centrifuge tube rinsing the concentrator tube with a small amount of PE and adding the rinsate to the centrifuge tube. If the volume in the centrifuge tube is greater than 1 mL, evaporate to 1 mL using nitrogen. Mix the tube contents by tapping the bottom of the tube causing a vortex which will rinse the sides of the tube. A Vortex

Genie mixer may be used for this step. Transfer the extract to a clean labeled culture tube and cap.

9.14 Repeat for 15% (tissue only) and 50% extracts. The extracts are ready for analysis by GC-ECD and GC-MSMS.

10.0 Analytical Procedure

10.1 Before the sample extracts can be analyzed, a sequence listing the order of calibration standards, second source check standards, initial and continuing calibration blanks, initial and continuing calibration verification standards and sample extracts is written using Agilent Chemstation (GC) or Varian (GC-MSMS) Software.

10.2 Each sequence includes a minimum of seven calibration standards. The calibration curve concentration for chlorinated hydrocarbons differs for different analytes, but in general the range is 0.5 ppb to 500 ppb. The calibration curve concentration range for polychlorinated biphenyl congeners (PCBs) is 0.5 ppb to 100 ppb. Higher concentrations of PCB standards (50 ppb to 1000 ppb) are analyzed with samples containing higher concentrations of PCBs.

10.3 To verify the calibration standards, second source pesticide check standards (Radian Corp., Pesticide Check Standard Mix A, ERP-009L; Pesticide Check Standard Mix B, ERP-011L) and PCB congener check standard (Ultra Scientific, RPC-EPA) are analyzed. The second source analytes and their concentrations are listed in Table 5 (pesticides) and Table 6 (PCB congeners).

Table 5. Radian Pesticide Calibration Check Standards (Mix A and B)

Mix A	Certified Concentration (ng/µL)
Aldrin	10.0
Gamma-HCH	5.00
DDT, p,p’	20.0
Dieldrin	10.0
Endosulfan I	10.0
Endosulfan II	20.0
Heptachlor	10.0
Heptachlor epoxide	10.0
Methoxychlor	80.0
Mix B	
Alpha-HCH	5.00
Beta-HCH	20.0
Delta-HCH	10.0
Cis-chlordane	10.0

Trans-chlordane		10.0
DDD, p,p'		20.0
DDE, p,p'		10.0
Endosulfan sulfate	20.0	
Endrin		20.0

Table 6. Ultra Scientific PCB Congener Check Standard

RPC-EPA	Certified Concentration (ng/µL)*
PCB 8	4.0
PCB 18	4.0
PCB 28	4.0
PCB 52	4.0
PCB 44	4.0
PCB 66	4.0
PCB 101	4.0
PCB 118	4.0
PCB 153	4.0
PCB 105	4.0
PCB 138	4.0
PCB 187	4.0
PCB 128	4.0
PCB 180	4.0
PCB 170	4.0
PCB 195	4.0
PCB 206	4.0
PCB 209	4.0

* Initial concentration of RPC-EPA is 0.2 µg/mL in iso-octane. This solution is diluted 2:100 in iso-octane

10.4 An initial calibration blank and initial calibration verification standard is analyzed after the calibration standards and prior to the first sample extract. For the 6% Fraction and 15% Fraction runs, continuing calibration blanks (CCBs) and calibration verification standards (CCVs) are analyzed after ten sample extracts have been analyzed. The 50% Fraction extracts contain more lipid material and can cause the CCVs to fail to meet the % recovery criteria, therefore the CCBs and CCVs are analyzed after every five sample extracts. If a CCV fails, the five samples prior to the failed CCV and the five samples after the failed CCV are re-analyzed after a new calibration curve is analyzed.

10.5 The CCV analyte concentrations are mid-range of the calibration curve (5 – 10 ppb).

10.6 As the run proceeds, sample extracts are monitored for analyte concentrations that are greater than the calibration curve and need dilution.

10.7 Instrumentation

Gas Chromatographs with Electron Capture Detectors:

10.7.1 Agilent 6890*plus* gas chromatograph equipped with two ^{63}Ni micro-electron capture detectors with EPC and autosampler. Two 60 meter, 0.25 mm ID, 0.25 µm (film thickness) fused silica columns (J&W) are used. A 5 meter length of DB-5 column is connected to a press fit "Y" union which splits the column effluent into two 60 m columns, a DB-5 and a DB-17MS. The injector is a split-splitless injector with EPC.

10.7.2 Chromatograph conditions:
The injector is operated isothermal at 240°C. The oven has an initial temperature of 80°C which is held for 1 minute and then temperature programmed to 210°C at a rate of 15°C/min and held for 10 min. It is then programmed to 280°C at a rate of 2°C/min and is held for 51 min (for PBDE analysis the oven is held at 280°C for 110 min). Helium is used as the carrier gas at a linear velocity of 35 cm/sec. Nitrogen is used for the detector makeup at 30 mL/min.

10.7.3 Sample volume:
Three microliters of samples and standards are injected and split approximately 50/50 onto the 60 m DB-5 and the 60 m DB-17MS.

10.7.3 Instrument calibration:
External standard calibration is used.

10.7.4 Data acquisition and processing:
Detector signals are acquired and processed with a Agilent 3365 Series II Chemstation. Data processing may also be done using Enviroquant Software.

Gas Chromatograph-Triple Quadrupole Mass Spectrometer:

10.7.5 Varian Model 3800/1200L gas chromatograph/triple quadrupole mass spectrometer equipped with a Model 1177 split-splitless injector with EPC and CombiPal autosampler. A J&W 60 meter, 0.25 mm ID, 0.25 µm (film thickness) XLB fused silica columns (J&W) is used. The injector is a split-splitless injector with EPC.

10.7.6 Chromatograph Conditions:
The injector is operated isothermal at 280°C in splitless mode with pressure pulse (45 psi for 1.05 min). The oven has an initial temperature of 80°C which is held for 1 minute and then temperature programmed to 210°C at a rate of 15°C/min and held for 10 min. It is then programmed to 280°C at a rate of 2°C/min and is held for 8 min.

Helium is used as the carrier gas at a constant column flow of 1 mL/min.

10.7.7 Mass Spectrometer Conditions:
The mass spectrometer is operated in electron impact (EI) ionization and MSMS mode using argon as the CID gas. A collision energy of 10 to 30 volts is used depending on the analyte. Q1 and Q3 mass fragments were selected to optimize selectivity and sensitivity. See Table 7.

Table 7. Varian 1200 MS collision energies and mass fragments (Q1 and Q3) for targeted analytes.

	Segment	Q1	Q3	Collision Energy	Internal Standard
DBOB	1	296	246	-20	HCH, alphaC^{13}
HCH, alpha	2	219	183	-10	HCH, alphaC^{13}
HCH, alphaC^{13}	2	223	187	-10	Internal Std
HCB	3	284	214	-30	HCB C^{13}
HCBC^{13}	3	290	220	-30	Internal Std
HCH, gamma	4	219	183	-15	HCH, alphaC^{13}
HCH, beta	4	219	183	-15	HCH, alphaC^{13}
Heptachlor	5	272	237	-15	HeptachlorC^{13}
HeptachlorC^{13}	5	277	242	-15	Internal Std
Chlorpyrifos	6	314	258	-10	ChlorpyrifosC^{13}
ChlorpyrifosC^{13}	6	325	260	-15	Internal Std
Aldrin	6	293	258	-10	ChlorpyrifosC^{13}
Oxychlordane	7	387	263	-10	Nonachlor, transC^{13}
Heptachlor epoxide	7	387	353	-10	HeptachlorC^{13}
DDE, o,p’	8	318	246	-10	DDE, p,p’C^{13}
DDMU, p,p’	9	284	212	-15	DDE, p,p’C^{13}
Chlordane, trans	9	373	266	-15	Nonachlor, transC^{13}
Chlordane, cis	9	373	266	-15	Nonachlor, transC^{13}
Nonachlor, trans	10	409	310	-15	Nonachlor, transC^{13}
Nonachlor, transC^{13}	10	418	310	-15	Internal Std
DDE, p,p’	12	318	246	-15	DDE, p,p’C^{13}

DDE, p,p'C^{13}	12	329	258	-15	Internal Std
DDD, o,p'	13	235	165	-20	DDE, p,p'C^{13}
DDT, o,p'	15	235	165	-15	DDT, p,p'C^{13}
Nonachlor, cis	16	409	275	-15	Nonachlor, transC^{13}
DDD, p,p-deuterated	16	243	173	-20	DDT, p,p'C^{13}
DDD, p,p'	16	235	165	-15	DDT, p,p'C^{13}
DDT, p,p'	17	235	165	-25	DDT, p,p'C^{13}
DDT, p,p'C^{13}	17	248	177	-20	Internal Std
Methoxychlor	18	227	169	-20	DDT, p,p'C^{13}
Mirex	20	272	237	-15	DDE, p,p'C^{13}

10.7.8 Instrument Calibration:

Internal standard calibration is used. Internal standards are added to the standards and sample extracts just prior to analysis. The following internal standards are used at 1.0 ng/µL:

<u>PCB Internal Standards</u>
PCB 52 (C^{13}) – 4Cl congeners
PCB 97 (C^{13}) – 5Cl congeners
PCB 128 (C^{13}) – 6Cl and 7Cl congeners
PCB 194 (C^{13}) – 8Cl congeners
PCB 206 (C^{13}) – 9Cl congeners
PCB 209 (C^{13}) – 10Cl congeners

<u>OC Internal Standards</u>
HCH, alpha (C^{13})
HCB, (C^{13})
Heptachlor, (C^{13})
Chlorpyrifos, (C^{13})
Nonachlor, trans (C^{13})
DDE, p,p' (C^{13})
DDT, p,p' (C^{13})

Nine target analyte calibration levels are used (0.25, 0.50, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, 100 ng/µL).

10.7.9 Sample volume:

Two microliters of samples and standards are injected.

10.7.10 Data processing:

Mass spectrometer signals are acquired and processed using Varian 1200L software .

11.0 References

Tetra Tech, Inc. 1986. Bio Accumulation monitoring Guidance: 4. Analytical Methods for U.S. Priority Pollutants and 301 (h) Pesticides in tissues from Estuarine and Marine Organisms. TC-3953-03. U.S. EPA Washington, DC.

U.S. Environmental Protection Agency. 1993. Guidance For Assessing Chemical Contaminant Data For Use In Fish Advisories, Volume I, Fish Sampling and Analysis. EPA 823-R-93-002. U.S. EPA, Office of Water, Washington D.C.

U.S. Food and Drug Administration. 1994. Pesticide Analytical Manual. Volume 1, Chapter 3, Multiclass Multiresidue Methods. U.S. Food and Drug Administration, Rockville, MD.

U.S. Environmental Protection Agency, Office of Solid Waste, SW-846 On-Line,
Method 3545A, *Pressurized Fluid Extraction*, Revision 1, February 2007,
http://www.epa.gov/epawaste/hazard/testmethods/sw846/pdfs/3545a.pdf [11/10/08]
Method 3620C, *Florisil Cleanup*, Revision 3, February 2007,
http://www.epa.gov/epawaste/hazard/testmethods/sw846/pdfs/3620c.pdf [11/10/08]
Method 3640A, *Gel Permeation Cleanup*, Revision 1, September 1994,
http://www.epa.gov/epaoswer/hazwaste/test/pdfs/3640a.pdf [03/29/07]

SOP Section Approval: _____ Date: _____

SOP Final Approval: _____ Date: _____

SOP QA Officer Approval: _____ Date: _____

Method # MPSL-105

LABORATORY PREPARATION OF TRACE METAL AND SYNTHETIC ORGANIC SAMPLES OF TISSUES IN MARINE AND FRESHWATER BIVALVES AND FISH

1.0 Scope and Application

1.1 The following procedures describe techniques for the laboratory preparation of marine and freshwater tissues for trace metal (TM) and synthetic organic (SO) analysis.

2.0 Summary of Method

2.1 Laboratory processing is carried out under "clean room" conditions, with a positive pressure filtered air supply, non-contaminating laboratory surfaces, and a supply of deionized (DI) and Type II water (MilliQ).

2.2 All tools that come in contact with the sample are washed with Micro and water, rinsed with tap water and then DI. It is important to use tap water because DI alone will not remove Micro detergent.

2.3 Dissection information (initial jar weight, total weight, and tissue weight) is recorded in individual log books as well as project specific dissection sheets. Other information specific to each type of dissection is also recorded.

2.4 Personnel MUST wear polyethylene gloves at all times when handling samples and prepared dissection equipment.

2.5 All samples are dissected and placed in prepared containers appropriate for the analyses requested.

2.6 Any anomalies (parasites, injuries, etc) are recorded in all cases.

2.7 Dissected samples are homogenized to obtain a uniform sample. Aliquots of homogenate are distributed according to analyte and are acid-digested or solvent-extracted.

3.0 Interferences

3.1 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or elevated baselines, causing inaccurate analytical results. All materials should be demonstrated to be free from interferences under the conditions of the analysis by running method blanks initially and with each sample lot.

3.2 Polypropylene and polyethylene surfaces are a potential source of contamination for SO specimens and should not be used whenever possible.

3.3 TO MINIMIZE CONTAMINATION, ALL SAMPLES ARE PROCESSED UNDER "CLEAN ROOM" CONDITIONS. Criteria enumerated in Flegal (1982) are recommended. Shoe covers and lab coats are worn in the laboratory to minimize transport of contaminants into the laboratory. The trace metal laboratory has no metallic surfaces, with bench tops, sinks and fume hoods constructed of acid resistant plastic to avoid metal contamination. A filtered air supply (class 100) which provides a positive pressure clean air environment is an important feature for reducing contamination from particulates.

4.0 Apparatus and Materials

Procedures for equipment preparation can be found in Method # MPSL-101.

4.1 Brinkmann Polytron model PT 10-35

4.2 Büchi Mixer B-400

4.3 Disposable Scalpel, #10: Fisher Scientific Part # 08-927-5A

4.4 Ear Protection

4.5 Fillet knives

4.6 Glass Jar Class 100, 500 mL, prepared

4.7 Glass Jar Class 200, 500 mL, prepared

4.8 Glass Jar Class 300, 500 mL, prepared

4.9 Glass Jar Class 100, 125 mL, prepared

4.10 Glass Jar Class 200, 125 mL, prepared

4.11 Glass Jar Class 300, 125 mL, prepared

4.12 Glass Jar Class 200, 60 mL: I-Chem Part # 220-0060

4.13 Glass Jar Class 300, 60 mL: I-Chem Part # 320-0060

4.14 Heavy Duty Beakers, 1000 mL

4.15 Heavy Duty Beakers, 400 mL

4.16 Garbage Bags, Clear 30 gallon

4.17 Lab Coats

4.18 Plastic Knives, prepared

4.19 Polyethylene Gloves: VWR Part # 32915-166, 32915-188, and 32915-202

4.20 Polyethylene (HDPE) jar, 30 mL, prepared

4.21 Polyethylene (HDPE) jar, 125 mL, prepared

4.22 Shoe Covers: Cellucap Franklin Part # 28033

4.23 Teflon Forceps, prepared

4.24 Titanium Bars

4.25 Titanium Generator: Brinkmann Part # PTA 20

5.0 Reagents

5.1 Tap water (Tap)

5.2 Deionized water (DI)

5.3 Type II water (ASTM D1193): Use Type II water, also known as MilliQ, for the preparation of all reagents and as dilution water.

5.4 Micro Detergent: ColeParmer Part # 18100-20

5.5 Methanol: VWR Part # JT9263-3

5.6 Petroleum Ether: VWR Part # JT9265-3

5.7 Hydrochloric Acid (HCl), BAKER ANALYZED, 36.5-38.0%: VWR Part # JT9535-3

5.8 Hydrochloric Acid (HCl), 50%: prepared by adding 1 part Baker HCl to 1 part MilliQ

5.9 Nitric Acid (HNO_3), BAKER INSTRA-ANALYZED'*, 69.0–70.0%: VWR Part # JT9598-34

5.10 Nitric Acid (HNO_3), 50%: prepared by adding 1 part Baker HNO_3 to 1 part MilliQ

6.0 Sample Collection, Preservation and Handling

6.1 Samples should be collected according to Method # MSPL-102a, # MPSL-102b, and EPA 1669, modified.

6.2 All dissection equipment and containers must be prepared according to Method # MPSL-101.

6.3 Tissue dissections should be carried out by or under the supervision of a competent biologist. Each organism should be rinsed free of dirt with deionized water and handled with prepared stainless steel, quartz, or Teflon instruments. Fish or other samples processed as "whole body" must only come in contact with MilliQ water to reduce contamination. The SO specimens should come in contact with prepared glass, aluminum foil or Teflon surfaces only (Method # MPSL-101).

6.4 Samples should be maintained at -20°C and extracted or digested as soon as possible.

7.0 Procedure

7.1 Dissection

7.1.1 Bivalve Dissection

7.1.1.1 For both TM and SO: Frozen mussels are thawed, removed from the bags, and cleaned of epiphytic organisms, byssal threads and debris under running DI. Dissections are conducted on cleaned Teflon cutting boards.

7.1.1.2 The gametogenic condition of each sample is recorded in the logbook and dissection sheet a "ripe", "partial" or "not ripe".

7.1.1.3 For both TM and SO: The first 15 shell lengths are recorded. Lengths are measured across the longest part of each shell.

7.1.1.4 TM Bivalve Dissection

7.1.1.4.1 Forty-five mussels are dissected per sample. These are divided into 3 groups of 15. Each group of 15 creates A, B, and C replicates. If there are fewer than 45 mussels the mussels are divided into three equal samples. The total number of mussels in each jar is recorded.

7.1.1.4.2 The adductor muscle is severed with a scalpel and the shell is pried open with the plastic end of the scalpel. The gonads are then excised. The weight of the gonads from the first 15 mussels is recorded. These and all subsequent gonads can then be thrown away.

Note: Gonads are not removed from clams.

7.1.1.4.3 The remainder of the soft part is removed from shell and placed in a pre-weighed, prepared polypropylene 125mL jar. The final sample weight for each jar is recorded. All jars must be properly labeled on both the lid and the jar itself.

7.1.1.5 SO Bivalve Dissection

7.1.1.5.1 The adductor muscle is severed and the shell is pried open with clean titanium blade. The entire body, including gonads, is placed in a pre-weighed, prepared glass jar. All forty-five individuals are placed in the same jar. All jars must be properly labeled on both the lid and the jar itself.

7.1.1.6 "Split" Bivalve Dissection

7.1.1.6.1 Samples are dissected as TM samples with the following exceptions:

7.1.1.6.1.1 All gonads from each sample of 45 mussels are excised and retained in prepared 125mL glass jar. The combined weight of all 45 gonads is recorded.

7.1.1.6.1.2 The remainder of the tissue from each of the 3 replicates is dissected into prepared 125mL glass jars.

7.1.2 Fish Dissection

7.1.2.1 Large fish requiring dissection are partially thawed, then washed with DI water. It may be necessary to rub more vigorously in order to remove mucous. Place the rinsed fish in a clean, Teflon lined bin.

7.1.2.2 Total fish length and fork length are measured to the nearest millimeter. The body is then placed on a clean Teflon sheet on the balance and weighed. All lengths and weights are recorded.

7.1.2.3 Scaly fish (Large Mouth Bass, Perch, etc.) are de-scaled from the tail to the operculum above the lateral line with the titanium rod, and are dissected "skin-on". The skin is removed from scale-less fish in the same section as above, and the fish are dissected "skin-off". (EPA Guidelines) If the contract requires aging, 10 scales are taken from the appropriate region of the fish and placed in labeled coin envelopes for later age determination.

7.1.2.4 Fish are filleted to expose the flesh. It is important to maintain the cleanliness of the tissue for analysis, therefore any "skin-off" flesh that has been in direct contact with the skin or with instruments in contact with skin must be eliminated from the sample.

Trim the edges of the fillet with a clean scalpel or fillet knife to remove this contaminated tissue.

7.1.2.5 Fillets are cut into small pieces, less than 1 square inch for homogenization purposes.

7.1.2.6 Record the individual fillet weight. For composite samples, equal fillet weights are taken from each individual.

7.1.2.7 As much flesh as possible should be removed for each sample to meet the requirements for each analysis as well as have tissue retained for archive. Generally, 150-200g total sample weight is ideal.

7.1.2.8 If possible, the sex of each individual is determined and recorded.

7.1.2.9 If the contract requires liver analysis, the livers are removed from the predator species by opening the body cavity with the incision scalpel. The liver is freed by cutting with a fresh dissection scalpel and removed with a clean forceps. The livers are rinsed with MilliQ and placed in a prepared, pre-weighed sample jar. Individual liver weights recorded.

7.1.2.10 At this time vertebrae may be taken from ictalurids for aging. The first unfused vertebra is removed and placed in a 25mL beaker, covered with water and placed in the refrigerator until the flesh has broken down enough to be cleaned away. The vertebrae are placed in a coin envelope and may later be used for age determination.

7.1.2.11 Sections of fish, rather than whole body, may be delivered from the sampling crew. The lengths and weight will have already been recorded by the collection team. Tissue is dissected as before, however any exposed flesh must be eliminated from the sample.

7.1.2.12 Whole-bodied fish are thawed under MilliQ. They may be stripped of mucous by using prepared forceps. At no time may the whole body fish touch any unclean surface or instrument.

7.1.2.13 Total length, fork length and weight are recorded.

7.1.2.14 The body is cut into pieces smaller than 1 square inch for homogenization. It may be necessary to use a prepared bone saw to cut through larger vertebrae.

7.1.2.15 All samples are refrozen after dissection and maintained at -20°C until homogenization and/or analysis. It may be possible to homogenize fish samples immediately after dissection, but is not necessary.

7.2 Homogenization

7.2.1 TM Bivalve Homogenization

7.2.1.1 Samples are homogenized in the original sample jar using the Polytron and Titanium Generator.

Note: Ear Protection should be worn when operating any homogenizer.

7.2.1.2 Clean the generator by running it in a dilute Micro/Tap Solution. Rinse by running the generator in a 2 separate Tap baths, followed by 3 DI baths and 1 MQ bath. Allow to dry. Extra rinses may be necessary if tissue can be seen in any of the baths. If tissue is found in the DI or MQ baths, begin again with Tap water.

7.2.1.3 The tissue is homogenized to a paste-like consistency. No chunks of clearly defined tissue should be left in homogenate.

Note: operate the Polytron at the lowest speed possible to avoid heating the sample or splattering tissue.

7.2.1.4 The generator is cleaned with new solution baths between reps as well as between stations.

7.2.1.5 Samples must be refrozen at -20°C until acid-digestion can take place.

7.2.2 SO Bivalve Homogenization

7.2.2.1 Samples are homogenized in the original sample jar using the Polytron and either Stainless Steel or Titanium Generator.

Note: Ear Protection should be worn when operating any homogenizer.

7.2.2.2 Clean the generator by running it in 3 separate DI baths and 1 MQ bath, followed by 3 wash bottle rinses each with Methanol and Petroleum Ether. Extra rinses may be necessary if tissue can be seen in any of the baths. If tissue is found in the MQ bath, begin again with DI water.

7.2.2.3 The tissue is homogenized to a paste-like consistency. No chunks of clearly defined tissue should be left in homogenate.

Note: operate the Polytron at the lowest speed possible to avoid heating the sample or splattering tissue.

7.2.2.4 The generator is cleaned with new solution baths between stations.

7.2.2.5 Samples must be refrozen at -20°C until transfer to analytical lab and solvent extraction can occur.

7.2.3 "Split" Bivalve (TM and SO) Homogenization

7.2.3.1 Samples are homogenized as TM with the following exceptions:

7.2.3.1.1 The TM cleaned titanium generator is washed 3 times with 6% HNO_3 prior to the 3 MQ rinses, and is further rinsed 3 times each with Methanol and Petroleum Ether.

7.2.3.1.2 The retained gonads are homogenized in addition to the 3 replicates.

7.2.3.2 Homogenized samples are aliquoted for SO, ensuring enough tissue remains for TM analysis. Equal portions of body tissue are taken from each of the 3 replicates. The ratio of gonad:body weight is calculated for the entire sample, and the ratio is applied to the SO aliquot body weight to determine the amount of gonad material to add back in. Once all tissue is present in the SO sample, it is homogenized by hand with a prepared titanium rod.

7.2.4 Fish

7.2.4.1 Fish samples are removed from the freezer and are allowed to thaw long enough to be transferred to split-clean Büchi sample jar.

7.2.4.2 Prior to and after homogenization the blades and drive shaft of the Buchi are scrubbed with Micro, and rinsed 3 times each in tap and DI.

7.2.4.3 To TM clean the titanium blades, rinse 3 times in MilliQ.

7.2.4.4 To SO clean the steel blades, rinse 3 times in MilliQ, followed by 3 rinses each in methanol and PE. Air dry.

7.2.4.5 To split clean titanium blades, rinse 3 times in 6% HNO_3, followed by 3 rinses in MilliQ. Follow up with 3 rinses each in methanol and PE. Air dry.

7.2.4.6 Assemble the homogenizer according to manufacturer specifications.

7.2.4.7 Place sample jar on tray; close and lock the homogenizer door.

7.2.4.8 Raise the sample jar into position with the on/off toggle. When the jar reaches the appropriate height, the blades will begin rotation and come in contact with the sample.

7.2.4.9 It is important to PULSE the cutting unit in the sample by briefly releasing the toggle. This allows the entire sample to be homogenized, and not get pushed against the sides of the container, as well as keeping the friction to a minimum. It is imperative the sample not get hot.

7.2.4.10 Once the sample has fully homogenized, it may be aliquoted with a prepared titanium rod into the appropriate prepared sample containers for each analysis.

7.2.4.11 Samples are frozen at -20°C until acid-digestion or transfer to analytical lab and solvent extraction can occur.

8.0 Analytical Procedure

8.1 Trace Metal and Mercury Only digestion procedures can be found in EPA 3052, modified, and Method # MPSL-106, respectively.

8.2 Trace Metals are analyzed with ICP-MS according to EPA 200.8.

8.3 Mercury samples are analyzed by FIMS according to Method # MPSL-103 or by DMA and EPA 7473.

8.4 Methylmercury tissue samples are extracted and analyzed according to Method # MPSL-109.

9.0 Quality Control

9.1 Sample Archive: All remaining sample homogenates and extracts can be archived at -20°C for future analysis.

9.2 A record of sample transport, receipt and storage is maintained and available for easy reference.

9.3 All samples are prepared in a clean room to avoid airborne contamination.

10.0 Method Performance

10.1 See individual analytical methods.

11.0 References

11.1 Flegal, R.A. 1982. In: Wastes in the Ocean, Vol VI: Near Shore Waste Disposal. B.H. Ketchum (ed.). John Wiley and Sons Inc. Publishers, New York, 1982.

11.2 Goldberg, E.D., ed. 1980. The International Mussel Watch. National Academy of Sciences Publ., Washington, D.C.

11.3 Gordon, R.M., G.A. Knauer and J.H. Martin. 1980a. *Mytilus californianus* as a bioindicator of trace metal pollution: variability and statistical considerations. Mar. Poll. Bull. 9:195-198.

11.4 Hayes, S. P. and P. T. Phillips. 1986. California State Mussel Watch: Marine water quality monitoring program 1984-85. State Water Resources Control Board Water Quality Monitoring Report No. 86-3WQ

DFG REQUEST FOR ANALYSIS AND CHAIN OF CUSTODY RECORD

Page_____ of _____

Sampler	Ph #	Send Results To	Lab Number
Address		Address	Field Number
City	Zip CA	City / Zip CA	Lab Storage
		Copies To	Spill Title
Date Required/Reason		Address	Suspect
Shipped Via		City / Zip CA	Index-PCA

☐ Fish & Wildlife Loss Date:_____ Region:_____

☐ DFG Code Violation:_____

☐ Suspected or Potential Problem

☐ Routine Analysis

Water Temp: F or C pH: DO: mg/L Conductivity: *u* mhos/cm

Analysis Requested >>>

Sample Identification/Location (Draw map on separate sheet if necessary)	Collection Date	Collection Time	Petroleum Fingerprint	Trace Elements (Specify Below)	Pesticides (Specify Below)							Sample Type: Water	Sample Type: Filtered Water	Sample Type: Soil	Sample Type: Tissue	Number of Containers: Plastic	Number of Containers: Glass	Number of Containers: VOA Vial		Preservation: Temp	Preservation: Acid	

Problem Description

Suspect/Incident Location

Comments/Special Instructions

Pollution Action Kit: Yes☐ No☐

Glove Size: Large ☐ Medium ☐

Hazmat Shipper Requested: Yes☐ No☐

Samples Reliquished By (Signature)	Print Name	Date	Received By (Signature)	Print Name	Date

Pesticide Investigations Lab
1701 Nimbus Road
Rancho Cordova, CA 95670
(916) 358-2950

☐

Petroleum Chemistry Lab
1995 Nimbus Road
Rancho Cordova, CA 95670
(916) 358-2803

☐

Water Pollution Control Lab
2005 Nimbus Road
Rancho Cordova, CA 95670
(916) 358-2858

☒

LAB COPIES: WHITE, CANARY, PINK **SUBMITTER: GOLDENROD**

FG 1000 (Rev. 9/01)

Method # MPSL-102a

SAMPLING MARINE AND FRESHWATER BIVALVES, FISH AND CRABS FOR TRACE METAL AND SYNTHETIC ORGANIC ANALYSIS

1.0 Scope and Application

1.1 The following procedures describe techniques of sampling marine mussels and crabs, freshwater clams, marine and freshwater fish for trace metal (TM) and synthetic organic (SO) analyses.

2.0 Summary of Method

2.1 Collect mussels, clams, crabs, or fish. Mussels or clams to be transplanted are placed in polypropylene mesh bags and deployed. Mussels and clams to be analyzed for metals are double-bagged in plastic zipper-closure bags. Bivalves to be analyzed for organics are wrapped in PE cleaned aluminum foil prior to placement in the zipper-closure bags. Fish are wrapped whole or proportioned where necessary in cleaned Teflon sheets or aluminum foil and subsequently placed into zipper-closure bags. Crabs for TM and/or SO are double-bagged in plastic zipper-closure bags.

2.2 Each sample should be labeled with Date, Station Name, and any other information available to help identify the sample once in the lab.

2.3 After collection, samples are transported back to the laboratory in coolers with ice or dry ice. If ice is used, care must be taken to ensure that ice melt does not come into direct contact with samples.

3.0 Interferences

3.1 In the field, sources of contamination include sampling gear, grease from ship winches or cables, ship and truck engine exhaust, dust, and ice used for cooling. Efforts should be made to minimize handling and to avoid sources of contamination.

3.2 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or elevated baselines, causing inaccurate analytical results. All materials should be demonstrated to be free from interferences under the conditions of the analysis by running method blanks initially and with each sample lot.

3.3 Polypropylene and polyethylene surfaces are a potential source of contamination for SO specimens and should not be used whenever possible.

4.0 Apparatus and Materials

Procedures for equipment preparation can be found in Method # MPSL-101.

4.1 Anchor Chains

4.2 Backpack Shocker (electro-fishing)

4.3 Boats (electro-fishing and/or for setting nets)

4.4 Bone Saw

4.5 Camera, digital

4.6 Cast Nets (10' and 12')

4.7 Data Sheets (see MPSL QAP Appendix E for example)

4.8 Daypacks

4.9 Depth Finder

4.10 Dip Nets

4.11 Dry Ice or Ice

4.12 Gill Nets (various sizes)

4.13 GPS

4.14 Heavy Duty Aluminum Foil, prepared

4.15 Heavy Duty plastic bags, Clear 30 gallon

4.16 Inflatable Buoy

4.17 Labels, gummed waterproof: Diversified Biotech Part #: LCRY-1258

4.18 Nylon Cable Ties, 7/16" wide x 7" long

4.19 Other (minnow traps, set lines, throw nets, etc)

4.20 Otter Trawl (various widths as appropriate)

4.21 Permanent Marking Pen

4.22 Plastic bucket, 30 gallon

4.23 Plastic Ice Chests

4.24 Polyethylene Gloves: VWR Part # 32915-166, 32915-188, and 32915-202

4.25 Polypropylene Mesh, 76mm wide with 13mm mesh

4.26 Polypropylene Mesh, 50mm wide with 7mm mesh

4.27 Polypropylene Line, 16mm

4.28 Rods and Reels

4.29 Screw in Earth Anchor, 4-6” diameter

4.30 Scuba Gear

4.31 Seines (various size mesh and lengths as appropriate)

4.32 Stainless Steel Dive Knives

4.33 Trap Nets (hoop or fyke nets)

4.34 Teflon Forceps

4.35 Teflon Sheet, prepared

4.36 Teflon Wash Bottle, 500 mL

4.37 Wading Gear

4.38 Zipper-closure Polyethylene Bags, 4milx13”x18”: Packaging Store Part # zl401318redline

5.0 Reagents

5.1 Tap water (Tap)

5.2 Deionized water (DI)

5.3 Type II water (ASTM D1193): Use Type II water, also known as MilliQ, for the preparation of all reagents and as dilution water.

5.4 Micro Detergent: ColeParmer Part # 18100-20

5.5 Methanol: VWR Part # JT9263-3

5.6 Petroleum Ether: VWR Part # JT9265-3

6.0 Sample Collection, Preservation and Handling

6.1 All sampling equipment will be made of non-contaminating materials and will be inspected prior to entering the field. Nets will be inspected for holes and repaired prior to being used. Boats (including the electroshocking boat) will be visually checked for safety equipment and damage prior to being taken into the field for sample collection.

6.2 To avoid cross-contamination, all equipment used in sample collection should be thoroughly cleaned before each sample is processed. Ideally, instruments are made of a material that can be easily cleaned (e.g. Stainless steel, anodized aluminum, or borosilicate glass). Before the next sample is processed, instruments should be washed with a detergent solution, rinsed with ambient water, rinsed with a high-purity solvent (methanol or petroleum ether), and finally rinsed with MilliQ. Waste detergent and solvent solutions must be collected and taken back to the laboratory.

6.3 Samples are handled with polyethylene-gloved hands only. The samples should be sealed in appropriate containers immediately.

6.4 Mussels and clams to be analyzed for metals are double-bagged in zipper-closure bags. Bivalves to be analyzed for organics are wrapped in prepared aluminum foil prior to placement in zipper-closure bags.

6.5 Fish are wrapped in part or whole in prepared Teflon sheets and subsequently placed into zipper-closure bags.

6.6 Crabs analyzed for metals and/or organics are double-bagged in plastic zipper-closure bags.

6.7 Data is recorded for each site samples are transplanted to or collected from. Data includes, but is not limited to station name, sample identification number, site location (GPS), date collected or transplanted, collectors names, water depth, photo number, ocean/atmospheric conditions (if appropriate), description of site, and drawing if necessary.

6.8 A chain of custody form (MPSL QAP Appendix E) will accompany all samples that are brought to the lab. All samples that are processed in the lab MUST be checked in according to Method # MPSL-104.

6.9 Samples are maintained at -20°c and extracted or digested as soon as possible.

7.0 Procedure

7.1 Sample collection - mussels and clams

7.1.1 The mussels to be transplanted (*Mytilus californianus*) are collected from Trinidad Head (Humboldt Bay Intensive Survey), Montana de Oro (Diablo Canyon Intensive Survey), and Bodega Head (all other statewide transplants). The freshwater clam (*Corbicula fluminea*) source is Lake Isabella or the Sacramento River. Analyze mussel and clam samples for background contaminates prior to transplanting.

7.1.2 Polyethylene gloves are worn while prying mussels off rocks with dive knives. Note: polyethylene gloves should always be worn when handling samples. Mussels of 55mm to 65mm in length are recommended. Fifty mussels are collected for each TM and each SO sample.

7.1.3 Collected mussels are carried out of collection site in zipper-closure bags placed in cleaned nylon daypacks. For the collection of resident samples where only one or two samples are being collected the mussels are double bagged directly into a labeled zipper-closure bag. Samples for SO are wrapped first in prepared aluminum foil.

7.1.4 Clams (*Corbicula fluminea*) measuring 20 to 30mm are collected by dragging the clam dredge along the bottom of the lake or river. The clams are poured out of the dredge into a 30 gallon plastic bag. Clams can also be collected by gloved hands in shallow waters and placed in labeled zipper-closure bags. 25-200 clams are collected depending on availability and necessity for analyses.

7.1.5 Data is recorded for each site samples are collected from. Data includes, but is not limited to station name, date collected, collectors names, water depth, GPS readings, photo, ocean/atmospheric conditions (if appropriate), description of site, and drawing if necessary.

7.2 Transplanted sample deployment

7.2.1 With polyethylene gloves, fifty transplant mussels are placed in each 76mm X 13mm polypropylene mesh bag. Each bag represents one TM or one SO sample. A knot is tied at each end of mesh bag and reinforced with a cable tie. On one end another cable tie is placed under the cable tie which will be used to secure the bag to the line for transplant deployment. The mussels in the mesh bag are divided into three groups of approximately equal size and sectioned with two more cable ties.

7.2.2 Once bagged, the mussels are placed in a 30 gallon plastic bag and stored in a cooler (cooled with ice) for no more than 48 hours. The ice is placed in zipper-closure bags to avoid contamination.

7.2.3 If marine samples are held for longer than 48 hours they are placed in holding tanks with running seawater at the lab. Control samples for both SO and TM are also held in the tank.

7.2.4 For freshwater clams: clams (25-200) are placed in 50mm X 7mm polypropylene mesh bags using identical procedures to those used with mussels (section 7.2.1). If clams need to be stored for more than 48 hours, the mesh bags are deployed either in a clean source or in holding tanks with running freshwater at the lab until actual sample deployment.

7.2.5 The mussels are attached to an open water transplant system that consists of a buoy system constructed with a heavy weight anchor (about 100lbs) or screw-in earth anchor, 13mm polypropylene line, and a 30cm diameter subsurface buoy. The sample bags are attached with cable ties to the buoy line about 15 feet below the water surface. In some cases the sample is hung on suspended polypropylene lines about 15 feet below the water surface between pier pilings or other surface structures. Creosote-coated wooden piers are avoided because they are a potential source of contamination. In some cases the mussels are hung below a floating dock. In shallow waters a wooden or PVC stake is hammered into the substrate and the mussel bags are attached by cable ties to the stake.

7.2.6 The clams are deployed by attaching the mesh bag with cable ties to wooden or PVC stakes hammered into substrate or screw in earth anchors. The bags containing clams are typically deployed 15cm or more off the bottom. In areas of swift water, polypropylene line is also attached to the staked bags and a permanent object (piling, tree or rock).

7.2.7 Transplants are usually deployed for 1-4 months. Ideally mussels are transplanted in early September and retrieved in late December and early January. Clams are usually transplanted in March or April and retrieved in May or June.

7.2.8 Data is recorded for each site samples are transplanted to or collected from. Data includes, but is not limited to station name, date collected or transplanted, collectors names, water depth, GPS readings, photo, ocean/atmospheric conditions (if appropriate), description of site, and drawing if necessary.

7.3 Sample Retrieval

7.3.1 The transplanted or resident and control mussels analyzed for TM are double bagged in appropriately sized and labeled zipper-closure bags.

7.3.2 All mussels to be analyzed for SO are wrapped in prepared aluminum foil (Method # DFG 101). The foil packet is double bagged in appropriately sized and labeled zipper-closure bags. Note: samples should only contact the dull side of the foil.

7.3.3 The bags containing samples are clearly and uniquely identified using a water-proof marking pen or pre-made label. Information items include ID number, station name, depth (if from a multiple sample buoy), program identification, date of collection, species and type of analysis to be performed.

7.3.4 The samples are placed in non-metallic ice chests and frozen using dry ice or regular ice. (Dry ice is used when the collecting trip takes more than two days.) At the lab, samples should be stored at or below -20°c until processed.

7.4 Sample Collection – Fish

7.4.1 Fish are collected using the appropriate gear for the desired species and existing water conditions.

7.4.1.1 Electro-fisher boat- The electro-fisher boat is run by a trained operator, making sure that all on board follow appropriate safety rules. Once on site, adjustment of the voltage, amps, and pulse for the ambient water is made and recorded. The stainless steel fish well is rinsed with ambient water, drained and refilled. The shocked target fish are placed with a nylon net in the well with circulating ambient water. The nylon net is washed with a detergent and rinsed with ambient water prior to use. Electro-fishing will continue until the appropriate number and size of fish are collected.

7.4.1.2 Backpack electro-fisher- The backpack shocker is operated by a trained person, making sure that all others helping follow appropriate safety rules. The backpack shocker is used in freshwater areas where an electro-fisher boat can not access. Once on site, adjustment of the voltage, amps, and pulse for the ambient water is made and recorded. The shocked target fish are captured with a nylon net and placed in a 30 gallon plastic bag. The nylon net is washed with a detergent and rinsed with ambient water prior to use. Electro-fishing will continue until the appropriate number and size of fish are collected.

7.4.1.3 Fyke or hoop net- Six-36 inch diameter hoops connected with 1 inch square mesh net is used to collect fish, primarily catfish. The net is placed parallel to shore with the open hoop end facing downstream. The net is placed in areas of slow moving water. A partially opened can of cat food is placed in the upstream end of the net. Between 2-6 nets are placed at a site overnight. Upon retrieval a grappling hook is used to pull up the downstream anchor. The hoops and net are pulled together and placed on a 30

gallon plastic bag in the boat. With polyethylene gloves the desired fish are placed in a 30 gallon plastic bag and kept in an ice chest with ice until the appropriate number and size of fish are collected.

7.4.1.4 Otter-trawl- A 14 foot otter trawl with 24 inch wooden doors or a 20 foot otter trawl with 30 inch doors and 80 feet of line is towed behind a boat for water depths less than 25 feet. For water depths greater than 25 feet another 80 feet of line is added to capture fish on or near the substrate. Fifteen minute tows at 2-3 knots speed are made. The beginning and ending times are noted on data sheets. The trawl is pulled over the side of the boat to avoid engine exhaust. The captured fish are emptied into a 30 gallon plastic bag for sorting. Desired fish are placed with polyethylene gloves into another 30 gallon plastic bag and kept in an ice chest with ice.

7.4.1.5 Gill nets- A 100 yard monofilament gill net of the appropriate mesh size for the desired fish is set out over the bow of the boat parallel to shore. The net is retrieved after being set for 1-4 hours. The boat engine is turned off and the net is pulled over the side or bow of the boat. The net is retrieved starting from the down-current end. If the current is too strong to pull in by hand, then the boat is slowly motored forward and the net is pulled over the bow. Before the net is brought into the boat, the fish are picked out of the net and placed in a 30 gallon plastic bag and kept in an ice chest with ice.

7.4.1.6 Beach seines- In areas of shallow water, beach seines of the appropriate length, height, and mesh size are used. One sampler in a wetsuit or waders pulls the beach seine out from shore. The weighted side of the seine must drag on the bottom while the float side is on the surface. The offshore sampler pulls the seine out as far as necessary and then pulls the seine parallel to shore and then back to shore, forming a half circle. Another sampler is holding the other end on shore while this is occurring. When the offshore sampler reaches shore the two samplers come together with the seine. The seine is pulled onto shore making sure the weighted side drags the bottom. When the seine is completely pulled onshore, the target fish are collected with polyethylene gloves and placed in a 30 gallon plastic bag and kept in an ice chest with ice. The beach seine is rinsed off in the ambient water and placed in the rinsed 30 gallon plastic bucket.

7.4.1.7 Cast net- A 10 or 12 foot cast net is used to collect fish off a pier, boat, or shallow water. The cast net is rinsed in ambient water prior to use and stored in a covered plastic bucket. The target fish are sampled with polyethylene gloves and placed in a 30 gallon plastic bag and kept in an ice chest with ice.

7.4.1.8 Hook and line- Fish are caught off a pier, boat, or shore by hook and line. Hooked fish are taken off with polyethylene gloves and placed in a Ziploc™ bag or a 30 gallon plastic bag and kept in an ice chest with ice.

7.4.1.9 Spear fishing- Certain species of fish are captured more easily by SCUBA divers spearing the fish. Only appropriately trained divers following the dive safety program guidelines are used for this method of collection. Generally, fish in the kelp beds are more easily captured by spearing. The fish are shot in the head area to prevent the fillets from being damaged or contaminated. Spear tips are washed with a detergent and rinsed with ambient water prior to use.

7.4.2 As a general rule, five fish of medium size or three fish of larger size are collected as composites for analysis. The smallest fish length cannot be any smaller than 75% of the largest fish length. Five fish usually provides sufficient quantities of tissue for the dissection of 150 grams of fish flesh for organic and inorganic analysis. The medium size is more desirable to enable similar samples to be collected in succeeding collections.

7.4.3 When only small fish are available, sufficient numbers are collected to provide 150 grams of fish flesh for analysis. If the fish are too small to excise flesh, the whole fish, minus the head, tail, and guts are analyzed as composites.

7.4.4 Species of fish collected are chosen for their importance as indicator species, availability or the type of analysis desired. For example, livers are generally analyzed for heavy metals. Fish without well-defined livers, such as carp or goldfish, are not collected when heavy metal analyses are desired.

7.4.5 Fish collected, too large to fit in clean bags (>500 mm) are initially dissected in the field. At the dock, the fish are laid out on a clean plastic bag and a large cross section from behind the pectoral fins to the gut is cut with a cleaned bone saw or meat cleaver. The bone saw is cleaned (micro, DI, methanol) between fish and a new plastic bag is used. The internal organs are not cut into, to prevent contamination. For bat rays, a section of the wing is cut and saved. These sections are wrapped in prepared Teflon sheets, double bagged and packed in dry ice before transfer to the freezer. During lab dissection, a subsection of the cross section is removed, discarding any tissue exposed by field dissection.

7.4.6 Field data (MPSL QAP Appendix E) recorded include, but are not limited to site name, sample identification number, site location (GPS), date of collection, time of collection, names of collectors, method of collection, type of sample, water depth, water and atmospheric conditions, fish total lengths (fork lengths where appropriate), photo number and a note of other fish caught.

7.4.7 The fish are then wrapped in aluminum foil or Teflon sheets if thylates are analyzed. The wrapped fish are then double-bagged in zipper-closure bags with the inner bag labeled.

The fish are put on dry ice and transported to the laboratory where they are kept frozen until they are processed for chemical analysis.

7.5 Sample Collection- Crabs

7.5.1 Crab/lobster traps- Polyethylene traps are baited to collect crabs or lobsters. Traps are left for 1-2 hours. The crabs are placed in a zipper-closure bag or a 30 gallon plastic bag and kept in an ice chest with ice.

8.0 Analytical Procedure

8.1 Tissue Preparation procedures can be found in Method # MPSL-105.

8.2 Trace Metal and Mercury Only digestion procedures can be found in EPA 3052, modified, and Method # MPSL-106, respectively.

8.3 Trace Metals are analyzed with ICP-MS according to EPA 200.8.

8.4 Mercury samples are analyzed by FIMS according to Method # MPSL-103 or by DMA and EPA 7473.

8.5 Methylmercury tissue samples are extracted and analyzed according to Method # MPSL-109.

9.0 Quality Control

9.1 Field Replicates: project specific requirements are referenced for field replication.

9.2 A record of sample transport, receipt and storage is maintained and available for easy reference.

10.0 References

10.1 Flegal, R.A. 1982. In: Wastes in the Ocean, Vol VI: Near Shore Waste Disposal. B.H. Ketchum (ed.). John Wiley and Sons Inc. Publishers, New York, 1982.

10.2 Goldberg, E.D., ed. 1980. The International Mussel Watch. National Academy of Sciences Publ., Washington, D.C.

10.3 Gordon, R.M., G.A. Knauer and J.H. Martin. 1980a. *Mytilus californianus* as a bioindicator of trace metal pollution: variability and statistical considerations. Mar. Poll. Bull. 9:195-198.

10.4 Hayes, S. P. and P. T. Phillips. 1986. California State Mussel Watch: Marine water quality monitoring program 1984-85. State Water Resources Control Board Water Quality Monitoring Report No. 86-3WQ.

10.5 EPA. 1995. Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories. Volume 1: Fish Sampling and Analysis. EPA 823-R-95-007.

CDFG FISH AND WILDLIFE WATER POLLUTION CONTROL LABORATORY STANDARD OPERATING PROCEDURE FOR THE MANAGEMENT OF SAMPLES RECEIVED FOR CHEMICAL ANALYSIS

1.0 Scope and Application

This method describes the procedures to be followed for the receipt, handling, scheduling, storage, and disposal of samples received by the laboratory.

2.0 Summary of Method

The WPCL sample receiving area is located in the sample storage room at the back of the main laboratory. All samples are immediately unpacked, checked for temperature, logged-in using the sample receipt log book, entered in Labworks (LIMS), labeled, checked for required preservation and preserved as necessary, checked for appropriate holding time limitations and properly stored (refrigerated or frozen). If samples are delivered frozen, they should be immediately transferred to the freezer after they are logged-in. DFG request for analysis and chain-of-custody records (Form FG 1000 Rev. 9/01) or chains of custody submitted with samples are completed and then given to the appropriate section leader for scheduling. After the analyses are completed, samples are stored until data review and reporting have been completed.

Samples are then disposed using the evaporation pond (non-hazardous samples only) or logged into the hazardous waste storage area for scheduled pickup by a licensed hazardous waste contractor.

Deleted: ¶
Deleted:
Deleted: ¶
Deleted:
Deleted: ¶
Deleted:
Deleted: ¶
Deleted: Sample request forms
Deleted:
Deleted: ¶
Deleted:
Deleted: ¶
Deleted:
Deleted: ¶
Deleted:
Deleted: ¶
Deleted: ¶

3.0 Sample Receipt

3.1 Samples are delivered to the Laboratory by DFG personnel, United Parcel Service (UPS), U.S. Postal Service, Federal Express, and by other commercial courier companies. Samples are shipped in Pollution Action Kit (PAK) boxes (non-hazardous samples only), hazardous materials shipping containers, and various sizes of ice chests.

3.2 Samples received by the Laboratory should be **immediately** taken by qualified Laboratory personnel to the sample log-in station located in the sample storage room at the back of the main laboratory building for unpacking. Samples should **not** be left in the sample receiving area or removed to office areas or laboratories. Samples addressed to individuals should not be left unattended if the addressee is not available.

3.3 All samples received by the Laboratory should be considered to be potentially hazardous and **caution** should be used when opening packages containing samples. Even non-hazardous samples can be a safety hazard to the person unpacking the samples (ie. broken glass sample containers). All samples shipped

in hazardous material shippers should be considered hazardous and should always be unpacked in a well ventilated area (under a fume extractor) or in a fume hood. Personnel unpacking hazardous samples should wear appropriate protective clothing, which at a minimum would include:

safety glasses
nitrile gloves
laboratory coat or apron
closed toe shoes

3.4 Cut packaging tape with a knife or scalpel. Open package or ice chest and remove paperwork which should be on top. Open laboratory log book and record the letter “L” followed by the next consecutive number followed by the last two numbers of the current year. Below the laboratory "L" number, write the date and below the date write your initials. The "L" number will be used for the entire set of samples. Record this number on the line labeled laboratory number in the upper right hand corner of the Form FG 1000 Rev. 9/01, on the chain-of-custody form (if applicable), and any other paperwork that accompanied the samples. All writing must be in ink (preferably ball point pen).

3.5 Remove samples from the shipping container and line them up on the counter. Check each sample container for cracks or breakage. Make sure that each sample is labeled. Labworks LIMS can be used to print labels for sample containers. See 3.14 for instructions. Labels may be handwritten by writing the laboratory number on each container with waterproof marker followed by consecutive numbers. For example:

sample 1 = L-345-07-01 **Deleted:** 3
sample 2 = L-345-07-02 **Deleted:** 3

3.6 Record the following information about the samples in the log book next to the laboratory number:

number of containers
type of sample (eg. sediment, water, oil, etc.)
condition of sample (broken, leaking, etc.) if necessary
where samples were collected
person requesting analyses and DFG region or other agency name
type of analysis
Index-PCA code, if given by collector

3.7 Check the Form FG 1000 Rev. 9/01 for sample descriptions (Identification/Location), if this is not filled out, do so using the information on the sample labels. Check the Form FG 1000 Rev. 9/01 for analysis requested. If instructions are unclear, contact the person who collected the samples.

3.8 If any problems are found with the samples when they are received (eg. broken containers, missing samples, samples have been shipped that are not recorded on the chain-of-custody documents), notify the individual who shipped the samples immediately and inform them of the problem.

3.9 Sign, print name and date the Form FG 1000 Rev. 9/01 and chain of custody card (if used) next to "received by". Give the submitter the goldenrod copy. Put the pink copy in the binder at the log-in area. The pink copy is used to enter all sample information into the laboratory information management system (LIMS).

3.10 Check the holding time/sample preservation table for the analysis requested and record the holding time expiration on the Form FG 1000 Rev. 9/01 if applicable. If preservation is required, do so immediately and record the type of preservative, date preserved, and initial the Form FG 1000 Rev. 9/01. Preservation of inorganics samples is also entered in a separate log book located in the inorganic lab (this is done by inorganic lab staff). Each container should receive a label indicating that the sample was preserved and the type of preservative.

3.11 Determine where the samples will be stored and record refrigerator or freezer number in the space provided on the Form FG 1000 Rev 09/01. Keep samples together as a set. Samples should be stored as follows:

VOA samples only – Elmo (front lab, left side)
petroleum samples – Grover (front lab, left side)
inorganic samples – WPCL R5, R7, WPCL R2 (machine room), TSM F2 (sample storage room), walk in R2
ambient monitoring samples – TSM R3 (left side), TSM F2, F4, WPCL F1 (sample storage room), walk in F1
pesticides (F&W Loss) – WPCL F3 (sample storage room), WPCL R1, R2, R3, R4 (back lab), walk in R3, walk in F1

F = freezer; R = refrigerator

Enforcement and regulatory samples must be stored in a locked refrigerator/freezer.

3.12 Give all paperwork to the appropriate lead analyst:

Petroleum/semi-VOA/VOA - Bob Todd
Inorganic/hatchery monitoring - Patty Bucknell
Pesticide (F&W Loss) investigations - Abdou Mekebri
Pesticides (pesticides contracts) - Abdou Mekebri
Tissue / Sediment Monitoring programs (PCBs, PBDEs, OC pesticides) - Kathleen Regalado

Deleted: database
Deleted:
Deleted: (machine room)
Deleted:
Deleted: R6
Deleted: ,
Deleted:
Formatted: Indent: Left: 36 pt
Deleted: ¶
Formatted: Indent: Left: 180 pt, Hanging: 66 pt, Tabs: 246 pt, Left + Not at 180 pt + 252 pt
Deleted:
Deleted: all samples – Walk-in R2 ¶ ¶
Deleted: Martice Vasquez
Deleted: WQ
Deleted:
Deleted:

3.13 If samples were received in a PAK, photocopy the Form 1000 and write "PAK" in large letters on the front of the photocopy or if a Form FG 1000 Rev 09/01 was used, check the box labeled "PAK Requested" if not already done. Give the photocopy to Bob Todd so that a new PAK will be sent out to replace the one received.

3.14. Using Labworks for log-in samples

1. Go into Multilog
2. Load the project pre-log in group and enter site location, collection date, collection time and checking analytes, matrix, report address against COC
3. Labels can be printed from Labworks with L#, site location, analyses, collection date and time
   - 3a. Go to Labworks explore and pick L #'s for the labels
   - 3b. Highlight the accession for labels. Choose LABEL 30251.CEF and click OK
   - 3c. The program is automatically connected to access. Pick report feature and click LABEL-30251-inorg
4. Place the labels on samples

Formatted: Bullets and Numbering

Formatted: Indent: Left: 72 pt

Deleted: ¶

4.0 Scheduling

4.1 Laboratory analyses are scheduled by priority. Priorities are ranked as follows:

1-Enforcement samples with regulatory holding time
2-Spill and/or wildlife loss in progress
3-Routine samples with regulatory holding time
4-Enforcement samples with completion date requested
5-Routine samples with completion date requested
6-Enforcement samples with no holding time
7-Routine samples with no holding time

4.2 Samples should be completed as soon as possible after receipt. If a delay is anticipated for the completion of an analysis, the person requesting the analysis should be advised of the delay.

5.0 Sample Storage

5.1 Samples remain refrigerated or frozen until they are needed for analysis. Samples are removed from storage for analysis and then are returned to refrigerated storage. Tissue samples are always returned to a freezer after they are analyzed. Samples remain refrigerated/frozen until results are reported.

5.2 Samples that will not spoil may be moved to locked non-refrigerated storage after the report has been completed. Retain chain-of-custody cards or the original copy of the Form FG 1000 Rev. 09/01 for all stored samples.

5.3 Samples retained by the laboratory for six months that are not required as physical evidence should be disposed using a hazardous materials disposal contractor. The person submitting the samples should be contacted and told the samples will be disposed unless a request is made to store the samples at the laboratory for a longer period of time. When samples are disposed, the word "Disposed" and the disposal date are recorded next to the logbook entry (lab accession number) for that sample with the person's initials that authorized the disposal. Inorganics samples are also entered in separate disposal log book, located in the inorganic lab (this is done by inorganic lab staff).

Deleted: ¶
¶

QA Officer Approval:_____ Date: _____

SOP Final Approval:_____ Date: _____

CDFG FISH AND WILDLIFE WATER POLLUTION CONTROL LABORATORY STANDARD OPERATING PROCEDURE FOR THE MANAGEMENT OF SAMPLES RECEIVED FOR CHEMICAL ANALYSIS

1.0 Scope and Application

This method describes the procedures to be followed for the receipt, handling, scheduling, storage, and disposal of samples received by the laboratory.

2.0 Summary of Method

The WPCL sample receiving area is located in the sample storage room at the back of the main laboratory. All samples are immediately unpacked, checked for temperature, logged-in using the sample receipt log book, entered in Labworks (LIMS), labeled, checked for required preservation and preserved as necessary, checked for appropriate holding time limitations and properly stored (refrigerated or frozen). If samples are delivered frozen, they should be immediately transferred to the freezer after they are logged-in. DFG request for analysis and chain-of-custody records (Form FG 1000 Rev. 9/01) or chains of custody submitted with samples are completed and then given to the appropriate section leader for scheduling. After the analyses are completed, samples are stored until data review and reporting have been completed.

Samples are then disposed using the evaporation pond (non-hazardous samples only) or logged into the hazardous waste storage area for scheduled pickup by a licensed hazardous waste contractor.

3.0 Sample Receipt

3.1 Samples are delivered to the Laboratory by DFG personnel, United Parcel Service (UPS), U.S. Postal Service, Federal Express, and by other commercial courier companies. Samples are shipped in Pollution Action Kit (PAK) boxes (non-hazardous samples only), hazardous materials shipping containers, and various sizes of ice chests.

3.2 Samples received by the Laboratory should be **immediately** taken by qualified Laboratory personnel to the sample log-in station located in the sample storage room at the back of the main laboratory building for unpacking. Samples should **not** be left in the sample receiving area or removed to office areas or laboratories. Samples addressed to individuals should not be left unattended if the addressee is not available.

3.3 All samples received by the Laboratory should be considered to be potentially hazardous and **caution** should be used when opening packages containing samples. Even non-hazardous samples can be a safety hazard to the person unpacking the samples (ie. broken glass sample containers). All samples shipped

in hazardous material shippers should be considered hazardous and should always be unpacked in a well ventilated area (under a fume extractor) or in a fume hood. Personnel unpacking hazardous samples should wear appropriate protective clothing, which at a minimum would include:

safety glasses
nitrile gloves
laboratory coat or apron
closed toe shoes

3.4 Cut packaging tape with a knife or scalpel. Open package or ice chest and remove paperwork which should be on top. Open laboratory log book and record the letter “L” followed by the next consecutive number followed by the last two numbers of the current year. Below the laboratory "L" number, write the date and below the date write your initials. The "L" number will be used for the entire set of samples. Record this number on the line labeled laboratory number in the upper right hand corner of the Form FG 1000 Rev. 9/01, on the chain-of-custody form (if applicable), and any other paperwork that accompanied the samples. All writing must be in ink (preferably ball point pen).

3.5 Remove samples from the shipping container and line them up on the counter. Check each sample container for cracks or breakage. Make sure that each sample is labeled. Labworks LIMS can be used to print labels for sample containers. See 3.14 for instructions. Labels may be handwritten by writing the laboratory number on each container with waterproof marker followed by consecutive numbers. For example:

sample 1 = L-345-07-01
sample 2 = L-345-07-02

3.6 Record the following information about the samples in the log book next to the laboratory number:

number of containers
type of sample (eg. sediment, water, oil, etc.)
condition of sample (broken, leaking, etc.) if necessary
where samples were collected
person requesting analyses and DFG region or other agency name
type of analysis
Index-PCA code, if given by collector

3.7 Check the Form FG 1000 Rev. 9/01 for sample descriptions (Identification/Location), if this is not filled out, do so using the information on the sample labels. Check the Form FG 1000 Rev. 9/01 for analysis requested. If instructions are unclear, contact the person who collected the samples.

3.8 If any problems are found with the samples when they are received (eg. broken containers, missing samples, samples have been shipped that are not recorded on the chain-of-custody documents), notify the individual who shipped the samples immediately and inform them of the problem.

3.9 Sign, print name and date the Form FG 1000 Rev. 9/01 and chain of custody card (if used) next to "received by". Give the submitter the goldenrod copy. Put the pink copy in the binder at the log-in area. The pink copy is used to enter all sample information into the laboratory information management system (LIMS)..

3.10 Check the holding time/sample preservation table for the analysis requested and record the holding time expiration on the Form FG 1000 Rev. 9/01 if applicable. If preservation is required, do so immediately and record the type of preservative, date preserved, and initial the Form FG 1000 Rev. 9/01. Preservation of inorganics samples is also entered in a separate log book located in the inorganic lab (this is done by inorganic lab staff). Each container should receive a label indicating that the sample was preserved and the type of preservative.

3.11 Determine where the samples will be stored and record refrigerator or freezer number in the space provided on the Form FG 1000 Rev 09/01. Keep samples together as a set. Samples should be stored as follows:

VOA samples only – Elmo (front lab, left side)
petroleum samples – Grover (front lab, left side)
inorganic samples – WPCL R5, R7, WPCL R2 (machine room), TSM F2 (sample storage room), walk in R2
ambient monitoring samples – TSM R3 (left side), TSM F2, F4, WPCL F1 (sample storage room), walk in F1
pesticides (F&W Loss) – WPCL F3 (sample storage room), WPCL R1, R2, R3, R4 (back lab), walk in R3, walk in F1

F = freezer; R = refrigerator

Enforcement and regulatory samples must be stored in a locked refrigerator/freezer.

3.12 Give all paperwork to the appropriate lead analyst:

Petroleum/semi-VOA/VOA - Bob Todd
Inorganic/hatchery monitoring - Patty Bucknell
Pesticide (F&W Loss) investigations - Abdou Mekebri
Pesticides (pesticides contracts) - Abdou Mekebri
Tissue / Sediment Monitoring programs (PCBs, PBDEs, OC pesticides) - Kathleen Regalado

3.13 If samples were received in a PAK, photocopy the Form 1000 and write "PAK" in large letters on the front of the photocopy or if a Form FG 1000 Rev 09/01 was used, check the box labeled "PAK Requested" if not already done. Give the photocopy to Bob Todd so that a new PAK will be sent out to replace the one received.

3.14. Using Labworks for log-in samples

1. Go into Multilog
2. Load the project pre-log in group and enter site location, collection date, collection time and checking analytes, matrix, report address against COC
3. Labels can be printed from Labworks with L#, site location, analyses, collection date and time
   - 3a. Go to Labworks explore and pick L #'s for the labels
   - 3b. Highlight the accession for labels. Choose LABEL 30251.CEF and click OK
   - 3c. The program is automatically connected to access. Pick report feature and click LABEL-30251-inorg
4. Place the labels on samples

4.0 Scheduling

4.1 Laboratory analyses are scheduled by priority. Priorities are ranked as follows:

1-Enforcement samples with regulatory holding time
2-Spill and/or wildlife loss in progress
3-Routine samples with regulatory holding time
4-Enforcement samples with completion date requested
5-Routine samples with completion date requested
6-Enforcement samples with no holding time
7-Routine samples with no holding time

4.2 Samples should be completed as soon as possible after receipt. If a delay is anticipated for the completion of an analysis, the person requesting the analysis should be advised of the delay.

5.0 Sample Storage

5.1 Samples remain refrigerated or frozen until they are needed for analysis. Samples are removed from storage for analysis and then are returned to refrigerated storage. Tissue samples are always returned to a freezer after they are analyzed. Samples remain refrigerated/frozen until results are reported.

5.2 Samples that will not spoil may be moved to locked non-refrigerated storage after the report has been completed. Retain chain-of-custody cards or the original copy of the Form FG 1000 Rev. 09/01 for all stored samples.

5.3 Samples retained by the laboratory for six months that are not required as physical evidence should be disposed using a hazardous materials disposal contractor. The person submitting the samples should be contacted and told the samples will be disposed unless a request is made to store the samples at the laboratory for a longer period of time. When samples are disposed, the word "Disposed" and the disposal date are recorded next to the logbook entry (lab accession number) for that sample with the person's initials that authorized the disposal. Inorganics samples are also entered in separate disposal log book located in the inorganic lab (this is done by inorganic lab staff).

QA Officer Approval:_____ Date: _____

SOP Final Approval:_____ Date: _____

Water

	Silver	Aluminum	Arsenic	Cadmium	Chromium	Copper	Manganese
Unit	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
MDL	**0.02**	**1.70**	**0.04**	**0.01**	**0.10**	**0.04**	**0.03**
RL	**0.04**	**5.00**	**0.06**	**0.03**	**0.30**	**0.10**	**0.05**

Tissue

	Silver	Aluminum	Arsenic	Cadmium	Chromium	Coppoer	Manganese
unit	µg/g dw	µg/g dw	µg/g dw	µg/g dw	µg/g dw	µg/g dw	µg/g dw
MDL	**0.02**	**9.40**	**0.08**	**0.01**	**0.82**	**0.34**	**0.19**
RL	**0.06**	**25.0**	**0.25**	**0.03**	**2.00**	**1.00**	**0.50**
unit	µg/g ww	µg/g ww	µg/g ww	µg/g ww	µg/g ww	µg/g ww	µg/g ww
MDL	**0.003**	**1.00**	**0.02**	**0.002**	**0.10**	**0.07**	**0.03**
RL	**0.010**	**3.00**	**0.06**	**0.006**	**0.30**	**0.20**	**0.10**

Nickel	Lead	Selenium	Zinc
µg/L	µg/L	µg/L	µg/L
0.01	**0.015**	**0.60**	**0.50**
0.03	**0.030**	**1.00**	**0.70**

Nickel	Lead	Selenium	Zinc
µg/g dw	µg/g dw	µg/g dw	µg/g dw
0.02	**0.01**	**0.98**	**4.97**
0.06	**0.03**	**2.00**	**15.0**

µg/g ww	µg/g ww	µg/g ww	µg/g ww
0.003	**0.002**	**0.10**	**0.70**
0.010	**0.006**	**0.30**	**2.00**

SAMPLING AND ANALYTICAL ACTIVITIES

State of California
Department of Fish and Game
Office of Spill Prevention and Response
Scientific Program
Fish and Wildlife Water Pollution Control Laboratory

Approvals:

OSPR Scientific Branch Chief	Julie Yamamoto, Ph.D.
OSPR Scientific Branch Asst. Chief and Laboratories Manager	John Turner
Laboratory Director, Fish and Wildlife Water Pollution Control Laboratory	David Crane
Laboratory Quality Assurance Program Manager	Tom Lew
Contract Program Quality Assurance Officer	Loc Nguyen

STATE OF CALIFORNIA
DEPARTMENT OF FISH AND GAME
OFFICE OF SPILL PREVENTION AND RESPONSE

LABORATORY QUALITY ASSURANCE PROGRAM PLAN

California Department of Fish and Game
Fish and Wildlife Water Pollution Control Laboratory
2005 Nimbus Road
Rancho Cordova, CA 95670

SAMPLING AND ANALYTICAL ACTIVITIES

State of California
Department of Fish and Game
Office of Spill Prevention and Response
Scientific Program
Fish and Wildlife Water Pollution Control Laboratory

Approvals:

OSPR Scientific Branch Chief	_____ Julie Yamamoto, Ph.D.
OSPR Scientific Branch Asst. Chief and Laboratories Manager	_____ John Turner
Laboratory Director, Fish and Wildlife Water Pollution Control Laboratory	_____ David Crane
Laboratory Quality Assurance Program Manager	_____ Tom Lew
Contract Program Quality Assurance Officer	_____ Loc Nguyen

DEPARTMENTAL QUALITY ASSURANCE PROGRAM POLICY

The Fish and Game Departmental (DFG) quality assurance program describes the requirements, controls and responsibilities for implementation of quality assurance principles specified in applicable regulations, codes, and standards applied to the environmental laboratory activities. The program begins with quality assurance training for all new employees, and an orientation to the Departmental quality assurance/quality control practices. The importance of quality assurance is recognized by Department management and is documented within the Office of Spill Prevention and Response for all laboratory operations.

The primary commitment of the Departmental Quality Assurance/Quality Control Program is to implement the program activities and requirements committing time and resources ensuring that data are as precise, accurate and complete as required by the data quality objectives of the projects involved.

OFFICE OF SPILL PREVENTION AND RESPONSE
LABORATORY QUALITY ASSURANCE PROGRAM PLAN

TABLE OF CONTENTS

Section	Title
1	Title Page
2	Table of Contents
3	Quality Assurance Description
4	Quality Assurance Organization and Responsibilities
5	Data Quality Objectives and Assessment Methods
6	Sampling Procedures
7	Sample Custody
8	Calibration Procedures and Preventive Maintenance
9	Analytical Procedures
10	Data Reduction, Validation and Reporting
11	Internal Quality Control Checks and Frequency
12	System Audits
13	Preventive Maintenance
14	Routine Assessment of Data Precision, Accuracy, and Completeness
15	Corrective Action
16	Quality Assurance Communication with Management
17	Staff Training and Documentation
18	Glossary

Appendices

A	Organizational Chart
B	Qualifications and Specifications of Key Personnel
C	SOP's
D	Sample Containers, Preservation and Holding Time
E	Forms
F	Analytical Methods and Reference Sources
G	Method Detection Limit Revision 1.11, Federal Register, Vol. 49, No. 209, Friday October 26, 1984.

3.0 QUALITY ASSURANCE DESCRIPTION

3.1 Overview

The purpose of this document is to describe the State of California Department of Fish and Game's Quality Assurance Program as implemented within the Office of Spill Prevention and Response (OSPR) Laboratories. This program plan summarizes those quality assurance and quality control (QA/QC) elements which ensure the accurate and precise development of Department sampling and analytical results, as is consistent with project objectives. The program plan has been designed to meet requirements of many projects and specifically addresses all elements of the Environmental Protection Agency Office of Environment Information "Guidance for Quality Assurance Project Plans" EPA QA/G-5, EPA/240/R-02/009 December 2002 and "Specifications for Preparing Quality Assurance Project Plans" QAMS-005/80. This plan establishes the quality assurance and quality control procedures common to most of the Laboratory services. When necessary, particular project protocols or Standard Operating Procedures (SOPs) will be used to define any project-specific requirements.

3.1.1 Department Quality Assurance System

Quality assurance is a system for integrating the quality planning, quality assessment and quality improvement efforts of various sections to enable operations to meet specified project needs. Quality assurance of field and laboratory systems is concerned with all activities that have an important effect on the quality of measurements as well as the establishment of methods and techniques to monitor the performance of these systems. In addition, quality assurance is composed of those activities performed on a routine basis to gain an independent assessment of the operation and validity of the product. In summary, quality assurance is an essential system of activities to provide the confidence that quality control methods are performing adequately.

3.1.2 Department Quality Control System

In contrast, quality control is the system of activities which provide a quality product for a data user, consisting of internal laboratory operations which document product quality.

3.2 Summary

In summary, this Quality Assurance and Quality Control Program Plan is designed to satisfy the requirements and concerns of the analyst, management, and regulatory agencies concerned with the project.

4.0 QUALITY ASSURANCE ORGANIZATION AND RESPONSIBILITIES

4.1 OSPR Scientific Branch Chief

The Scientific Branch Chief is responsible for administrative and financial oversight of all activities within the OSPR Scientific Branch. Organizational chart can be found in Appendix A.

4.2 OSPR Scientific Branch Environmental Program Manager (Laboratories)

The EPM is responsible for administrative and financial oversight of all activities within the OSPR Scientific Branch's laboratory system.

4.3 Laboratory Directors

Laboratory directors are designated for each of the laboratories within OSPR. The laboratory directors are accountable for all operational activities, including examination of all analytical data, quality assurance parameters, and report preparation and review.

4.4 Laboratory Quality Assurance Program Manager

The Quality Assurance Manager is responsible for all aspects of the laboratory QA program including laboratory certification, performance evaluation studies, and document control**.**

4.5 Contract Program Quality Assurance Officer

The Project Quality Assurance Officer is responsible for the evaluation of all sample logging/numbering procedures, final evaluation of quality control data for all contract projects, and preparation of QA summary reports.

4.6 Project/Section Leaders

Project Leaders are responsible for daily laboratory activities relating to their individual project assignments. Responsibilities include: making daily work assignments for laboratory staff, generation and review of data and preparation and initial review of all laboratory data reports.

4.7 Laboratory Staff

The responsibilities of the laboratory staff include sample container and glassware preparation, calibration standard and reagent preparation, sample preparation, analysis, and preparation of analytical reports with quality control data for the

project/section leaders and laboratory supervisor. Staff members will be familiar with all general laboratory procedures and quality assurance objectives.

5.0 DATA QUALITY OBJECTIVES AND ASSESSMENT METHODS

5.1 Overview

The primary data quality objective is to provide a product that fulfills all project and/or agency requirements. The requirements for projects are established prior to their commencement. In the absence of specific data requirements, standard methods or verified alternative protocols will be routinely applied.

5.2 Objectives

The data that is produced from the laboratory must be scientifically valid, defensible, comparable, and of known precision and accuracy. Objective measures of data quality such as method blanks, duplicates, spikes, reference materials, standards, and recoveries will be employed. Acceptance limits will be established for data accuracy and precision. Whenever possible, statistical methods such as confidence limits, significance tests and/or variability measures will be used to evaluate precision and accuracy of data as well as conformance to acceptance limits. Corrective action will be initiated when the quality of the data does not meet established quality standards.

5.3 Standard Operating Procedures

5.3.1 Standard Quality Control Procedures

Where appropriate, standard quality control procedures, data reduction, and reporting will be in compliance with requirements in Standard Methods for the Examination of Water and Wastewater, 18th edition (1992), with requirements in USEPA Handbook for Analytical Quality Control in Water and Waste Water Laboratories: EPA-600/4-79-019, and with the requirements in USEPA Test Methods for Evaluation of Solid Waste: Physical/Chemical Methods, SW-846, 3rd edition, Update III, 1996.

5.3.2 Standard Operating Procedures

Written standard operating procedures (SOPs) for receipt of samples, tracking of custody, sample preparation and analysis, use of equipment and instrumentation shall be followed. These SOPs shall include use of standard data logging formats, logbook/worksheet entry procedures, and other written or printed documents relevant to the samples. These SOPs are available on request.

6.0 SAMPLING PROCEDURES

6.1 Objectives

The reason for sampling and the parameters of concern for each sampling event establish the requirements for sample container type and preparation, sample amount and preservation, and the sampling technique. Information on the sampling site is assembled so that a project work plan can be developed for the collection of representative samples.

6.2 Preparation for Sampling

Prior to conducting project field sampling operations, pre-cleaned sample containers and sampling devices are assembled along with the necessary equipment and portable instrumentation. A team of trained personnel with appropriate protective equipment will then conduct actual sampling using established procedures.

6.2.1 Field Quality Measures

Field quality measures such as trip blanks for water control samples, field blanks, duplicates, and background references are employed to assure data quality. Sample filtration, when required, can be performed in the field. Sample preservation is routinely provided by using sample containers with pre-added preservatives. Sampling record sheets and chain-of-custody or record-of-custody forms are completed at the time of sampling to document collection operations. Samples are carefully placed in suitable containers or coolers for prompt transportation to the laboratory. Appendix D summarizes the type of sample container and preservation methods used, as well as the maximum acceptable holding time between sampling and analysis for various types of analyses and matrices. When available and applicable, the holding times, sample container type and preservatives will follow regulatory guidance.

6.2.2 Sampling Site Identification

Sampling sites will be identified in a field logbook or project sampling form used for recording information during the conduct of sampling activities. Each sampling site will be identified by exact location, which may include address, GPS coordinates, well number, or site name. A unique sample site name and/or number is recorded in the field logbook and the sample collection form. The sample site name and/or number is also used to identify the sample on the project sampling form and chain-of-custody form.

6.2.3 Sample Container Inspection

Inspect sample containers for good closure, proper labeling, and correct number and type required for the site. Where split samples are being collected, additional containers will be needed.

6.3 Sample Collection

6.3.1 Sample Identification

When appropriate each sample will be uniquely identified by a number previously designated by the project/section officer. This number will also be used on the project sampling forms. The numbers assigned to splits, duplicate samples, and spiked samples will be coded in such a way to prevent easy identification as blind quality control samples when handled in the laboratory. Labels with adhesive backings and with the sample number on the face will be used. Extra labels will be available. Should more labels be required, they may be prepared with a permanent marking pen in the field, or a permanent marking pen may be used on the sample container. In the latter case, an adhesive label should be prepared and attached to the sample container as soon as the sample is returned to the laboratory. Ziplock bags used to carry samples will be labeled by writing appropriate identification directly on the bag using a permanent marking pen.

6.3.2 Collection of Field Replicate Quality Control Samples

Quality control criteria require that more than one set of samples be collected at a selected number of sampling events. These samples will be used to verify the consistency of results. Appropriate type and number of quality control samples will be specified with each project.

6.3.3 Field Storage of Samples

All sample containers will be kept in chilled storage in the field unless specific sampling protocol stipulates otherwise. Insulated ice chests and frozen plastic-encased coolants (Blue Ice, for example) will be used. For long term field storage of biological samples, dry ice will be used. Ice may also be used in sealed ziplock bags. The sampling team will have sufficient number of ice chests and frozen coolants to assure that samples remain chilled throughout the day. The samples must always be kept in the possession of the sampling team until they are transferred to the custody of the laboratory. Since the ice chests will have to be kept in a locked car or truck, the vehicle should be parked in the shade to the extent possible. Sampling vehicles

should use unleaded fuels. Ice chests will be cleaned with water and stored uncovered after each day. Sealed refrigerants will be washed with water and put into a freezer for reuse. The vehicle will be refilled with fuel after samples are transferred when possible.

6.3.4 Storing and Shipping Samples

6.3.4.1 Storage at the Laboratory

The samples received at the laboratory will be kept in refrigerators or freezers. Temperature will be kept as close as possible to the storage temperature required for each sample matrix and type of analysis. Generally, refrigerators will be kept at 4 +/-2 degrees C, freezers will be kept at -15 +/-5 degrees C or colder. Storage shall be in an environment where the sample identification numbers will remain attached. Mechanical refrigeration units shall be used. The use of ice as a refrigerant for sample storage at the laboratory is not allowed.

6.3.4.2 Shipping

All samples will be refrigerated or frozen during shipment through the use of ice, cold packs, or dry ice. Samples will be shipped in insulated containers. All caps and lids will be checked for tightness prior to shipping. To the extent possible, transporting vehicles will use unleaded fuel.

7.0 SAMPLE CUSTODY

The Department of Fish and Game's chain-of-custody procedures for sample tracking are initiated during the time of actual sample collection by field personnel and maintained throughout the time the samples are in their possession. Chain of custody documents must be initiated and maintained for all samples received by the laboratory.

7.1 Chain of Custody

The person responsible for sample collection must originate the chain-of-custody record. The sampler will clearly label the sample with the project name, sample location, field identification number, the date and time of sampling, and his/her own name and initials. The same information will be entered on the chain-of-custody record along with information concerning the sample type, the analyses to be performed and the sample container. The individual collecting the samples will be responsible for the custody of samples until they are transferred or properly dispatched. If samples are hand-carried to the laboratory by Fish and Game personnel, custody of samples will be transferred to laboratory staff. Shipping containers (ice chests) transported by commercial carrier will be secured with strapping tape. Documentation of the shipment will be kept with a copy of the chain-of-custody record by the person shipping the samples. The original copy of the chain-of-custody record will accompany the sample(s) when transported to a departmental or commercial laboratory.

The laboratory staff person logging the sample(s) in will carefully inspect each sample for chain-of-custody documentation, sample labeling, packing lists, and for the condition of the custody seals, sample packing materials, and the sample containers. Any discrepancies or problems associated with sample shipment will be documented on the chain-of-custody form. In the case of a discrepancy between information on the container and the COC form, the information written on the container will be used and the sample collector or project manager will be notified of the discrepancy.

After inspection, the samples will be entered into the laboratory sample receiving logbook, and will be assigned a unique sample identification number. The following information shall be included when samples are logged-in:

- Laboratory number (assigned when samples are submitted)
- Laboratory storage location (refer or freezer no.)
- Spill Title (if applicable)
- Suspects name (if applicable)
- Index-PCA code (if applicable)
- Sampler’s name, address and phone number
- Name and address of person who will receive report

- Date samples received by the laboratory
- Analysis requested
- Sample identification/location
- Sample type (matrix)
- Number of containers and container type
- Sample preservation
- Required report completion date
- Signatures of person submitting samples and person receiving samples for the laboratory
- Problem description (if applicable)
- Incident location (if applicable)
- Special instructions (if applicable)

The person logging the samples in will ensure that the samples are either retained in secure storage or are given directly to an authorized analyst. A copy of the chain-of-custody form will be used to provide analysis requirements to the analyst(s). This form will accompany the sample containers and/or prepared extracts as each authorized employee performs a required task on the samples.

After all analyses have been completed and disposal of the sample is authorized, a designated sample custodian will make proper disposition of the sample with appropriate documentation. Disposal method and approximate disposal date will be noted in the laboratory log-in records. The completed chain-of-custody form(s) will be retained as a permanent part of the project record.

7.2 Sample Handling, Storage, and Holding Times

All samples will be handled, prepared, transported, and stored in a manner designed to minimize bulk loss, analyte loss, contamination or biological degradation. The sample containers will be clearly labeled with permanent marker. Soil and tissue samples for organic constituents must be frozen to prevent degradation or volatilization.

Samples will be stored for the maximum sample holding time for the required analyses as specified for the analysis. Samples which do not have a maximum holding time specified, will be stored for the duration of the research or study activity unless the sample is consumed entirely for analysis. Thereafter, the laboratory director or project leader will determine if the sample will be archived.

When the holding time interval has passed and samples are approved for disposal, samples and the sample containers will be disposed of properly. It is the sole

responsibility of the laboratory personnel to ensure that all applicable regulations are followed in the disposal of samples or related chemicals. If the contracting officer should request return of a sample prior to the maximum holding time, it will be returned in a manner that meets Department of Transportation regulations.

8.0 CALIBRATION PROCEDURES AND PREVENTIVE MAINTENANCE

All laboratory instruments and equipment that are used for laboratory measurements will be maintained and calibrated for good operating conditions that meet laboratory accuracy requirements.

The calibration/maintenance techniques will be performed according to a specific calibration standard operating procedure (SOP) which has been specified by the manufacturer's recommendations, an analytical or agency requirement, or by good laboratory practices.

Analytical standards to be used for instrument calibration are obtained from sources that have demonstrated accuracy levels, and are properly stored to ensure accuracy integrity. Calibration chemicals will be logged in and assigned a specific shelf life based on chemical stability. Non-chemical standards, instruments and equipment used for calibration purposes will be re-certified per an established schedule.

Routine maintenance will be performed by qualified laboratory personnel with recommended parts and supplies kept in stock. For some items, the services of an outside vendor will be used. The correct operation of instruments/equipment repaired by vendor services will be verified prior to use on projects.

An established schedule for the routine calibration or maintenance of the instrumentation and equipment will be developed based on manufacturer's recommendations, operational experience, procedural requirements and good laboratory practices. A maintenance log-book will be established for each instrument in which all maintenance will be recorded. Calibration results, which serve as a measure of instrument condition, must be kept with the project data files.

9.0 ANALYTICAL PROCEDURES

For analysis mandated by regulatory agencies, specific methods have been designated for routine analyses with a particular range of concentrations and matrices. If methods are not stipulated, standard methods from a recognized authoritative source will be used for the tests. Analytical methods and reference sources routinely used at the Department of Fish and Game are listed in Appendix F.

9.1 Standard Procedures

All procedures developed and routinely used by the laboratory are documented with laboratory standard operating procedure (SOPs). In addition to the actual test procedure, SOPs include applicable references, acceptance limits, health and safety precautions, trouble-shooting guidelines, quality control requirements, sample preparation and documentation criteria, calculation methods and reporting protocol. Analysts are trained to perform sampling and testing tasks per the established SOP. Each analyst has access to a current copy of the procedure for the methods they perform. When it is not possible to perform an analysis per established procedures, the section supervisor is promptly notified and corrective action may be initiated.

9.2 Method Development

If a suitable standard method is not available, the laboratory technical staff will develop appropriate methods to meet the project requirements. Procedures developed within the laboratory are thoroughly validated to assure accurate, consistent results. Reference materials, replicate analyses, matrix spikes, and procedural blanks are some of the techniques applied to validate procedure development. Based on these techniques, project-specific acceptance limits can be developed, and SOPs can be written.

SOPs are reviewed and updated annually or when a change in procedure is made. The revisions are coordinated through the laboratory Quality Assurance Officer and are distributed to appropriate personnel. Prior versions of the SOPs are retrieved and archived or destroyed.

9.3 Analytical Methods

Analytical methodology described in one of the following approved methodology manuals will be used as guidelines for all analytical methods used:

- EPA Methods for the Chemical Analysis of Water and Wastes, EPA-600/4-79-020

- EPA Test Methods for the Evaluation of Solid Waste, Physical/Chemical Methods, SW-846, third edition, Update III, 1996
- EPA Test Methods for Chemical Analysis of Municipal and Industrial Wastewater, EPA-600/4-82-057
- EPA EMAP - Estuaries QAPP, EPA/600/x-93/xxx, May 1993
- Standard Methods for the Examination of Water and Wastewater,18th Ed., 1992
- Manual for Association of Analytical Chemists, 15th Ed.,1990
- USFWS, Patuxent Wildlife Research Center Analytical Manual (PWRCAM)
- Pesticide Analytical Manual (PAM Vol. I and II), USFDA
- Quality Assurance of Chemical Measurements by Dr. John Keenan Taylor
- Manual of Analytical Methods for the Analysis of Pesticides in Humans and Environmental Samples, EPA-600/8-80-038.

Modifications of the above approved methods together with methodology developed by DFG personnel will be documented in SOPs. This methodology may be used with approval in advance, in writing, by the Contracting Officer or the Contracting Officer's Technical Representative. The limit of quantitation of any method which is used must meet a Method Detection Limit (MDL) consistent with the methodology being used. Analytical control will be maintained by strictly following written SOPs. If for some reason the SOP cannot be followed, deviations will be noted and reported in the data submital package. Deviations from the approved analytical methodology must meet requirements based on the method validation test outlined subsequently in this section.

9.4 Analytical Method Validation

9.4.1 Limit of Detection

Determine, for each method, the limit of detection (LOD), defined as the lowest concentration level that can be determined to be statistically different from a blank, and the limit of quantification (LOQ), defined as the level above which quantitative results may be obtained with a specified degree of confidence. Calculate the MDL and RL, the Federal Register, Vol. 49, No. 209, Friday, October 26, 1984 (Appendix G).

9.4.2 Standard Reference Material Test

To the extent that a standard reference material (NIST or National Research Council of Canada) can be obtained and when appropriate or required, it will be analyzed with each set of samples or every twenty samples for sets greater than twenty. To validate a new or non-standard method for limited use, comparability data will be generated.

All results must be within 65-135% of the 95% certified confidence interval for the reference materials unless otherwise stated.

9.4.3 Sample Duplicate Test

For each set of samples, analyze one sample in duplicate for each analyte in question or one duplicate for every twenty samples.

9.4.4 Spike Recovery Test

One spike recovery test will be run for each set of twenty samples for each analyte on each matrix type analyzed. For method validation, each matrix will be fortified with analyte at 1-10x reporting limit. In general, recovery values for sample spikes must be greater than 50 percent for method validation unless otherwise stated.

9.4.5 Method Documentation

Maintain a file containing all validation and modification reports. Upon completion of the validation tests, prepare a data report detailing the results.

9.5 Round-Robin Studies

The DFG laboratories will participate in round-robin studies and/or other standard reference sample programs as an on-going laboratory QC effort.

9.6 Organization of Laboratory

Qualifications of personnel is acknowledged to be very important to the laboratory. When hired, chemists and technicians must have knowledge of laboratory protocol. Such experience can be obtained from laboratory experience in another facility or satisfactory completion of suitable college course work. Anyone conducting analytical procedures in the laboratory is responsible for the accuracy of those procedures and is answerable to the Laboratory Director. New personnel will be trained by a qualified analyst and will report to the appropriate

Project/Section Leader. All personnel are expected to be familiar with and carefully follow the appropriate laboratory standard operating procedures (SOPs) developed for use in the laboratory when conducting analyses on samples received.

9.7 Laboratory Operating Practices

9.7.1 Sample Receipt

The following procedure will be followed immediately upon receiving a sample shipment:

- Record sample number in sample log book and check that the sample number is clearly marked on the sample container and on accompanying custody forms or sample worksheets.
- Record the requested analytical information in the sample log book.
- If sample is not going to be run immediately, follow appropriate sample storage procedures.
- Copies of COC will be distributed to the appropriate laboratory staff.

9.7.2 Laboratory Procedures

The individual analyst upon receiving a sample shipment will proceed as follows:

- Check the sample collection date and analysis holding time. This will determine the priority for sample preparation and analysis.
- Record date and procedure to be used.
- Record all pertinent information regarding instrument and/or materials in the lab book or on the data sheet.
- Examine glassware routinely to confirm cleanliness.
- Check to make sure that the reagents used are the correct grade and type for the analysis to be done.
- If a new lot of reagent is put into use, reagent blanks or other checks should be run to demonstrate continuity of the required quality.

- Follow designated procedure (SOP) exactly, including all quality control requirements.

9.7.3 Record Keeping

The following logs will be maintained by laboratory personnel:

- Sample log: record date of receipt, number of samples, laboratory sample number, analyses to be completed.

- Run log: record each analytical run by run number, date, and analysis. Data printouts shall be referenced Laboratory number (log-in or L#).

- Standards preparation log: record all weights and volumes used to prepare standards, solvent, source and purity or concentration of neat or concentrated standards, date prepared, final concentration, and preparer's initials. All standard storage containers will be labeled to reference the standard preparation log.

9.7.4 Reports

Procedures for analytical reporting will be as follows:

- Retain all appropriate computer printouts and strip chart recordings in a binder (be sure information includes sample number, date and time).

- Have all information clearly recorded so that a written data report can be made and reviewed.

- Final data reports shall be given to the QA officer for review and laboratory director for final review and signature.

9.7.5 Instruments

Instrument check procedures will be implemented as follows:

- The instrument is calibrated for the range in which work is intended. The calibration must meet required linearity or curve specifications for the method (eg. $R^2 \geq 0.995$)

- The accessory equipment (such as syringes or autosampler tubes) must be the correct ones for the instrument and method being used and they must be CLEAN.

- The reagents being used are the proper ones, and they have been checked for interferences by running a blank.

- Analytical results (raw data) are stored in hardcopy or electronically with the project data files.

- If the instrument or other equipment does not appear to be working properly, contact the section supervisor IMMEDIATELY.

9.7.6 Quality Assurance Procedure

The following quality assurance procedures form the foundation for quality control practices in the laboratory. These procedures will be practiced as a matter of routine unless superceded or modified by specific quality assurance requirements of a given project.

9.7.6.1 Chemical Analysis – General

- At least one method blank will be run for each set of samples of one matrix type to determine whether interferences are introduced. Method blanks will be run through the complete analytical method along with the sample set. Blanks shall be run with a minimum frequency of one blank per 20 samples unless otherwise specified.

- At least one sample will be fortified or one SRM of similar matrix to that of the samples will be used and run with each set of samples to measure the analytical accuracy. When appropriate, the sample will be spiked with the analyte(s) at the expected level in the sample or at mid- range of the standard curve or spiked at 1-10x reporting limit. A minimum of one fortified sample or SRM will be run with each sample set of 20 samples or less.

- At least one sample or fortified sample will be prepared in duplicate and analyzed with each set of samples of one matrix type to measure analytical precision. A minimum of one duplicate will be analyzed for each set of 20 samples or less.

- Prepare new standards as needed according to the guidelines in section 8.0. Record all the required information in the standards preparation log. Freshly-made standards should be compared to the response of existing calibration standards or reference standards before using in order to verify the reliability of the new standard.

- When washing glassware, follow procedures outlined in method SOPs.

- All QA procedures used will be documented and stored with the results in the project files.

- Additional periodic checks of accuracy or precision will be required as deemed necessary by laboratory supervisor.

9.7.6.2 Review of Quality Control/Analytical Data

- All quality control/analytical data will be reviewed for correctness of the analytical, calibration, and data reduction procedures used, and initialed by the section supervisor, QAO or laboratory director before the accompanying data may be reported.

- If after being reviewed, a set of data are determined to be out of control, the laboratory director shall be notified and an appropriate course of corrective action will be prescribed. (See sections 11, 14, and 15 for data evaluation criteria and corrective measures.) The analyst shall keep records of the corrective measures taken. No additional analytical data will be generated until the problem has been identified and corrected.

9.7.7 Laboratory Safety

- Safety glasses will be worn in designated laboratory areas at all times. Laboratory visitors will be issued safety glasses when necessary.

- Lab coats or aprons will be worn while working with any solvents, acids, caustics, condensers, or designated instrumentation.

- Walkways, exits, and safety shower/eyewash stations will be clear of debris at all times.

- No horseplay of any kind will be tolerated.

- No food or drink will be allowed in laboratory areas.

- Additional safety and emergency procedures outlined in the Standard Operating Procedures will be followed.

- Refer to laboratory Injury and Illness Prevention Plan for additional safety measures.

9.7.8 Laboratory Cleanliness

- Glassware will be washed and prepared for use in accordance with the procedures set forth the method SOPs.

- Clean glassware will be returned to its proper place as soon as it has been cleaned appropriately and properly capped.

- Counter tops will be kept clean.

- Spills will be cleaned up immediately.

- Broken glassware will be placed in a specified container.

9.7.9 Records

Records on all relevant data are to be easily located in files that pertain to a specific analysis, question, or project.

10.0 DATA REDUCTION, VALIDATION AND REPORTING

10.1 Analysis

The conversion of raw data into functional values and the presentation of these values is a critical process in the laboratory function. In order to assure the production of data that is scientifically valid, defensible, comparable and of known precision and accuracy, the following steps are required.

10.2 Validation

Reduction of raw data is accomplished using established techniques. The calculations required to perform the reduction of raw data are performed manually or with the aid of automated data processing systems, as specified by the SOP for the particular testing method. If manual processing is to be used, the SOP will provide the calculation method and the units for reporting derived values. For automated data reduction, the accuracy of calculations will be verified through the use of standards or raw data inputs of known values.

Raw data, related quality control information and derived values are carefully evaluated prior to final reporting. The initial evaluation is performed by the analyst/specialist performing the work. Statistical methods, such as precision and accuracy acceptance limits and/or control charts, are employed to assess data acceptability. The QAO or second analyst provides a second evaluation of the data and conclusion contained in the final report. The laboratory director provides a final evaluation prior to release of the report.

10.3 Reporting

Analytical detection limits will be experimentally developed either as instrument detection limits (IDL) or method detection limits (MDL). For applications requiring a greater degree of statistical confidence, the reporting limit (RL) will be used to establish a minimum reporting concentration which will be at or above the MDL. The minimum reporting limit will be based upon project requirements and proven laboratory capabilities.

10.4 Final Reports

The final reports contain an outline of the scope of the project, sample identification, methodologies performed, a discussion of any unusual circumstances regarding the project, and tabulated analytical results. This report is reviewed and may be signed by the analyst or when multiple analysts are involved, each analyst's initials are recorded on the report and the report is signed by the lead chemist and/or the technical reviewer, QA officer and laboratory director.

10.5 Record Keeping and Maintenance

Instrument logbooks will be maintained with each instrument. A record will be made of any conditions or incidents the analyst encounters which are in any way unusual, or deviate from the SOP.

When maintenance is required, a record will be made of the symptom, the repair performed, and the individual performing the repair.

All observations, electronic records, and most printouts, and other raw material generated in the course of any analysis will be saved. They will be filed with reference to laboratory log number, date, batch number, analyst, and other information deemed pertinent. Also recorded in the laboratory notebook or bench sheets will be all weights or other types of raw data generated in the laboratory but not printed on a hard copy by the data generating device. All documentation in the laboratory notebook or bench sheets will be made in ink. Corrections to notebooks or other data records will be made by crossing a single inked line through the error, entering and initialing the correction, and recording the date.

The records to be maintained in the laboratory include such items as sample tracking records, notebooks, bench sheets, instrument read-out records, computer printouts, quality control data, and raw data. The records will be maintained for the life of the project and they will be provided to the organizations contracting for the research or studies upon request.

11.0 INTERNAL QUALITY CONTROL CHECKS AND FREQUENCY

Quality control checks are routinely performed in the WPCL operations. These checks may be increased or modified to meet the needs of a particular analysis or project.

11.1 QA Samples

Internal quality assurance samples (fortified samples and duplicates, appropriate reference materials, duplicate samples, and method or procedural blanks) will be analyzed with each set or every twenty analyses being performed. These internal quality assurance analyses are conducted for the parameters being monitored by that analytical procedure. In addition, the compounds contained in the quality assurance sample will be representative of those compounds being monitored. Accuracy is measured by calculating percent recovery for laboratory control spikes (fortified reagent sample) and matrix spikes (fortified samples) and certified reference materials (CRMs or SRMs). Accuracy is also determined for CRMs by comparing the analysis results with the certified values. CRM results are acceptable if they are within 65-135% of the 95th percentile confidence interval of the consensus values for the certified materials.

The results of all QA analyses and the percent recoveries for fortified samples and reference materials will be calculated and documented.

11.2 Duplicate Samples

One duplicate sample and/or a matrix spike duplicate or laboratory control spike duplicate will be analyzed for each set of twenty samples analyzed. The relative percent difference for each constituent is calculated as follows:

$$RPD = \{(D_1-D_2)/[(D_1+D_2)/2]\}x100$$

Where, RPD = Relative Percent Difference
D_1 = First Sample Value
D_2 = Second Sample Value (duplicate)

The results of all duplicate determinations and the calculated relative percent difference will be reported with the data sets. For RPD, use a control limit of 25 percent unless otherwise specified by a project specific QAPP.

If either sample value is less than the MDL, the notation of "ND" (not detected) will be reported. If the precision falls outside the control limits, the analysis results will be reported with the appropriate data qualifier.

11.3 Fortified Matrix (MS/MSD) Sample Analyses

When required, matrix spike and matrix spike duplicate analyses will be conducted at a rate of five percent. The spike will be added prior to any digestion, extraction, or distillation steps as a check on the sample preparation and analysis. An amount of analyte will be added to the sample that is five to ten times the reporting limit for the analyte of interest. Recovery values are calculated as follows:

$$\text{Recovery} = [(D_a - D)/D_s] \times 100$$

Where, Recovery = Percent Recovery

D_a = Analysis value of fortified sample
D = Analysis value of sample without spike
D_s = Amount of spike added

Recovery values for fortified samples must be greater than 50 percent except where a specific method (SOP) or project specific QAPP require a different acceptable range. Exceptions shall be noted in the project specific data quality objectives. When a specific method and analyte require a different acceptable recovery range, as determined by actual spike recovery runs, the acceptable range shall be noted in the Standard Operating Procedure for that method. If the recovery falls outside of the acceptable recovery range, the analysis results will be qualified or rejected. If the results are rejected, the batch of samples associated with the rejected results may need to be re-analyzed. When sample concentrations are less than the MDL, the value of "0" will be used as the sample result concentration for purposes of calculating spike recoveries. All fortified sample results will be reported with the data package.

If the percent recovery for matrix spike is unacceptable, there might be an interference due to the matrix. The sample may be diluted to lower the interference and re-analyzed. If dilution doesn't work, the method of standard additions will be used, if appropriate. If matrix interference is determined to be the cause of unacceptable recoveries, the data will be qualified.

11.4 Method Blanks

Method blanks will be analyzed at a minimum of once for every batch of samples. Blank concentrations should not exceed the reporting limit for the analyte. If blank values exceed the reporting limit, the source of the contamination should be investigated and corrected, and the results associated with the contaminated blank re-analyzed or qualified. All blank analysis results will be reported with the data package.

12.0 SYSTEM AUDITS

The system audit is an on-site review which provides a qualitative appraisal of a project data set.

12.1 System Audit

The Quality Assurance Officer or person acting in that capacity conducts a QA/QC evaluation of selected project data reports prior to reporting data. This evaluation includes a review of QC data. Findings are reviewed by the laboratory director. The project lead chemist may be required to re-analyze samples associated with the unsatisfactory findings.

13.0 PREVENTATIVE MAINTENANCE

Maintenance on analytical instruments will be performed by WPCL chemists or by manufacturer's service personnel. An inventory of critical spare parts (for gas chromatographs - septa, syringes, column ferrules, backup column, etc.; for atomic adsorption spectrophotometers -- AA lamps, quartz cell, plastic tubing, etc.) will be maintained on hand.

Fume hoods will be checked quarterly. The results and the ventilation capacity across the face of the fume hood and the inspection date will be posted on an exterior wall of the fume hoods.

Equipment manuals containing trouble-shooting SOPs will be kept near the instruments.

Instrument operators are responsible for daily maintenance and for maintaining instrument logs. These logs will contain the date, operator's initials and description of routine maintenance procedures. Each entry into the run log will be initialed by the individual making the entry.

14.0 ROUTINE ASSESSMENT OF DATA PRECISION, ACCURACY, AND COMPLETENESS

14.1 Precision

Precision shall be assessed with each sample set for each analysis type. Precision will be expressed in terms of relative error as the percent deviation of the duplicate results from the original results obtained. The equation for determining precision is:

$$RPD = (D_1-D_2)/[(D_1+D_2)/2]x100$$

Where RPD = Relative Percent Difference
D_1 = First Sample Value
D_2 = Second Sample Value (duplicate)

14.2 Accuracy

Accuracy will be assessed on a regular basis in each set of samples for each analysis type by comparison of the analytical results of internal QA samples provided or approved by the QA Officer with accepted concentrations. Accuracy will be expressed in terms of percent recovery. Percent recovery is calculated as follows:

$$\text{Percent Recovery} = [(D_a-D)/D_s]x100$$

Where, D_a = Analysis value of fortified sample
D = Analysis value of sample without spike
D_s = Amount Spiked

14.3 Completeness

Completeness shall be assessed for each sample set and for each analysis type. The comparison for completeness will consist of a comparison of the number and type of analyses scheduled to be performed with those analyses successfully completed. Completeness shall be expressed as the percentage of analyses successfully completed relative to the number of analyses scheduled to be performed for each analysis type.

15.0 CORRECTIVE ACTION

Corrective action includes a variety of activities starting with the individual analyst applying the elements of quality control to a particular task. At this level, the corrective action takes the form of problem identification based on spike, calibration, or recovery results that exceed acceptance limits. Appropriate action taken at this stage includes checking calculations or calibrations, preparing new standards or spiking solutions, re-analyzing samples or re-extracting and re-analyzing samples. If the above actions do not correct the matter, the laboratory director is notified. Project work requiring the use of the problem method or defective instrument will be suspended until the problem has been resolved.

A review of data from spiked samples, reference standards, duplicates, method blanks and standards may also indicate a necessity for corrective action. In these instances, corrective action for out-of-limit values are normally requested by the Quality Assurance officer.

The data generated during the period of problem identification will not be reported, unless additional analysis is not possible due to restricted sample availability, or time constraints. In this case, the result will be reported with qualifications that have been clearly identified and approved by project officials.

All corrective actions are recorded in laboratory notebooks, instrument logbooks, or electronically with project data packages. Corrective action forms will be filed with QAO. These records are maintained at least eight years (unless otherwise required by project specific QAPP) in files at the laboratory. These are always available for review during external audits. These records include the analyst's comments on the corrective action such as calibrations, preparing new standards or spiking solutions, or re-analyzing samples and the results of corrective action.

The final disposition of documents is consistent with agency record-keeping procedures. Paper copies of all laboratory notebooks, benchsheets, instrument logbooks, and electronic data packages are part of the permanent archives.

16.0 QUALITY ASSURANCE COMMUNICATION WITH MANAGEMENT

Communication with all levels of management concerning quality subjects is an ongoing process and routine quality issues are communicated to appropriate levels of management. The results of performance audit evaluation sample analyses are provided to the laboratory director, as well as to the lead chemists and analysts. If significant QA problems are experienced or observed in any aspect of lab operations, the laboratory director is promptly notified.

17.0 STAFF TRAINING AND DOCUMENTATION

17.1 Hiring Process

People often begin their careers at the Water Pollution Control Laboratory (WPCL) as temporary employees. Under these circumstances, screening and reference confirmation is undertaken by the laboratory director and/or lead chemists. If/when the employee becomes permanently hired by CDFG or SJSUF, she/he completes and signs the agency employment documents, and those papers are retained in the employee's personnel file.

New employees hired by CDFG and SJSUF provide documentation of skills they already possess via publications, detailed resumes, letters of recommendation, and self-certification. Written management and/or peer performance reviews are maintained in the individual's personnel files. Every staff member is formally evaluated annually using a combination of self and supervisor evaluation. Job descriptions are reviewed and may be updated at that time.

17.2 Safety Training and Meetings

On a yearly basis, all staff attend at least one safety seminar. In addition, staff attend the laboratory's regular safety meetings. The agendas from all staff meetings which include health and safety concerns will be signed by the attendees, and copies will be retained in the safety meeting file.

18.0 Glossary

Accuracy - combination of bias and precision of an analytical procedure, which reflects the closeness of a measured value to a true value.

Bias - consistent deviation of measured values from the true value, caused by systematic errors in a procedure.

Calibration check standard - standard used to determine the state of calibration of an instrument between periodic recalibrations.

Confidence coefficient - the probability, %, that a measurement result will lie within the confidence interval or between the confidence limits.

Confidence interval - set of possible values within which the true value will lie with a specified level of probability.

Confidence limit - one of the boundary values defining the confidence interval.

Detection limits - Various limits in increasing order are:

Instrument detection limit (IDL)-the constituent concentration that produces a signal greater than five times the signal/noise ratio of the instrument. This is similar in many respects, to "critical level" and "criterion of detection." The latter limit is stated as 1.645 times the s of blank analyses.

Lower limit of detection (LLD) - the constituent concentration in reagent water that produces a signal 2(1.645) s above the mean of blank analyses. This sets both Type I and Type II errors at 5 %. Other names for this limit are "detection limit" and "limit of detection" (LOD).

Method detection limit (MDL) - the constituent concentration that, when processed through the complete method, produces a signal with a 99% probability that it is different from the blank. For seven replicates of the sample, the mean must be 3.14s above the blank where it is the standard deviation of the seven replicates. The MDL will be larger than the LLD because of the few replications and the sample processing steps and may vary with constituent and matrix.

Limit of quantization (LOQ) - the constituent concentration that produces a signal sufficiently greater than the blank that it can be detected within specified limits by good laboratories during routine operating conditions. Typically it is the concentration that produces a signal 10s above the reagent water blank.

Duplicate - usually the smallest number of replicates (two) but specifically herein refers to duplicate samples, i.e. two samples taken at the same time from one location.

Internal standard - a pure compound added to a sample extract just before instrumental analysis to permit correction for inefficiencies.

Laboratory control standard - a standard, usually certified by an outside agency, used to measure the bias in a procedure. For certain constituents and matrices, use National Institute of Standards and Technology (NIST)* Standard Reference Materials when they are available.

Precision - measure of the degree of agreement among replicate analyses of a sample, usually expressed as the standard deviation.

Quality assessment - procedure for determining the quality of laboratory measurements by use of data from internal and external quality control measures.

Quality assurance - a definitive plan for laboratory operation that specifies the measures used to produce data of known precision and bias.

Quality control - set of measures within a sample analysis methodology to assure that the process is in control.

Random error - the deviation in any step in an analytical procedure that can be treated by standard statistical techniques.

Replicate - repeated operation occurring within an analytical procedure.

Surrogate standard - a pure compound added to a sample in the laboratory just before processing so that the overall efficiency of a method can be determined.

Type I error - also called alpha error, is the probability of deciding a constituent is present when it actually is absent.

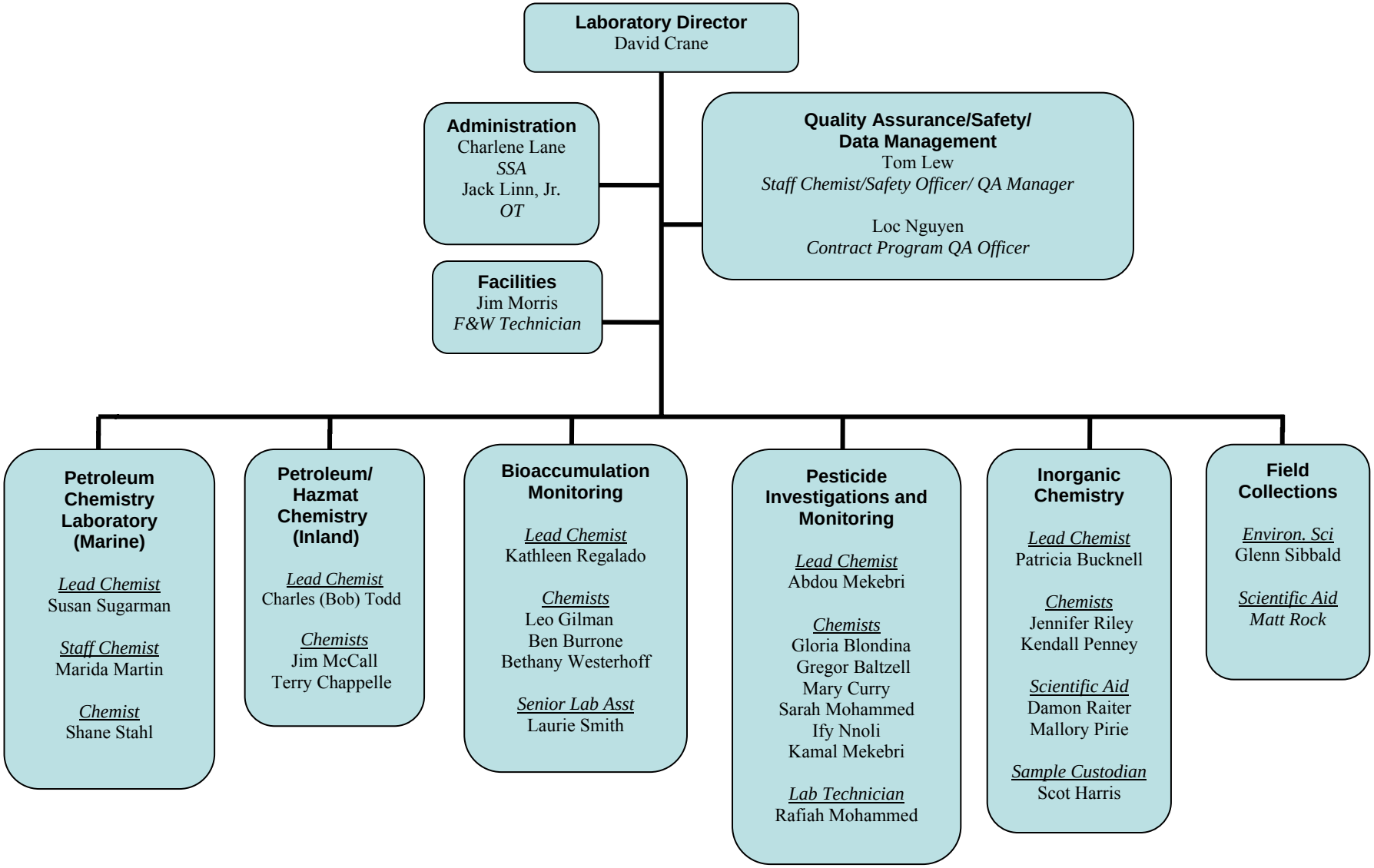
Type II error - also called beta error, is the probability of not detecting a constituent when it actually is present.

---

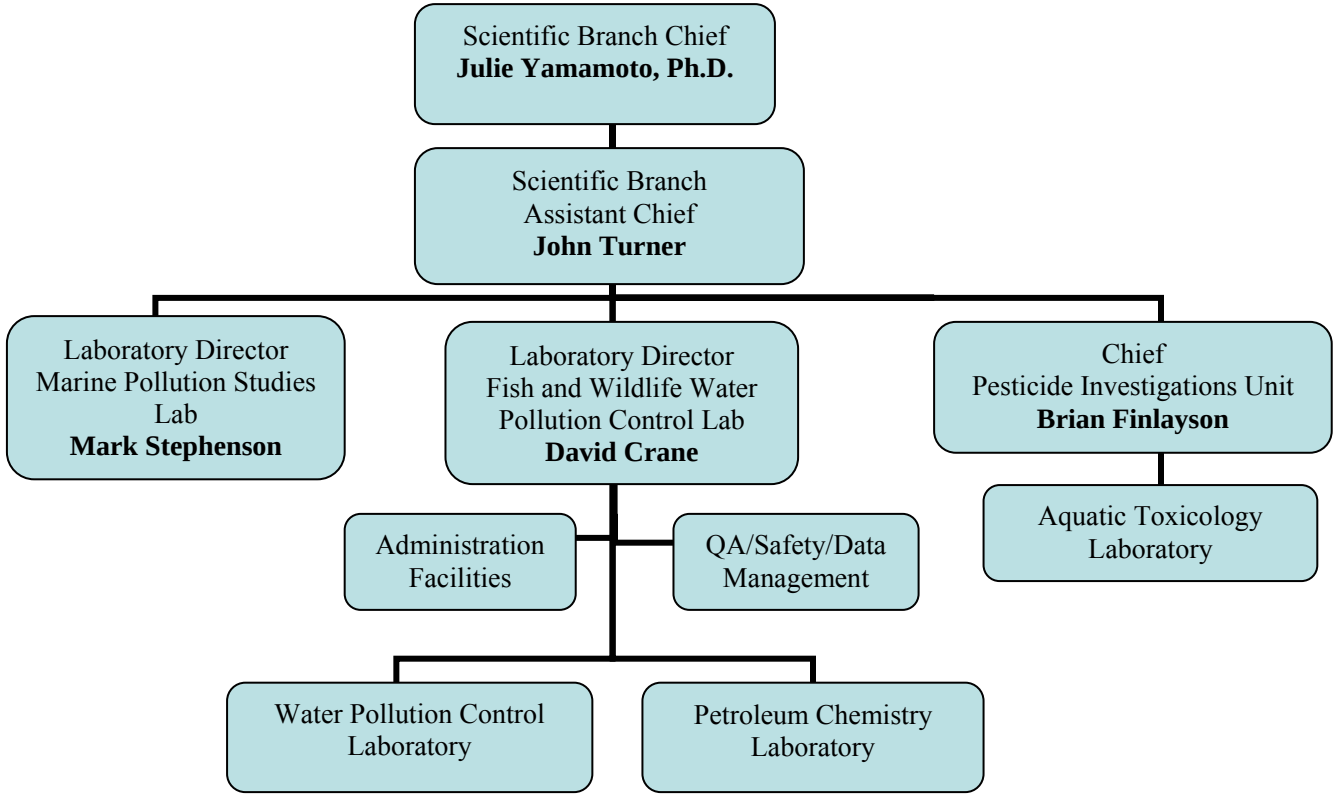
*Formerly National Bureau of Standard (NBS).

APPENDIX A

ORGANIZATIONAL CHART


Laboratory Director
David Crane
Administration
Charlene Lane
SSA
Jack Linn, Jr.
OT
Facilities
Jim Morris
F&W Technician
Quality Assurance/Safety/ Data Management
Tom Lew
Staff Chemist/Safety Officer/ QA Manager
Loc Nguyen
Contract Program QA Officer
Petroleum Chemistry Laboratory (Marine)
Lead Chemist
Susan Sugarman
Staff Chemist
Marida Martin
Chemist
Shane Stahl
Petroleum/ Hazmat Chemistry (Inland)
Lead Chemist
Charles (Bob) Todd
Chemists
Jim McCall
Terry Chappelle
Bioaccumulation Monitoring
Lead Chemist
Kathleen Regalado
Chemists
Leo Gilman
Ben Burrone
Bethany Westerhoff
Senior Lab Asst
Laurie Smith
Pesticide Investigations and Monitoring
Lead Chemist
Abdou Mekebri
Chemists
Gloria Blondina
Gregor Baltzell
Mary Curry
Sarah Mohammed
Ify Nnoli
Kamal Mekebri
Lab Technician
Rafiah Mohammed
Inorganic Chemistry
Lead Chemist
Patricia Bucknell
Chemists
Jennifer Riley
Kendall Penney
Scientific Aid
Damon Raiter
Mallory Pirie
Sample Custodian
Scot Harris
Field Collections
Environ. Sci
Glenn Sibbald
Scientific Aid
Matt Rock

California Dept of Fish and Game Office of Spill Prevention and Response Scientific Branch Laboratories Organizational Chart



APPENDIX B

QUALIFICATIONS AND SPECIFICATIONS OF KEY PERSONNEL
(Resumes Available upon Request)

SPECIFICATION FOR KEY PERSONNEL

Specifications for QC Officer

1. Respected person, have authority
2. Laboratory experience 5 - 10 years
3. Safety committee candidate

Specifications for Organic Laboratory Project Leader

1. Laboratory experience 5 - 10 years
2. Gas chromatography experience 2 - 3 years
3. Mass spectral interpretation experience 2 - 3 years
4. Communication skills
5. Computer skills
6. Dedicated to improvement

Specifications for Inorganic Laboratory Project Leader

1. Laboratory experience 3 - 5 years
2. Atomic absorption experience 2 years
3. Communication skills
4. Computer skills
5. Dedicated to improvement

Specifications for Chemists

1. Bachelors degree in chemistry or related sciences
2. Organic extraction experience 1 year
3. Inorganic digestion experience 6 mo.
4. Synthetic organic residue experience 2 years

APPENDIX C

SOP's
(Standard Operating Procedures for specific methods are available on request)

APPENDIX D

SAMPLE CONTAINERS, PRESERVATION AND HOLDING TIME

Samples Analyzed for Synthetic Organics
Polynuclear Aromatic Hydrocarbons or Petroleum Hydrocarbons

Sample Type	Sample Size	Containers	Preservation	Holding Time
Water	One gallon	Glass [1,2]	4° C, pH 5-9	7 days [3]
Animal	Whole	Al foil	-20° C	6 mo.
Vegetation	One pint	Al foil	-20° C	6 mo.
Sediment	One pint	Glass [1]	-20° C	14 days [2]

1. Previously rinsed with petroleum ether and dried, with Teflon liner in lids.

2. Sample must be extracted within the specified days and analyzed within 40 days of extraction.

3. PAHs are light sensitive, therefore, sample extracts and standards must be stored in foil wrapped containers.

Samples Analyzed for Trace Elements

Sample Type	Sample Size	Containers	Preservation	Holding Time
Water	500 ml	LPE[1]	HNO_3 to pH<2	6 mo.[2]
Animal	Whole	Plastic bag	-20° C	6 mo.
Sediment	One pint	LPE[1] [1]	-20° C	6 mo.

1. Previously soaked and rinsed with 1N HNO_3.

2. Six months except mercury and TBT which are 28 days.

APPENDIX E

FORMS

APPENDIX F

ANALYTICAL METHODS AND REFERENCE SOURCES

APPENDIX G

METHOD DETECTION LIMIT AND REPORTING LIMIT