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Northern and Central California, Nevada, and Utah
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DEPARTMENT OF THE NAVY
Ernesto Galang, Remedial Project Manager
Engineering Field Activity West
Naval Facilities Engineering Command
San Bruno, California

PHASE II ECOLOGICAL RISK ASSESSMENT
FINAL QUALITY ASSURANCE PROJECT PLAN
NAVAL STATION TREASURE ISLAND

February 3, 1997

Prepared By:

PRC ENVIRONMENTAL MANAGEMENT, INC.
135 Main Street, Suite 1800
San Francisco, CA 94105
(415) 543-4880

Sharon Tobias

Sharon Tobias, Project Manager

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QUALITY ASSURANCE PROJECT PLAN
FINAL

FOR

NAVAL STATION TREASURE ISLAND
SAN FRANCISCO, CALIFORNIA

Contract Task Order No. 0199
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Prepared for

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Prepared by: James H. Baker, Ph.D., CEP — Date 1/27/97

PRC Review and Approvals:

Project Quality Assurance Officer: Thorsten Anderson — Date 1/13/97

PRC Project Manager: Sharon Tobias — Date 1/31/97

Navy Review and Approvals:

Quality Assurance Officer: for Mary Obland — Date 1/13/97

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ABBREVIATIONS AND ACRONYMS

≥	Greater than or equal to
≤	Less than or equal to
±	Plus or minus
A2LA	American Association for Laboratory Accreditation
AA	Atomic absorption spectroscopy
ANOVA	Analysis of Variance
ASTM	American Society for Testing and Materials
AVS/SEM	Acid Volatile Sulfides/Simultaneously Extracted Metals
BOD	Biological Oxygen Demand
BTAG	Biological Technical Assistance Group
BTEX	Benzene, toluene, ethylbenzene, xylene
°C	Degrees Celsius
$CdCl_2$	Cadmium chloride
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
CLEAN	Comprehensive Long-Term Environmental Action Navy
CLP	Contract Laboratory Program
cm	Centimeter
COC	Chain-of-custody
COE	U.S. Army Corps of Engineers
CRDL	Contract required detection limits
CRQL	Contract required quantitation limits
CTO	Contract task order
CVAA	Cold vapor atomic absorption
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DERP	Defense Environmental Restoration Program
DOT	Department of Transportation
DQO	Data quality objective
DTSC	California Department of Toxic Substances Control
EA	Ecological risk assessment
EC	Electrical conductance
EC50	Effective concentration fifty
ECD	Electron capture detector
EDL	Electrodeless discharge lamp
EFA WEST	Engineering Field Activity West, Naval Facilities Engineering Command
EPA	U.S. Environmental Protection Agency
FID	Flame ionization detector

ABBREVIATIONS AND ACRONYMS (Continued)

FPD	Flame photometric detector
FSP	Field sampling plan
ft-c	Foot candles
FTL	Field team leader
g	Grams
GC	Gas chromatography
GC/MS	Gas chromatography/mass spectrometry
GFAA	Graphite furnace atomic absorption
GPC	Gel permeation chromatography
HCl	Hydrochloric acid
HDPE	High density polyethylene
Hg	Mercury
HNO_3	Nitric acid
HR/MS	High resolution/gas chromatography mass spectrometer
H_2SO_4	Sulfuric acid
HSP	Health and safety plan
HSPM	Health and safety program manager
HSV	High toxicity screening value
ICP	Inductively coupled plasma atomic emission spectroscopy
IRP	Installation Restoration Program
IR	Installation Restoration
KCl	Potassium chloride
kg	Kilogram
L	Liter
LC50	Lethal concentration fifty
LCS	Laboratory control sample
LIMS	Laboratory information management system
LOEC	Lowest observed effective concentration
LSV	Low toxicity screening value
LUFT	Leaking Underground Fuel Tank
MDL	Method detection limit
μg	Microgram
μg/kg	Microgram per kilogram
μm	Micrometers
μmhos	Micromhos
mg/L	Milligram per liter
mL	Milliliter
mm	Millimeter
MPR	Monthly progress report

ABBREVIATIONS AND ACRONYMS (Continued)

MS	Matrix spike
MSD	Matrix spike duplicate
MSD	Minimum significant difference
NaOH	Sodium hydroxide
NAVSTA TI	Naval Station Treasure Island
NEESA	Naval Energy and Environmental Support Activity (Now called NFESC)
NFESC	Naval Facilities Engineering Support Center (Formerly NEESA)
ng	Nanograms
NOAA	National Oceanic and Atmospheric Administration
NOEC	No observed effective concentrations
NSTP	National Status and Trends Program
NTU	Nephelometric turbidity unit
OC	Organochlorine (pesticides)
OSHA	Occupational Safety and Health Administration
PAH	Polynuclear aromatic hydrocarbons
PARCC	Precision, accuracy, representativeness, completeness, and comparability
PCB	Polychlorinated biphenyl
PE	Performance evaluation
PHSC	Project health and safety coordinator
PID	Photoionization detector
ppb	Parts per billion
ppt	Parts per thousand
PRC	PRC Environmental Management, Inc.
QA	Quality assurance
QAPP	Quality assurance project plan
QA/QC	Quality assurance and quality control
QC	Quality control
QCSR	Quality control summary report
RAB	Restoration advisory board
RI	Remedial investigation
RI/FS	Remedial Investigation/Feasibility Study
RPD	Relative percent difference
RPM	Remedial Project Manager
RWQCB	California Regional Water Quality Control Board
SARA	Superfund Amendments and Reauthorization Act
SDG	Sample delivery group
SIM	Selective ion monitoring or selected ion monitoring
SMEWW	Standard Methods for the Examination of Water and Wastewaters
SOD	Sediment oxygen demand

ABBREVIATIONS AND ACRONYMS (Continued)

SOP	Standard operating procedure
SVOC	Semivolatile organic compound
TAL	Target analyte list
TCL	Target compound list
TI	Treasure Island
TIC	Tentatively identified compound
TOC	Total organic carbon
TPH	Total petroleum hydrocarbons
uv	Ultraviolet
VOA	Volatile organic analysis
VOC	Volatile organic compound
WP	Work plan
YBI	Yerba Buena Island

1.0 INTRODUCTION

The Department of the Navy, Naval Facilities Engineering Command, Engineering Field Activity West (EFA WEST), is conducting an investigation to identify and evaluate hazardous waste sites at Naval Station Treasure Island (NAVSTA TI) under the Installation Restoration Program (IRP) of the Defense Environmental Restoration Program (DERP). The Navy has implemented a remedial investigation and feasibility study (RI/FS) at NAVSTA TI in accordance with the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), as amended by the Superfund Amendments and Reauthorization Act (SARA). PRC Environmental Management, Inc. (PRC) is providing technical support for RI/FS activities, including an assessment of ecological risks. The ecological risk assessment (EA) is being conducted under Contract Task Order (CTO) No. 0199, Modification 10, of the Comprehensive Long-Term Environmental Action Navy (CLEAN) Contract No. N62474-88-D-5086. PRC will implement the phase II EA under Modification 10 to CTO 0199.

The phase I EA at NAVSTA TI was conducted in 1993. The objective of phase I was the development of a preliminary characterization of the facility based on existing data, biotic surveys, and fate and transport analysis. The phase I EA report (PRC 1993) provides the preliminary problem formulation, exposure assessment, and ecological effects assessment. The phase II EA will be conducted based on the conclusions reached during phase I. The phase II EA will consist of sampling and analysis to develop a terrestrial risk characterization and aquatic risk characterization for NAVSTA TI upon which remediation decisions can be made. This approach is designed to initiate cleanup at sites as quickly as possible when chemical analyses indicate the sampling location fails to meet the screening criteria.

On April 24, 1995, EFA WEST presented to the Biological Technical Assistance Group (BTAG) a screening level approach to address ecological risk assessment of offshore sediments at Naval facilities in San Francisco Bay. The approach is designed to bring sites to cleanup as quickly as possible when chemical analyses indicate that the sampling site fails to meet screening criteria. The purpose is to focus monies on cleanup and subsequent monitoring instead of further study. EFA WEST has decided to implement the approach at NAVSTA TI and during the phase II activities covered by this QAPP.

The phase II EA work plan (WP) and the field sampling plan (FSP) and the quality assurance project plan (QAPP) are companion documents supporting the ecological assessment activities at NAVSTA TI.

The phase II ERA WP summarizes the existing data and presents the conceptual approach for the ecological assessment. The FSP describes the sampling design and methods, analyses to be conducted, and the interpretation of chemical and biological data. This QAPP discusses field protocols for sample collection and handling, equipment decontamination, field documentation, and sample chain of custody. Protocols for sample receipt, handling, tracking, and storage, as well as analytical methods and quality assurance (QA) procedures for conventional variables and organic and inorganic contaminants, are also described and are inclusive of all procedures anticipated for the project at this time.

The field measurement and laboratory protocols prescribed for this project are based primarily on methods approved by the U.S. Environmental Protection Agency (EPA). EPA Contract Laboratory Program (CLP) procedures will be used for analysis and reporting of volatile organic compounds (VOC), polynuclear aromatic hydrocarbons (PAH) (by a modified CLP method), organochlorine (OC) pesticides/polychlorinated biphenyls (PCB), and metals in sediment and pore water samples (EPA 1993a, b). EPA methods will also be used for analysis of additional organic and inorganic contaminants in sediment and pore water samples. Specific parameters are discussed in Section 4.0, Sampling Procedures. Analysis of tissue residue will follow CLP and EPA-approved methods for metals and the National Oceanic and Atmospheric Administration (NOAA) National Status and Trends Program (NSTP) guidelines for organics analysis. EPA guidelines will be followed for preparation of invertebrate and fish tissue for analysis. Pore water extractions will follow the technique presented in Appendix F.

Water samples were collected in Clipper Cove to comply with the California Regional Water Quality Control Board (RWQCB) Order 93-130. Only lead was analyzed following CLP and EPA-approved methods.

Bioassay protocols for the sediment and pore water toxicity tests will follow EPA and American Society for Testing and Materials (ASTM) methods; any differences or inconsistencies between the EPA and ASTM protocols are identified in the standard operating procedures in Appendices E, G, J, I, and K to provide clear instruction to the testing laboratories.

Because the phase II EA at NAVSTA TI follows the guidelines of the IRP, CERCLA, and SARA, preparation of a QAPP is required as part of the phase II EA planning process. The QAPP describes

the policy, organization, functional activities, and quality assurance and quality control (QA/QC) activities to be implemented during the phase II EA. In an effort to avoid duplication among the project documents, references are made herein to sections of the associated project plans where supplemental or detailed information can be found.

2.0 PROJECT MANAGEMENT

A project team will help to ensure consistent quality throughout the phase II EA. This section presents the key members and describes their responsibilities within the project team organization.

2.1 PROJECT AND TASK ORGANIZATION

This section describes the project and task organization for the phase II EA.

2.1.1 Project Organization

The project team for the phase II EA consists of the following:

- Navy Remedial Project Manager (RPM): Ernesto Galang
- PRC Navy CLEAN Program Manager: Daniel Chow
- PRC Project Manager: Sharon Tobias
- PRC Technical Reviewers: Mary Gleason, Ph.D., and John Collins, Ph.D.
- PRC QA Program Manager: Ken Partymiller
- PRC Project QA Officer: Thorsten Anderson
- PRC Health and Safety Program Manager: Conrad Sherman
- PRC Project Health and Safety Coordinator: To be determined
- PRC On-Site Safety Officer: To be determined
- PRC Ecological Task Manager: James Baker

- PRC Analytical Coordinator: Thorsten Anderson
- PRC Field Team Leader (FTL): Kris Gade

Figure 1 presents the organizational chart for this project.

2.1.2 Project Responsibilities

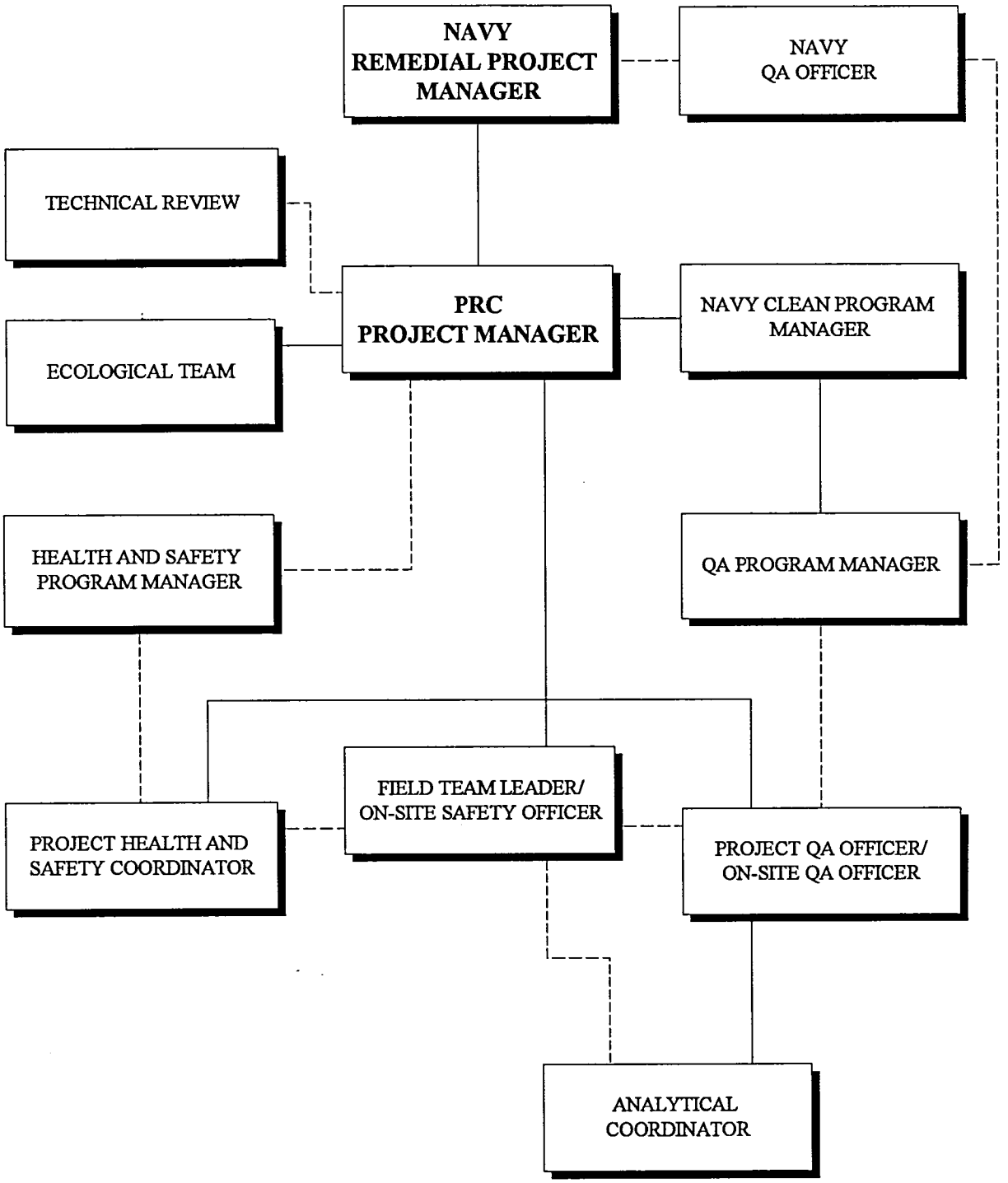
The specific responsibilities of the team members listed in Section 2.1 are described in this section.

Navy Remedial Project Manager: The Navy RPM is responsible for the following:

- Providing site information and history
- Providing logistical assistance
- Specifying sites requiring investigation
- Reviewing all results and recommendations and providing management and technical oversight
- Ensuring proper review and distribution of all documents
- Communicating comments from technical reviewers to contractors
- Ensuring that contractors address all comments and take appropriate corrective actions
- Coordinating with regulatory agencies

PRC Navy CLEAN Program Manager: The program manager is responsible for the following activities:

- Ensuring that all contract requirements are met
- Providing necessary resources to the project team to ensure adequate responses to all requirements of the investigation
- Maintaining consistency in procedures and work products with all other task orders



LEGEND

——— Lines of Authority

- - - - - Lines of Communication

Naval Station Treasure Island
Project Organization Chart
Figure 1

- Establishing and maintaining communication between the Navy RPM, QA program manager, health and safety program manager, and project manager
- Providing technical oversight, as necessary
- Providing guidance to the project manager as needed

PRC Project Manager: The project manager is ultimately responsible for the timely completion of the project. The responsibilities of the project manager include the following:

- Assigning technical staff
- Ensuring the completion of all requirements by team members
- Supervising the document control process
- Approving all deliverables and associated documents prior to transmittal
- Establishing and maintaining communication among technical staff, program managers, QA officer, health and safety coordinator, and regulatory agencies
- Implementing all programs and protocols related to the project
- Coordinating with the Navy RPM

PRC Technical Reviewers: The technical reviewers will provide assistance in the detailed review of deliverables for technical content and accuracy. Editorial, technical, and quality control evaluations will be used to ensure document clarity and completeness. The reviewers will assist the project managers in the approval process for selected deliverables.

PRC QA Program Manager: The QA program manager is responsible for ensuring that the phase II EA at NAVSTA TI has appropriate overall QA. The QA program manager reviews laboratory QA plans and audits field and laboratory reports. Other responsibilities include the following:

- Meeting regularly with the program manager, project manager, and project QA officer
- Developing and revising the QA program, as required
- Supervising the QA responsibilities of the project QA officer

- Identifying nonconformance situations to management, as required
- Providing guidance in the correction of nonconformances
- Ensuring that all deliverables meet the requirements of the CLEAN QA/QC program
- Making recommendations to the program manager, project manager or assistant project manager, and project QA officer regarding QA/QC topics and corrective action
- Conducting field and laboratory audits to ensure that sampling and analysis activities are performed in accordance with the QAPP

PRC Project QA Officer: Responsibilities of the project QA officer include, at a minimum, the following:

- Ensuring that all protocols described in the QAPP are met
- Providing guidance or assistance and resolving problems on QA/QC topics
- Verifying that the specified data collection methods comply with all QA/QC requirements and will yield data of desired quality and integrity
- Reviewing and evaluating quality-related changes to the FSP and QAPP
- Ensuring that all nonconformances are identified and appropriate corrective actions are taken. Providing assistance to the project manager with regard to corrective action and, if necessary, soliciting involvement by the program manager
- Communicating regularly with the project manager, QA program manager, and analytical coordinator to ensure the progress of the QA tasks for the phase II EA
- Acting as the main contact for all project QA matters, and providing guidance on appropriate procedures to the project manager and support personnel

PRC Health and Safety Program Manager: The CLEAN health and safety program manager is responsible for the following:

- Reviewing site-specific health and safety plans (HSP)
- Ensuring that each HSP meets the requirements of the CLEAN Health and Safety Program

- Providing assistance and guidance to the project health and safety coordinator, as needed
- Maintaining communication with the program manager, project manager, and project health and safety coordinator
- Performing responsibilities specified in the NAVSTA TI site-specific HSP

PRC Project Health and Safety Coordinator: The responsibilities of the project health and safety coordinator include the following:

- Preparing site-specific HSPs
- Ensuring that the HSP complies with all federal, state, and local health and safety requirements
- Establishing and maintaining communication between the on-site safety officer, project manager, and health and safety program manager
- Verifying that site personnel adhere to the site safety requirements
- Providing guidance to the project manager and support personnel on appropriate corrective action procedures
- Performing responsibilities specified in the site-specific HSP
- Conducting field audits to ensure field compliance with the HSP

PRC On-Site Safety Officer: The on-site safety officer is responsible for field implementation of the HSP and has the authority to correct and change site control measures and the required health and safety protection. The on-site safety officer has primary on-site enforcement authority for the policies and provisions of the CLEAN Health and Safety Program and HSP. Additional responsibilities are included in the site-specific HSP.

PRC Ecological Task Manager: The ecological task manager is responsible for the design and completion of the EA. The responsibilities of the ecological task manager include the following:

- Assigning technical staff to EA tasks

- Designing an approach for information gathering which will complement the information obtained for the remedial investigation (RI)
- Implementing all project programs and protocols related to the phase II EA
- Providing updates to the project manager as necessary
- Preparing an EA report

PRC Analytical Coordinator: The responsibilities of the analytical coordinator include the following:

- Ensuring that the laboratory implements the requirements of the QAPP and FSP
- Coordinating with the contract laboratory on QA/QC matters
- Reviewing laboratory data prior to release
- Coordinating data validation activities
- Providing updates to the project manager with regard to QA/QC data

PRC Field Team Leader: The FTL is responsible for the field program. The FTL will direct all on-site activities, including those of subcontractors, and will ensure that all procedures described in the FSP are followed in the field. The FTL will be responsible for ensuring that field equipment is properly calibrated and maintained and that individual samples are properly handled and documented to allow for tracing the possession and handling of samples from collection to laboratory receipt.

2.2 PROBLEM DEFINITION AND BACKGROUND

This section provides information concerning the project purpose and scope and background history concerning NAVSTA TI.

2.2.1 Project Purpose and Scope

The phase I EA at NAVSTA TI was conducted in 1993. The objective of phase I was to develop a preliminary characterization of the facility based on existing data, biotic surveys, and fate and transport analysis. The phase I EA report (PRC 1993) provides the preliminary problem formulation, exposure

assessment, and ecological effects assessment. The phase II EA will be conducted based on the conclusions reached during phase I. The phase II EA will consist of sampling and analysis to develop a terrestrial risk characterization and aquatic risk characterization for NAVSTA TI upon which remediation decisions can be made. This approach is designed to initiate cleanup at sites as quickly as possible when chemical analysis indicate the sampling location fails to meet the screening criteria.

2.2.2 NAVSTA TI Background

Military activities at NAVSTA TI date back to 1866 when the U.S. government took possession of Yerba Buena Island (YBI) for defensive fortifications; Treasure Island (TI) had not yet been constructed. YBI was occupied by the U.S. Army until 1896 when the Navy assumed control. TI was built in 1936 and 1937 on the Yerba Buena shoals, a sand spit extending from the northwest point of YBI. It was used initially for the Golden Gate International Exposition in 1939. TI was leased to the Navy in 1941, which has since operated the facility; troop and family housing; personnel support; Naval Readiness Command, Region Twenty; a Navy brig; and a Navy and Marine Corps Museum.

Today, NAVSTA TI is an active, fully operational Naval facility headed by a commanding officer. However, the base has been designated for closure and will be turned over to other government or civilian groups.

2.3 PROJECT AND TASK DESCRIPTION

The phase I EA report (PRC 1993) presented an initial, qualitative analysis of ecological risk using existing data on chemical contamination in environmental media at NAVSTA TI. The objectives of the analysis were to (1) evaluate analytical chemical data associated with the various media, (2) characterize terrestrial and aquatic receptors, (3) identify chemicals that may cause adverse effects, and (4) make a preliminary evaluation of the adverse effects associated with potential receptors.

The objectives of the phase II EA are to (1) further develop the problem formulation phase, exposure assessment, and ecological effects assessment for the aquatic and terrestrial sites, (2) further characterize offshore contamination by using quantitative measurements to determine ecological effects

in the aquatic environment, and (3) develop a detailed terrestrial risk characterization and aquatic risk characterization for NAVSTA TI upon which remediation decisions can be made.

2.4 PROJECT NARRATIVE

The primary objective of the phase II EA is to collect samples of sediment, pore water, and tissue that will be used to further define the extent of contamination in the offshore area of NAVSTA TI. The approach used to meet this objective is designed to initiate cleanup at sites as quickly as possible when chemical analyses indicate that the respective sampling location fails to meet certain screening criteria. The purpose is to focus the available funds toward cleanup and subsequent monitoring instead of further study.

The first step of the approach is a chemical screening assessment to identify sampling locations that (1) clearly require remedial action and can be moved directly into a feasibility study or (2) clearly do not require any further action. The whole sediment total chemistry values will be compared to the San Francisco Bay specific ambient values and the effect range values of Long and others (1995) as described in Section 7.1 of the EA WP. The contaminant results from the pore water analysis will be compared to the federal ambient water quality criteria (AWQC) (EPA 1994e) or the Great Lakes water quality initiative tier II values (EPA 1996) for chemicals without AWQC vaules.

The second step in this approach is to conduct additional study at sampling locations where the risk to ecological receptors cannot be clearly determined in the first step. For NAVSTA TI, bioassays will be performed and used in conjunction with the chemical analysis and other measures of bioavailability (sediment pore water analysis and acid volatile sulfide/simultaneously extracted metals [AVS/SEM] analysis) to determine risk at these sampling locations. If, after the first two steps are completed, some sampling locations still require additional risk characterization, a cost-benefit analysis will be performed to assess the benefits of further investigation.

Several issues in the EA approach remain undefined. The Navy is studying these issues which are as follows:

- Performance of a cost-benefit analysis to conduct further investigation rather than proceeding directly to a feasibility study
- Validation of the no further action alternative through planned monitoring over 5 years
- A review of possible remediation methods, impacts of remediation, and cost-benefit analysis as part of the feasibility study
- Definition of the criteria for determining how many times the concentrations of specific contaminants must exceed the screening values to initiate further evaluation

Upon approval by the regulators, these issues will be automatically adopted for use in this phase II EA at NAVSTA TI.

Sampling locations were chosen from areas of potential contamination from activities at NAVSTA TI, including (1) storm water outfall discharge zones and (2) areas offshore from the IR sites. The sampling locations have been grouped into seven sections based on chemicals detected as well as potential onshore sources. Samples will be collected along transects, and the farthest offshore sampling location will be about 1,500 feet offshore of NAVSTA TI. The grouping of sampling locations will facilitate statistical comparisons of near-shore samples to samples collected farther offshore, and will result in a determination of the nature and extent of offshore contamination. Additional details of the sampling design are discussed in Sections 5.0 and 6.0 of the EA WP.

Sample collection procedures are described in Section 11.0 of the EA WP. Sample handling and custody requirements are discussed in Section 5.0 of this QAPP. Special detection limits are required so that sample data can be compared to the screening criteria described above. The analytical methods were specifically selected to meet these detection limits and are discussed in section 8.0 of this QAPP. The calibration and maintenance of instrumentation used during sample collection are discussed in Sections 6.0 and 7.0 of this QAPP. The instrumentation and calibration requirements for the analytical methods are listed in Sections 6.0 and 8.0.

All project plans prepared for the phase II EA sampling were prepared as draft, draft final, and final documents. At each stage of document preparation, the project plans underwent significant peer review including three separate internal PRC QC reviews, reviews by regulatory agencies, and review by the community representatives on the Restoration Advisory Board (RAB) for NAVSTA TI.

2.5 SPECIAL TRAINING REQUIREMENTS AND CERTIFICATION

Personnel who work at a hazardous waste project site facility are required to meet the Occupational Safety and Health Administration (OSHA) training requirements of 29 Code of Federal Regulations (CFR) 1910.120(e), "Training," as described in the following sections. Since the ecological samples will be collected from a boat using sediment sampling equipment, PRC personnel and subcontractors should be familiar with the use and handling of such equipment. The following sections describe the training requirements for PRC personnel and subcontractors.

2.5.1 Personnel Health and Safety Training

All PRC personnel working on hazardous waste project sites, who are responsible for the project or site activities, are required to undergo specific training prior to participating in, managing, or supervising field activities. This training will thoroughly cover the following:

- Names of personnel and alternates responsible for health and safety at a hazardous waste project site
- Health, safety, and hazards present onsite
- Selection of the appropriate personal levels of protection
- Use of personal protective equipment (PPE)
- Work practices to minimize risks from hazards
- Safe use of engineering controls and equipment onsite
- Medical surveillance requirements, including recognition of symptoms and signs that might indicate over exposure to hazardous substances
- Contents of the site-specific HSP which will be developed before offshore sampling begins, which is not expected to begin until March 1997

Initially, all PRC personnel engaged in hazardous waste site activities that expose or could expose workers to hazardous substances and health hazards will receive a minimum of 40 hours of formal instruction offsite and a minimum of 3 days of actual field experience onsite under the supervision of a trained, experienced field supervisor.

All field personnel directly responsible for, or who supervise employees engaged in, hazardous waste operations also will also receive the 40 hours of initial training, 3 days of supervised onsite field experience under a trained supervisor, and at least 8 additional hours of specialized supervisor training. The specialized training will include the requirements of the CLEAN II Health and Safety Program, training requirements, PPE and personal level of protection programs, spill containment program, and health hazard monitoring procedures and techniques. PRC's project health and safety coordinator (PHSC) also will receive the additional 8 hours of supervisor training. PHSCs will receive additional health and safety training, including training in operating monitoring instrumentation.

Written certificates will be given to all employees who successfully complete this training. A letter documenting 24 hours (3 days) of supervised onsite experience will be provided. All PRC employees engaged in work at hazardous waste sites also are required to undergo 8 hours of annual refresher training to maintain certification.

The PRC FTL, who is the onsite manager with authority delegated by the project manager to direct field operations, will be fully trained in hazardous waste field operations and will ensure that all necessary preparation and coordination is complete prior to initiating onsite work. This preparation generally consists of drafting project documents such as the WP, sampling plan, QAPP, and requests for bids, and assisting in the preparation of the site-specific HSP under the guidance of the project manager. In some instances, the FTL trains for this position by working onsite as a team member before replacing the original FTL.

At least one member of every PRC field team will maintain current certification in the American Red Cross "Multimedia First Aid" and "Cardiopulmonary Resuscitation Modular," or equivalent. The health and safety program manager (HSPM) ensures that appropriate field personnel maintain their current certification in both first aid and CPR.

Copies of PRC's health and safety training records, including course completion certifications for the initial hazardous waste site health and safety training, first aid, CPR, and refresher training will be maintained in project files. The HSPM implements the training requirements by notifying employees when they are due for recertification, disseminating information about appropriate courses, conducting or assisting in refresher training, and performing other such tasks.

2.5.2 Subcontractor Training

Subcontractors who work onsite will certify that their own employees have been trained for work on hazardous waste project sites. The training will meet the OSHA training requirements of 29 CFR 1910.120(e), "Training." Before beginning work at the project site, the subcontractors will submit to the HSPM, certification of such training for each employee who will perform field work. The subcontractors also will ensure that these employees attend a pre-entry safety briefing.

The pre-entry safety briefing is designed to inform subcontractor employees of the potential risks of working with hazardous materials, site-specific hazards, required level of personal protection, and the use of PPE. This safety briefing is conducted by the PHSC or other qualified person designated by the HSPM. All employees of associate and professional services firms and technical services subcontractors will attend a safety briefing and complete the "Safety Meeting Sign-Off Sheet" prior to performing onsite work. Construction services subcontractors are responsible for conducting their own safety briefings. PRC personnel may audit these briefings.

3.0 QUALITY OBJECTIVES AND CRITERIA FOR MEASUREMENT DATA

The data quality objectives, the data uses, and the data quality indicators are discussed in the following sections in relation to the goals of the investigation and the sampling design.

3.1 DATA QUALITY OBJECTIVES

The data quality objective (DQO) process outlined by the EPA (1994f) has been addressed in planning the sampling effort for the offshore sediments at NAVSTA TI. The EPA guidance outlines a seven-step process for setting the objectives in remedial investigations. The seven steps are as follows:

- State the problem
- Identify the decision
- Identify the inputs to the decision
- Define the study boundaries
- Develop the decision rule

- Specify limits on decision errors
- Optimize the design for obtaining data

The following subsections discuss the seven-step process for achieving the data quality objectives.

3.1.1 State the Problem

The overall objective of the phase II EA sampling activities is to characterize the nature and extent of contamination present in the offshore sediments at NAVSTA TI and to perform an ecological risk assessment using the data collected. The characterization will be completed by collecting and analyzing pore water, sediment, and tissue samples for different parameters and by performing a separate series of bioassays for sediment and sediment pore water from each location. This characterization will provide the information necessary to (1) develop and evaluate effective remedial alternatives; (2) conduct a risk assessment to characterize the impact of NAVSTA TI contamination on the environment; and (3) support feasibility study activities. The sampling objectives will be met by designing and implementing the sampling program and by collecting a sufficient number of samples from appropriate locations. The rationale for the selection of sampling locations, the number of samples to be collected, and the methods for collecting samples are explained in the phase II EA WP (PRC 1996).

Naval operations at NAVSTA TI have caused the release of various organic and inorganic contaminants into the groundwater and into the storm water discharge via surface soil contamination. Groundwater contaminants consist of volatile and semivolatile organics, TPH, and metals. Storm water discharges were found to contain some semivolatiles, pesticides, TPH, and some metals. Section 8.0 of this QAPP describes the analytical procedures that will be used to detect contaminants present.

These contaminants enter into the offshore environment where they settle and collect in the sediment. They are then available for uptake by the benthos, such as polychaetes, crustaceans, and mollusks, via ingestion or dermal exposure. Avian receptors, such as shorebirds, which feed on the benthos in the intertidal and tidal areas around NAVSTA TI, are then exposed to these contaminants via ingestion of

contaminated prey. Section 6.0 of the EA final WP discuses the assessment endpoint or the receptors in more detail.

3.1.2 Identification of the Decisions

The main decision in this project is to determine the toxicity of the offshore sediments at NAVSTA TI. The ecological risk assessors will use the data collected to evaluate the level of risk at the sampled locations. The data for offshore and near shore sediments will also be statistically compared to determine if NAVSTA TI is the source of any contaminant determined to pose a risk. In addition, the data can be used to develop the feasibility studies and remediation alternatives. To make these decisions, the Navy must collect data to:

- Determine if NAVSTA TI is the source of contaminants
- Determine if these contaminants pose adverse effects to aquatic organisms
- Evaluate whether the risk posed is sufficiently significant to warrant removal of sediments

3.1.3 Inputs to the Decision

The sampling stations have been chosen to track contamination from onshore sources to offshore areas around storm water outfall discharge areas and areas offshore from the IR sites. Sampling locations have been grouped into seven sections based on chemicals detected as well as potential onshore sources. Samples will be collected along transects, and the farthest offshore sampling location will be about 1,500 feet offshore of NAVSTA TI. The sampling locations in the Clipper Cove area were chosen to provide aerial coverage and not to evaluate a concentration gradient. Near-shore samples will be statistically compared to samples collected further offshore to determine a contaminant concentration gradient. Section 5.0 of the EA WP discusses sampling design in more detail. Sampling is expected to begin in February 1997 and should be completed in 10 to 12 weeks.

The data needed to answer the questions posed in Section 3.1.2 are (1) results of bulk sediment and pore water chemistry analyses for all nearshore and offshore samples collected, and (2) results of whole sediment and pore water bioassays performed on half of the total number of samples collected.

The analytical chemistry results will provide information on the nature of the concentration gradients in the offshore sediments and provide a basis for comparison to ecologically based screening criteria. The bioassay results will provide toxicity information to determine the effects of the any contaminants detected and which exceed the screening criteria.

3.1.4 Definition of Study Boundary

Study boundaries were defined to incorporate: (1) areas identified for further study by the phase I EA, (2) locations of stormwater outfalls, and (3) sediments beyond the suspected maximum extent of impact from onshore sources (up to 1,500 feet from shore). Sampling stations selected focus on areas of potential contamination from activities at NAVSTA TI. Proposed stations are located near storm water outfall discharge zones and areas offshore from the IR sites. The sampling locations have been grouped into seven sections based on chemicals detected during the phase I EA as well as potential onshore sources. The sampling sections are identified in Section 5.1 of the phase II EA WP (PRC 1996). The grouping will facilitate statistical comparisons of near-shore samples to samples collected farther offshore, as well as statistical comparisons of one section to another. These comparisons will be used to evaluate the nature and extent of offshore contamination.

3.1.5 Decision Rule

Data to be collected includes total contaminant concentrations for both the sediments and extracted sediment pore water. The total contaminant concentrations in the sediment will be compared to the San Francisco Bay ambient values (RWQCB 1995) and the effect range values of Long and others (1995). The total contaminant concentrations in the pore water will be compared to the federal AWQC (EPA 1994e) or the Great Lakes water quality initiative tier II values (EPA 1996). This preliminary screening process will separate the sampling locations into "no further action," "feasibility studies," or "further investigation." Section 7.0 of the EA final WP describes this process in more detail. Sampling locations requiring further investigation will be evaluated for toxicity through the use of bioassays and other measures of bioavailability (sediment pore water and AVS/SEM analysis). A cost-benefit analysis will be performed throughout the process to determine cost-effective appropriation of monies. Section 7.1.3.1 of the EA final WP describes how the cost-benefit analysis will be conducted.

There are two independent decisions that need to be made with the data collected during this investigation. The first decision determines if NAVSTA TI is the potential source of contaminants. The second decision determines if the concentrations detected in the sediments causes adverse effects to aquatic organisms. The hypotheses are as follows:

- Null hypothesis (H_o) = The offshore sediments pose a risk and NAVSTA TI is the potential source of the contaminants posing the risk.

- Alternative hypothesis 1 (H_1) = The offshore sediments do not pose a risk because of low toxicity, volume, or potential exposure, but NAVSTA TI is the potential source of the nontoxic contaminants.

- Alternative hypothesis 2 (H_2) = The offshore sediments do not pose a risk because of low toxicity, volume, or potential exposure, and NAVSTA TI is not the source of the nontoxic contaminants.

- Alternative hypothesis 3 (H_3) = The offshore sediments pose a risk, but NAVSTA TI is not the source of the contaminants posing the risk.

Once a choice is made between the competing hypotheses, a risk mangement decision will be made regarding the need for action. There are multiple sources of error in making a decision between the alternate hypotheses; however, the two primary errors which would affect this decision and ultimately affect whether or not action is taken are:

- Decision error type a (false positive or Type I error): No action is taken because the offshore sediments are assessed not to pose a risk (when in fact they do) or that NAVSTA TI is not the potential source of contaminants (when in fact it is). If the sediments pose an unacceptable risk and NAVSTA TI is the primary source and no action is taken, some resources may be saved, but environmental risk would increase. Future damage to the environment may occur. This decision error is more severe.

- Decision error type b (false negative or Type II error): Action is taken because the offshore sediments are assessed to pose a risk and NAVSTA TI is identified as the potential source (when in fact the sediments do not pose a risk or NAVSTA TI is not the source). Costs from further study and potential actions would increase, which wastes resources and time. Offsetting this, to some degree, would be the marginal reduction in risk to the environment if an action is taken

3.1.6 Limits on Decision Errors

The intent is to minimize the probability of making either false positive or false negative types of errors. There is no guidance on how to assign the error rate for this type of project, since many different criteria will be used to determine toxicity at a sampling location. The minimum acceptable error rate is 5 percent, and the maximum is 10 percent. Measurement objectives for precision, accuracy, representativeness, completeness, and comparability are discussed in Sections 3.4.1 through 3.4.5.

Section 2.4 of this QAPP lists issues that the Navy is still considering for inclusion at a later date.

3.1.7 Design Optimization

During several scoping meetings, the Navy met with representatives of the regulatory agencies to discuss and refine sampling methods for the offshore area at NAVSTA TI. In addition, the comment and review process for this document is intended to optimize the project plan.

3.2 DATA TYPES AND USES

Sediment and sediment pore water will be collected from various locations and analyzed for multiple parameters as discussed in Section 8.0 of this QAPP. Anticipated uses of these data include the following:

- Characterization of the nature and extent of contamination
- Confirmation of the presence of suspected contaminants or concentrations of contaminants in off-shore sediments
- Design of an additional sampling scheme
- Comparison of investigation data with established criteria
- Assessment of exposure to, endangerment from, and risk from the presence of identified contaminants
- Screening or selection of remedial alternatives

- Input to the design of remedial technologies for contaminated sediments
- Reporting to regulatory agencies or in litigation

3.3 DATA QUALITY NEEDS

This section discusses the site contaminants, appropriate analytical levels and detection limits, and data quality indicators.

3.3.1 Identification of Site Contaminants

During the phase I EA activities (PRC 1993) both storm water and sediments, which were collected adjacent to the storm water outfalls, were sampled and analyzed. A range of organics and inorganics were identified. Since offshore sediments were not evaluated during phase I, phase II will address the contamination in offshore sediments, which will be analyzed for contaminants that were identified in the storm water and adjacent sediments during phase I (PRC 1993).

3.3.2 Appropriate Analytical Level

At NAVSTA TI, data quality needs primarily consist of definitive data subjected to formal QC checks to support RI, remedial action, and risk assessment activities. All analytical data collected in support of activities at NAVSTA TI will be generated in accordance with NEESA Level D QC protocols (equivalent to EPA definitive data). Screening data are not expected to be used for the Phase II EA at NAVSTA TI, and are not discussed here.

Definitive data, as defined by the EPA Data Quality Objectives Process for Superfund are data generated at the site or off-site in analytical laboratories using rigorous analytical methods, such as approved EPA reference methods (EPA 1993). Samples selected for definitive data will be analyzed by Navy- and California-certified laboratories, following procedures specified in the PRC statement of work (SOW) for analytical services (PRC 1995). Data are analyte-specific, with confirmation of analyte identity and concentration, and analytical error or total error determined. Methods produce

tangible raw data in the form of paper printouts or computer-generated electronic files. QA/QC elements required for definitive data include the following:

- Sample documentation (such as location, date and time collected and batch)
- Chain of custody (when appropriate)
- Sampling design approach (for example, systematic, simple or stratified random, or judgmental)
- Initial and continuing calibration
- Determination and documentation of detection limits
- Analyte identification
- Analyte qualification
- QC blanks (trip, method, rinsate)
- Matrix spike (MS) recoveries
- Performance evaluation (PE) samples (when specified)
- Matrix duplicate. For two or more aliquots, this is also called the analytical error determination (measures precision of analytical method): An appropriate number of replicate aliquots, as specified in Section 9.3.2, is taken from at least one thoroughly homogenized sample. The replicate aliquots are then analyzed, and standard laboratory QC parameters are calculated and compared to method-specific performance requirements defined in Appendix A of the QAPP
- Field duplicates or total measurement error determination (measures overall precision of measurement system, from sample acquisition through analysis): An appropriate number of colocated samples are independently collected from the same location and analyzed following standard operating procedures. The variance, mean, coefficient of variation, or relative percent difference are calculated for each matrix under investigation.

The QC samples are collected in addition to the field samples and are used in conjunction with laboratory QC samples to evaluate the quality of the data produced from the field sampling program. The QC samples serve to meet the DQOs by meeting EPA CLP and the laboratory's established acceptance criteria. QC samples that do not meet the criteria may indicate unacceptable data,

resulting in the laboratory implementing corrective action procedures or in the data being qualified. Sections 3.4.1 through 3.4.5 describe these criteria.

3.3.3 Levels of Concern and Analytical Detection Limits

The detection limit is the minimum concentration of an analyte that can be distinguished from the normal random electronic "noise" of an analytical instrument. The quantitation limit is the lowest concentration at which an analyte can be accurately and reproducible quantified. Quantitation limits will vary depending on instrument sensitivity and sample matrix effects. Usually, quantitation limits are equal to the instrument detection limits multiplied by a factor of 3 to 5. Section 8.0 lists the proposed detection limits and quantitation limits for sediment, water, pore water, and tissue.

3.4 DATA QUALITY INDICATORS

This section discusses precision, accuracy, representativeness, completeness, and comparability (PARCC).

3.4.1 Precision

Precision refers to the reproducibility of measurements of the same characteristic, usually under a given set of conditions. For duplicate measurements, precision is expressed as the relative percent difference (RPD) of the pair and is calculated using the following equation:

$$RPD = \frac{|D_1 - D_2|}{1/2\ (D_1 + D_2)} \times 100$$

where

D_1 = concentration of analyte in the field sample

D_2 = concentration of analyte in the duplicate sample

The precision of field measurements will not be evaluated as part of this project. Sediment and tissue samples are not routinely analyzed with field duplicates in CERCLA investigations at NAVSTA TI.

The precision of chemical analyses will be assessed through the analysis of matrix spike/matrix spike duplicate (MS/MSD) samples, and matrix duplicate samples. Each QC sample type will provide unique information regarding the precision of the sample collection and laboratory analytical programs, as described below:

- Field duplicate samples: Sampling and intralaboratory analytical precision
- MS/MSD samples: Intralaboratory analytical precision for organic analyses
- Matrix duplicate samples: Intralaboratory analytical precision for inorganic/physical parameters

General precision control limits for duplicate and MS/MSD samples are presented in Appendix A. When analytes are present in samples either near the method detection limit or substantially above the detection limit, these objectives may not be appropriate. Due to the process by which the RPD is calculated, concentrations near the detection limit or substantially above the detection limit skew the RPD values. If precision objectives are not met, an anomaly will be noted, and other QC data will be evaluated to determine the validity of the data.

3.4.2 Accuracy

Accuracy refers to the degree that a measurement agrees with the true value. The accuracy of a measurement system is impacted by errors introduced through the sampling process, field contamination, sample preservation, sample handling, sample matrix, sample preparation, and analytical techniques. Sampling accuracy will be evaluated based on the results of the analysis of field blank samples. Analytical accuracy will be evaluated on the basis of MS samples, laboratory control samples (LCS) or blank spike samples, reference standards such as internal and surrogate standards, and method blank samples. MS samples and LCS or blank spike samples are analyzed at a frequency of one for every sample delivery group (SDG), or every 20 samples of a similar matrix, whichever is more frequent. Surrogate standards and internal calibration standards, where available, are added to every sample analyzed for organic constituents.

Accuracy is expressed in terms of percent recovery which is calculated by the following equation:

$$\% \text{ Recovery} = \frac{\text{Measured Spike Value} - \text{Unspiked Value}}{\text{Known Spike Value}} \times 100$$

The results of blank samples will provide information on positive bias resulting from field or laboratory artifacts. The results of spiked samples and reference standards are expressed as percent recovery and provide information on positive and negative bias. Objectives for reference standards will be based on the type analyzed. Appropriate spike and reference standard compounds and concentration levels are specified in the analytical methods. When MS compounds are not specified, they will be selected such that the group of analytes is fairly represented (chemical characteristics, retention times, and other appropriate criteria). If a surrogate is desired but not specified by the analytical methods, a non-target analyte that is chemically similar to target analytes may be used. In cases where the spiking levels for MS and/or surrogate standards are not provided, the spiking will be conducted at a mid-calibration concentration level. Accuracy objectives for MS samples and surrogate compounds, expressed in percent recovery, are presented in Appendix A.

3.4.3 Representativeness

Representativeness is a qualitative expression of the degree to which sample data accurately represent the characteristics of a population, parameter variations at a sampling point, or an environmental condition they are intended to represent. Representativeness is maximized by selecting the appropriate number of samples and sampling locations and using appropriate and established sample collection, handling, and analysis techniques to provide information that reflects "true" site conditions.

3.4.4 Completeness

Completeness is defined as the percentage of measurements that are judged valid compared to the number of samples needed for the project. The project completeness value will be determined at the conclusion of the data validation phase and will be calculated by dividing the number of complete, valid sample results by the total number of sample analyses listed in the WP. As described in Section 10.2, the data validation process will determine whether a particular data point is a valid result that is acceptable for use, an estimated result that is acceptable for use, or a rejected result that is unacceptable for use. Complete results are defined as results that are considered acceptable and usable when compared to QC

criteria such as sample holding times and acceptable surrogate recoveries. Sample results that are considered rejected, unacceptable, and unusable when compared to QC criteria are listed as incomplete and will not be used. The completeness objective for field samples is 90 percent for this project.

3.4.5 Comparability

Comparability is a qualitative parameter that expresses the confidence that one data set may be compared to another. This goal is achieved through the use of standardized techniques to collect and analyze samples and appropriate units to report analytical results. These techniques are described in the WP and this QAPP. Generally, analytical techniques that will be used for this ecological assessment are comparable to techniques used for previous investigations at NAVSTA TI.

QA objectives setting requirements for precision, accuracy, and completeness have been established for each measurement variable where possible. The precision and accuracy requirements are listed in Appendix A and the completeness requirements are stated in Section 3.4.4.

4.0 SAMPLING PROCEDURES

The quality of data collected in an environmental study depends largely on the quality of sampling activities. Field operations must be well conceived and carefully implemented. Detailed procedures and protocols for sample collection, handling, preservation, shipping, and storage must be specified and documented.

Samples will be collected using a variety of sampling methods, all of which are described in the WP. Sample collection procedures for bioassays and the bioaccumulation test will follow the SOPs in Appendices E, G, J, K, and I, respectively. With the exception of field analyses, all sediment, pore water, tissue analyses, and bioassays will be conducted at the contracted laboratories. Samples will be handled in a manner appropriate for the intended analyses. Table 1 summarizes the analytical parameters. Tables 2 through 5 summarize sample container, holding time, and preservative requirements for all sampling parameters. Analytical details, including procedures and analyte reporting limits, are presented in Section 8.0.

The WP contains details on the sampling design for the sites and includes the following information:

- Site sampling rationale
- Sampling techniques and equipment
- Sample selection criteria
- Sample documentation, handling, and shipment
- Sampling equipment preparation and decontamination

Potential hazards and correlative health and safety measures to be implemented during the phase II EA sampling activities are described in the HSP.

4.1 CHANGES IN PROCEDURE

Any changes in the sampling procedures will be documented in the field logbook. Modifications of the sampling design or procedures must be approved by the project manager before the change is implemented.

4.2 ANALYTICAL PARAMETERS

Refer to Table 1 for the suite of analytical parameters required for each sample type.

5.0 SAMPLE CUSTODY PROCEDURES

Documentation during sampling activities is essential to ensure proper sample identification, integrity, and custody. Standard sample custody procedures will be used to maintain and document sample integrity during collection, transportation, storage, and analysis. The FTL is responsible for proper sample handling and documentation that will allow for tracking the possession and handling of individual samples from the time of collection to laboratory receipt. The laboratory QA manager is responsible for establishing a sample control system that will allow for tracking sample possession from laboratory receipt to final sample disposition.

TABLE 1

ANALYTICAL AND TOXICOLOGICAL TESTING PARAMETERS

Analytical Procedures[a]	Matrices				
	Sediment Samples[b]	Water Samples[c]	Elutriate Samples[b]	Pore Water[b]	Tissue Samples
Organic					
VOC[d]	X				
SVOC/PAHs	X			X	X
Pest/PCBs	X			X	X
Organotins	X			X	X
TPH-Gasoline/BTEX	X			X	
TPH-Diesel	X			X	
Inorganic					
Total Metals	X	X (Lead)		X	X
AVS/SEM	X				
Toxicological					
Amphipod	X				
Polychaete	X				
Echinoderm			X	X	
Bivalve			X		
Bioaccumulation	X				
General					
Sulfide				X	
Total Organic Carbon	X				
Grain Size	X				
Sediment Oxygen Demand	X				
Ammonia	X			X	
Dissolved Organic Carbon				X	

Notes:

[a] The analytical methods are provided in Tables 8, 9, and 10 (Section 8.0).

[b] Sediments, extracted pore water, and elutriates will be used for bioassays. As specified in Appendices E, G, J, and K the following physical parameters will be measured in the process of conducting the bioassays: dissolved oxygen, pH, ammonia (total and unionized), sulfide, salinity, and temperature.

[c] Water collected only in the water column in Clipper Cove.

[d] VOCs only will be analyzed in the sediments of selected sampling locations.

TABLE 2
SAMPLE CONTAINER, HOLDING TIME, AND PRESERVATIVE REQUIREMENTS FOR SEDIMENT SAMPLES

Parameter	Method Number[a]	Sample Container	Preservative	Holding Time[b]
ORGANIC ANALYSES:				
Volatile Organic Compounds (VOC)	CLP[c]	8 oz. glass	Cool, 4 °C	14 days
Polynuclear Aromatic Hydrocarbons (PAH)	Modified CLP	8 oz. glass	Cool, 4 °C	14 days/40 days
Organochlorine Pesticides and Polychlorinated Biphenyls (PCB)	CLP	8 oz. glass	Cool, 4 °C	14 days/40 days
Total Petroleum Hydrocarbons-Purgeable	CA LUFT[d] & EPA 8015A	8 oz. glass	Cool, 4 °C	14 days
Total Petroleum Hydrocarbons-Extractable	CA LUFT & EPA 8015A	8 oz. glass	Cool, 4 °C	14 days/40 days
Benzene, Toluene, Ethylbenzene, and Xylenes (BTEX)	EPA 8020	8 oz. glass	Cool, 4 °C	14 days
INORGANIC/PHYSICAL ANALYSES:				
Metals	CLP	8 oz. glass	Cool, 4 °C	Hg, 28 days; others, 6 months
Acid volatile sulfide/simultaneously extracted metals (AVS/SEM)	Boothman and Helmstetter[e]	8 oz glass	Cool, 4 °C	21 days
Total Organic Carbon (TOC)	ASTM D2974[f]	8 oz. glass	Cool, 4 °C	28 days
Grain Size	ASTM D422-63	8 oz. glass	NA	NA
Sediment Oxygen Demand (SOD)	Tetra Tech[g]	8 oz. glass	Cool, 4 °C	7 days
Ammonia (total and unionized)[h]	EPA 350.1, 350.2, 350.3	8 oz. glass	Cool, 4° C	28 days

Notes:

[a] Complete method references are presented in Section 8.0, Table 8.
[b] Maximum number of days from sampling to extraction/maximum number of days from extraction to analysis
[c] CLP = "Statement of Work for Organic Analyses Multi-Media Multi Concentration," OLM03.1 (EPA 1994d); or "Statement of Work for Inorganics Analyses Multi-Media Multi Concentration," ILM04.0 (EPA 1995); as appropriate
[d] CA LUFT = California Leaking Underground Fuel Tank Manual (1989)
[e] Boothman and Helmstetter (1993)
[f] ASTM (1990)
[g] Tetra Tech (1986)
[h] Analysis involves making a 1- to 1-slurry with water, decanting liquid, and testing liquid using EPA method(s).

TABLE 3
SAMPLE CONTAINER, HOLDING TIME, AND PRESERVATIVE REQUIREMENTS FOR PORE WATER AND WATER SAMPLES

Parameter	Method Number[a]	Sample Container[b]	Sample Volume	Preservatives	Holding Time[c]
ORGANIC ANALYSES:					
Polynuclear Aromatic Hydrocarbons (PAH)	Modified CLP[d]	G	2 L	Cool, 4 °C	7 days/40 days
Organochlorine Pesticides and Polychlorinated Biphenyls (PCB)	CLP[e]	G	2 L	Cool, 4 °C	7 days/40 days
Total Petroleum Hydrocarbons-Purgeable	CA LUFT[f] & EPA 8015A	V	80 mL	HCl to pH<2, 4 °C	14 days
Total Petroleum Hydrocarbons-Extractable	CA LUFT & EPA 8015A	G	2 L	Cool, 4 °C	7 days/40 days
INORGANIC/PHYSICAL ANALYSES:					
Metals[g]	CLP[d]	P	1 L	HNO_3 to pH<2	Hg, 28 days; others, 6 months
pH	CLP or EPA 150.1	P	100 mL	Cool, 4 °C	2 days
Temperature	EPA 170.1	P	SMEWW	None	NA[h]
Salinity	SM 2520B	P	SMEWW	None	NA[h]
Dissolved Oxygen	SM 4500	P	SMEWW	None	NA[h]
Sulfide	EPA 376.1,376.2	P,G	500 mL	NaOH to pH>9, 4° C	7 days
Ammonia (total and unionized)	EPA 350.1; 350.2;350.3	P,G	500 mL	Cool, 4° C	28 days

Notes:

[a] Complete method references are presented in Section 8.0, Table 9

[b] Container Types: G = Amber glass with Teflon-lined lid, sized according to sample volume
P = Polyethylene container, sized according to sample volume
V = 40 ml VOA with Teflon Septa cap

[c] Maximum number of days from sampling to extraction/maximum number of days from extraction to analysis

[d] CLP = "Statement of Work for Organic Analyses Multi-Media Multi-Concentration," OLM03.1 (EPA 1994d); "Statement of Work for Inorganic Analyses Multi-Media Multi Concentration," ILM04.0 (EPA 1995), as appropriate

[e] CLP for OC Pesticides and PCBs = "Superfund Analytical Methods for Low Concentration Water for Organic Analysis" (EPA 1992b)

[f] CA LUFT = California Leaking Underground Fuel Tank Manual (CA 1989)

[g] Only lead will be measured in water in Clipper Cove.

[h] Analysis for temperature, salinity, and dissolved oxygen are for monitoring water quality of bioassays and should be measured immediately after sample is taken from overlying water.

L = Liter; mL = Milliliter

TABLE 4

SAMPLE CONTAINER, HOLDING TIME, AND PRESERVATIVE REQUIREMENTS FOR TISSUE RESIDUE ANALYSIS

Parameter	Method Number[a]	Sample Container[b]	Sample Volume[c]	Preservatives[d]	Holding Time
INVERTEBRATES:					
Polynuclear Aromatic Hydrocarbons (PAH)	NOAA[e]	G	4 g	Frozen	1 year
Organochlorine Pesticides and Polychlorinated Biphenyls (PCB)	NOAA	G	4 g	Frozen	1 year
Metals	CLP[f]	G	2.5 g	Frozen	6 months (except Hg - 28 days)
FISH TISSUE:					
Polynuclear Aromatic Hydrocarbons (PAH)	NOAA	G	4 g	Frozen	1 year
Organochlorine Pesticides and Polychlorinated Biphenyls (PCB)	NOAA	G	10 g	Frozen	1 year
Metals	CLP[f]	G	2.5 g	Frozen	6 months (except Hg - 28 days

Notes:

[a] Complete method references are presented in Section 8.0, Table 10.
[b] Container Type: G = Glass jars with headspace to allow for expansion of water in the sample.
[c] Sample volumes do not include provision for volume loss due to handling for preparation of QC samples.
[d] Samples should be frozen within 8 hours of sampling
[e] NOAA, National Status and Trends Program (Lauenstein and Cantillo 1993)
[f] CLP = "Statement of Work for Inorganic Analyses Multi-Media Multi Concentration," ILMO3.0 (EPA 1994d)
g = Gram

TABLE 5

SAMPLE CONTAINER, HOLDING TIME, AND PRESERVATIVE REQUIREMENTS FOR SEDIMENT, PORE WATER, AND ELUTRIATE BIOASSAYS

Bioassay	Method Number[a]	Sample Container[b]	Sample Volume	Preservatives[d]	Holding Time[e]
Echinoderm Abnormal Development	EPA[f]	HDPE/Plastic	3 L[c]	Cool, 4 °C	48 hours[h]
Solid Phase Amphipod (Survival and Reburial)	EPA[g]	HDPE/Plastic	4 L	Cool, 4 °C	14 days
Solid Phase Polychaete (Survival and Growth)	Battelle[i]	HDPE/Plastic	4 L	Cool, 4 °C	14 days
Bivalve Shell Development	EPA[j]	HDPE/Plastic	4 L	Cool, 4 °C	14 days

Notes:

[a] Complete method references are presented in the work plan (PRC 1996)
[b] Container Type: 4-L High density polyethylene (HDPE) container
[c] Three L of sediment is required to extract enough pore water for the bioassay testing; for chemical analysis, extra sample volume is required.
[d] Sediment and pore water samples to be used for bioassays should be stored in the dark.
[e] Holding time refers to the maximum number of days from sampling to start of bioassay for toxicity testing of sediments.
[f] Method based on Chapman and others (1995). See Appendix E.
[g] Method based on ASTM (1992) and EPA (1994c). See Appendix G.
[h] Pore water should be extracted from the sediment within 48 hours of collection. The pore water will be prepared and analyzed within the holding time appropriate for each analysis.
[i] Method based on Battelle Marine Science Laboratory (1994). See Appendix J.
[j] Method based on Chapman and others (1995). See Appendix K.

L = Liter

5.1 FIELD PROCEDURES

The sample custody and documentation procedures employed in the field are discussed below. All sample custody and documentation material will be completed in ballpoint or permanent ink by field personnel. Corrections will be made by drawing one line through the incorrect entry, entering the correct information, and initialling and dating the change.

5.1.1 Sample Custody

Sample custody materials discussed below include sample labels, custody seals, and chain-of-custody (COC) records.

5.1.1.1 Sample Labels

A sample label (Figure 2) will be affixed to all sample containers sent to the laboratory. This identification label will be completed with the following information:

- Project name
- Site name
- Sample identification number
- Sample type (matrix)
- Container type
- Analysis required
- Date and time of sample collection
- Preservative used (if any)
- Filtering (if applicable)

After labeling, each sample will be refrigerated or placed in a cooler containing ice to maintain the sample temperatures at 4 degrees Celsius (°C) plus or minus (±) 2 degrees.

5.1.1.2 Custody Seals

Before sample shipment, two seals will be placed on each sample cooler so that they must be broken to gain access to the content. Custody seals used during the course of the project will consist of security tape with the date and initials of the sampler (Figure 3). Clear tape will be placed over the custody seals to protect them from accidental breakage.

PRC Environmental Management Inc. 135 Main Street, Suite 1800 San Francisco, CA 94105 415-543-4880			
Project:	0440199RI2BMW		
Site:	Treasure Island		
Sample ID:	199Q4		
Sample Type:	Water		
Container Type:	1 Liter Plastic		
Analysis:	CLP Metals		
Date:		Time:	
Preservative:	HNO3	Filtered (y/n):	N

Naval Station Treasure Island
Sample Label
Figure 2

Inchcape Testing Services
Aquatec Laboratories

55 South Park Drive, Colchester, Vermont 05446
TEL: 802/655-1203

CUSTODY SEAL

SEALED BY

_____

DATE _____ TIME _____

Naval Station Treasure Island
Custody Seal
Figure 3

5.1.1.3 Chain-of-Custody Records

COC procedures provide an accurate written record tracking the possession of individual samples from the time of field collection through laboratory analysis. A sample is considered in custody if one of the following applies:

- It is in a person's possession.
- It is in view after being in physical custody.
- It is in a secure area after having been in physical custody.
- It is in a designated secure area, restricted to authorized personnel.

The COC record (Figure 4) will be used to document the samples taken and the analyses requested. Information that field personnel will record on the COC record includes the following:

- Project name and number
- PRC technical contact (analytical coordinator)
- PRC project manager
- Laboratory name
- Printed name and signature of samplers
- Sample identification number
- Sampling description/notes
- Date and time of collection
- Sample matrix
- Number and type of containers filled
- Preservatives used, if any
- Analyses requested
- Signatures of individuals involved in custody transfer (including printed name, company name, date, and time of transfer)
- Turnaround time/remarks (including airbill number, if appropriate)

Unused lines on the COC record will be crossed out. COC records initiated in the field will be signed, placed in a plastic bag, and taped to the inside of the shipping cooler used for sample

6036

PRC

Environmental Management Inc.
120 Howard Street, Suite 700
San Francisco, CA 94105
415-543-4880
Fax 415-543-5480

Chain of Custody Record

Page _____ of _____

Project name:	PRC technical contact:	Lab:	
Project number:	PRC project manager:	Field samplers:	
		Field samplers' signatures:	

Sample ID	Sample Description/Notes	Date	Time	Matrix	40 ml VOA	1 Liter Amber	1 Liter Poly	Brass Tube	Glass Jar				CLP VOA	CLP SVOA	CLP Pest/PCBs	CLP Metals	TPH Purgeables	TPH Extractables	BTEX (8020)	TRPH (418.1)								

	Name (print)	Company Name	Date	Time
Relinquished by:				
Received by:				
Relinquished by:				
Received by:				
Relinquished by:				
Received by:				
Relinquished by:				
Received by:				

Turnaround time/remarks:

WHITE - Laboratory Copy YELLOW - Project Office Copy

Naval Station Treasure Island
Chain of Custody Record
Figure 4

transport. Signed COC records or airbills will serve as evidence of custody transfer between the field sampler and courier and between the courier and laboratory. Copies of the COC record and the airbill will be retained and filed by the sampler prior to shipment. COC forms are not signed by Federal Express-type couriers.

Occasionally, multiple coolers will be sent in one shipment to the laboratory. A single COC record may be used for samples distributed among multiple coolers. The outside of the coolers will be marked to indicate how many coolers are in the shipment.

5.1.2 Sample Documentation

Sampling activities during the field effort require several forms of documentation. While some custody documentation was discussed in Section 5.1.1 (sample labels, custody seals, and COC records), additional documentation is mandatory. The documents discussed in Section 5.1.1 are prepared to maintain sample identification and COC, as well as to provide records of significant events or observations. Other documents that will be prepared during this phase II EA include the following:

- Logbooks
- Daily field progress reports
- Notification of field variance

5.2 SHIPPING PROCEDURES

During the phase II EA at NAVSTA TI, all samples taken during the field effort are expected to be identified as environmental samples. Environmental samples are defined as soil or groundwater that is not saturated or mixed with product material. All Department of Transportation (DOT) regulations will be followed for sample packaging and shipment. The procedures outlined below meet these requirements, which are taken from EPA guidance on field operations methods (EPA 1987).

- The cooler will be filled with "bubble wrapped" sample bottles and packing material such as vermiculite or styrofoam "peanuts." Sufficient packing material will be used to

prevent sample containers from making contact during shipment. Enough ice will be added to maintain sample temperatures at 4 °C ± 2 degrees.

- Each cooler will have a "temperature blank" included with the environmental samples. The temperature blank will consist of an unpreserved 40-milliliter (mL) volatile organic analysis (VOA) vial at least half-filled with tap water. The temperature blank will be used by laboratory personnel to take an accurate thermometer reading of the cooler's temperature at the time of sample receipt.

- As mentioned in Section 5.1.1.3, the COC records going to the laboratory will be placed inside a plastic bag. The bag will be sealed and taped to the inside of the cooler lid. The laboratory will be notified if the sampler suspects that the sample contains any substance for which the laboratory personnel should take safety precautions.

- The cooler will be closed and taped shut with strapping tape (filament-type) around both ends. If there is a drain in the cooler, it will be taped shut both inside and outside of the cooler.

- Two signed custody seals will be placed on the cooler, one on the front and one on the back. Wide clear tape will be placed over the seals to ensure against accidental breakage.

- The cooler will be handed over to the overnight carrier, such as Federal Express. A standard freightbill is necessary for shipping environmental samples. Figure 5 shows an example of the standard Federal Express airbill.

- If a laboratory courier is used, the same packing method described above will be used. The courier will sign the COC record to document transfer of samples. The cooler will be taped shut, and two signed custody seals will be placed on the cooler.

No samples will be held on site for more than 24 hours except during weekend field activities. Samples collected on the weekend will be stored under refrigeration and shipped the following Monday. Sampling for analytes with extremely short holding times (such as 24 hours) will not be scheduled as a weekend activity.

5.3 LABORATORY PROCEDURES

Upon receipt of a cooler, laboratory personnel will review the contents and will sign and retain the COC record and the freightbill. Information that will be recorded on the COC record, or other appropriate document, at the time of sample receipt will include the following:

FedEx *USA Airbill* Tracking Number 0677383475

Sender's Copy
129 200 63805632 6121M

1 From (please print)

Date _____ Sender's FedEx Account Number [redacted]

Sender's Name _____ Phone (415-543-4880

Dept/Floor/Suite/Room

Company PRC ENVIRONMENTAL MGMT INC/CRC

Address 135 MAIN STREET STE 1800

City SAN FRANCISCO State CA Zip 94105

2 Your Internal Billing Reference Information (Optional) (First 24 characters will appear on invoice) _____

3 To (please print)

Recipient's Name _____ Phone ( )

Dept/Floor/Suite/Room

Company _____

Address _____
(To "HOLD" at FedEx location, print FedEx address here) (We Cannot Deliver to P.O. Boxes or P.O. Zip Codes)

City _____ State _____ Zip _____

For HOLD at FedEx Location check here
☐ **Hold Weekday** (Not available with FedEx First Overnight)
☐ **Hold Saturday** (Not available at all locations) (Not available with FedEx First Overnight or FedEx Standard Overnight)

For Saturday Delivery check here
☐ (Extra Charge. Not available to all locations) (Not available with FedEx First Overnight or FedEx Standard Overnight)

Service Conditions, Declared Value, and Limit of Liability – By using this Airbill, you agree to the service conditions in our current Service Guide or U.S. Government Service Guide. Both are available on request. See back of Sender's Copy of this airbill for information and additional terms. We will not be responsible for any claim in excess of $100 per package whether the result of loss, damage, or delay, non-delivery, misdelivery, or misinformation, unless you declare a higher value, pay an additional charge, and document your actual loss in a timely manner. Your right to recover from us for any loss includes intrinsic value of the package, loss of sales, interest, profit, attorney's fees, costs, and other forms of damage, whether direct, incidental, consequential, or special, and is limited to the greater of $100 or the declared value but cannot exceed actual documented loss. The maximum declared value for any FedEx Letter and FedEx Pak is $500. Federal Express may, upon your request, and with some limitations, refund all transportation charges paid.
See the FedEx Service Guide for further details.

Questions?
Call 1·800·Go·FedEx (1-800-463-3339)

The World On Time

4 Service Delivery commitment may be later in some areas

☐ FedEx Priority Overnight (Next business morning)
☒ ~~FedEx Standard Overnight~~ (Next business afternoon)
☐ FedEx 2Day* (Second business day)
☒ FedEx Govt. Overnight (Authorized user only)
☐ (EXCEPTION)
☐ FedEx Overnight Freight
☐ FedEx 2Day Freight
(For packages over 150 pounds. Call for delivery schedule.)

☐ NEW FedEx First Overnight (Earliest next business morning delivery to select locations) (Higher rates apply)

*FedEx Letter Rate not available. Minimum charge: One pound FedEx 2Day rate.

5 Packaging

☐ FedEx Letter* ☐ FedEx Pak* (Declared value limit $500) ☐ FedEx Box ☐ FedEx Tube ☐ Other Packaging

6 Special Handling

Does this shipment contain dangerous goods? ☐ Yes (As per attached Shipper's Declaration) ☐ Yes (Shipper's Declaration not required)

☐ Dry Ice
Dry Ice, 9, UN 1845 III _____ x _____ kg 904
(Dangerous Goods Shipper's Declaration not required)

CA ☐ Cargo Aircraft Only

7 Payment

Bill to: ☒ Sender (Account no. in section 1 will be billed) ☐ Recipient ☐ Third Party ☐ Credit Card ☐ Cash/Check
(Enter FedEx account no. or Credit Card no. below)

FedEx Account No. _____

Credit Card No _____ Exp. Date _____

Total Packages	Total Weight	Total Declared Value*	Total Charges
		$ .00	$

*When declaring a value higher than $100 per package, you pay an additional charge. See **SERVICE CONDITIONS, DECLARED VALUE AND LIMIT OF LIABILITY** section for further information.

8 Release Signature

Your signature authorizes Federal Express to deliver this shipment without obtaining a signature and agrees to indemnify and hold harmless Federal Express from any resulting claims.

232

FORM ID NO 0200

WCSL 0396
Rev. Date 10/95 • PART #147381
©1994-95 FedEx • PRINTED IN U.S.A.

Naval Station Treasure Island
Standard Federal Express Airbill
Figure 5

- Status of the custody seals
- Temperature of the ice chest upon receipt
- Identification number of any broken sample containers
- Description of discrepancies between the COC records, sample labels, and requested analyses
- Observations of visible headspace in VOA vials indicating inadequate sample collection
- pH of water samples upon receipt; pH of VOA water samples will be documented at the time of analysis
- Storage location of the sample and sample extracts

Laboratory personnel will contact the analytical coordinator regarding discrepancies in paperwork and sample preservation and will document nonconformances and corrective actions according to the laboratory's SOPs. These procedures will be on file at the laboratory. Once samples have been accepted by the laboratory, checked, and logged in, they must be maintained in a manner consistent with custody and security requirements specified in the laboratory QA plan.

All samples and sample extracts will be assigned to a specific refrigerator within the laboratory. All laboratory refrigerators will be assigned a number, and the refrigerator number will be recorded on an appropriate document for reference of the sample and extract locations. Only laboratory personnel will have access to the samples and will be required to sign a log sheet when removing or replacing samples and extracts from the refrigerators. These log sheets will provide a COC record as the sample moves within the laboratory. A COC record, similar in form to the COC record used for sampling procedures, will be completed for samples removed from the laboratory for disposal or other purposes.

6.0 CALIBRATION PROCEDURES AND FREQUENCY

Standard calibration procedures exist for all field equipment to be used for on-site monitoring and testing. Laboratory equipment used for sample analysis also has prescribed calibration procedures. These procedures, along with the required frequency of calibration, are discussed below.

6.1 FIELD MEASUREMENT EQUIPMENT

Measurement equipment to be used during field activities will be calibrated at the beginning of the field effort and at prescribed intervals. The FTL will be responsible for ensuring that the field equipment is properly calibrated. The frequency of calibration is dependent on the type and stability of equipment, the analytical methods employed, the intended use of the equipment, and the recommendations of the manufacturer. A summary of calibration requirements for the field equipment to be used at NAVSTA TI is presented in Table 6. Some equipment, such as the dissolved oxygen meter, will be calibrated before being taken to the field and the calibration will be checked before use. More detailed calibration procedures for equipment listed in Table 6 are provided in the specific manufacturer's instruction manuals.

All calibration information will be recorded in the site logbook. Additionally, a label specifying the date of the next calibration will be attached to the equipment. If this identification is not feasible, calibration records for the piece of equipment will be readily available for reference.

Should a piece of equipment become inoperable, it will be removed from service and tagged to indicate that repair, recalibration, or replacement is needed. The FTL will be notified so that prompt service can be performed or substitute equipment can be obtained. Any action of this type will be reported in a corrective action status report. Additional details on corrective action procedures are presented in Section 12.0.

6.2 LABORATORY MEASUREMENT EQUIPMENT

Laboratory instrument calibration procedures and frequency will be performed in accordance with the referenced analytical method requirements. All laboratory calibration procedures and frequencies are listed in the laboratory QA plan and the analytical methods referenced in Section 8.0.

TABLE 6

FIELD EQUIPMENT CALIBRATION

Instrument Type	Standard Reference	Calibration Technique	Calibration Frequency	Acceptance Specifications
Specific electrical conductance (EC) meter	(1) KCl[a] standard solution	Manufacturer's user manual	Once daily[b] (prior to use)	Standard solution
	(2) Calibration line	Manufacturer's user manual	Before each use	Red line value
pH meter	Two buffers	Set range and span based on anticipated field conditions	Once daily[b] (prior to use)	Buffer solution values
Temperature meter with compensation probe	Electronic thermometer	Manufacturer's user manual	Once daily (prior to use)	Mercury thermometer value
Photoionization detector	Gas standard kit (isobutylene)	Manufacturer's user manual	Twice daily (prior to use and at end of daily use)	Indication of standard ppm concentrations and zero setting
Water level indicator	Measurement tape	Manufacturer's user manual	Before commencement of field activities	Measurement tape value
Turbidity meter	Standard solution	Manufacturer's user manual	Before each use	Standard solution value
Combustible gas indicator	Standard calibration gas (pentane)	Manufacturer's user manual	Twice daily (prior to use and at end of daily use)	Indication of standard percent of lower explosive limit and zero settings

TABLE 6

FIELD EQUIPMENT CALIBRATION
(Continued)

Instrument Type	Standard Reference	Calibration Technique	Calibration Frequency	Acceptance Specifications
Dissolved oxygen meter	Iodine/thiosulfate titration endpoint	Winkler titration	Before field use	Meter matches titration endpoint within 5 percent
	Atmospheric air	Manufacturer's user manual	Daily	Atmospheric oxygen level

Notes:

[a] KCl = Potassium chloride
[b] Once daily calibration of field equipment will consist of a morning calibration on each day of field activities. Recalibration will be performed as necessary.

7.0 PREVENTIVE MAINTENANCE PROCEDURES

Regularly scheduled preventive maintenance will be performed to keep all field and laboratory equipment in good working condition.

7.1 FIELD EQUIPMENT

Detailed information regarding maintenance and servicing is available in the operation manual of the specific instrument to be used. General service and maintenance information will be performed by the equipment rental company, who will record the information about the date and type of service performed. Instrument problems encountered during the field program will be recorded in the site logbook and, if possible, remedied in the field. Spare batteries will be kept on hand for replacement, if needed. Specific preventive maintenance practices will follow manufacturer's recommendations.

Regularly scheduled general maintenance tasks for each type of field equipment are outlined in Table 7. Common spare parts for all field equipment will be kept on site, readily available to all field personnel. Preventive maintenance tasks will be the responsibility of the FTL and the equipment rental company. Additional details on equipment maintenance procedures are presented in EPA guidance (EPA 1987) and the operation manual for the specific instrument.

7.2 LABORATORY EQUIPMENT

A description of specific preventive maintenance procedures for laboratory equipment will be available in the laboratory QA plan or in written SOPs maintained by the selected laboratory, as required by Navy Installation Restoration Laboratory Quality Assurance Guide (Chief of Naval Operations [CNO] Environmental Laboratory Advisory Council [ELAC] 1996). These documents will identify the personnel responsible for major, preventive, and day-to-day maintenance; the frequency and type of maintenance; and the documentation procedures.

TABLE 7

FIELD EQUIPMENT PREVENTIVE MAINTENANCE

Instrument Type	Maintenance Tasks
Specific Electrical Conductance (EC) Meter	• Check charge on battery regularly. Recharge or replace, as appropriate. • Clean cell cup or electrode with deionized water after each reading. Rinse well.
pH Meter	• Check that pH electrode contains sufficient liquid. • Ensure that the outside of the probe stays moist. • Check sensor bulb for development of film if drifting is a problem - lightly clean with liquid cleanser. • Rinse electrode with deionized water and replace storage cap after each use.
Temperature Meter	• Regularly clean and maintain the instrument and any accessories.
Photoionization Detector	• Check charge on battery regularly. Recharge or replace as appropriate. • Check UV lamp and ion chamber for cleanliness. • Clean probe if deposits develop on UV lamp surface or in ion chamber. • Clean air fan and/or pump if sand grains or dirt are present. • Test for leaks by plugging the inlet. • Regularly clean and maintain the instrument and accessories.
Water Level Indicator	• Check charge on battery regularly. Recharge or replace, as appropriate. • Rinse probe and tape after use. • Inspect cable and all electrical connections for breaks and/or bare wire.
Turbidity Meter	• Check charge on battery regularly. Recharge or replace, as appropriate. • Regularly clean and maintain the instrument and accessories.
Combustible Gas Indicator	• Check charge on battery regularly. Recharge or replace, as appropriate. • Check alarm horn to ensure correct operation. • Test for leaks by plugging the inlet. Inspect intake hose and nozzle.

TABLE 7

FIELD EQUIPMENT PREVENTIVE MAINTENANCE
(Continued)

Instrument Type	Maintenance Tasks
Noise Dosimeter	• Check battery level indicator. Recharge or replace, as appropriate. • Regularly clean and maintain the instrument and accessories.
Dust Monitor	• Check battery level indicator. Recharge or replace, as appropriate. • Regularly clean and maintain the instrument and accessories.
Ground Penetrating Radar (GPR)	• Check charge on battery regularly. Recharge or replace, as appropriate. • Regularly clean and maintain the instrument and accessories.
Metal Detector	• Check charge on battery regularly. Recharge or replace, as appropriate. • Regularly clean and maintain the instrument and accessories.
Magnetometer	• Check charge on battery regularly. Recharge or replace, as appropriate. • Regularly clean and maintain the instrument and accessories.
Geiger Counter	• Check charge on battery regularly. Recharge or replace, as appropriate. • Regularly clean and maintain the instrument and accessories.

8.0 ANALYTICAL PROCEDURES AND REPORTING LIMITS

Analytical methods that will be used for the analysis of sediment, water, pore water, and tissue samples collected at NAVSTA TI are presented in Tables 8, 9, and 10. Other EPA and Navy-approved analytical methods may be selected, with approval from the Navy RPM, if existing DQOs are met or exceeded. A description of any modifications to analytical methods presented in this section will be appended to the QAPP and submitted for regulatory agency review. Appendix H presents SOP for preparing tissue for analysis.

The subcontract laboratory will be certified under the Environmental Laboratory Accreditation Program of the California Department of Health Services and approved by NFESC. The analyses, data reporting, and validation will be performed according to the specified Navy QA program (CNO ELAC 1996). Data will be reported as full EPA CLP or CLP-like data packages. The CLP-like data packages are prepared for non-CLP analytical methods. The documentation requirements for CLP-like data packages are designed to mimic the information required by CLP data packages to obtain the necessary laboratory documentation required for data validation (EPA Region 9 1990). The following sections provide details regarding the specific procedures that will be used to analyze samples.

8.1 VOLATILE ORGANIC COMPOUNDS

The contract-required quantitation limits (CRQL) for CLP VOC analysis are shown in Table 11. CLP VOC analysis will be performed on NAVSTA TI sediment samples. In addition, if one of the initial calibration standards is not at the required quantitation limit, a quantitation limit standard at that level will be required to verify instrument sensitivity and linearity. When required, the quantitation limit standard will be analyzed daily after the calibration standards. All calibration compounds will be detected and will be within 25 percent of the true value.

CLP VOC analysis is a purge-and-trap gas chromatography/mass spectrometry (GC/MS) method for determining purgeable organics in solid or aqueous samples. An inert gas is bubbled through a specially designed purging chamber at 40 °C for sediments and tissues and at ambient temperatures for the pore water samples. The gas chromatography (GC) instrument is temperature-programmed to separate the purgeables that are then detected by the mass spectrometer. The target compound list

TABLE 8

ANALYTICAL METHODS FOR SEDIMENT SAMPLES

Parameter	Method Number[a]	Extraction Technique[b]	Reference[c]	Analyte List[d]	Technique[e]
ORGANIC ANALYSES:					
Volatile Organic Compounds (VOC)	CLP	Purge and Trap	OLM03.1	TCL + 10 TIC	GC/MS
Polynuclear Aromatic Hydrocarbons (PAH)	Modified CLP	Sonication and GPC	Modified OLM03.1	17 PAH	GC/MS/SIM
Organochlorine Pesticides and Polychlorinated Biphenyls (PCB)	CLP	Sonication and GPC	OLM03.1	TCL	GC/ECD
Total Petroleum Hydrocarbons-Purgeables/ BTEX	CA LUFT & EPA 8015A/ 8020	Purge and Trap	CA LUFT & SW-846	Gasoline, BTEX	GC/FID,PID
Total Petroleum Hydrocarbons-Extractables	CA LUFT & EPA 8015A	Sonication	CA LUFT & SW-846	Diesel, Motor Oil	GC/FID
INORGANIC/PHYSICAL ANALYSES:					
Metals	CLP	Acid Digestion	ILM04.0	TAL	ICP & AA
Acid volatile sulfide/simultaneously extracted metals (AVS/SEM)	Boothman and Helmstetter	Volatilization and Acid Digestion	Boothman and Helmstetter	TAL	ICP & AA
pH	CLP or EPA 9045A	NA	SW-846	NA	pH Meter
Total Organic Carbon (TOC)	ASTM D2974	NA	ASTM	TOC	
Grain Size	ASTM D422-63	NA	ASTM	NA	
Sediment Oxygen Demand (SOD)	Tetra Tech	NA	Tetra Tech 1986	SOD	
Ammonia (total and unionized)	EPA 350.1; 350.2; 350.3	Distillation	SMEWW	Ammonia Nitrogen	Colorimetric

TABLE 8

ANALYTICAL METHODS FOR SEDIMENT SAMPLES
(Continued)

Notes:

[a] Other EPA and Navy-approved methods may be selected with approval from the Navy RPM. All method changes will be documented in writing and be reviewed by the regulatory agencies.

[b] The extraction procedures for CLP analyses are specified in the CLP statement of work, but are based on the listed EPA method.
GPC: Gel permeation chromatography extract cleanup

[c] CLP: Contract laboratory program
"Statement of Work for Organic Analyses Multi-Media Multi-Concentration," OLM03.1 (EPA 1994d)
"Statement of Work for Inorganic Analyses Multi-Media Multi-Concentration, ILM04.0 (EPA 1995)

SW-846: EPA Test Methods for Evaluating Solid Waste, 1986 with updates through 1990 (EPA 1986)
CA LUFT: State of California Leaking Underground Fuel Tank Field Manual (CA 1989)
ASTM: American Society for Testing and Materials (ASTM 1990)
SMEWW: Standard Methods for the Examination of Water and Wastewater, 18th Edition (APHA 1989)

[d] BTEX: Benzene, toluene, ethylbenzene, xylenes
TAL: Target analyte list
TCL: Target compound list
TIC: Tentatively identified compounds
TOC: Total organic carbon
NA: Not applicable

[e] AA: Atomic absorption spectroscopy
ECD: Electron capture detector
FID: Flame ionization detector
FPD: Flame photometric detector
GC: Gas chromatography
ICP: Inductively coupled plasma emission spectroscopy
MS: Mass spectrometry
PID: Photoionization detector
SIM: Selective ion monitoring

TABLE 9

ANALYTICAL METHODS FOR PORE WATER AND WATER SAMPLES

Parameter	Method Number[a]	Extraction Technique[b]	Reference[c]	Analyte List[d]	Technique[e]
ORGANIC ANALYSES:					
Polynuclear Aromatic Hydrocarbons (PAH)	Modified CLP	Liquid-Liquid	OLM03.1	17 PAHs	GC/MS/SIM
Organochlorine Pesticides and Polychlorinated Biphenyls (PCB)	CLP	Liquid-Liquid	OLM03.1	TCL	GC/ECD
Total Petroleum Hydrocarbons-Purgeables	CA LUFT & EPA 8015A/ 8020	Purge and Trap	CA LUFT & SW-846	Gasoline, BTEX	GC/FID,PID
Total Petroleum Hydrocarbons-Extractables	CA LUFT & EPA 8015A	Sonication	CA LUFT & SW-846	Diesel, Motor Oil	GC/FID
INORGANIC/PHYSICAL ANALYSES:					
Metals (Total)[f]	CLP	Acid Digestion	ILM04.0	TAL	ICP & AA
pH	CLP or EPA 150.1	NA	SW-846	pH	pH Meter
Temperature	EPA 170.1	NA	SMEWW	Temperature	Thermometer
Salinity	SM 2520B	NA	SMEWW	Salinity	Meter
Dissolved Oxygen	SM 4500	NA	SMEWW	Dissolved Oxygen	Meter
Ammonia (Total and Unionized)	EPA 350.1; 350.2; 350.3	Distillation	SMEWW	Ammonia	Colorimetric
Sulfide	EPA 376.1	Acidification	SMEWW	Sulfide	Titration

TABLE 9

ANALYTICAL METHODS FOR PORE WATER AND WATER SAMPLES (Continued)

Notes:

[a] Other EPA and Navy-approved methods may be selected with approval from the Navy RPM. All method changes will be documented in writing and be reviewed by the regulatory agencies.

[b] The extraction procedures for CLP analyses are specified in the CLP statement of work but are based on the listed EPA method.

GPC: Gel permeation chromatography extract cleanup

[c] CLP: Contract laboratory program
"Statement of Work for Organic Analyses Multi-Media Multi-Concentration," OLM03.1 (EPA 1994d)
"Statement of Work for Inorganic Analyses Multi-Media Multi-Concentration," ILM04.0 (EPA 1995)
"Superfund Analytical Methods for Low Concentration Water for Organic Analysis" (EPA 1992b)

SW-846: EPA Test Methods for Evaluating Solid Waste, 1986 with updates through 1990 (EPA 1986)

CA LUFT: State of California Leaking Underground Fuel Tank Field Manual (CA 1989)

ASTM: American Society for Testing and Materials (ASTM 1990)

SMEWW: Standard Methods for the Examination of Water and Wastewater, 18th Edition (APHA 1989)

[d] TAL: Target analyte list
TCL: Target compound list
TIC: Tentatively identified compounds
TOC: Total organic carbon
NA: Not applicable

[e] AA: Atomic absorption spectroscopy
ECD: Electron capture detector
FID: Flame ionization detector
FPD: Flame photometric detection
GC: Gas chromatography
ICP: Inductively coupled plasma emission spectroscopy
MS: Mass spectrometry
PID: Photoionization detector
SIM: Selective ion monitoring

[f] Only lead will be measured in Clipper Cove water.

TABLE 10

ANALYTICAL METHODS FOR TISSUE SAMPLES

Parameter	Method Number[a]	Extraction Technique[b]	Reference[c]	Analyte List[d]	Technique[e]
ORGANIC ANALYSES:					
Polynuclear Aromatic Hydrocarbons (PAH)	NA	Sonication and GPC	NOAA	25 PAHs	GC/MS/SIM
Organochlorine Pesticides and Polychlorinated Biphenyls (PCB)	NA	Sonication	NOAA	28 Pesticides 19 Congeners	GC/MS
INORGANIC/PHYSICAL ANALYSES:					
Metals	CLP	Acid Digestion	ILMO4.0	TAL	ICP/CVAA

Notes:

Appendix H presents the standard operating procedure for preparing tissue for analysis.

[a] Other EPA and Navy-approved methods may be selected with approval from the Navy RPM. All method changes will be documented in writing and submitted to the regulatory agencies.

[b] The extraction procedures for analyses are specified in the NOAA National Status and Trends Program (Lauenstein and Cantillo 1993).
GPC: Gel permeation chromatography extract cleanup

[c] CLP: Contract Laboratory Program
"Statement of Work for Inorganic Analyses Multi-Media Multi-Concentration," ILMO4.0 (EPA 1995)
NOAA: National Status and Trends Program (Lauenstein and Cantillo 1993)

[d] TAL: Target analyte list
PAH: Polynuclear aromatic hydrocarbon

[e] CVAA: Cold vapor atomic absorption spectroscopy
ICP: Inductively coupled plasma emission spectroscopy
GC: Gas chromatography
MS: Mass spectrometry
SIM: Selected ion monitoring

TABLE 11

VOLATILE ORGANIC COMPOUNDS
CONTRACT-REQUIRED QUANTITATION LIMITS (CRQL)

Volatile Organic Compound	Sediment (μg/kg) CRQL[a]
Chloromethane	10
Bromomethane	10
Vinyl chloride	10
Chloroethane	10
Methylene chloride	10
Acetone	10
Carbon disulfide	10
1,1-Dichloroethene	10
1,1-Dichloroethane	10
1,2-Dichloroethene	10
Chloroform	10
1,2-Dichloroethane	10
2-Butanone	10
1,1,1-Trichloroethane	10
Carbon tetrachloride	10
Bromodichloromethane	10
1,2-Dichloropropane	10
cis-1,3-Dichloropropene	10
Trichloroethene	10
Dibromochloromethane	10
1,1,2-Trichloroethane	10
Benzene	10
trans-1,3-Dichloropropene	10
Bromoform	10
4-Methyl-2-pentanone	10
2-Hexanone	10
Tetrachloroethene	10
Toluene	10
1,1,2,2-Tetrachloroethane	10
Chlorobenzene	10
Ethylbenzene	10
Styrene	10
Total Xylenes	10

Notes:

[a] Quantitation limits listed for sediment analysis are based on wet weight. The quantitation limits reported by the laboratory for sediment, calculated on dry weight basis as required by the contract, will be higher.

μg/kg = Micrograms per kilogram

(TCL) components are identified by mass spectra and retention time. The TCL will be quantified in both sediment and pore water samples. In addition to the TCL, library searches will be performed to identify the 10 nontarget compounds selected at the highest concentrations in each sample; these are commonly known as the tentatively identified compounds (TIC). CLP CRQL for TCL VOCs are listed in Table 11.

8.2 SEMIVOLATILE ORGANIC COMPOUNDS

Three different methods will be used for the analysis of semivolatile organic compounds (SVOC) in sediment, tissue, and pore water samples. Selective ion monitoring (SIM) GC/MS will be used for the analysis of PAH, the most commonly detected subset of SVOCs, in sediment, pore water, and tissue samples. A modified CLP SVOC method will be used to analyze pore water samples. Details about these methods are discussed below.

8.2.1 Polynuclear Aromatic Hydrocarbons in Sediment and Pore Water

A modified version of the CLP SVOC method will be used for the analysis of PAHs in sediment and pore water samples. The modification includes the use of SIM GC/MS to achieve lower quantitation limits than the normal CLP method. A measured amount of sediment sample is solvent-extracted by sonication (sediment) or continuous liquid-liquid extraction (pore water) in the same manner required by the CLP extraction procedure. The extract is concentrated, processed through a gel permeation chromatography (GPC) cleanup, and analyzed by SIM GC/MS. The GC instrument is temperature programmed to separate the extractables, which are then detected by the mass spectrometer. Because the mass spectrometer is programmed to focus on a few selected mass ions instead of scanning the entire range of masses, the lower quantitation limits are achievable. The mass spectra and retention times are used to identify target compounds. Table 12 presents the TCL and CRQLs for PAHs. The CRQLs given in Table 12 are generally one-tenth of the CLP CRQLs.

The SIM GC/MS method is only capable of analyzing a maximum of 20 compounds in a single sample run. Therefore, the method targets only PAHs and cannot detect any of the other SVOCs (for example, phenols and phthalates) or the nontarget compounds (TICs). The method is the most useful

TABLE 12

POLYNUCLEAR AROMATIC HYDROCARBON QUANTITATION LIMITS

Polynuclear Aromatic Hydrocarbons	Quantitation Limits		
	Tissue (μg/kg)	Pore Water (μg/L)	Sediment (μg/kg)
Acenaphthene	50	1.0	0.026
Acenaphthylene	50	1.0	0.026
Anthracene	50	1.0	0.026
Benzo(a)anthracene	50	1.0	0.026
Benzo(a)pyrene	50	1.0	0.026
Benzo(e)pyrene	50	NA	NA
Benzo(b)fluoranthene	50	1.0	0.026
Benzo(k)fluoranthene	50	1.0	0.026
Benzo(g,h,i)perylene	50	1.0	0.026
Biphenyl	50	NA	NA
Chrysene	50	1.0	0.026
Dibenzothiophene	50	NA	NA
Dibenzo(a,h)anthracene	50	1.0	0.026
2,6-Dimethylnaphthalene	50	NA	NA
Fluoranthene	50	1.0	0.026
Fluorene	50	1.0	0.026
Indeno(1,2,3-cd)pyrene	50	1.0	0.026
2-Methylnaphthalene	50	1.0	0.026
1-Methylnaphthalene	50	NA	NA
1-Methylphenanthrene	50	NA	NA
Naphthalene	50	1.0	0.026
Phenanthrene	50	1.0	0.026
Perylene	50	NA	NA
Pyrene	50	1.0	0.026
2,3,5-Trimethylnaphthalene	50	NA	NA

Notes:

Quantitation limits listed for sediment are based on wet weight. The quantitation limts reported by the laboratory for sediment, calculated on a dry weight basis, will be higher.

μg/kg = Micrograms/kilogram
μg/L = Micrograms/liter
NA = Not applicable

choice for analysis of sediment samples because of the need for lower quantitation limits, and because none of the other SVOCs have previously been detected in the sediment at NAVSTA TI.

8.2.2 Polynuclear Aromatic Hydrocarbons in Tissue

SIM GC/MS will also be used to achieve low quantitation limits in the PAH analysis of tissue samples. The protocol proposed was developed for the NOAA NSTP. A measured amount of tissue sample is solvent-extracted by sonication. While the NSTP method specifies a sample size of 40 grams of tissue to achieve low part-per-billion (ppb) detection of contaminants, a sample size of 2 to 4 grams is sufficient to achieve the target detection limits of 5 to 10 micrograms per kilogram (μg/kg).

The extract is concentrated, processed through a GPC cleanup, and analyzed by SIM GC/MS. The GC instrument is temperature programmed to separate the extractables, which are then detected by the mass spectrometer. The mass spectrometer is programmed to focus on a few selected mass ions instead of scanning the entire range of masses. The mass spectra and retention times are used to identify target compounds. Table 12 presents the TCL and CRQLs for PAHs. As with the analysis of sediment samples, this method will target only PAHs and cannot detect any of the other SVOCs (for example, phenols, phthalates, and TICs), but is useful for achieving low quantitation limits.

8.3 ORGANOCHLORINE PESTICIDES AND POLYCHLORINATED BIPHENYLS

The standard CLP procedure will be used for analysis of OC pesticides/PCBs in sediment and pore water samples, while the NSTP protocol will be used for the analysis of OC pesticides/PCBs in tissue samples.

8.3.1 Pesticides/PCBs in Sediment and Pore Water

The standard CLP method will be used to analyze OC pesticides/PCBs in sediment and pore water samples. In this CLP method, a measured aliquot of sample is solvent-extracted using sonication (sediment) and a separatory funnel or continuous liquid-liquid extraction (pore water). The extract is solvent-exchanged and concentrated. The extract is directly injected onto a GC which is temperature-programmed to separate the analytes and uses an electron capture detector (ECD) to detect the

analytes. The GC analysis is performed using two dissimilar columns, which produce unique retention times for each analyte. A retention time match on both columns is used to identify target analytes. Compounds and CRQLs for OC pesticides and PCBs are presented in Table 13. For sediment analysis, the CRQLs given in Table 13 are generally one-half of the CLP CRQL. These CRQLs are achievable by increasing the sample volume by a factor of 2. In addition, the CRQLs may be further reduced by concentrating the final extract to a volume of less than 1 mL. These attempts at reducing the CRQLs are highly matrix dependent; an increase in sample size increases the matrix effect, and the concentration of the extract concentrates interferences. For pore water analysis, a five-fold improvement in the CRQL can be achieved by using "Superfund Analytical Methods for Low Concentration Water for Organic Analysis" (EPA 1992b). If one of the initial calibration standards is not at the required quantitation limit, a quantitation limit standard at that level will be required to verify instrument sensitivity and linearity. When required, the quantitation limit standard will be analyzed daily after the calibration standards. All calibration compounds in this standard will be detected and will be within 25 percent of the true value.

8.3.2 Pesticides/PCBs in Tissue

The NSTP protocol will be used for the analysis of PCBs and pesticides in tissue samples. A measured aliquot of tissue is solvent-extracted using sonication. The extract is solvent-exchanged and concentrated. The NSTP method specifies a sample size of 10 grams of tissue to achieve the specified detection limits. However, the same detection limits are achievable by decreasing the sample size to 5 grams and concentrating the final extract volume to 2 mL instead of 4 mL. The extract is directly injected onto a GC which is temperature-programmed to separate the analytes and uses an ECD to detect the analytes. The GC analysis is performed using two dissimilar columns, which produce unique retention times for each analyte. A retention time match on both columns is used to identify target analytes. The primary difference between the NSTP method and the CLP method is that, in the NSTP method, the PCBs are quantitated as individual compounds (known as “congeners”) rather than by product mixture (that is, Aroclor group). Known concentrations of appropriate Aroclor standards will be analyzed by the NSTP method to determine the relative congener concentrations in the Aroclor mixture. Then, the congener concentrations from each sample will be used to calculate an estimated Aroclor concentration in the tissue sample. The target

TABLE 13

ORGANOCHLORINE PESTICIDES AND PCBs QUANTITATION LIMITS

Analyte	Quantitation Limits		
	Tissue (µg/kg)	Pore Water (µg/L)	Sediment (µg/kg)
Organochlorine Pesticides			
alpha-BHA	0.5	0.01	0.85
beta-BHC	0.5	0.01	0.85
gamma-BHC (Lindane)	0.5	0.01	0.85
Heptachlor	0.5	0.01	0.85
Aldrin	0.5	0.01	0.85
Heptachlor epoxide	0.5	0.01	0.85
Endosulfan I	0.5	0.01	0.85
Dieldrin	0.5	0.02	1.65
4,4'-DDE	0.5	0.02	1.65
2,4'-DDE	0.4	NA	NA
Endrin	0.5	0.02	1.65
Endosulfan II	0.5	0.02	1.65
4,4'-DDD	0.5	0.02	1.65
2,4'-DDD	0.4	NA	NA
Endosulfan sulfate	0.5	0.02	1.65
4,4'-DDT	0.5	0.02	1.65
2,4'-DDT	0.4	NA	NA
Methoxychlor	0.5	0.10	8.50
Endrin Ketone	0.5	0.02	1.65
Endrin Aldehyde	0.5	0.02	1.65
alpha-Chlordane	0.5	0.01	0.85
gama-Chlordane	0.5	0.01	0.85
Toxaphene	80.0	1.00	85.00
Technical Chlordane	20.0	NA	NA
Hexachlorobenzene	0.4	NA	NA
Mirex	0.4	NA	NA
PCBs			
Aroclor-1016	NA	0.20	16.50
Aroclor-1221	NA	0.20	33.50
Aroclor-1232	NA	0.20	16.50

TABLE 13

ORGANOCHLORINE PESTICIDES AND PCBs QUANTITATION LIMITS (Continued)

Analyte	Quantitation Limits		
	Tissue (µg/kg)	Pore Water (µg/L)	Sediment (µg/kg)
Aroclor-1242	NA	0.20	16.50
Aroclor-1248	NA	0.20	16.50
Aroclor-1254	NA	0.20	16.50
Aroclor-1260	NA	0.20	16.50
2,4'-Dichlorobiphenyl (BZ 8)	0.5	NA	NA
2,2',5-Trichlorobiphenyl (BZ 18)	0.5	NA	NA
2,4,4'-Trichlorobiphenyl (BZ 28)	0.5	NA	NA
2,2',3,5'-Tetrachlorobiphenyl (BZ 44)	0.5	NA	NA
2,2',5,5'-Tetrachlorobiphenyl (BZ 52)	0.5	NA	NA
2,3',4,4'-Tetrachlorobiphenyl (BZ 66)	0.5	NA	NA
2,2',4,5,5'-Pentachlorobiphenyl (BZ 101)	0.5	NA	NA
2,3,3',4,4'-Pentachlorobiphenyl (BZ 105)	0.5	NA	NA
2,3',4,4',5-Pentachlorobiphenyl (BZ 118)	0.5	NA	NA
3,3',4,4',5-Pentachlorobiphenyl (BZ 126)	0.5	NA	NA
2,2',3,3',4,4'-Hexachlorobiphenyl (BZ 128)	0.5	NA	NA
2,2',3,4,4'5'-Hexachlorobiphenyl (BZ 138)	0.5	NA	NA
2,2',4,4',5,5'-Hexachlorobiphenyl (BZ 153)	0.5	NA	NA
2,2'3,3',4,4',5-Heptachlorobiphenyl (BZ 170)	0.5	NA	NA
2,2',3,4,4',5,5'-Heptachlorobiphenyl (BZ 180)	0.5	NA	NA
2,2',3,4',5,5',6-Heptachlorobiphenyl (BZ 187)	0.5	NA	NA

TABLE 13

ORGANOCHLORINE PESTICIDES AND PCBs QUANTITATION LIMITS (Continued)

Analyte	Quantitation Limits		
	Tissue (μg/kg)	Pore Water (μg/L)	Sediment (μg/kg)
2,2'3,3',4,4',5,6-Octachloribiphenyl (BZ 195)	0.5	NA	NA
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl (BZ 206)	0.5	NA	NA
Decachlorobiphenyl (BZ 209)	0.5	NA	NA

Notes:

Quantitation limits listed for sediment and tissue are based on wet weight. The quantitation limts reported by the laboratory for sediment, calculated on a dry weight basis, will be higher.

μg/kg = Micrograms/kilogram
μg/L = Micrograms/liter
NA = Not applicable

compounds and quantitation limits for the OC pesticides and PCBs analyzed by the NSTP method are presented in Table 13.

8.4 METALS

Sediment, tissues, and pore water samples will be analyzed for metals. Analyses will be performed according to CLP procedures, and metal analytes on the target analyte list (TAL) will be measured in the samples. A measured aliquot of sample is digested using appropriate combinations of hydrochloric acid (HCl), nitric acid (HNO_3), and hydrogen peroxide. Digested samples are analyzed by inductively coupled plasma atomic emission spectroscopy (ICP), graphite furnace atomic absorption (GFAA), and cold vapor atomic absorption (CVAA).

ICP analysis allows the simultaneous multielemental determination of the CLP metals by measuring the element-emitted light by optical spectrometry. Element-specific atomic line emission spectra are dispersed by a grating spectrometer, and the intensities of the lines are monitored by photo multiplier tubes.

GFAA analysis allows the individual analysis of arsenic, lead, selenium, and thallium to provide lower detection limits. In the furnace, the sample is evaporated to dryness, charred, and atomized. A light beam from a hollow cathode lamp or an electrodeless discharge lamp (EDL) is directed through the tube into a monochromator and onto a detector that measures the amount of light. Because the wavelength of the light beam is characteristic of a single metal, the light energy absorbed is a measure of that metal's concentration.

These analytes may be analyzed by a trace ICP analyzer that is capable of reaching the required detection limits. The CVAA technique is based upon the absorption by mercury vapor of radiation at 253.7 nanometers. The mercury is reduced to the elemental state and aerated from solution in a closed system. The mercury vapor passes through a cell positioned in the light path of an atomic absorption spectrophotometer. Absorbance is measured as a function of mercury concentration. The contract required detection limits (CRDL) for all metals are presented in Table 14. The following analytes have quantitation limits lower than the normal CLP CRDLs: aluminum, antimony, beryllium, and thallium. If one of the initial calibration standards is not at the required detection

TABLE 14

METALS
CONTRACT REQUIRED DETECTION LIMITS (CRDL)

	Pore Water or Water[a] (μg/L)	Sediment or Tissue[b] (mg/kg)
Analyte	**CRDL**	**CRDL**
Aluminum	16.3	1.60[c]
Antimony	2.1	0.21[c]
Arsenic	2.8	0.28
Barium	0.3	0.30
Beryllium	0.1	0.01
Cadmium	0.2	0.02
Calcium	14.4	1.40
Chromium	1.8	0.18
Cobalt	0.5	0.05
Copper	0.8	0.08
Iron	15.7	1.60
Lead	1.5	0.15
Magnesium	16.4	1.60
Manganese	0.6	0.06
Mercury	0.1	0.05
Nickel	3.3	0.33
Potassium	27.8	2.80
Selenium	3.5	0.34
Silver	0.6	0.06
Sodium	127.9	12.80
Thallium	2.0	0.20[c]
Vanadium	0.6	0.06
Zinc	0.3	0.03

Notes:

[a] These are the instrument detection levels using an ICP-trace analyzer.

[b] Detection limits listed for sediment and tissue are based on wet weight. The detection limits reported by the laboratory for sediment, calculated on dry weight basis as required by the contract, will be higher.

[c] Detection limits for sediment samples are based on the CRDL established for water samples.

μg/L = Micrograms per liter
mg/kg =Milligrams per kilogram

limit, a detection limit standard at that level will be required to verify instrument sensitivity and linearity. When required, the detection limit standard will be analyzed daily after the calibration standards. All calibration compounds will be detected and will be within 25 percent of the true value.

8.5 TOTAL PETROLEUM HYDROCARBONS-PURGEABLES AND AROMATIC VOLATILES

The procedures described in Appendix D of the Leaking Underground Fuel Tank (LUFT) field manual (CA 1989) and in SW-846 Method 8015A (EPA 1986) will be used for analysis of total petroleum hydrocarbons (TPH)-purgeables and the aromatic volatile compounds of benzene, toluene, ethylbenzene, and xylene (BTEX) (EPA 8020) in NAVSTA TI sediments and pore water samples. The purge-and-trap extraction technique, described in SW-846 Method 5030A (EPA 1986), will be used for preparation of samples. An inert gas is bubbled through a specially designed purging chamber, and the analytes are retained on a column until purging is complete. They are then released to the GC which is temperature-programmed to separate the analytes and uses a flame ionization detector (FID) and photoionization detector (PID) to detect the analytes. Hydrocarbons detected at retention times of less than those of dodecane (containing 12 carbon atoms--C12) and a pattern comparison to a gasoline standard will be used to identify gasoline hydrocarbons. Other unknown purgeable hydrocarbons may also be identified. The CRDL for TPH-purgeables is presented in Table 15.

8.6 TOTAL PETROLEUM HYDROCARBONS-EXTRACTABLES

The procedures described in Appendix D of the LUFT field manual (CA 1989) and in SW-846 Method 8015A (EPA 1986) will be used for analysis of TPH-extractables in NAVSTA TI sediment and pore water samples. A measured aliquot of sample is solvent-extracted using sonication (sediment) and a separatory funnel or continuous liquid-liquid extraction (pore water). The extract is solvent-exchanged and concentrated. The extract is directly injected onto a GC which is temperature-programmed to separate the analytes and uses a FID to detect the analytes. Retention times between

TABLE 15

MISCELLANEOUS ANALYSES
CONTRACT REQUIRED DETECTION LIMITS (CRDL)

	Sediment or Tissue[a]	Pore Water or Water
Analyte	**CRDL**	**CRDL**
AROMATIC VOLATILES		
Benzene	0.5 µg/kg	0.5 µg/L
Ethylbenzene	0.5 µg/kg	0.5 µg/L
Toluene	0.5 µg/kg	0.5 µg/L
Xylenes	1.0 µg/kg	1.0 µg/L
TPH-Purgeable	0.5 mg/kg	50 µg/L
TPH-Extractable	10 mg/kg	100 µg/L
OTHER PARAMETERS		
Total Organic Carbon	1 mg/kg	NM
Grain Size	0.0001 gram dry weight	NM
Sediment Oxygen Demand	0.1 mg/kg	NM
Sulfide	NM	0.01 mg/L
Salinity	NM	0 % salinity
Dissolved Oxygen	NM	0.1 mg/L
Ammonia (Total and unionized)	0.01 mg/kg	0.01 mg/L
Dissolved Organic Carbon	NM	1 mg/L

Notes:

[a] Detection limits listed for sediment are based on wet weight. The detection limits reported by the laboratory for sediment, calculated on dry weight basis as required by the contract, will be higher.

NA = Detection limits not applicable
NM = Parameter not measured
µg/L = Micrograms per liter
µg/kg = Micrograms per kilogram
mg/kg = Milligrams per kilogram
mg/L = Milligrams per liter

those of decane (containing 10 carbon atoms--C10) and n-tricosane (C23), as well as a pattern comparison to a diesel standard, will be used to identify diesel hydrocarbons. Motor oil (consisting of hydrocarbons eluting a retention time after n-tricosane [C23]) and other unknown extractable hydrocarbons may also be identified. The CRDL for TPH-extractables is presented in Table 15.

8.7 ADDITIONAL PARAMETERS

Sediment from NAVSTA TI will also be analyzed for sediment oxygen demand (SOD), total organic carbon (TOC), ammonia (total and unionized), and grain size. Water from Clipper Cove will be analyzed for lead only. Pore water will be analyzed for sulfides, ammonia (total and unionized), and dissolved organic carbon. The protocols for determination of TOC and sediment particle size are presented in Appendices B and C, respectively. The CRQLs for these analyses are presented in Table 15. Conductivity, temperature, pH, salinity, and dissolved oxygen will be measured in the field according to instrument manufacturer instructions.

8.8 BIOASSAY PROTOCOLS

Specific bioassay protocols are described in detail in the SOPs in Appendices E, G, J, and K. The SOPs will be provided to the laboratory chosen to perform the work. The general QA/QC and test procedures are discussed briefly below. Every test series with a particular organism should include one test chamber containing clean, inert material to serve as a negative control. The complete bioassay series must be repeated if more than 10 to 50 percent (depending on the respective toxicity test requirements as presented in Sections 8.8.1, 8.8.2, 8.8.3, and 8.8.4) of the control animals die or show evidence of sublethal effects. For sediment bioassays, sediment samples from an area known to be free of chemical contamination should be tested so that toxicant effects can be partitioned from unrelated effects such as those of sediment grain size. Inert material that mimics the grain size at the site can also be used by the laboratory. Reference toxicants, to serve as positive controls, shall be included in all bioassays. The bioassays include those for amphipod whole sediment, echinoderm development, polychaete whole sediment, and bivalve shell development and are described in the following sections.

8.8.1 Amphipod Whole Sediment Test

Test procedures for *Eohaustorius estuarius* are as follows (ASTM 1992; EPA 1994c):

Test Type:	Whole-sediment toxicity test, static
Temperature:	15 °C ± 2 °C
Salinity:	20 parts per thousand (ppt)
Light Quality:	Wide-spectrum fluorescent lights
Illuminance:	500 - 1,000 foot-candles (ft-c)
Photoperiod:	24L:0D
Test Chamber:	1-Liter (L) glass beaker or jar with an inner diameter of about 10 centimeters
Sediment Volume:	175 mL (2 cm)
Overlying Water Volume:	800 mL
Renewal of Overlying Water:	None
Size of Amphipods:	3 to 5 millimeters (mm)
Number of Organisms/Chamber:	20
Number of Replicates:	5
Feeding Regime:	None
Overlying Water Aeration:	Aerate overnight before start of test, and throughout to maintain greater than or equal to (≥) 90 percent saturation
Overlying Water Quality:	Temperature, dissolved oxygen, salinity, and pH in overlying water daily; ammonia (total and unionized) and sulfides at days 2 and 8 in the overlying water; and pH of pore water at beginning and end of test
Test Duration:	10 days
Endpoints:	Survival and reburial

Test Acceptability:	Minimum survival of 90 percent in controls and satisfaction of performance-based criteria as outlined below

Test acceptability requirements are as follows:

- The amphipods must range from 3 to 5 mm long at the end of the test.
- Average survival in the control sediment must be greater than or equal to 90 percent at the end of the test.
- Salinity, pH, and ammonia in the overlying water and sediment grain size must be within tolerance limits of *E. estuarius*.
- All organisms must be from the same population.
- Tests should begin within 10 days after receipt of amphipods.
- All test chambers should be identical and should contain the same amount of sediment and overlying water.
- Negative-control sediment must be included in a test.
- The daily mean temperature must be within $\pm$ 1 °C of the desired temperature. The instantaneous temperature must always be within $\pm$ 3° of the desired temperature.
- The dissolved oxygen must not fall below 6.0 mg/L in any chamber.
- Natural physico-chemical characteristics of the test sediment collected from the field should be within the tolerance limits of the test organisms.
- Treatments must be randomly assigned to individual test chambers which should be randomly placed within the test area.
- Test amphipods must be randomly distributed to test chambers.

The report should document the following information, in electronic and hard copy formats:

- Name of test and investigator(s), name and location of laboratory, and dates of start and end of test

- Source of negative control, reference sediment, and test sediment with the method of collection, handling, shipping, storage, and disposal of sediment
- Source of overlying water, its chemical characteristics, and a description of any pretreatment
- Results of any demonstration of the ability of the species to survive, grow, or reproduce in the overlying water
- Source, history, and age of test organisms
- Source and date of collection of the test organisms, scientific name, name of person who identified the organisms, and the taxonomic key used. In addition, age, life-stage, and means and ranges of weights and lengths should be provided. Observed diseases or unusual appearance, treatment, holding, and acclimation procedures should also be recorded.
- Source and composition of food, procedure used to prepare food, feeding method, frequency, and ration
- Description of the experimental design and test chambers used; the depth and volume of sediment and overlying water in the chambers; lighting; number of replicates and number of test organisms per replicate
- Records of temperature, salinity, pH, dissolved oxygen, and ammonia tested in overlying water and in pore water
- Biological endpoints used along with appropriate measurements
- A table of the biological data for each test including the control(s) in sufficient detail to allow independent statistical analysis
- Statistical test used and results of analysis of the data
- Results of reference toxicant tests
- Information on calibration of equipment and instruments used during the test
- Copy of the COC record, the sample log-in sheet, the original bench sheets for the test organism responses during the sediment test(s), and original signatures and dates of laboratory personnel performing the test(s)
- Summary of general observations made during the test
- Any deviations from the required procedures and any other relevant information

8.8.2 Echinoderm Development Test

The echinoderm development test will be used to test both the sediment pore water and the elutriate from petroleum-contaminated soils. A dilution series will be implemented for the analysis of the elutriate to calculate an effect concentration (EC)10, EC25, EC50, no observed effect concentration (NOEC), and lowest observed effect concentration (LOEC). These values provide information about the dose response relationship. Tests of single concentrations, providing pass/fail information, are used during the analysis of the sediment pore water, in which case the test concentration requirement is not applicable.

Test procedures for *Strongylocentrotus purpuratus* are as follows (Chapman and others 1995):

Test Type:	Pore water toxicity test, static, nonrenewal
Temperature:	15 °C ± 1 °C
Salinity:	34 ± 2 ppt
Light Quality:	Wide-spectrum fluorescent lights
Illuminance:	50 to 100 foot-candles (ft-c)
Photoperiod:	16L:8D
Test Chamber:	20 mL minimum
Test Solution Volume:	10 mL minimum
Renewal of Test Solution:	None
Age of Test Organisms:	Less than or equal to (≤) 1 hour embryos
Number of Organisms/Chamber:	25 per mL
Number of Replicates:	4
Test Concentrations:	5 concentrations for each site and control water
Dilution Series:	100 percent, 50 percent, 25 percent, 12.5 percent, and 6.35 percent

Feeding Regime:	None
Aeration:	None; dissolved oxygen must not fall below 4.0 mg/L
Overlying Water Quality:	Temperature daily; pH, salinity, and dissolved oxygen at beginning and end of test; ammonia (total and unionized) at beginning and end of test in 100 per cent pore water and control
Test Duration:	72 hours
Endpoints:	Normal development
Test Acceptability:	Greater than 80 percent normal development in controls, must achieve a percent minimum significant difference (MSD) of less than 20 percent in controls, and satisfaction of performance-based criteria as outlined below
Sample Volume Required:	500 mL of sediment pore water for one series of tests (approximately 1 gallon of sediment is required to produce this volume of sediment pore water)

Test acceptability requirements are as listed below. Performance criteria for testing are as follows:

- The age of the echinoderm larvae must be less than 1 hour at the start of the test.
- Average survival in the control must be greater than or equal to 80 percent at the end of the test.
- Salinity and ammonia in the test solution must be within tolerance limits of *S. purpuratus*.
- All organisms must be from the same population.
- Tests should begin within 10 days after receipt of echinoderms.
- All test chambers should be identical and should contain the same amount of test water.
- The daily mean temperature must be within $\pm$ 1°C of the desired temperature. The instantaneous temperature must always be within $\pm$ 3° of the desired temperature.
- The dissolved oxygen must not fall below 4.0 mg/L in any chamber.

- Treatments must be randomly assigned to individual test chambers which should be randomly placed within the test area.

- Test echinoderm larvae must be randomly distributed to test chambers.

The report should document the following information, either directly or by reference to available documents in electronic and hard copy formats:

- Name of test and investigator(s), name and location of laboratory, and dates of start and end of test

- Source of reference sediment and test sediment used for extraction of pore water with the method of collection, handling, shipping, storage, extraction, and disposal of sediment

- Source of test solution, its chemical characteristics, and a description of any pretreatment

- Results of any demonstration of the ability of the species to survive, grow, or reproduce in the test solution

- Source and history of test organisms

- Source and date of collection of the test organisms, scientific name, name of person who identified the organisms, and the taxonomic key used. Observed diseases or unusual appearance, treatment, holding, and acclimation procedures should also be recorded

- Source and composition of food, procedure used to prepare food, feeding method, frequency, and ration

- Description of the experimental design and test chambers used; the volume of water in the chambers; lighting; number of replicates and number of test organisms per replicate

- Records of temperature, salinity, pH, dissolved oxygen, and ammonia tested in test solution

- Biological endpoints used along with appropriate measurements

- A table of the biological data for each test including the control(s) in sufficient detail to allow independent statistical analysis

- Statistical test used and results of analysis of the data

- Results of reference toxicant tests

- Information on calibration of equipment and instruments used during the test
- Copy of the COC record, the sample log-in sheet, the original bench sheets for the test organism responses during the test(s), and original signatures and dates of laboratory personnel performing the test(s)
- Summary of general observations made during the test
- Any deviations from the required procedures and any other relevant information

8.8.3 Polychaete Whole Sediment Test

Test parameters for *Neanthes arenaceodentata* are as follows (Battelle Marine Sciences Laboratory 1994; PTI 1990):

Test Type:	Static renewal
Test Duration:	20 days
Temperature:	20 ± 1°C
Salinity:	28 ± 2 ppt
Light Quality:	Ambient laboratory
Light Intensity:	50 to 100 ft-c
Photoperiod:	24L/0D
Test Chamber:	1 L
Overlying Water Volume:	750 mL total volume
Sediment Depth:	2.0 cm (~210 mL)
Renewal of Test Solutions:	Every third day
Age of Test Organisms:	2 to 3 weeks post-emergence or dry weight of 0.5 to 1.0 gram
Number of Organisms per Chamber:	5

Number of Replicate Chambers per Treatment:	5
Number of Organisms per Treatment:	25
Feeding Regime:	Feed 8 mg (dry weight) of TetraMarin® as a slurry per worm every other day. Maintain dose regardless of mortality (40 mg per container).
Overlying Water Aeration:	Trickle-flow (less than 100 bubbles per minute)
Dilution Water:	Natural seawater or GP-2.
Test Treatments:	Site sediment and control sediment
Dilution Series:	Not applicable
Overlying Water Quality:	Ammonia (total and unionized), hydrogen sulfide, salinity, pH, and dissolved oxygen measured at the beginning and end of the test and every third day in at least one replicate. Temperature should be measured daily. Interstitial salinity should be measured at the beginning and end of test.
Endpoints:	Survival, biomass, and growth rate
Test Acceptability:	Minimum mean control survival of 90 percent. Survival in any single sediment replicate should not be less than 80 percent. Performance criteria specifications must be met.

Test acceptability requirements for (1) a 20-day sediment toxicity test with *N. arenaceodentata* and (2) test organisms suitability are listed below. Performance criteria for testing are as follows:

- Age of *N. arenaceodentata* at the start of the test must be between 2 and 3 weeks. Organisms must weigh between 0.5 to 1.0 gram dry weight.
- Average survival of *N. arenaceodentata* in the control sediment must be greater than or equal to 90 percent at the end of the test.
- The mortality in any sediment replicate should not exceed 20 percent.
- Temperature, salinity, pH, and dissolved oxygen in the overlying water within a treatment should not vary by more than 50 percent during the test.

Performance-based criteria for test organisms are as follows:

- All polychaetes should be from the same population and species.
- If the organism supplier does not have reference toxicant test data, then the testing laboratory must conduct five reference toxicant tests before conducting the bioassays. The laboratory must conduct reference toxicant tests on each batch of test organisms or once a month. The laboratory should perform monthly 96-hour water-only reference-toxicity tests to assess the sensitivity of organisms. If reference-toxicity tests are not conducted monthly, the lot of organisms used to start a sediment test must be evaluated using the reference toxicant cadmium chloride ($CdCl_2$).
- Records should be kept on the frequency of restarting cultures and the age of brood organisms.
- Laboratories should record the following water-quality characteristics of the cultures at least weekly and the day before the start of a sediment test: pH, salinity, temperature, and ammonia (total and unionized). Dissolved oxygen and temperature should be measured weekly.

Additional requirements are as follows:

- Tests must be started within 2 to 8 weeks after collection of the sediment.
- All test chambers should be identical and contain the same amount of sediment and overlying water.
- The daily mean test temperature must be within $\pm$ 1 °C of the desired temperature. The instantaneous temperature must always be within $\pm$ 3 °C of the desired temperature.
- The dissolved oxygen must not fall below 4.0 mg/L in any chamber.
- Treatments must be randomly assigned to individual test chambers which are also randomly placed in the testing area.
- Test polychaetes must be randomly distributed to test chambers.

The report should document the following information either directly or by reference to available documents:

- Name of test and investigator(s), name and location of laboratory, and dates of start and end of test

- Source of reference sediment and test sediment with the method of collection, handling, shipping, storage, and disposal of sediment.

- Source of overlying water, chemical characteristics, and a description of any pretreatment

- Source, history, and age of test organisms

- Source, history, and age of brood stock, culture procedure or source and date of collection of the test organisms, scientific name, name of person who identified the organisms, and the taxonomic key used. In addition, age, life-stage, means and ranges of weights and lengths should be provided. Observed diseases or unusual appearance, treatments, holding and acclimation procedures should also be recorded.

- Source and composition of food, procedure used to prepare food, feeding methods, frequency, and ration

- Description of the experimental design and test chambers used; the depth and volume of sediment and overlying water in the chambers; lighting; number of replicates and number of test organisms per replicate

- Records of temperature, salinity, pH, and ammonia; dissolved oxygen concentration (as percent saturation), and any aeration used prior to initiating the test and during the conduct of the test

- Biological endpoints used along with appropriate measurements

- A table of the biological data for each test including the control(s) in sufficient detail to allow independent statistical analysis

- Statistical tests used and results of analysis of the data

- Results of reference toxicant tests

- Information of calibration of equipment and instruments used during the test

- Copy of the COC record, the sample log-in sheet, and the original bench sheets for the test organism responses during the test(s), with original signatures and dates of laboratory personnel performing the test(s)

- Summary of general observations made during the test

- Any deviations from the required procedures and any other relevant information

8.8.4 Bivalve Shell Development Test

The bivalve shell development test is being used to test the elutriate from petroleum-contaminated soils as part of the procedure to develop cleanup goals for petroleum. A dilution series is used to calculate an EC10, EC25, EC50, NOEC, and LOEC and will be used to develop a cleanup goal. Test parameters for *Mytilus edulis* are as follows (Chapman and others 1995):

Test Type:	Static nonrenewal
Temperature:	18 °C ± 1 °C
Salinity:	30 ± 2 ppt
Light Quality:	Ambient laboratory light
Light Intensity:	50 to 100 ft-c
Photoperiod:	16L/8D
Test Chamber:	30 mL
Test Solution Volume:	10 mL
Renewal of Test Solution:	None
Age of Test Organism:	1.5- to 2.5-hour embryos
Number of Organisms/ Chamber:	150 to 300
Number of Replicates:	4
Test Concentrations:	100 percent, 50 percent, 25 percent, 12.5 percent, and 6.35 percent
Dilution Series:	0.5
Feeding Regime:	None
Aeration:	None; dissolved oxygen must not fall below 4.0 mg/L
Test Solution Water Quality:	Temperature, pH, salinity, and dissolved oxygen at the start of test and daily; ammonia (total and unionized) and sulfides at the beginning and end of test

Test Duration:	48 hours or until complete development up to 54 hours
Endpoints:	Survival and normal shell development
Test Acceptability:	Control survival must be ≥ 50 percent for mussels in control chambers; ≥ 90 percent normal shell development in surviving controls; and must achieve a percent minimum significant difference of less than 25 percent relative to the control for survival and normal shell development.
Sample Volume Required:	1 L per test

Test acceptability requirements for a 48-hour bivalve embryo development bioassay are listed below. Performance criteria for testing are as follows:

- The age of the mussel larvae must be from 1.5 to 2.5 hours old at the start of the test.
- Control survival must be greater than or equal to 50 percent.
- Normal shell development in surviving controls should be greater than or equal to 90 percent.
- A percent minimum significant difference of less than 25 must be achieved relative to the control relative to the control for survival and normal shell development.

Performance criteria for field collection are as follows:

- Reference-toxicant tests must be performed on each batch of field-collected mussels used in the tests.
- Mussels must not have spawned as a result of the collection and transportation.

Additional requirements are as follows:

- All organisms must be from the same population.
- All test chambers should be identical and should contain the same amount of test water.
- The daily mean temperature must be within ± 1 °C of the desired temperature. The instantaneous temperature must always be within ± 3 °C of the desired temperature.

- The dissolved oxygen must not fall below 4.0 mg/L in any chamber.
- Treatments must be randomly assigned to individual test chambers which should be randomly placed within the test area.
- Test mussel larvae must be randomly distributed to test chambers.

The report should document the following information either directly or by reference to available documents.

- Name of test and investigator(s), name and location of laboratory, and dates of start and end of test
- Source of reference sediment/soil and test sediment/soil used for making the elutriate with the method of collection, handling, shipping, storage, and disposal of sediment
- Source of test solution, chemical characteristics, and a description of any pretreatment
- Results of any demonstration of the ability of the species to survive, grow, or reproduce in the test solution
- Source and history of test organisms
- Source and date of collection of the test organisms, scientific name, name of person who identified the organisms, and the taxonomic key used. Observed diseases or unusual appearance, treatment, holding and acclimation procedures should also be recorded.
- Source and composition of food, procedure used to prepare food, feeding method, frequency, and ration
- Description of the experimental design and test chambers used; the volume of water in the chambers; lighting; number of replicates and number of test organisms per replicate
- Records of temperature, salinity, pH, dissolved oxygen, sulfides, and ammonia tested in test solution
- Biological endpoints used along with appropriate measurements
- A table of the biological data for each test including the control(s) in sufficient detail to allow independent statistical analysis
- Statistical test used and results of analysis of the data
- Results of reference toxicant tests

- Information on calibration of equipment and instruments used during the test
- Copy of the COC record, the sample log-in sheet, the original bench sheets for the test organism responses during the test(s), including original signatures and dates of laboratory personnel performing the test(s)
- Summary of general observations made during the test
- Any deviations from the required procedures and any other relevant information

For all bioassays, information regarding test organism source, condition, and mortality of test organisms upon arrival at the laboratory, maintenance of organisms in the laboratory before testing, light regime, and dates of test initiation and completion will be reported in addition to test results.

All treatment and bioassay containers should be randomized; testing should proceed without laboratory personnel knowing sample identities. Water quality variables such as salinity, dissolved oxygen, pH, and temperature should be measured at the beginning and end of testing to ensure that proper bioassay conditions have been maintained.

SOPs for the 10-day amphipod whole sediment toxicity test with *Eohaustorius estuarius*, 72-hour abnormal development test with *Strongylocentrotus purpuratus*, 20-day polychaete whole sediment toxicity test with *Neanthes arenaceodentata*, the 48-hour embryo-larval development toxicity test with *Mytilus edulis*, and extraction of pore water are attached as Appendices G, E, J, K, and F, respectively, of this QAPP and will be provided to the contract laboratory.

8.9 BIOACCUMULATION TEST PROTOCOLS

Specific protocols for the polychaete bioaccumulation test using *Nepthys caecoides* are described in detail in the SOP in Appendix I. The SOP will be provided to the laboratory chosen to perform the work. The general QA/QC and test procedures are discussed briefly below. Every test series with a particular organism should include one test chamber containing clean, inert material to serve as a negative control. The complete test series must be repeated if more than 20 percent of the control animals die or show evidence of sublethal effects. Reference toxicants, to serve as positive controls, shall be included in all tests.

Test parameters for this SOP are as follows (Battelle Marine Sciences Laboratory 1994):

Test Type:	Static renewal
Test Duration:	28 days
Temperature:	20 ± 1°C
Salinity:	20 ppt ± 2 ppt
Light Quality:	Ambient laboratory
Light Intensity:	10 to 20 $\mu E/m^2/s$ (50 to 100 ft-c)
Photoperiod:	16L/8D
Test Chamber Size:	37 L
Test Solution Volume:	16.1 L overlying water
Sediment Depth:	3 cm (3.9 L)
Renewal of Test Solutions:	Daily
No. Organisms per Test Chamber:	30
No. Replicate Chambers per Treatment:	5
No. Organisms per Treatment:	150
Feeding Regime:	None
Overlying Water Aeration:	Trickle-flow (< 100 bubbles/minute)
Dilution Water:	Natural seawater or GP-2
Test Treatments:	Site sediment and native sediment
Dilution Series:	Not applicable
Overlying water quality Monitoring:	Temperature, salinity, pH, and dissolved oxygen measured daily; ammonia measured at the beginning and end of test and weekly.
Endpoints:	Survival and bioaccumulation

Test Acceptability:	Minimum mean survival in native control sediment is not less than 80 percent; minimum mean survival of 70 percent in any single control sediment; adequate biomass at test completion for detection of target analytes; and performance criteria specifications as outlined in Section 10 of Appendix I.

Test acceptability requirements for a 28-day sediment toxicity test with *N. caecoides* are listed below. Performance criteria for testing are as follows:

- Size of *N. caecoides* at the start of the test must be the same.
- Average survival of *N. caecoides* in the native control sediment must be greater than or equal to 80 percent, and survival in any control sediment replicate must be at least 70 percent at the end of the test.
- Adequate biomass must be present at the test completion for detection of target analytes.
- Temperature, salinity, pH, and dissolved oxygen in the overlying water within a treatment should not vary by more than 50 percent during the test.

Performance-based criteria for culturing *N. caecoides* are as follows:

- All polychaetes should be from the same population and species.
- The laboratory should perform monthly 96-hour water-only reference toxicity tests to assess the sensitivity of culture organisms. If reference toxicity tests are not conducted monthly, the lot of organisms used to start a sediment test must be evaluated using the reference toxicant $CdCl_2$.
- Records should be kept on the frequency of restarting cultures and the age of brood organisms.

Additional requirements are as follows:

- All test polychaetes must be the same size.
- Tests must be started within 2 weeks after collection of the sediment.

- All test chambers should be identical and contain the same amount of sediment and overlying water.

- The daily mean test temperature must be within ± 2 °C of the desired temperature. The instantaneous temperature must always be within ± 3 °C of the desired temperature.

- The dissolved oxygen must not fall below 4.0 mg/L in any chamber.

- Treatments must be randomly assigned to individual test chambers.

- Test polychaetes must be randomly distributed to test chambers.

The test report should document the following information, either directly or by reference to available documents:

- Name of test and investigator(s), name and location of laboratory, and dates of start and end of test

- Source of reference sediment and test sediment with the methods of collection, handling, shipping, storage, and disposal of sediment

- Source of overlying water, its chemical characteristics, and a description of any pretreatment

- Results of any demonstration of the ability of a species to survive, grow, or reproduce in the overlying water

- Date and location of collection, scientific name, name of person who identified the organisms, and the taxonomic key used. Size of test organisms and any observed diseases or unusual appearance, treatments, holding, and acclimation procedures should also be provided.

- Description of the experimental design and test chambers used; the depth and volume of sediment and overlying water in the chambers; lighting; number of replicates and number of test organisms per replicate

- Records of temperature, salinity, pH, and ammonia; dissolved oxygen concentration (as percent saturation); and any aeration used prior to initiating the test and during the conduct of the test

- Biological endpoints used along with appropriate measurements

- A table of the biological data for each test including the control(s) in sufficient detail to allow independent statistical analysis
- Statistical tests used and results of analysis of the data
- Results of reference toxicant tests
- Information on calibration of equipment and instruments used during the test
- Copy of the COC record and sample log-in sheet. Original bench sheets for the test organism responses during the sediment test(s), with original dated signatures of laboratory personnel performing the test(s) should be included.
- Summary of general observations made during the test
- Any deviations from the required procedures and any other relevant information

9.0 INTERNAL QUALITY CONTROL CHECKS AND FREQUENCY

Internal quality control checks were developed to ensure accuracy and precision during field sampling and measurement as well as laboratory analysis. As described below, field checks will be conducted on a regularly scheduled basis. Laboratory checks will be conducted according to referenced analytical method protocols. A discussion of measurements and procedures for internal QC is presented in this section.

9.1 FIELD QUALITY CONTROL SAMPLES

Field QC samples are collected for laboratory analysis to check sampling and analytical precision, accuracy, and representativeness. The QC samples for this project include the samples described in the following sections and listed in Table 16. These samples are consistent with guidelines presented in the Navy QA requirements (NEESA 1988). Additionally, the requirements presented in this section meet or exceed those presented in regulatory guidance (EPA Region 9 1990).

9.1.1 Field Duplicate Samples

A field duplicate sample is collected at the same time and from the same source as the original sample but submitted to the laboratory separately to assess the accuracy of the overall sampling and analytical system. However, field duplicates are not collected for sediment samples and, therefore, will not be collected for this project.

9.1.2 Field Blanks

Field blanks consist of the source water used in detergent wash and steam cleaning decontamination activities. San Francisco Bay water will be used for decontamination procedures during the investigation. This water may be obtained at taps or fire hydrants as approved by the public works department. Distilled water, obtained from a commercial supplier, will be used for the final rinse of all sampling equipment. This water will be used to collect field blanks. At a minimum, one field blank from each sampling event will be collected and analyzed for the same parameters as the samples collected during the event. A sampling event is defined as a period of time during which drilling and sampling activities occur. An extended absence followed by a return to the site would constitute two events.

9.1.3 Equipment Rinsate Blanks

Equipment rinsate blanks are used to evaluate sampling device cleanliness. The equipment rinsate blanks will be collected after a sample collection device is subjected to standard decontamination procedures. Appropriate water for the intended analysis will be poured over or through the sampling device, collected in a sample container, and sent blind to the laboratory for analysis. Deionized distilled water will be used and will be analyzed for all parameters that were collected using the equipment. The equipment rinsates will be collected for 1 in every 10 sampling locations.

TABLE 16

FIELD QUALITY CONTROL SAMPLES

Sample Type	Frequency of Sampling and Analysis
Field Duplicate	Not collected for sediment/tissue/pore water 10% for water samples
Referee Duplicate	Variable[a]
Field Blank	1/event for all analytes[b]
Equipment Rinsate Blank	1/10 sampling locations
Trip Blank[c]	NC

Notes:

[a] Referee duplicates will be collected and sent to the referee QA laboratory only in the case where regulatory agencies collect split samples or if a special problem occurs in sample collection or analysis.

[b] A sampling event is defined as a period of time during which drilling and sampling activities occur. An extended absence, followed by a return to the site (between bimonthly sampling rounds, for example) would constitute two events.

[c] Trip blanks accompany water VOC samples only. Not collected (NC) in this project.

9.2 FIELD MEASUREMENT QUALITY CONTROL PRACTICES

Field measurements will be conducted as part of the sampling program. Results from field measurements and QC checks will be compared to the PARCC criteria presented in Section 3.0. If the results fail to meet the objectives set for data quality, calibration of the instrument in question will be conducted as described in Section 6.1. Should discrepancies continue, general maintenance tasks listed in Section 7.1 will be performed. If the instrument still fails to meet the QC criteria, it will be removed from service and replaced.

9.3 LABORATORY QUALITY CONTROL SAMPLES

In addition to the QC samples collected in the field, the laboratory will analyze other QC samples that measure the laboratory's analytical accuracy, precision, and representativeness. Laboratory QC samples will be analyzed at the frequency specified in the analytical methods employed. The following laboratory QC samples will be analyzed as appropriate. The specific schedule for the analysis of laboratory QC samples will be included in the laboratory QA plan or in laboratory SOPs.

9.3.1 Method Blanks

Method blanks provide a measure of the combined contamination from the laboratory water, the instrument, the reagents, and the sample preparation steps. They are subjected to the entire preparation process. Method blanks will be analyzed at a frequency established by the referenced analytical methods. The method blanks aid in distinguishing between low-level field contamination and laboratory contamination. Concentrations that are suspected to be the result of laboratory contamination will be evaluated as part of the data validation process.

9.3.2 Matrix Spike, Matrix Spike Duplicate, and Matrix Duplicate Samples

MS/MSD samples will be selected in advance by the FTL in coordination with the laboratory to ensure ample sample volume. MS/MSD pairs (organic) or MS and matrix duplicate (inorganic) will be analyzed at a rate of 5 percent, or at a frequency of one for every SDG, or type of matrix, or 20 samples, whichever is more frequent (EPA 1987). MS/MSD sediment samples will be obtained from

the same container as the original sample. MS/MSD samples for pore water will be determined by the testing laboratory based on volumes of extracted pore water.

Before sample preparation at the laboratory, the organic MS/MSD and inorganic MS samples will be spiked with appropriate analytes and analyzed according to the referenced method. Results from the analysis of the MSD and matrix duplicate samples will be used to evaluate the effect of the matrix on precision and accuracy. The percent recoveries will be calculated for each of the spiked analytes detected and used to assess analytical accuracy in the MS analysis. The RPD between MS and MSD samples or original and matrix duplicate samples will be calculated and used to assess analytical precision.

9.3.3 Laboratory Control Samples

The LCS or blank spike sample is spiked with a known concentration of target analytes added to a controlled interference-free matrix. The LCS is used to measure the laboratory accuracy in the absence of matrix interferences. In general, an LCS will accompany each MS/MSD (organic) or MS (inorganic) sample analysis.

9.4 BIOASSAY QUALITY CONTROL

This section discusses the use of negative and positive controls to be used for QC of bioassay analyses.

9.4.1 Negative Controls

All bioassays must be conducted using negative (clean) controls. Such controls are clean, nontoxic sediment samples taken from outside each study area or using inert sediment or water. The sediment used for the negative control will duplicate conditions such as grain size and temperature in the sample sediment to ensure that other factors are not responsible for additional biological effects. For every test series with a particular organism, one bioassay test chamber or series of chambers must contain clean material. Bioassay analysis should be repeated if more than a specified percentage of corresponding control organisms exhibits the response of interest.

The water used for the negative control will consist of artificial sea water of approximately the same temperature, salinity, and dissolved oxygen content as the water at the sites to be tested. The artificial sea water will be prepared using ACS reagent grade chemical salts and deionized water. The recipe for synthetic GP-2 sea water was published by the U.S. Environmental Protection Agency (EPA 1988) and is listed in Table E-1 of Appendix E. Salinity adjustments are made, if necessary, with distilled water (to decrease salinity) or a brine prepared from distilled water and artificial sea salts (to increase salinity). Dissolved oxygen is maintained above 40 percent saturation.

9.4.2 Positive Controls

All bioassays will be conducted using well-established positive (toxic) controls. Reference toxicants are used to provide information on mortalities or increased sensitivity that may occur as a result of disease, changes in tolerance sensitivity, or loading density. The laboratory must have a reference toxicant control chart for each source of the test organisms, which may be obtained from the organism supplier. In addition, the testing laboratory must conduct reference toxicity test for each new batch of test organisms or one per month, whichever comes first.

Standard laboratory procedures must be followed in all testing. These include proper documentation, proper cleaning, avoidance of contamination, and maintenance of appropriate test conditions. All unusual observations or deviations from the established procedures described in the protocols in Appendices E, G, J, and K must be recorded and reported.

9.4.3 Assessment of Extended Storage Time

Because of the nature of this project the sediment and sediment pore water samples used for toxicity testing may be stored for a period of 8 weeks until the chemistry results have been received from the analytical laboratory. If extended storage of the samples is required, split samples from five sampling locations will be used to assess the effect of the extended holding time for both sediments and sediment pore water, as stated in the work plan (see Section 7.1.2.3). The split samples will be prepared by extracting a double volume of pore water. The chemical and biological tests will immediately be performed using half of the split pore water sample. The other half of the split pore water sample will be frozen and analyzed at the end of the 8-week holding period.

10.0 DATA REPORTING, VALIDATION, AND USABILITY

Sections 10.1 and 10.2 discuss the requirements and methods for data reporting, and data verification and validation. Section 10.3 discusses the process for reconciling the data generated with the DQOs for the task.

10.1 DOCUMENTATION AND RECORDS

Documentation of all activities is critical for evaluating the success of any activity. The following sections discuss the requirements for laboratory data packages. Field documentation, including sample handling and custody requirements, is discussed in Section 5.1.

10.1.1 Laboratory Documentation and Records

All NAVSTA TI data will be reported in full, definitive data package format. The documentation requirements for these data packages are designed to provide information similar to that in CLP data packages, which present laboratory documentation required for data validation. The following subsections discuss the requirements of laboratories for preparing definitive data packages. All requirements for analytical data can be found in the PRC SOW.

Each definitive data package will consist of a case narrative, copies of all associated COC records, sample results, QA/QC summaries, and all associated raw data for the samples in one SDG. Data qualifiers described in the CLP SOW for organic and inorganic analyses, will also be included. For ease of use, the data packages should be separated by analysis and bound, if possible. The subcontract laboratory will provide PRC with up to two copies of the data package within 35 days of receipt of the last sample in the SDG.

In general, definitive data package requirements include, but are not limited to, the categories of information listed below.

- Sample custody
- Instrument performance

- Parameter identification and quantitation
- Sample integrity, precision, and accuracy (QC checks)

10.1.1.1 Summary Data Package

The CLP and CLP-type summary data packages will be required for all analyses and will contain sample results (Form I) and all QA/QC summary forms (Forms II through X for organics and Forms II through XIV for inorganics) for all associated samples in one SDG. Form I will include all sample results, corrected for dilution and soil moisture as appropriate. If the client sample identifier has been truncated because of software limitations, the complete sample identifier will appear on Form I — either in the comments form or by being hand-printed. Data qualifiers, as described in the CLP SOW for organics analysis and inorganics analysis, will be included.

An SDG is a group of 20 or fewer samples, for the same project order, that are received over a period of 14 days or less. An SDG is mainly a reporting format and is not limited to sample receipt groups, preparation batches, or analytical batches. The SDG name will be a unique number that is not an actual sample identifier or a part of an actual sample identifier. Data for all samples in the SDG will be submitted concurrently. Partial submittals are unacceptable. The subcontractor will provide PRC with two copies of the summary data package within 28 days of receipt of the last sample in the SDG; this package will be part of the standard analytical service set forth herein (at no additional cost). When a full data package is also required, the summary data package will be provided to PRC within 35 days of receipt of the last sample in the SDG.

The subcontractor will prepare summary data packages in accordance with instructions provided in Section II.D, Exhibit B, in the CLP SOWs for organics analysis and inorganics analysis. For ease of use, the summary data packages should not be bound but should be separated by analysis. The summary data package will consist of a case narrative, copies of all associated COC records, sample results, and QA/QC summaries. The case narrative will include the following information:

- Subcontractor name; project name; CTO (project) number; project order number; SDG number; and a table that cross-references client and laboratory sample identifiers

- Detailed documentation of all sample shipping/receiving, preparation, analytical, and quality deficiencies, including analyses performed without an A2LA-certified standard
- Thorough explanation of all instances of manual integration
- Carbon ranges for each sample TPH-purgeables and/or TPH-extractables
- Tabular presentation of the pH of each sample for which volatiles analyses are performed
- Copies of all associated nonconformance and corrective action forms, which will describe the nature of the deficiency and the corrective action taken
- Copies of all associated sample receipt notices

The following outlines describe the format of the summary data package for organics and inorganics analyses.

<u>Summary Data Package — Organics Analyses</u>

<u>Section I</u> **Case Narrative**

1. Case narrative
2. Copies of nonconformance/corrective action forms
3. COC forms
4. Copies of sample receipt notices
5. Internal tracking documents, as applicable

<u>Section II</u> **Sampling Results** — Form I for the following —

1. Environmental samples, including dilutions and reanalyses
2. Tentatively identified compounds (TICs) (Volatile organic analysis [VOA] and semivolatile organic analysis [SVOA] only)

<u>Section III</u> **QA/QC Summaries** — Forms II through VIII for the following —

1. System monitoring compound/surrogate recoveries (Form II)
2. MS/MSD recoveries and RPDs (Forms I and III)
3. Blank spike or LCS recoveries (Forms I and III-Z)
4. Method blanks (Forms I and IV)
5. Performance check (Form V)
6. Initial calibrations, with retention time information (Form VI)
7. Continuing calibrations, with retention time information (Form VII)
8. Quantitation limit standard (Form VII-Z)
9. Internal standard areas and retention times (Form VIII)
10. Analytical sequence (Forms VIII-D and VIII-Z)

11. Gel permeation chromatography (GPC) calibration (Form IX)
12. Single component analyte identification (Form X)
13. Multicomponent analyte identification (Form X-Z)
14. Matrix-specific MDL (Form XI-Z)

Summary Data Package - Inorganics Analyses

Section I **Case Narrative**

1. Case narrative
2. Copies of nonconformance/corrective action forms
3. COC forms
4. Copies of sample receipt notices
5. Internal tracking documents, as applicable

Section II **Sampling Results** — Form I for the following —

1. Environmental samples (including dilutions and reanalyses)

Section III **QA/QC Summaries** — Forms II through XIV for the following —

1. Initial and continuing calibration verifications (Form II)
2. Contract required detection limit (CRDL) standard (Form II)
3. Detection limit standard (Form II-Z)
4. Method blanks, continuing calibration blanks, and preparation blanks (Form III)
5. Inductively coupled plasma (ICP) interference check samples (Form IV)
6. MS and post-digestion spikes (Forms V and V-Z)
7. Sample duplicates (Form VI)
8. LCSs (Form VII)
9. Method of standard additions (Form VIII)
10. ICP serial dilution (Form IX)
11. Instrument detection limits (IDLs) (Form X)
12. ICP interelement correction factors (Form XI)
13. ICP linear range (Form XII)
14. Preparation log (Form XIII)
15. Analysis run log (Form XIV)

10.1.1.2 Full Data Package (CLP and CLP-Type)

For the NAVSTA TI project, full data packages will be required; this requirement will be stated on the project order issued by PRC. Full data packages will contain all of the information from the summary data package and all associated raw data for the associated samples in one SDG. Data qualifiers, as described in the CLP SOW for organics analysis and inorganics analysis, will be

included. When a full data package is required, the subcontractor will provide PRC with up to two copies of the full data package within 35 days of receipt of the last sample in the SDG and will be required to provide these in a manner that is consistent with the specifications set forth herein. Unless otherwise requested, the subcontractor will deliver one copy of the full data package.

The subcontractor will prepare full data packages in accordance with instructions provided in (1) Section II.E, Exhibit B of the CLP SOW for organics analysis (EPA 1994d) and (2) Section II.D, Exhibit B of the CLP SOW for inorganics analysis (EPA 1995). For ease of use, the full data packages should be separated by analysis and bound, if possible. The full data package will consist of a case narrative, copies of all associated COC records, sample results, QA/QC summaries, and all associated raw data. The following outlines describe the format of the full data package for organics and inorganics analyses.

Full Data Package — Organics Analyses

Sections I, II, and III **Summary Package**

Section IV **Sample Raw Data** — indicated form plus all associated raw data

1. Environmental samples, including dilutions and reanalyses (Forms I and X)
2. TICs (Form I - VOA and SVOA only)

Section V **QC Raw Data** — indicated form plus all associated raw data

1. Method blanks (Form I)
2. MS/MSD samples (Form I)
3. Blank spikes or LCSs (Form I)

Section VI **Standard Raw Data** — indicated form plus all associated raw data

1. Performance check (Form V)
2. Initial calibrations, with retention time information (Form VI)
3. Continuing calibrations, with retention time information (Form VII)
4. Quantitation limit standard (Form VII-Z)
5. GPC calibration (Form IX)

Section VII **Other Raw Data**

1. Percent moisture for soil samples
2. Sample extraction and cleanup logs
3. Instrument analysis log for each instrument used (Form VIII-Z)

4. Standard preparation logs, including initial and final concentrations for each standard used
5. Formula and a sample calculation for the initial calibration
6. Formula and a sample calculation for water and soil sample results

Full Data Package — Inorganics Analyses

Sections I, II, III **Summary Package**

Section IV **Instrument Raw Data** — Sequential measurement readout records for ICP, graphite furnace atomic absorption (GFAA), flame atomic absorption (AA), cold vapor atomic absorption (CVAA), mercury, cyanide, and other inorganics analyses, which will include the following information:

1. Environmental samples, including dilutions and reanalyses
2. Initial calibration
3. Initial and continuing calibration verifications
4. Detection limit standard(s)
5. Method blanks, continuing calibration blanks, and preparation blanks
6. ICP interference check samples
7. MS and postdigestion spikes
8. Sample duplicates
9. LCSs
10. Method of standard additions
11. ICP serial dilution

Section V **Other Raw Data**

1. Percent moisture for soil samples
2. Sample digestion, distillation, and/or preparation logs
3. Instrument analysis log for each instrument used
4. Standard preparation logs, including initial and final concentrations for each standard used
5. Formula and a sample calculation for the initial calibration
6. Formula and a sample calculation for water and soil sample results

10.1.1.3 Electronic Data Deliverable

EDDs are required for all NAVSTA TI analytical results. The EDD format and specifications are detailed in Task 8 of the PRC SOW. The subcontract laboratory will provide PRC with up to two copies of the EDD within 35 days of receipt of the last sample in the SDG.

An automated LIMS must be used to produce the EDD. Manual creation of the deliverable (that is, data entry by hand) is unacceptable. The subcontract laboratory will verify all EDDs internally before

issue. The EDD will exactly correspond to the hard copy data, and no duplicated data shall be submitted.

An EDD will be submitted for the results of the bioassay tests. However, due to the nature of the bioassay tests and because instrumentation is not usually automated, manual creation of the EDD may be required. The laboratory will be required to either 1) generate the hardcopy and EDD from the same source or 2) provide proof that the EDD has been verified.

10.1.1.4 Storage and Disposal

Electronic and hard copy data will be retained for a minimum of 3 and 10 years, respectively, after final data submittal. The subcontractor will use a magnetic tape storage device or other similar storage device that is capable of recording data for long-term, off-line storage. All raw data will be retained on magnetic tape in accordance with the appropriate instrument.

10.1.2 Field Data

Daily, the weather conditions and sediment temperature are recorded in the field log book. In addition, the sediment temperature is recorded on the COC forms for the pore water extraction laboratory. The pore water extraction laboratory, in its extraction of pore water, maintains the refrigerated centrifuge at the temperature of the sediment at the time of collection, thereby, minimizing degradation of the contaminants.

10.1.3 Tissue Residue Data

The laboratory reporting requirements for the tissue residue data are as follows:

- The laboratory will deliver a case narrative that includes a summary of any QC, sample, shipment, or analytical problems; how the tissue was prepared; and documentation of all internal decisions. Included in this document will be an outline of problems encountered and a description of solutions implemented. A copy of the signed COC form for each group of samples will also be included in the narrative packet.

- The sample concentrations will be reported on standard data sheets in proper units and to the appropriate number of significant figures (1 significant figure for concentrations less than 10, and 2 significant figures for concentrations greater than 10). For undetected values, the lower limit of detection of each compound will be reported separately for each sample. The date of sample analysis must be included.
- The surrogate percent recovery will be summarized for all organic analyses.
- The MS/MSD results will be reported.
- A summary of the method blank analysis will be reported.
- All check standard data will be reported.
- The results of all replicate samples will be reported.
- All sample concentration results will be reported on an EDD as specified in Section 10.1.1.3.

The data will be compared to the project DQO to determine whether the data are sufficient for project tasks.

When conducting organic analyses using GC/MS or the high resolution/gas chromatography mass spectrometer (HR/MS), the laboratories will also report and tentatively identify the 10 largest peaks in addition to those specified by the method as target analytes.

Sample holding times will be calculated by comparing the date of the sample collection shown on the summary sampling logs with the date of the sample analysis (and extraction, when appropriate) presented with sample results. Certificates of analysis with complete sample results will be provided by laboratories within 35 days after the laboratory receives each sample group.

10.2 DATA VALIDATION AND VERIFICATION REQUIREMENTS

One hundred percent of the data for the NAVSTA TI project will be reviewed and verified prior to input into the database. All of the analytical data will undergo cursory validation, and a minimum of 10 percent will be randomly selected for full validation. More than 10 percent of the data will be selected for full validation when severe analytical problems are observed. Full validation will also be

performed on the first data package submitted by the laboratory for each new analytical method or when a significant break, of 3 or more months, in field activities has occurred.

The validation and verification of the data generated during field activities is essential to ensuring that the data are defensible and of acceptable quality. Sections 10.2.1 and 10.2.2 present verification methods for field and laboratory activities. Validation of analytical data is further discussed in Section 10.2.3.

10.2.1 Field Data Verification

Field personnel will review field data to identify inconsistencies or anomalous values. Any inconsistencies discovered will be resolved immediately, if possible, by seeking clarification from the personnel responsible for data collection. All field personnel will be responsible for following the sampling and documentation procedures described in the FSP and this QAPP to ensure that defensible and justifiable data are obtained.

10.2.2 Laboratory Verification of Data

Laboratory personnel will assess data at the time of analysis and reporting through a review of raw data for any nonconformances in analytical method protocols. Detailed procedures for laboratory validation and corrective action are described in the laboratory's QA plan. The laboratory QA plan discusses sample control, methods of analyses, calibration procedures, document control, QC, corrective actions, QC checks, QA, and data review.

10.2.3 Analytical Data Validation

Data validation is a systematic process of reviewing and qualifying data against a set of criteria to ensure that the data are adequate for their intended use. During the validation process, all results will be identified as either acceptable for use, estimated and acceptable for limited use, or rejected and unacceptable for use. Results considered rejected will be retained in the database but will not be used in quantitative evaluations. Estimated and rejected data can result from improper sampling or analytical methodology, matrix interferences, errors in data transcription, and changes in instrument

performance. Erroneous results found during data validation will be identified and corrected. Validation methods for field and laboratory data are presented below.

Data validation will be performed by one or more of the following firms: Maxwell/S-Cubed, Alexandria, Virginia; ICF Kaiser Engineers, Inc., of San Francisco, California; Luce and Associates of Seattle, Washington; Quantalex, Inc., of Lakewood, Colorado; and Triangle Laboratories of Dublin, Ohio. Data validation will be performed by a subcontractor independent of the project laboratory. At a minimum, data will be validated in accordance with the CLP criteria outlined in the following documents:

- "National Functional Guidelines for Inorganic Data Review" (EPA 1994a)
- "National Functional Guidelines for Organic Data Review" (EPA 1994b)

The validation procedure for non-CLP methods includes (1) reviewing the requirements of the analytical method and this project QAPP, (2) reviewing the data package to ensure that the requirements of the analytical methods and this QAPP were met, and (3) reviewing the data package against applicable criteria from the EPA functional guidelines. Although the functional guidelines do not specifically address analyses other than CLP methods, the general concepts presented in the guidelines can be applied to numerous analytical methods.

Bioassay test data can also be validated versus a set of criteria to ensure that the data are adequate for their intended use. For bioassay data, the validation relies more on evaluating strict compliance with the conditions specified by the methodology. The methods being used for bioassay test during the Phase II ecological investigation are included in Appendices E through K. The document Guidance for Assessing the Validity of Biological Data (PRC 1996b) is useful in establishing criteria against which to validate the data.

10.2.3.1 Cursory Data Validation

Cursory validation shall be performed on the data summary packages for analyses of sediment and porewater samples by CLP and non-CLP methods. The data reviewer is required to notify PRC and request any missing information needed from the laboratory. The elimination of data from the review

process is not allowed. All data will continue through the validation process and be qualified and requalified as many times as they fail to meet the established criteria. Data summary packages consist of sample results and QA/QC summaries (equivalent to CLP Forms I through X for organic analyses, and Forms I through XIV for inorganic analyses), including calibration and internal standard data. There are no guaranteed minimum number of samples for a sample data group; however, the maximum number of samples shall not exceed 20.

10.2.3.2 Full Data Validation

Full validation shall be performed on data packages for analyses of sediment and porewater samples by CLP and non-CLP methods. The data reviewer is required to notify PRC and request any missing information needed from the laboratory. The elimination of data from the review process is not allowed. All data will continue through the validation process and be qualified and requalified as many times as they fail to meet the established criteria. Full validation shall be required on approximately 10 percent of a sample data group. Full data validation shall not be required for all project orders. Data packages consist of sample results, QA/QC summaries (equivalent to CLP Forms I through X for organic analyses, and Forms I through XIV for inorganic analyses), and all raw data associated with the sample results and QA/QC summaries.

10.2.3.3 Data Validation Criteria

The QC criteria for both cursory and full validations are as follows:

<u>Cursory Data Validation</u>

1. CLP Organic Analyses

- Holding times
- Calibration
- Blanks
- Surrogate recovery
- MS/MSD recovery
- Blank spike or LCS recovery
- Internal standard performance
- Overall assessment of data for an SDG
- Field duplicate sample analysis

2. CLP Inorganic Analyses

- Holding times
- Calibration
- Blanks
- LCS
- Matrix spike recovery
- Matrix Duplicate sample analysis
- Field Duplicate sample analysis
- ICP serial dilution
- Overall assessment of data for an SDG

3. Non-CLP Organic Analyses

- Method compliance
- Holding times
- Calibration
- Blanks
- Surrogate recovery
- MS/MSD recovery
- LCS or blank spike
- Internal standard performance
- Other laboratory QC specified by the method
- Overall assessment of data for an SDG
- Field duplicate sample analysis

4. Non-CLP Inorganic and Physical Analyses

- Holding times
- Calibration
- Blanks
- MS/MSD recovery
- Internal standard performance
- LCS
- Field duplicate sample analysis
- Other laboratory QC specified by the method
- Overall assessment of data for an SDG

<u>Full Data Validation</u>

1. CLP Organic Analyses

- Holding times
- GC/MS tuning
- Calibration
- Blanks
- Surrogate recovery

- MS/MSD recovery
- Internal standard performance
- Target compound list (TCL) identification
- Compound quantitation and reported detection limits
- TIC
- Field duplicate sample analysis
- Blank spike or LCS analysis
- System performance
- Overall assessment of data for an SDG

2. CLP Inorganic Analyses

- Holding times
- Calibration
- Blanks
- ICP interference check sample (ICS)
- LCS
- Matrix spike recovery
- Field duplicate sample analysis
- Matrix duplicate sample analysis
- Graphite furnace atomic absorption QC
- Sample result verification
- ICP serial dilution
- Overall assessment of data for an SDG

3. Non-CLP Organic Analyses

- Method compliance
- Holding times
- Calibration
- Blanks
- Surrogate recovery
- MS/MSD recovery
- LCS or blank spike
- Internal standard performance
- Field duplicate sample analysis
- Other laboratory QC specified by the method
- Detection limits
- Compound identification
- Compound quantitation
- Sample results verification
- Overall assessment of data for an SDG

4. Non-CLP Inorganic and Physical Analyses

- Holding times
- Calibration

- Blanks
- MS/MSD recovery
- Internal standard performance
- LCS
- Field duplicate sample analysis
- Other laboratory QC specified by the method
- Detection limits
- Analyte identification
- Analyte quantitation
- Sample results verification
- Overall assessment of data for an SDG

NAVSTA TI project personnel will review the data validation reports from the validation subcontractor to assess whether chemical measurement DQOs were met and to determine whether the data are usable for their intended purpose.

10.3 RECONCILIATION WITH DATA QUALITY OBJECTIVES

The DQO process is the scientific and statistical evaluation of data to determine if the data are of the right type, quality, and quantity to support their intended use. After various analytical and toxicological parameters are analyzed, the data will be subjected to various statistical tests as discussed in EPA (1993). Two questions will be addressed:

- Can a decision concerning the toxicity of a sampling location be made with the desired level of confidence, given the quality of the data set?

- How well does the sampling design proposed for this project perform over a wide range of possible outcomes? If this same sampling design were used again for a similar screening process at another Naval facility, would the data be expected to support the same intended use with the desired level of confidence, especially if the measured parameters were lower or higher than those observed in the current project?

Also, this process examines the uncertainty in the measured parameters due to sampling design error and measurement error. In addition, the DQO process will determine if the data provide conclusive evidence or if the evidence are inconclusive. The procedures to be followed are discussed in more detail in EPA (1993).

10.4 DATA ACQUISITION REQUIREMENTS

Analytical data will be reported following formats established by the PRC SOW and within the required deliverable schedule. All data from subcontract laboratories will be presented in a CLP hard copy or equivalent data package format and in EDD format as detailed in the PRC SOW.

The EDD submitted from the laboratory is an ASCII file of the results and sample identification information downloaded from the LIMS into a specific file structure. After the data files have been checked for completeness and proper structuring, the EDD is imported into the NAVSTA TI database. Validation qualifiers and changes to the data are entered manually once the validation reports have been completed and reviewed.

10.5 DATA MANAGEMENT

Field and analytical data collected from the phase II EA are critical to the completion of all site characterization efforts, comprehensive conceptual model development, risk assessments, and the selection of remedial actions to protect the environment. Data associated with laboratory analysis include laboratory hardcopy reports, EDD files, additonal backup data not submitted by the laboratory, and data validation reports. Data associated with field work include logbooks, boring logs, well sampling forms, well construction and development forms, other forms used to record field activities, and field analysis logs. An information management system is needed to ensure efficient access to this data so that the goals of real-time and on-site decision making can be achieved. This section describes the strategy to achieve short- and long-term data management goals.

10.5.1 Data Management Scheme

To satisfy long-term data management goals, after review and validation of the laboratory data, the data will be loaded into the database system at PRC for storage, further manipulation, and retrieval. Data collected in the field will also be verified and entered into the database for storage and future retrieval; however some information, such as field logbooks, will only be stored as hardcopy information. The database will be used to provide data for contamination and geologic analyses and for preparing reports and graphic representations of the data. The database files are generally

installation or project-specific to facilitate the tracking and association of the data. Figure 6 summarizes the data management scheme. Laboratory data packages and reports will be archived at PRC or Navy offices. The remaining data will be archived at the subcontract laboratory for a minimum of 10 years. All field data, forms, and logbooks will be archived with the project files at PRC or Navy offices. The laboratory and field data will be stored at PRC until the end of the CLEAN contract, at which time the files will revert to the Navy.

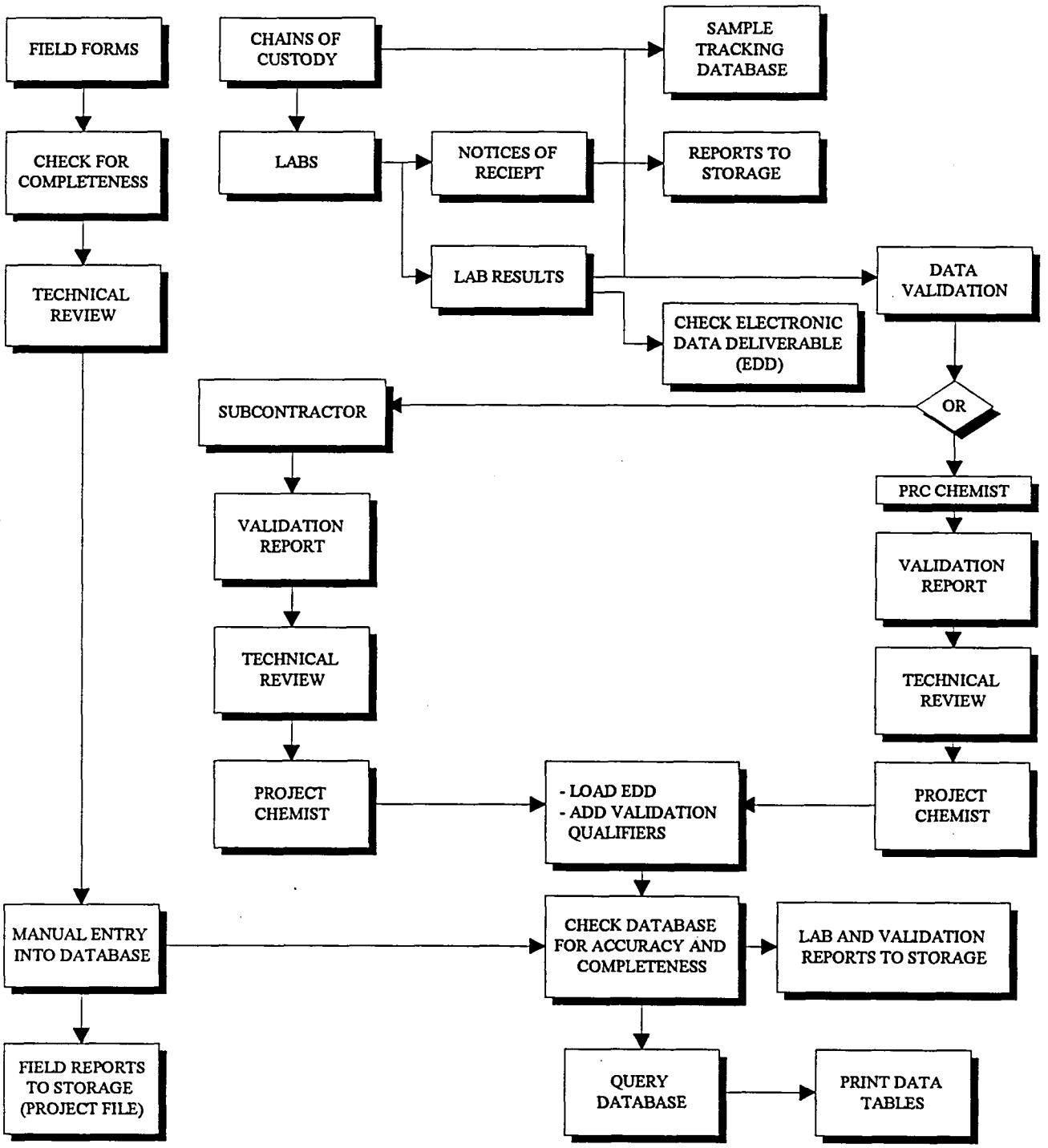
10.5.2 Data Management Strategy

Short- and mid-term strategies for data management require that the NAVSTA TI data set be updated monthly. These data consist of chemical data from Navy contractors. From this database, report data tables in various formats may be generated as well using available computer-aided drafting and design and contouring software.

11.0 PERFORMANCE, SYSTEM, AND FIELD AUDITS

An audit evaluates the capability and performance of a measurement system or its components, and identifies problems warranting correction. Three types of audits may be conducted during execution of this project: performance, system, and field audits. Audits will be performed at scheduled intervals by the QA program manager, project QA officer, or senior technical staff. At a minimum, one audit per year will be performed. All auditors will be independent of the activities audited. Technical expertise and experience in auditing will be considered in the selection of an auditor or audit team.

Audits may include reviews of project plan adherence, training status, health and safety procedures, activity performance and records, budget status, QC data, calibrations, conformance to SOPs, and compliance with laws, regulations, policies, and procedures. The QA program manager will coordinate a management review of any deficiencies that are noted.



Naval Station Treasure Island
Data Management Scheme
Figure 6

The auditor or audit team will issue a corrective action request to identify and schedule specific corrective actions to be undertaken and completed by the project manager. Completion of corrective action is verified by the auditor or the audit team. After acceptance and verification of all corrective actions, the corrective action request form will be used to close the audit. Corrective action is discussed in detail in Section 12.0. A flow chart depicting the QA audit pathways is presented as Figure 7.

11.1 PERFORMANCE AUDITS

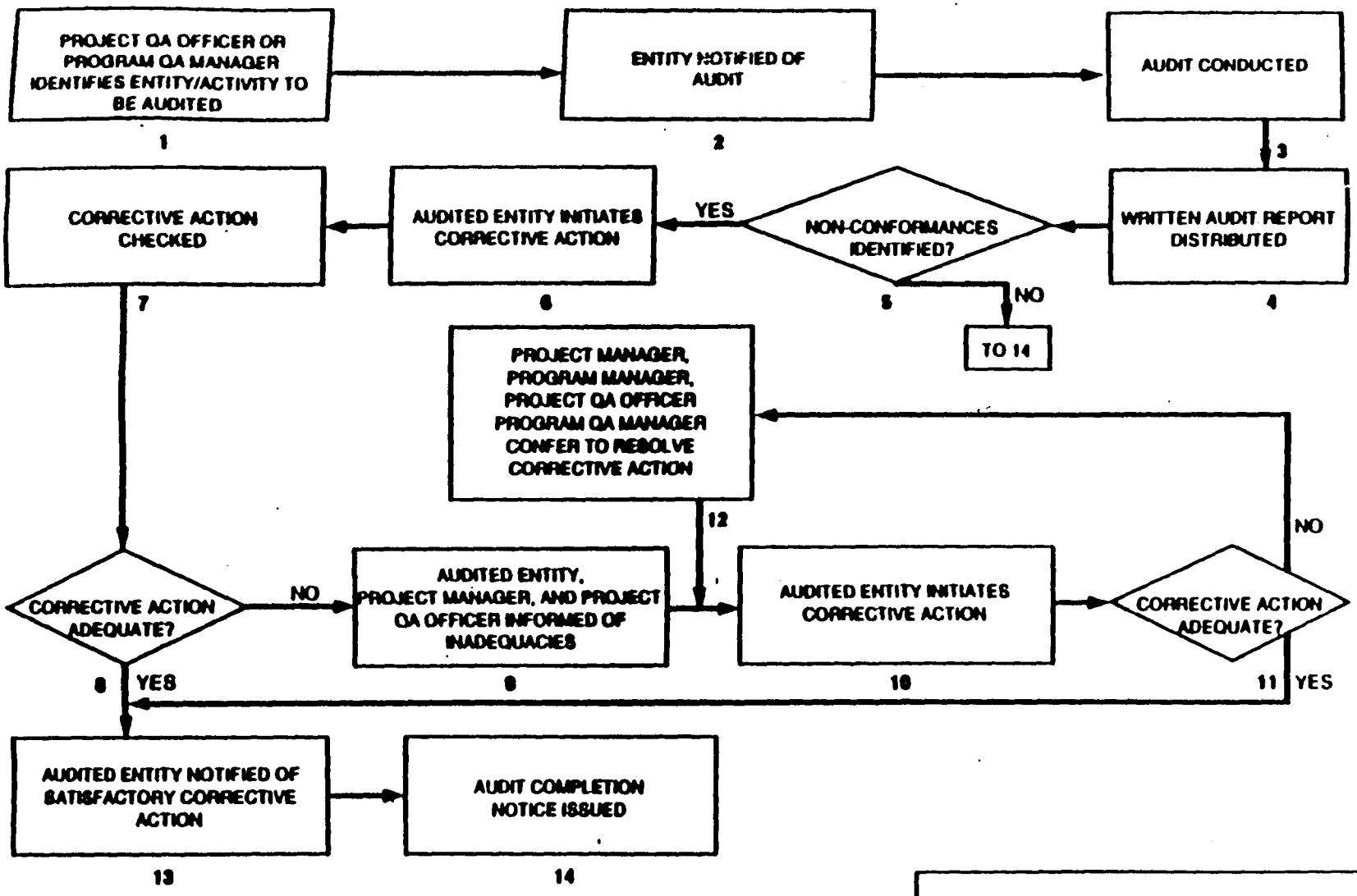
A performance audit is a review of the existing project and QC data to determine the accuracy of a total measurement system or a component of the system. Laboratory performance audits are conducted routinely by the Navy and PRC. Internal audit routines for the laboratory are described in the laboratory QA plan.

11.2 SYSTEM AUDITS

A system audit is used to verify adherence to QA policies and SOPs. This type of audit may consist of on-site review of measurement systems, including facilities, equipment, and personnel. Additionally, procedures for measurement, QC, and documentation may be evaluated. System audits are conducted on a regularly scheduled basis, with the first audit conducted shortly after a system becomes operational.

11.3 FIELD AUDITS

A field audit involves an on-site visit by the auditor or auditing team. Items to be examined include the availability and implementation of approved work procedures; calibration and operation of equipment; packaging, storage, and shipping of samples; documentation of procedures and instructions; and nonconformance documentation.



Naval Station Treasure Island
QA Audit Flow Chart
Figure 7

12.0 CORRECTIVE ACTION PROCEDURES

An effective QA program requires prompt and thorough correction of nonconformances affecting quality. Rapid and effective corrective action minimizes the possibility of questionable data or documentation.

Two types of corrective actions exist: immediate and long-term. Immediate corrective actions include the correction of documentation deficiencies or errors, the repair of inaccurate instrumentation, or the correction of inadequate procedures. Often, the source of the problem is obvious and can be corrected quickly. Long-term corrective actions are designed to eliminate the source of problems. Examples of long-term corrective actions include the correction of systematic errors in sampling or analysis or the correction of procedures producing questionable results. Corrections can be made through additional personnel training, instrument replacement, and/or procedural improvements.

All QA problems and corrective actions will be documented to provide a complete record of QA activities and help identify needed long-term corrective actions. Defined responsibilities are required for scheduling, performing, documenting, and ensuring the effectiveness of the corrective action. This section describes the corrective action procedures to be followed in the field and laboratory.

12.1 FIELD PROCEDURES

The definition of field nonconformances as well as the corrective action procedures that will be used to eliminate any nonconformances are presented in the following sections.

12.1.1 Definition of Field Nonconformances

Field nonconformances are defined as occurrences or measurements that are (1) either unexpected or do not meet established acceptance criteria and (2) will impact data quality if corrective action is not implemented. Nonconformances may include the following:

- Incorrect use of field equipment

- Improper sample collection, preservation, and shipment procedures
- Incomplete field documentation, including COC records
- Incorrect decontamination procedures
- Incorrect collection of QC samples

12.1.2 Field Corrective Action Procedures

Corrective action procedures will depend on the severity of the nonconformance. In cases where immediate and complete corrective action may be implemented by field personnel, corrective actions will be recorded in the field logbook and summarized in the daily field progress report and site logbook.

Nonconformances identified during an audit that have a substantial impact on data quality require the completion of a corrective action request form. This form may be filled out by an auditor or any individual who suspects that any aspect of data integrity is being affected by a field nonconformance. Each form is limited to a single nonconformance. If additional problems are identified, multiple forms will be used for documentation. A sample form is presented as Figure 8.

Copies of the corrective action request form will be distributed to the project manager, FTL, the project QA officer, and the project file. The project QA officer will forward forms to the program manager and the QA program manager as appropriate. Key personnel will meet to accomplish the following:

- Determine when and how the problem developed
- Assign responsibility for problem investigation and documentation
- Determine the corrective action needed to eliminate the problem
- Design a schedule for completion of the corrective action
- Assign responsibility for implementing the corrective action
- Document and verify that the corrective action has eliminated the problem
- Determine whether the Navy should be notified

Figure 9 presents a corrective action status report to be used by the project QA officer to monitor the status of all corrective actions. In addition to a brief description of the problem and the individual who identified it, the report will list personnel responsible for the determination and implementation of the corrective action. Completion dates for each phase of the corrective action procedure will also be listed, along with the due date for the QA program manager to review and check the effectiveness of the solution. A follow-up date, or "poke date," will also be listed to check that the problem has not reappeared. This follow-up is conducted to ensure that the solution has adequately and permanently corrected the problem.

The QA program manager can require data acquisition to be limited or discontinued until the corrective action is complete and the nonconformance eliminated. The QA program manager can also request the reanalysis of any or all data acquired since the system was last in control.

12.2 LABORATORY PROCEDURES

The internal laboratory corrective action procedures and a description of out-of-control situations requiring corrective action are contained in the laboratory QA plan. At a minimum, corrective action will be implemented when control chart warning or control limits are exceeded, method QC requirements are not met, or sample holding times are exceeded. Out-of-control situations will be reported to the project analytical coordinator within 2 working days of identification. In addition, a corrective action report, signed by the laboratory director or project managers and the laboratory QC coordinator, will be provided to the project analytical coordinator.

The corrective action procedures require that the laboratory identify all out-of-control situations that would result in significant amounts of qualified data and perform a corrective action designed to reduce the amount of qualified data. This corrective action is often the reanalysis of samples once the cause of the out-of-control situation has been identified and corrected.

Naval Station Treasure Island
Corrective Action Request Form
Figure 8

CTO Project/
CTO Project No.: _____ Date: ____/____/____

Location: _____

To (CTO Project Manager): _____

From (Audit Team Members): _____

Description of Problem:

_____

_____

_____

_____

_____

Corrective Action Requested:

_____

_____

_____

_____

_____

The above correction action must be completed by: _____

Corrective Action Taken:

_____

_____

_____

_____

_____

RAW(SF):044-0001FBOC)PRC_CARF.AI - 04/18/95:R000 [ORG - 04/18/95]

Naval Station Treasure Island
Corrective Action Request Form (Continued)
Figure 8

CTO Project Manager: _____
(Subcontractor QA Manager)

Acknowledgement of Receipt	Correction Action Completed
_____	_____
(Date and Initial)	(Date and Initial)

Audit Team Members:

Corrective action *is / is not* satisfactory

_____
(Date and Initial)

Remarks: _____

QC Coordinators:

Corrective action *is / is not* satisfactory

_____
(Date and Initial)

Remarks: _____

cc: PRC Program QA Manager
PRC Corporate QA Manager

RAW[SF]:[044-0...BOC]PRC_CARF.AI - 04/14/95;R000[ORG - 04/14/95]

Naval Station Treasure Island
Corrective Action Status Report
Figure 9

CTO Project/
CTO Project No.: _____ QC Level: _____

Project Manager: _____ Date: ___/___/___

Corrective Action No.	Brief Description	Originator Initials and Date	Determination and Implementation				Effectiveness	
			Initials	Due Date	Initials	Due Date	Due Date	Follow-up Date

13.0 QUALITY ASSURANCE REPORTS TO MANAGEMENT

Several reports will be prepared concerning QA during the course of the phase II EA at NAVSTA TI. Each of these reports is summarized in this section.

13.1 DAILY FIELD PROGRESS REPORT

The field progress report will summarize the daily field activities during each field sampling excursion. This will consist of a Daily Field and QA Report which will summarize work performed, QA/QC activities, health and safety activities, problems encountered, and corrective actions taken. Copies of the Daily Field and QA Report shall be made available by the FTL for review by the PRC project manager, the Navy RPM, and the PRC QA project officer. During preparation of the Phase II EA report, field logbooks will be made available to the report writers. The content of the field logbook will be summarized and included in the final RI report.

The program QA manager will prepare a monthly report and may prepare reports to management that concern program and project QA. These reports may include the following:

- QA audit and other inspection results
- Instrument, equipment, or procedural problems that affect quality
- Past due corrective actions
- Objectives from the previous report that were achieved
- Objectives from the previous report that were not achieved
- Work planned for the next month

This information will also be required from subcontractors and will be included in the monthly report.

13.2 QUALITY CONTROL SUMMARY REPORT

A quality control summary report (QCSR) will be prepared by PRC and submitted to the Navy RPM with the final Phase II EA report. The QCSR will contain a data assessment based on the analyses

performed on the samples collected for this project. In addition, the report will list data validation requirements with appropriate qualifier definitions.

13.3 PHASE II ECOLOGICAL RISK ASSESSMENT REPORT

The phase II EA report will contain a section summarizing data quality information collected during the project. Information in this section may include the following:

- Results of performance audits
- Results of systems audits
- Significant QA problems encountered
- Corrective actions taken
- Summary of data quality
- Modifications in the FSP

Particular emphasis will be placed on determining whether project DQOs were met and whether data are of sufficient quality to support required decisions.

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APPENDIX A

PRECISION AND ACCURACY OBJECTIVES

TABLE A-1

VOLATILE ORGANIC COMPOUNDS - CLP METHOD
MATRIX SPIKE AND SURROGATE COMPOUND CONTROL LIMITS

MATRIX SPIKE RECOVERY AND RELATIVE PERCENT DIFFERENCE (RPD) LIMITS

		Sediment	
Fraction	**Matrix Spike Compound**	**Percent Recovery**	**RPD**
VOC	1,1-Dichloroethene	59-172	22
VOC	Trichloroethane	62-137	24
VOC	Chlorobenzene	60-133	21
VOC	Toluene	59-139	21
VOC	Benzene	66-142	21

SURROGATE RECOVERY LIMITS

		Sediment
Fraction	**Surrogate Compound**	**Percent Recovery**
VOC	Toluene-d_8	84-138
VOC	4-Bromofluorobenzene	59-113
VOC	1,2-Dichloroethane-d_4	70-121

Note:

RPD = Relative percent difference

TABLE A-2

POLYNUCLEAR AROMATIC HYDROCARBONS - NOAA METHOD
MATRIX SPIKE AND SURROGATE COMPOUND CONTROL LIMITS

MATRIX SPIKE RECOVERY AND RELATIVE PERCENT DIFFERENCE (RPD) LIMITS

	Pore Water		Sediment	
Matrix Spike Compound	Percent Recovery	RPD	Percent Recovery	RPD
Acenaphthene	70-120	30	70-120	30
Acenaphthylene	70-120	30	70-120	30
Anthracene	70-120	30	70-120	30
Benzo(a)anthracene	70-120	30	70-120	30
Benzo(a)pyrene	70-120	30	70-120	30
Benzo(e)pyrene	70-120	30	70-120	30
Benzo(b)fluroanthene	70-120	30	70-120	30
Benzo(k)fluoranthene	70-120	30	70-120	30
Benzo(g,h,i)perylene	70-120	30	70-120	30
Biphenyl	70-120	30	70-120	30
Chrysene	70-120	30	70-120	30
Dibenzothiophene	70-120	30	70-120	30
Dibenzo(a,h)anthracene	70-120	30	70-120	30
2,6-Dimethylnaphthalene	70-120	30	70-120	30
Fluoranthene	70-120	30	70-120	30
Fluorene	70-120	30	70-120	30
Indeno(1,2,3-cd)pyrene	70-120	30	70-120	30
2-Methylnaphthalene	70-120	30	70-120	30
1-Methylnaphthalene	70-120	30	70-120	30
1-Methylphenanthrene	70-120	30	70-120	30
Naphthalene	70-120	30	70-120	30
Phenanthrene	70-120	30	70-120	30
Perylene	70-120	30	70-120	30
Pyrene	70-120	30	70-120	30
2,3,5-Trimethylnaphthalene	70-120	30	70-120	30

TABLE A-2

POLYNUCLEAR AROMATIC HYDROCARBONS - NOAA METHOD
MATRIX SPIKE AND SURROGATE COMPOUND CONTROL LIMITS
(Continued)

SURROGATE RECOVERY LIMITS

Surrogate Compound	Pore Water Percent Recovery	Sediment Percent Recovery
Naphthalene-d_8	20-130	20-130
Acenaphthene-d_{10}	20-130	20-130
Phenanthrene-d_{10}	20-130	20-130
Chrysens-d_{12}	20-130	20-130
Perylene-d_{12}	20-130	20-130

TABLE A-3

ORGANOCHLORINE PESTICIDES AND PCBs - CLP METHOD MATRIX SPIKE AND SURROGATE COMPOUND CONTROL LIMITS

MATRIX SPIKE RECOVERY AND RELATIVE PERCENT DIFFERENCE (RPD) LIMITS

		Pore Water		Sediment	
Fraction	Matrix Spike Compound	Percent Recovery	RPD	Percent Recovery	RPD
Pest	Aldrin	50-150	50	50-150	50
Pest	alpha-BHC	50-150	50	50-150	50
Pest	beta-BHC	50-150	50	50-150	50
Pest	gamma-BHC (Lindane)	50-150	50	50-150	50
Pest	alpha-chlordane	50-150	50	50-150	50
Pest	gamma-chlordane	50-150	50	50-150	50
Pest	2,4'-DDD	50-150	50	50-150	50
Pest	4,4'-DDD	50-150	50	50-150	50
Pest	2,4'-DDE	50-150	50	50-150	50
Pest	4,4'-DDE	50-150	50	50-150	50
Pest	2,4'-DDT	50-150	50	50-150	50
Pest	4,4'-DDT	50-150	50	50-150	50
Pest	Dieldrin	50-150	50	50-150	50
Pest	Endosulfan sulfate	50-150	50	50-150	50
Pest	Endrin	50-150	50	50-150	50
Pest	Endrin ketone	50-150	50	50-150	50
Pest	Hexachlorobenzene	50-150	50	50-150	50
Pest	Heptachlor	50-150	50	50-150	50
Pest	Heptachlor epoxide	50-150	50	50-150	50
Pest	Methoxychlor	50-150	50	50-150	50
Pest	Mirex	50-150	50	50-150	50
Pest	trans-Nonachlor	50-150	50	50-150	50
PCB	BZ[a] No. 8	50-150	50	50-150	50
PCB	BZ No. 18	50-150	50	50-150	50
PCB	BZ No. 28	50-150	50	50-150	50
PCB	BZ No. 44	50-150	50	50-150	50
PCB	BZ No. 52	50-150	50	50-150	50
PCB	BZ No. 66	50-150	50	50-150	50
PCB	BZ No. 101	50-150	50	50-150	50
PCB	BZ No. 105	50-150	50	50-150	50

TABLE A-3

ORGANOCHLORINE PESTICIDES AND PCBs - CLP METHOD MATRIX SPIKE AND SURROGATE COMPOUND CONTROL LIMITS
(Continued)

PCB	BZ No. 118	50-150	50	50-150	50
PCB	BZ No. 126	50-150	50	50-150	50
PCB	BZ No. 128	50-150	50	50-150	50
PCB	BZ No. 138	50-150	50	50-150	50
PCB	BZ No. 153	50-150	50	50-150	50
PCB	BZ No. 170	50-150	50	50-150	50
PCB	BZ No. 180	50-150	50	50-150	50
PCB	BZ No. 187	50-150	50	50-150	50
PCB	BZ No. 195	50-150	50	50-150	50
PCB	BZ No. 206	50-150	50	50-150	50
PCB	BZ No. 209	50-150	50	50-150	50

SURROGATE RECOVERY LIMITS

		Pore Water	Sediment
Fraction	Surrogate Compound	Percent Recovery	Percent Recovery
Pest	Tetrachloro-m-xylene	60-150	60-150
PCB	BZ[a] No. 121	60-150	60-150
PCB	BZ No. 204	60-150	60-150

Notes:

[a] These BZ ("Ballschnitzer and Zell") numbers comprise a systematic numbering of individual polychlorinated biphenyl congeners and are provided for reference purposes.

Pest = Pesticide
PCB = Polychlorinated biphenyls

TABLE A-4

INORGANICS AND MISCELLANEOUS ANALYSES MATRIX SPIKE AND DUPLICATE SAMPLE CONTROL LIMIT

		Pore Water		Sediment[c]	
Analyses	Method[a]	Percent Recovery[b]	RPD[b]	Percent Recovery	RPD
Metals	CLP	75-125	20	75-125	35
Total Petroleum Hydrocarbons-Purgeable	CA LUFT & EPA 8015A	75-125	20	75-125	35
Total Petroleum Hydrocarbons-Extractable	CA LUFT & EPA 8015A	50-150	50	50-150	50
Aromatic Volatiles (BTEX)	EPA 8020	75-125	20	75-125	35
Total Organic Carbon	ASTM D2974 (sed.) SM 5310B (water)	75-125	20	75-125	35
Grain Size	ASTM D422-63	NA	NA	NA	10
Ammonia (Total and Unionized)	EPA 350.1	75-125	20	75-125	20
Sed. Oxy. Demand	Tetra Tech	NA	NA	NA	NA
Dissolved Oxygen	360.1[d]	NA	±0.1 mg/L	NA	NA
Conductivity	120.1[d]	NA	±10 μmhos	NA	NA
Temperature	170.1[d]	NA	1 C°	NA	NA
pH	CLP or EPA 9045A (sed.) EPA 150.1 (water)[d]	NA	10	NA	10

Notes:

[a] Complete method references are provided in the main text.
[b] Percent recovery and relative percent difference (RPD) control limits are based on spiked samples and duplicate samples, respectively.
[c] Criteria will apply to the sediment and tissue samples, as appropriate
[d] Indicates precision requirements for field parameters

BTEX = Benzene, toluene, ethylbenzene, and xylene
NA = Not applicable;
mg/L = Milligrams per liter
μmhos = unit of electrical resistivity

APPENDIX B

STANDARD OPERATING PROCEDURE
FOR
DETERMINATION OF TOTAL ORGANIC CARBON

APPENDIX B

STANDARD OPERATING PROCEDURE FOR DETERMINATION OF TOTAL ORGANIC CARBON

1.0 INTRODUCTION

Total organic carbon is a measure of the total amount of nonvolatile, volatile, partially volatile, and particulate organic compounds in a sample. Total organic carbon is independent of the oxidation state of the organic compounds and is not a measure of the organically bound and inorganic elements that contribute to the biochemical and chemical oxygen demand tests. Inorganic carbon should be removed because it interferes with total organic carbon determinations.

2.0 FIELD PROCEDURES

Field procedures for determining sediment total organic carbon (TOC) are described in the following sections.

2.1 COLLECTION

Samples can be collected in glass or plastic containers. A minimum sample size of 100 to 150 grams is recommended. If unrepresentative material is to be removed from the sample, it should be removed in the field under the supervision of the chief scientist and noted on the field log sheet.

2.2 PROCESSING

Samples should be stored frozen and can be held for up to 6 months before analysis. Excessive temperatures should not be used to thaw samples.

3.0 LABORATORY PROCEDURES

Laboratory procedures for determining sediment TOC are described in the following sections.

3.1 ANALYTICAL PROCEDURES

Analytical procedures for determining sediment TOC are discussed in the following sections.

3.1.1 Equipment

The following equipment should be used to determine sediment TOC:

- Induction furnace (e.g., Leco WR-12, Dohrmann DC-50, Coleman CH analyzer, Perkin Elmer 240 elemental analyzer, Carlo-Erba 1106)
- Analytical balance (0.1 milligrams [mg] accuracy)
- Desiccator
- Combustion boats
- 10 percent hydrochloric acid (HCl)
- Cupric oxide fines
- Benzoic acid

3.1.2 Equipment Preparation

Equipment should be prepared as follows:

- Clean combustion boats by placing them in the induction furnace at 950°C. After being cleaned, combustion boats should not be touched with bare hands.
- Cool boats to room temperature in a desiccator.
- Weigh each boat to the nearest 0.1 mg.

3.1.3 Sample Preparation

- Allow frozen samples to warm to room temperature.

- Homogenize each sample mechanically.
- Transfer a representative aliquot (30 grams) to a clean container.

3.1.4 Analytical Procedures

- Dry samples to constant weight at 70 ± 2°C. The drying temperature is relatively low to minimize loss of volatile organic compounds.
- Cool dried samples to room temperature in a desiccator.
- Grind sample using a mortar and pestle to break up aggregates.
- Transfer a representative aliquot (0.2 - 0.5 grams) to a clean, preweighed combustion boat.
- Determine sample weight to the nearest 0.1 mg.
- Add several drops of HCl to the dried sample to remove carbonates. Wait until the effervescing is complete and add more acid. Continue this process until the incremental addition of acid causes no further effervescence. Do not add too much acid at one time as this may cause loss of sample because of frothing. Exposure of small samples (i.e., 1 - 10 mg) having less than 50 percent carbonate to an HCl atmosphere for 24 - 48 h has been shown to be an effective means of removing carbonates (Hedges and Stern 1984). If this method is used for sample sizes greater than 10 mg, its effectiveness should be demonstrated by the user.
- Dry the HCl-treated sample to constant weight at 70 ± 2°C.
- Cool to room temperature in a desiccator.
- Add previously ashed cupric oxide fines or equivalent material (e.g., alumina oxide) to the sample in the combustion boat.
- Combust the sample in an induction furnace at a minimum temperature of 950 ± 0 °C.

3.1.5 Calculations

If an ascarite-filled tube is used to capture carbon dioxide (CO_2), the carbon content of the sample can be calculated as follows:

$$\text{Percent carbon} = \frac{A(0.2729)(100)}{B}$$

Where:

A	=	the weight (g) of CO_2 determined by weighing the ascarite tube before and after combustion
B	=	dry weight (g) of the unacidified sample in the combustion boat
0.2729	=	the ratio of the molecular weight of carbon to the molecular weight of carbon dioxide

A silica gel trap should be placed before the ascarite tube to catch any moisture driven off during sample combustion. Additional silica gel should be placed at the exit end of the ascarite tube to trap any water that might be formed by reaction of the trapped CO_2 with the sodium hydroxide (NaOH) in the ascarite.

If an elemental analyzer is used, the amount of CO_2 will be measured by a thermal conductivity detector. The instrument should be calibrated daily using an empty boat blank as the zero point and at least two standards. Standards should bracket the expected range of carbon concentrations in the samples.

3.2 QUALITY ASSURANCE/QUALITY CONTROL PROCEDURES

It is critical that each sample be homogenized thoroughly in the laboratory before a subsample is collected for analysis. Laboratory homogenization should be conducted even if samples were homogenized in the field.

Dried samples should be cooled in a desiccator and held there until they are weighed. If a desiccator is not used, the sediment will accumulate ambient moisture, and the sample weight will be overestimated. A color-indicating desiccant is recommended so that spent desiccant can be detected easily. Also, the seal on the desiccator should be checked periodically and, if necessary, the ground glass rims should be greased, or the "O" rings should be replaced.

It is recommended that triplicate analyses be conducted on one of every 20 samples, or on one sample per batch if less than 20 samples are analyzed. A method blank should be analyzed at the same

frequency as the triplicate analyses. The analytical balance should be inspected daily and calibrated at least once per week. The carbon analyzer should be calibrated daily with freshly prepared standards. A standard reference material should be analyzed at least once for each major survey.

4.0 DATA REPORTING REQUIREMENTS

TOC should be reported as a percentage of the dry weight of the unacidified sample to the nearest 0.1 unit. The laboratory should report the results of all samples (including quality assurance replicates, method blanks, and standard reference measurements) and should note any problems that may have influenced sample quality. The laboratory should also provide a summary of the calibration procedure and results (e.g., range covered, regression equation, coefficient of determination).

REFERENCES

Hedges, J.I. and J.H. Stern. 1984. "Carbon and Nitrogen Determinations of Carbonate-containing Solids." *Limnology and Oceanography*, Vol. 29, Pages 657-663.

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APPENDIX C

STANDARD OPERATING PROCEDURE
FOR
DETERMINATION OF SEDIMENT PARTICLE SIZE

APPENDIX C

STANDARD OPERATING PROCEDURE FOR DETERMINATION OF SEDIMENT PARTICLE SIZE

1.0 INTRODUCTION

Organisms in the aquatic environment are affected in their distribution by the size of the sediment particles. In addition, high levels of organics and contaminants are often found associated with finer particle sizes. Therefore, in order to interpret the distribution of the benthic organisms and the occurrence of high organics and pollutants, it is necessary to understand the distribution of the sediment particle sizes with which they are associated. Particle-size analysis is a measurement of the size distribution of individual particles in a soil or sediment sample. The major features of particle-size analysis are the destruction or dispersion of soil aggregates into discrete units by chemical, mechanical, or ultrasonic means and the separation of particles according to size limits by sieving and sedimentation. This procedure is based on the recommended protocols for the Puget Sound Estuary Program (Tetra Tech 1986).

2.0 FIELD PROCEDURES

Field procedures for determining sediment particle size are described in the following sections.

2.1 COLLECTION

Samples can be collected in glass or plastic containers. A minimum sample size of 100 - 150 grams (g) is recommended. If unrepresentative material is to be removed from the sample, it should be removed in the field under the supervision of the chief scientist and noted on the field log sheet.

2.2 PROCESSING

Samples should be stored at 4°C and can be held for up to 6 months before analysis. Samples must not be frozen or dried before analysis, as either process may change the particle size distribution.

2.0 LABORATORY PROCEDURES

Laboratory procedures for determining sediment particle size are described in the following sections.

3.1 ANALYTICAL PROCEDURES

Analytical procedures for determining sediment particle size are described in the following sections.

3.1.1 Equipment

The following equipment should be used to determine sediment particle size:

- Sieve shaker, Ro-Tap or equivalent
- Drying oven
- Constant temperature bath
- Analytical balance with 0.1 milligrams (mg) accuracy
- Desiccator
- Clock with second hand
- Standard sieves of appropriate mesh sizes
- Sieve pan and top
- Sieve brush
- Funnel
- 1-Liter (L) graduated cylinders
- 50-milliter (mL) beakers
- 20-mL pipettes
- Water pique or squirt bottle
- Glossy paper

- Dispersant (1 percent sodium hexametaphosphate equals 1 percent commercially available Calgon)
- Distilled water

3.1.2 Sample Preparation

Samples should be prepared as follows:

- Allow samples to warm to room temperature.
- Homogenize each sample mechanically.
- Remove a representative aliquot (approximately 25 g) and analyze for total solids content. This information can be used to estimate the dry weight of the aliquot used for particle size analysis. The efficiency of the entire analysis can then be evaluated by adding the dry weights of all sample fractions and comparing this sum with the estimated dry weight of the original aliquot.
- Remove a second representative aliquot for wet sieving. The aliquot can range from 20 g for muddy sediments to 100 g for sandy sediments. The critical factor for sample size determination is the weight of fine-grained material that will be used for the pipette analysis. Ideally the total dry weight of fine-grained material in the 1-L graduated cylinder should equal approximately 15 g. However, total weights between 5 and 25 g are considered acceptable. Total weights outside this range are not considered acceptable and it is recommended that aliquot size be modified to bring the amount of fine-grained material into the acceptable range.
- Weigh the wet sample to the nearest 0.01 g.

3.1.3 Organics Oxidation

This procedure (steps described below) removes organic material from the sample. It is optional and depends upon the objectives of each study.

- Place the sediment sample in a large beaker (greater than or equal to 2 L).
- Add 20 mL of 10 percent hydrogen peroxide solution.
- Let the sample stand until frothing stops.
- Once frothing stops, add an additional 10 mL of hydrogen peroxide solution.

- Continue adding 10-mL portions of hydrogen peroxide solution until no frothing occurs on addition.
- Boil the sample to remove any excess hydrogen peroxide.
- Be careful that material is not lost from the beaker during frothing and boiling.

3.1.4 Wet-sieving

This step separates the sample into size fractions greater than 62.5 μm (i.e., sand and gravel) and less than 62.5 μm (i.e., silt and clay)

- Place the 62.5-μm (4 phi) sieve in a funnel, with a 1-L graduated cylinder underneath. Moisten the sieve using a light spray of distilled water.
- Place the sample in a beaker, add 20 - 30 mL of distilled water, and stir to suspend find-grained material.
- Pour the sample into the sieve and thoroughly rinse the beaker and stirrer with distilled water.
- Wash the sediment on the sieve with distilled water using a water pique or squirt bottle with low pressure. Aggregates can be gently broken using a rubber spatula.
- Continue wet sieving until only clear water passes through the sieve. Try to ensure that the rinsate does not exceed approximately 950 mL. This can generally be accomplished by sieving a sample quantity that is not too large and by efficiently using the rinse water. Both of these techniques may require experimentation before routine wet sieving is started.

3.1.5 Gravel-sand Fraction

This fraction is subdivided further by mechanically dry sieving it through a graded series of screens.

- Wash the coarse fraction into a preweighted 50-mL beaker using distilled water. Rinse the sieve thoroughly.
- Dry the coarse fraction to constant weight at 90 $\pm$ 2 °C. The drying temperature is less than 100°C to prevent boiling and potential loss of sample.
- Cool the sample to room temperature in a desiccator.

- Weigh the cooled sample to the nearest 0.1 mg.

- Set up a nest of sieves that will divide the coarse fraction into the desired number of subfractions. Set up the sieves in a graded series of mesh sizes, with the coarsest mesh on top and the finest mesh on the bottom. The bottom sieve always should have a mesh size of 62.5 μm (4 phi). Place a solid pan on the bottom of the stack and a lid on top of the stack. At a minimum, the coarse fraction should be separated into gravel and sand fractions, using a sieve with a mesh size of 2 mm (-1 phi).

- Add the sample to the uppermost sieve. Complete transfer can be ensured by using a sieve brush to remove any material adhering to the beaker. The sieve brush can also be used to gently break up aggregated sediment.

- Shake mechanically for exactly 15 minutes using the Ro-Tap (or equivalent). A shaker having an automatic timer is preferable.

- After shaking, empty the contents of each sieve onto a glossy piece of paper (e.g., wax paper). To empty a sieve, invert it and tap it on the table several times while ensuring that all edges hit the table at the same time. If the sieve is not tapped evenly, the meshes may be distorted. After tapping the sieve, ensure complete removal of the sample by brushing the back of the screen. After brushing the back of the screen, turn the sieve over and brush out any particles adhering to the sides of the sieve or the inside of the screen.

- Add the fraction that passed through the bottom sieve (e.g., 4 phi) and was retained by the solid pan to the silt-clay fraction of that sample.

- Weigh each remaining size fraction to the nearest 0.1 mg.

- Sum the weights of all size fractions and compare the result with the initial weight of the coarse fraction. Losses and inaccuracies should be less than 1 percent of the initial weight.

- Large amounts of organically derived fragments (e.g., wood debris, grass, shells) or any unusual material in any size fraction should be noted on the laboratory log sheet.

3.1.6 Silt-clay Fraction

This fraction is subdivided further using a pipette technique that depends upon the differential settling rates of different particles. Because additions to this fraction may be made after mechanical sieving of the gravel-sand fraction (see above), it is recommended that the silt-clay analysis for each sample not be conducted until the gravel-sand analysis has been completed.

- Determine the weight of dispersant in a 20-mL aliquot by pipetting a 20-mL aliquot of dispersant into each of five tared beakers, drying the samples to constant weight at 90 plus or minus 2°C, cooling the samples in a desiccator, weighing the cooled samples, and calculating the mean weight of dispersant in the five samples. This weight will be subtracted from the weight of each sediment fraction at the end of the pipet analysis.

- Add 10 mL of the dispersant to each sample suspension in the 1-L graduated cylinders.

- Mix each suspension by either stoppering and inverting the cylinder or by using the up and down motion of a perforated disc plunger.

- Allow the mixed suspension to stand for 2 to 3 hours and check for signs of flocculation. Flocculation can be recognized by a curdling and rapid settling of lumps of particles or by the presence of a thick soupy layer on the bottom of the cylinder passing abruptly into clear water above.

- If flocculation occurs, add dispersant in 10-mL increments until no noticeable flocculation occurs. Record the volume of dispersant added.

- When ready to conduct the pipette analysis, bring the sample volume to 1 L by adding distilled water, mix the suspension thoroughly, and place the cylinder in a constant-temperature water bath. If the volume is greater or less than 1 L, the factor for converting the weight of the sediment in each 20-mL aliquot to that in the total volume must be modified accordingly.

- After 20 seconds, withdraw a 20-mL aliquot from a depth of 20 cm below the surface of the suspension using a pipette. The pipette should be marked for the specified sampling depths and should be inserted vertically into the settling cylinder when the aliquot is taken. A suction bulb may be used on the open end of the pipette to facilitate sampling. It is critical that the suspension be disturbed as little as possible when pipette aliquots are taken.

- Transfer the 20-mL aliquot to a preweighted 50-mL beaker. Rinse the pipette into the beaker using 20 mL of distilled water.

- Withdraw 20-mL aliquots at a depth of 10 cm below the surface of the suspension at the appropriate time(s). A formula for calculating withdrawal times is given by Folk (1968) and Buchanan (1984). If a withdrawal is missed, the suspension can be stirred again and the missed withdrawal can be taken at the appropriate time after settling begins. It is not necessary to withdraw the initial 20-mL aliquot when this corrective action is conducted.

- Transfer these additional 20-mL aliquots to 50-mL preweighted beakers, each time rinsing the pipette into the respective beaker using 20 mL of distilled water.

- Dry all aliquots to constant weight at 90 plus or minus 2°C. A drying temperature less than 100°C is used to prevent boiling and potential loss of sample.

- Cool dried samples to room temperature in a desiccator.
- Weigh cooled samples to the nearest 0.1 mg.

3.1.7 Calculations

The total weight of a phi-size interval in the 1-L graduated cylinder is determined as follows:

Phi weight (g dry weight) - 50[(A-C)-(B-C)]

Where:

A = weight (g) of residue in a 20-mL aliquot for a given phi-size boundary

B = weight (g) residue in a 20-mL aliquot for the next larger phi-size boundary

C = mean weight (g) of dispersant in a 20-mL aliquot

3.2 QUALITY ASSURANCE/QUALITY CONTROL PROCEDURES

It is critical that each sample be homogenized thoroughly in the laboratory before a subsample is collected for analysis. Laboratory homogenization should be conducted even if samples were homogenized in the field.

After dry-sieving a sample, all material must be removed from the sieve. This can be accomplished by tapping the rim of the sieve evenly on a hard surface and by brushing the screen.

The total amount of fine-grained material used for pipette analysis should be 5 - 25 g. If more material is used, particles may interfere with each other during settling and the possibility of flocculation may be enhanced. If less material is used, the experimental error in weighing becomes unacceptably large.

Before pipette extractions can be made, the sample must be homogenized thoroughly within the settling cylinder. Once the pipette analysis begins, the settling cylinders must not be disturbed, as this will alter particle settling velocities. Care must be taken to disturb the sample as little as possible when pipette extractions are made.

After a pipette extract has been transferred to a drying beaker, any sample adhering to the inside of the pipette must be removed. This can be accomplished by drawing 20 mL of distilled water into the pipet and adding this rinse water to the drying beaker.

Dried samples should be cooled in a desiccator and held there until they are weighed. If a desiccator is not used, the sediment will accumulate ambient moisture, and the sample weight will be overestimated. A color-indicating desiccant is recommended so that spent desiccant can be detected easily. Also, the seal on the desiccator should be checked periodically, and, if necessary, the ground glass rims should be greased, or the "O" rings should be replaced.

It is recommended that triplicate analyses be conducted on one of every 20 samples, or on one sample per batch if less than 20 samples are analyzed. It is also recommended that the analytical balance, drying oven, and temperature bath be inspected daily and calibrated at least once per week.

4.0 DATA REPORTING REQUIREMENTS

The weight of each sediment fraction should be reported to the nearest 0.0001 g dry weight. The laboratory should report the results of all samples analyzed (including quality assurance replicates) and should note any problems that may have influenced data quality.

REFERENCES

Buchanan, J.B. 1984. "Sediment Analysis." *Methods for the Study of Marine Benthos*. N.A. Holme and A.D. McIntyre (eds.). Blackwell Scientific Publications. Boston, Massachusetts.

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APPENDIX D

STANDARD OPERATING PROCEDURE
FOR
DETERMINATION OF SEDIMENT BIOCHEMICAL OXYGEN DEMAND

APPENDIX D

STANDARD OPERATING PROCEDURE FOR DETERMINATION OF SEDIMENT BIOCHEMICAL OXYGEN DEMAND

1.0 INTRODUCTION

Sediments are generally in a reduced chemical state and have the potential to remove oxygen from overlying water. This occurs as a result of (1) the migration of dissolved oxygen to the sediment water interface followed, by subsequent chemical reaction; or (2) the migration of reduced chemical species (ferrous iron, manganous manganese, sulfide) from the sediments to the overlying water, followed by subsequent oxidation. The sediment biochemical oxygen demand (BOD) procedure characterizes sediments in terms of rate of exertion of oxygen demand. This standard operating procedure is based on the Puget Sound Estuary Program (Tetra Tech 1986).

2.0 FIELD PROCEDURES

Field procedures for determining sediment BOD are described in the following sections.

2.1 COLLECTION

Samples can be collected in glass or plastic containers. A minimum sample size of 50 gram is recommended. If unrepresentative material is to be removed from the sample, it should be removed in the field under the supervision of the chief scientist and noted on the field log sheet.

2.2 PROCESSING

Samples should be stored at 4°C, and can be held for up to 7 days under that condition. Samples should be kept field-moist, and air contact should be prevented to minimize oxidation.

3.0 LABORATORY PROCEDURES

Laboratory procedures for determining sediment BOD are described in the following sections.

3.1 EQUIPMENT

The following equipment should be used to determine BOD:

- Incubator
- Thermostatically controlled at 20° ± 1° C.
- All light should be excluded to prevent the photosynthetic production of dissolved oxygen by algae in the sample.
- Incubation bottles (300 milliliter [mL] capacity, with ground glass stoppers)
- Distilled water (free of copper, chlorine, chloramines, caustic alkalinity, acids, and organic material)
- Phosphate buffer solution (Dissolve the following in distilled water: 8.5 g potassium dihydrogen phosphate (KH_2PO_4); 21.75 g dipotassium hydrogen phosphate (K_2HPO_4); 33.4 g disodium hydrogen phosphate heptahydrate ($NA_2HPO_47H_2O$); and 1.7 g ammonium chloride (NH_4Cl). Dilute to 1L. The pH of this buffer should be 7.2 without further adjustment. If dilution water is to be stored in the incubator, the phosphate buffer should be added just before using the dilution water).
- Magnesium sulfate ($MgSO_47H_2O$) solution (Dissolve 22.5 g $MgSO_47H_2O$ in distilled water and dilute to 1 L.)
- Calcium chloride ($CaCl_2$) solution (Dissolve 27.5 g anhydrous $CaCl_2$ in distilled water and dilute to 1 L.)
- Ferric chloride ($FeCl_36H_2O$) solution (Dissolve 0.25 g $FeCl_36H_2O$ in distilled water and dilute to 1 L.)
- Dilution water (Store distilled water in cotton-plugged bottles for a sufficient length of time to become saturated with dissolved oxygen. The water should be aerated by shaking a partially filled bottle or using a supply of clean compressed air. The distilled water used should be as near as possible to 20°C and of high purity. Place the desired volume of distilled water in a suitable bottle and add 1 mL each of phosphate buffer, magnesium sulfate, calcium chloride, and ferric chloride for each liter of water.)
- Seeding material (Satisfactory seed may sometimes be obtained by using the supernatant liquor from domestic sewage that has been stored at 20°C for 24 - 36 h. Use the seed that has been found by practical experience to be the most satisfactory for the particular material under study. Only past experience can determine the amount of seed to be added per liter, but the amount should give an oxygen depletion of

approximately 2 mg/L. The amount of seed required may vary with the source of the seed. If the sample contains organic compounds not amenable to oxidation by domestic sewage seed, it may be necessary to use seed prepared from soil, an acclimated seed developed in the laboratory, or sediments collected below a particular waste discharge (preferably 2 - 5 mi below the point of discharge). Seeded dilution water should be used the same day it is prepared.)

3.2 STANDARDS PREPARATION

Prepare a stock BOD standard solution by dissolving 0.150 g reagent grade glucose and 0.150 g reagent grade glutamic acid in 1 L of distilled water. The solids should be dried for 1 hr at 103°C before they are weighted.

3.3 SAMPLE PREPARATION

Samples should be prepared as follows:

- Allow samples to warm to room temperature.
- Homogenize each sample mechanically.
- Remove a representative aliquot (approximately 25 g) and analyze for total solids content.
- Remove a representative aliquot (approximately 5 g) and weigh it to the nearest 0.1 mg.
- Transfer the weighted aliquot to a BOD bottle for analysis.

3.4 ANALYTICAL PROCEDURES

The following analytical procedures should be performed:

- Fill each BOD bottle with dilution water and place the samples in the incubator. Ensure that air bubbles are not trapped in the BOD bottles. Prepare a blank consisting of dilution water in a separate BOD bottle. Ensure that there is a water seal in the neck of each sample bottle and blank when in the incubator. Replenish the water seals on all bottles each morning.

- Determine the initial dissolved oxygen concentration of each sample and blank using the Winkler titration method or a dissolved oxygen concentration in the dilution water. This method is recommended because sediment may cause a rapid consumption of oxygen, making it difficult to obtain a stable initial dissolved oxygen reading. If a probe is used for oxygen measurement, the same sample can be used for immediate dissolved oxygen demand and BOD.

- Incubate samples and blanks for 5 days at 20 ± 1° C. Analyze the second sample to determine initial dissolved oxygen concentration. Following the 5-day period, determine dissolved oxygen in the incubated sample. The oxygen depletion should not be more than 0.2 milligrams per liter (mg/L) and preferably not more than 0.1 nanograms per liter (ng/L). If these values are exceeded, the quality of the dilution water or the treatment of samples (e.g., filling of water seals) should be considered suspect.

- Prepare a working BOD standard solution by diluting 20 mL of the stock solution to 1 L with seeded dilution water. Fill three BOD bottles and incubate at 20 plus or minus 1°C for 5 days. The resulting BOD of these samples should be 218 plus or minus 11 mg/L. Any appreciable deviation from these expected results may raise questions on the quality of the dilution water, the viability or suitability of the seed material, or the analytical technique.

3.5 CALCULATIONS

Sediment BOD is calculated as follows:

$$\text{BOD (mg/kg dry weight)} = \frac{(0-F)(b)}{(g)(\text{percentS})}$$

Where:

0 = dissolved oxygen concentration at time zero, mg/L
F = dissolved oxygen concentration after 5 days, mg/L
b = volume of BOD bottle, mL
g = wet weight of sediment sample used, gram
percentS = percent solids in sediment sample (expressed as a decimal fraction).

3.6 QUALITY ASSURANCE/QUALITY CONTROL PROCEDURES

It is critical that each sample be thoroughly homogenized in the laboratory before a subsample is collected for analysis. Laboratory homogenization should be conducted even if samples were homogenized in the field.

It is recommended that triplicate analyses be conducted on one of every 20 samples, or on one sample per batch if less than 20 samples are analyzed. A dilution water blank and glucose-glutamic acid standard should be analyzed at the same frequency as the triplicate analysis.

4.0 DATA REPORTING REQUIREMENTS

BOD should be reported as mg/L dry weight, to the nearest 0.1 unit. The laboratory should report the results of all samples analyzed, including quality assurance replicates, seeded dilution water blanks, unseeded dilution water blanks, and glucose-glutamic acid standards. The laboratory should also note any problems that may have influenced sample quality.

REFERENCES

Tetra Tech, Inc. 1986. "Recommended Protocols for Measuring Conventional Sediment Variables in Puget Sound." TC-3991-04. Prepared for U.S. Environmental Protection Agency, Region 10.

APPENDIX E

STANDARD OPERATING PROCEDURE
FOR
72-HOUR DEVELOPMENT ABNORMALITY TOXICITY TEST USING THE ECHINODERM,
Strongylocentrotus purpuratus

APPENDIX E

STANDARD OPERATING PROCEDURE FOR 72-HOUR DEVELOPMENT ABNORMALITY TOXICITY TEST USING THE ECHINODERM, *Strongylocentrotus purpuratus*

1.0 APPLICATION

The echinoderm embryo bioassay is described by Dinnel and Stober (1985) as a rapid and sensitive technique for assessing the toxicity of marine sediments. Purple sea urchin (*Strongylocentrotus purpuratus*) is the recommended species for testing. During the first 48 to 96 hours of embryonic development, fertilized echinoderm eggs normally develop into the pluteus stage. Failure of the eggs to develop normally is used as the indicator of toxicity. Methods described in this SOP are based on Chapman and others (1995), *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms*.

The echinoderm development test will be used to test both the sediment pore water and the elutriate from petroleum-contaminated soils. A dilution series will be implemented for the analysis of the elutriate to calculate an effect concentration (EC)10, EC25, EC50, no observed effect concentration (NOEC), and lowest observed effect concentration (LOEC). These values provide information about the dose response relationship. Tests of single concentrations, providing pass/fail information, are used during the analysis of the sediment pore water, in which case the test concentration requirement is not applicable.

2.0 TEST ORGANISMS

Strongylocentrotus purpuratus is commonly found in the lower intertidal zone on rocky shores and pilings, typically in areas of moderate to strong wave action (Morris and others 1980). Subtidally the species is found to a depth of 160 m. This species occurs from Vancouver Island, British Columbia, to Isla Cedros, Baja California. The main reproductive season of *S. purpuratus* is during January, February, and March, throughout their entire range, although some individuals with ripe gametes may occur before and after that time period (Ricketts and others 1985).

Adult *S. purpuratus* (brood stock) should be obtained from the same source—either commercial harvesters or a chemically uncontaminated area. If the brood stock is obtained from a commercial source, the original collection area should be identified. Within 24 hours of collection or purchase, adults should be transported to the test laboratory and placed into flowing seawater similar in character to that from which they were taken. Because epidemic spawning can occur when echinoderms are transported in sea water, test animals can be transported in ice chests containing only kelp or other moist material. If animals are transported in sea water, the sea water should be kept cool (4°C) and, if necessary, aerated. Rough handling, extended periods of desiccation, and abrupt changes in temperature, salinity, or other water quality variables must be avoided as these induce premature spawning or render the stock useless for later controlled spawning or both. Upon receipt, adults should be cleaned of detritus and placed in flowing sea water.

If adults are to be conditioned for spawning out of season, a continuous supply of temperature-controlled, aerated sea water is needed. Laboratory facilities should be well ventilated and free of organic vapors. Holding and conditioning chambers preferably should not be in a room in which toxicity tests are conducted, stock or test solutions are prepared, or equipment is cleaned. Air used for aeration should be free of organic vapors, oil, and water. Raw sea water can be used for holding and conditioning, but feeding the adults a natural or cultivated alga is necessary to deter starvation. The flow rates used for adult conditioning must be high enough (typically greater than 28 liters/hour/individual) to prevent water quality degradation and provide as much food possible to the adults.

Tanks and trays are necessary for holding adults. These are placed in a water bath, incubator, or temperature-controlled room to maintain proper temperature. Adult holding tanks should be cleaned several times each week to prevent accumulation of organic matter and bacteria. Dead specimens should be removed immediately and the tanks cleaned. The tanks should be cleaned with detergent and rinsed with clean sea water, and if microbial contamination is present, they should be rinsed with 200 mg/L of hypochlorite and then sea water. With enriched waters and elevated conditioning temperatures, more frequent cleaning may be required.

Sea water used in the bioassay is maintained at a salinity of 30 to 32 parts per thousand (ppt). Water temperature should be maintained at 15 ± 1 °C for all test species. Sea water should be collected

from uncontaminated areas (e.g., deep or offshore waters) to avoid contamination and should be held at less than or equal to 15 °C for no longer than 2 days before inoculation. The bioassay sea water must be uncontaminated and of acceptable low toxicity. The biological criterion of acceptability is that the larvae, spawned by adults in the dilution water, must not incur more than 20 percent abnormal development or 30 percent mortality during 48 hours of exposure to the bioassay sea water.

3.0 CONTROL WATER

Artificial sea water of approximately the same temperature, salinity, and dissolved oxygen content as the water at the sites to be tested, may be prepared using ACS reagent grade chemical salts and deionized water. The recipe for synthetic GP-2 sea water was published by the U.S. Environmental Protection Agency (EPA 1988) and is listed in Table E-1.

Once the necessary volume of GP-2 has been prepared, it is aged, with aeration, for 2 weeks before it is used in bioassay testing. If a residue or precipitate is present after aging, the sea water is filtered through 0.45 μm filter, before it is used. Salinity adjustments are made, if necessary, with distilled water (to decrease salinity) or a brine prepared from distilled water and artificial sea salts (to increase salinity). Dissolved oxygen is maintained above 40 percent saturation.

4.0 TEST PARAMETERS

Test parameters for this SOP are as follows (Chapman and others 1992):

Test Type:	Pore water toxicity test, static, nonrenewal
Temperature:	15°C ± 1°C
Salinity:	34 ± 2 ppt
Light Quality:	Wide-spectrum fluorescent lights
Illuminance:	50 to 100 foot-candles (ft-c)
Photoperiod:	16L:8D
Test Chamber:	20 mL minimum

Test Solution Volume:	10 mL minimum
Renewal of Test Solution:	None
Age of Test Organism:	less than or equal to (≤) 1 hour old embryos
Number of Organisms per Chamber:	25 per mL
Number of Replicates:	4
Test Concentrations:	5 concentrations for each site and control water
Dilution Series:	100 percent, 50 percent, 25 percent, 12.5 percent, 6.35 percent
Feeding Regime:	None
Aeration:	None; dissolved oxygen must not fall below 4.0 mg/L
Overlying Water Quality Monitoring:	Temperature daily; pH, salinity, and dissolved oxygen at beginning and end of test; ammonia at beginning and end of test in 100 percent pore water and control.
Test Duration:	72 hours
Endpoints:	Normal development
Test Acceptability:	Greater than 80 percent normal development in controls, must achieve a minimum significant difference (MSD) of less than 20 percent in controls for survival and normal development, and satisfaction of performance-based criteria as outlined in Section 10 below.
Sample Volume Required:	500 mL

5.0 SEDIMENT PREPARATION AND PORE WATER EXTRACTION BEFORE TESTING

The tests will be conducted using pore water extracted from the sediment being tested. Sediments used for pore water analysis should be processed to remove the pore water as soon as possible after collection to minimize effects due to chemical changes (ASTM 1994). Extraction of pore water within 24 hours of collection is recommended, but in no case should the sediments be stored longer than 2 weeks after collection before extraction of pore water. The homogenized sediment sample should be centrifuged at ambient temperature (ASTM 1994) of the sediments at the time of collection, for 30 minutes at a speed of 3,200 gravity units (g) using a refrigerated centrifuge equipped with a swinging bucket type rotor capable of spinning 100 to 1000 mL bottles. A second centrifugation will be performed for 30 minutes at 10,000 g. Tubes or bottles of glass or polycarbonate are recommend in order to minimize adsorption of soluble contaminants on the container walls. The supernatant should be (re-) centrifuged to further remove any suspended particulate matter. A pipette should be used to transfer the supernatant to 1-L amber glass bottle for storage until used. An aerobic environment should be maintained. Pore water samples can be held at 4° C for in the dark until testing begins. Pore water samples may also be frozen but should be (re-) centrifuged after thawing to minimize artifactual responses resulting from precipitated suspended particulate matter in the sample (Carr and Chapman 1995). Pore water extraction should follow the protocol as defined in Appendix F.

6.0 BIOASSAY PROCEDURE

The procedures described below follow the guidance of Chapman and others (1995). Adult echinoderms, conditioned as necessary in the laboratory, are induced to spawn with chemical stimulation. Selected densities of the resulting embryos are exposed to the test for 72 hours, during which the embryos normally will develop into the four-armed pluteus stage. A slightly longer exposure period may be used if necessary to achieve adequate development of embryos. Exposure time should not exceed 96 hours for an acceptable test. Data from tests with longer exposures (greater than 48 hours) may not be comparable to those from tests conducted using the standard 48-hour exposure. Toxicity test endpoint is based on failure of the embryo to develop to the pluteus stage.

Adult sea urchins are spawned by injecting 1 mL of 0.5-molar potassium chloride (KCl) through the peristomal membrane into the coelomic cavity. Animals are rinsed with clean sea water and inverted

over individual 150 to 250 mL beakers filled with sea water for about 30 minutes until spawning is completed. As many as 12 females may need to be spawned to ensure and adequate quantity of eggs. The spawning beakers should be placed in a water bath or temperature-controlled room to maintain temperature at acclimation levels. Gametes should only be collected during the first 15 minutes after each animal starts releasing.

Eggs from females discharging relatively small numbers of eggs (e.g., less than 100,000) are discarded. The retained eggs are examined microscopically for viability and ripeness. Ripe, viable eggs are normally round, uniform in size, free of excessive debris, and appear slightly granular. Immature eggs contain a large, clear, spot (the germinal vesicle) in the cytoplasm; overripe eggs are usually less circular, have inconsistent granularity of the cytoplasm, and are often associated with increased debris. If the proportion of underripe or overripe eggs in a beaker exceeds 10 percent, the eggs are discarded. Eggs that are accepted are pooled together into a 1-liter beaker and washed three times by repeatedly decanting the water above the eggs and bringing the volume to 600 mL. The eggs are allowed to settle to the bottom of the beaker between washes. Using a plunger to mix the egg solution, remove a 10-mL sample and place in a 1-L graduated cylinder. Bring the volume up to 1-L with dilution water, mix well, remove a 1-mL sample, and place in a counting cell. Count all the eggs in the 1-mL sample twice and take the mean of the two counts. The final concentration if eggs in the diluted stock must be 250 eggs per 0.25 mL, which is equal to 1,000 eggs per mL. The total number if eggs in the test solutions must remain 0.25 eggs per mL.

The solutions of sperm from males producing thick, viscous discharges are combined to provide a stock solution. Sperm density is determined by immobilizing the sperm (i.e., by heat shock or exposure to 10-percent glacial acetic acid) and by counting on a hemocytometer. Fertilization should be initiated within 1 hour of spawning by adding sperm to the beaker containing the eggs, at a sperm to egg ratio of less than or equal to 500:1. A perforated plastic plunger is used to gently mix the contents of the beaker. Care should be taken that excessive amounts of sperm are not used. Fertilization should be monitored by examining successive 1-mL aliquots microscopically and determining the percentage of eggs with a raised fertilization membrane. When greater than 90 percent of the eggs show membrane formation (about 10 to 15 minutes), the developing eggs are counted and the density is adjusted to 2,000 per mL, either by diluting with sea water to decrease density or decanting excess surficial water to increase density.

Bioassay chambers are set up according to the test dilution series. Within 1 hour of fertilization, a 1-mL aliquot of the solution of embryos (about 2,000 embryos) is added to each bioassay chamber using an automatic pipette. The containers are covered with a watchglass and incubated for 48 hours (or longer if required) at 15 plus or minus 1°C. Test chambers generally are not aerated during the bioassay. However, if the dissolved oxygen concentration in any test chamber declines below 60 percent of saturation, the water in that chamber should be aerated gently for the remainder of the test. A random numbering method should be used to distribute the chambers in the water bath (or incubator or cold room).

The mean embryo concentration at 0 hours should be determined by collecting five replicate 1-mL samples from thoroughly mixed control cultures and preserving them in 5-percent buffered formalin. This method of determining the initial embryo concentration is one of three methods recommended by ASTM (1989) for larval bioassays of water and elutriates. The other two methods include: (1) direct subsampling of each test chamber after inoculation and (2) direct subsampling of the stock solution. According to ASTM (1989), the preferred method is direct subsampling of test chambers after inoculation. This method provides the best estimate of embryo densities within each chamber and the variability of densities among chambers.

Three replicates of the sea water control are included in all bioassays. These comprise negative (clean) controls that facilitate comparison among experiments and among laboratories of the validity of the procedures used in individual investigations. At least 70 percent of the larvae must survive the 48-hour exposure with sea water alone, and of these, at least 90 percent must show no abnormalities.

7.0 DAILY MONITORING OF THE TESTS

The following parameters should be monitored during the test in each test chamber.

- Measure and record the following water parameters just before introduction of test organisms and daily thereafter: temperature, salinity, dissolved oxygen, and pH.
- Measure the ammonia concentration at the start and end of the bioassay.

8.0 REFERENCE TOXICANT TESTING

The quality of field collected echinoderms should be verified by conducting a reference-toxicity test. Monthly reference-toxicity tests should be conducted on *S. purpuratus* cultures using procedures as outlined below. If reference-toxicity tests are not conducted monthly, a reference toxicant test is run simultaneously with the echinoderm pore water test to compare the relative health of the specimens being used. This involves determining 72-hour (or longer) LC_{50} and EC_{50} values for echinoderm larvae exposed to reference toxicants in clean, filtered or UV-treated sea water under the same general test conditions as the sediment pore water bioassays. Such data are necessary to determine the relative sensitivity of the larvae. Bioassays to establish an LC_{50} or an EC_{50} involve four or five logarithmic concentration series and a control. At least one treatment should give a partial response below the LC_{50} and EC_{50} and one above the LC_{50} and EC_{50}. Statistical procedures for LC_{50} and EC_{50} estimates are given in APHA (1985) and ASTM (1989).

This test is run in clean sea water, with a 72-hour exposure, and an LC50 value can be calculated. ACS Reagent-grade cadmium chloride, $CdCl_2$, is used as a reference toxicant. Test parameters are as follows:

Test Type:	Water-only
Dilution Series:	Control and at least five test concentrations (0.5 dilution factor)
Toxicant:	Cadmium Chloride ($CdCl_2$)
Temperature:	15°C ± 2°C
Salinity:	34 ± 2 ppt
Light Quality:	Ambient laboratory
Photoperiod:	16L:8D
Renewal of Water:	None
Age of Test Organisms:	≤ 1 hour embryos
Test Chamber:	20 mL glass beaker

Volume of Water:	10 mL
Number of Organisms/Chamber:	25/mL
Replicates:	3
Aeration:	None
Water Quality:	Temperature, salinity, pH, and dissolved oxygen daily; ammonia (total and unionized) at beginning and end of test
Test Duration:	72-hour
Endpoint:	Survival (LC50); embryo development (EC50)
Test Acceptability:	80 percent control survival

9.0 TEST COMPLETION

The bioassay is terminated when greater than 95 percent of the embryos in the duplicate sea water control have reached the four-armed pluteus stage (approximately 48 to 96 hours). Once this stage has been achieved in the control beakers, final water quality measurements are recorded, and the test is terminated.

The bioassay is terminated in the following manner. The water and larvae for each chamber are carefully poured into separate clean 50 mL beaker. The water is then mixed thoroughly using a perforated plunger, and 10-mL aliquots of the sample are removed by pipette and placed in 10-mL screw-cap vials. The contents of each vial are preserved in 5-percent buffered formalin.

Preserved samples (equal in volume to those containing 300 to 500 larvae in controls) are examined in Sedgewick-Rafter cells. Normal and abnormal larvae are enumerated to determine percent survival and percent abnormality. Percent survival for each replicate bioassay chamber is based on the number of larvae surviving in each test container relative to the initial number. Percent mortality is then calculated, including correction for mortality in the sea water control. Larvae that fail to transform into clearly defined pluteus with two well-developed arms and the second pair of arms budding are considered abnormal. A minimum sample size of 20 living larvae in each of the three replicate

bioassay chambers for test sediment pore water and reference area sediment pore water and 100 larvae in each replicate chamber for the sea water control should be scored for abnormalities. Aliquot counts should be completed even after the minimum sample size is achieved. Percent survival for each replicate bioassay chamber is based on the number of larvae surviving relative to the mean number of survivors in the sea water controls. Embryos that fail to transform to the four-armed pluteus stage are considered abnormal. Percent abnormality for each replicate bioassay chamber is based on the number of survivors that fail to reach the pluteus stage.

10.0 ACCEPTABILITY OF THE TEST

Test acceptability requirements for a 72-hour echinoderm embryo development bioassay are listed below. Performance criteria for testing are as follows:

- The age of the echinoderm larvae must be less than 1 hour at the start of the test.
- Normal development in the controls must be greater than or equal to 80 percent.
- The minimum significant difference is less than or equal to 20 percent relative to the controls.

Performance criteria for field collection:

- Reference-toxicant tests must be performed on each batch of field-collected echinoderms used in the tests.
- Echinoderms must not have spawned as a result of the collection and transportation activities.

Additional requirements are as follows:

- All organisms must be from the same population.
- Tests should begin within 10 days after receipt of echinoderms.
- All test chambers should be identical and should contain the same amount of test water.

- The daily mean temperature must be within ± 1 °C of the desired temperature. The instantaneous temperature must always be within ± 3 °C of the desired temperature.
- The dissolved oxygen must not fall below 4.0 mg/L in any chamber.
- Treatments must be randomly assigned to individual test chambers.
- Test echinoderm larvae must be randomly distributed to test chambers.

11.0 STATISTICAL METHODS

The data should be tabulated and summarized. The lethal concentration 50 (LC_{50}), effects concentration 50 (EC_{50}), lowest observed effects concentration (LOEC), and no observed effects concentration (NOEC) shall be determined for each test endpoint. Statistical comparisons among treatments shall be made using analysis of variance (ANOVA) and Dunnett's one-tailed *t*-test (which controls the experiment wise error rate) on the arcsine square-root-transformed data. An estimate of the pore water concentration which would cause a 50 percent toxic effect for mortality (LC_{50}) and abnormal development (EC_{50}) shall be calculated using Trimmed Spearman-Karber analysis with Abbott's correction (Hamilton, Russo, and Thurston 1977). The one-way ANOVA followed by Dunnett's Procedure (Dunnett 1955) to compare single treatments to the control shall be used in order to estimate no observed effect and lowest observed effect concentrations (NOEC and LOEC values) (EPA 1990).

12.0 POTENTIAL PROBLEM AREAS

The following are potential problem areas that may be encountered during tests.

- Caution should be taken during collection and transportation. Rough handling, extended periods of desiccation, and abrupt changes in temperature, salinity, or other water quality variables must be avoided as these induce premature spawning or render the stock useless for later controlled spawning or both.
- Pore water should be (re-) centrifuged after thawing, if frozen, to minimize artifactual responses resulting from precipitated suspended particulate matter in the sample (Carr and Chapman 1995).

13.0 RECORD KEEPING

The report should document the following information either directly or by reference to available documents in electronic and hard copy formats.

- Name of test and investigator(s), name and location of laboratory, and dates of start and end of test
- Source of reference sediment and test sediment used for extraction of pore water with the method of collection, handling, shipping, storage, extraction, and disposal of sediment
- Source of test solution, its chemical characteristics, and a description of any pretreatment
- Results of any demonstration of the ability of the species to survive, grow, or reproduce in the test solution
- Source and history of test organisms
- Source and date of collection of the test organisms, scientific name, name of person who identified the organisms, and the taxonomic key used. Observed diseases or unusual appearance, treatment, holding, and acclimation procedures should also be recorded
- Source and composition of food, procedure used to prepare food, feeding method, frequency, and ration
- Description of the experimental design and test chambers used; the volume of water in the chambers; lighting; number of replicates and number of test organisms per replicate
- Records of temperature, salinity, pH, dissolved oxygen, and ammonia tested in test solution
- Biological endpoints used along with appropriate measurements
- A table of the biological data for each test including the control(s) in sufficient detail to allow independent statistical analysis
- Statistical test used and results of analysis of the data
- Results of reference toxicant tests
- Information on calibration of equipment and instruments used during the test

- Copy of the chain-of-custody record, the sample log-in sheet, the original bench sheets for the test organism responses during the sediment test(s), and original signatures and dates of laboratory personnel performing the test(s)

- Summary of general observations made during the test

- Any deviations from the required procedures and any other relevant information

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TABLE E-1

SALTS USED IN THE PREPARATION OF GP-2 ARTIFICIAL SEA WATER (30 ppt SALINITY) (EPA 1988)

Compound	Concentration (g/L)
NaCl	21.03
Na_2SO_4	3.52
KCl	0.61
KBr	0.088
$Na_2B_4O_7 \circ 10H_2O$	0.034
$MgCl_2 \circ 6H_2O$	0.02
$CaCl_2 \circ 2H_2O$	1.32
$SrCl_2 \circ 6H_2O$	0.02
$NaHCO_3$	0.17

APPENDIX F

STANDARD OPERATING PROCEDURE
FOR
EXTRACTION OF PORE WATER

APPENDIX F

STANDARD OPERATING PROCEDURE FOR EXTRACTION OF PORE WATER

1.0 INTRODUCTION

Simple pore water (interstitial water, or water which exists between sediments) extraction techniques are used to collect and evaluate sediment pore waters during bio-geochemical and toxicological studies. The whole sediment centrifugation method uses centrifugal force without filtration to remove pore water from interstitial spaces. It may not be possible to obtain any pore water by centrifugation with a very sandy sample (Carr and Chapman 1995). Where the primary contaminants of concern are hydrophobic organic compounds, centrifugation is the method of choice for maximizing the sensitivity of any toxicity tests. Regardless of method used substantial losses of nonpolar contaminants (e.g. fluoranthene and p,p'-DDE) can occur (Carr and Chapman 1995). The following technique is the method utilized by the National Fisheries Contaminant Research Center, U.S. Fish and Wildlife Service, Columbia, Missouri (Kemble and others 1994) and the U.S. Environmental Protection Agency, Duluth, Minnesota (Ankley and Schubauer-Berigan 1994) as modified by ASTM Standard E 1391-94, *Collection, Storage, Characterization, and Manipulation of Sediments for Toxicological Testing* (ASTM 1994) and Carr and Chapman (1995).

1.1 SEDIMENT SAMPLING FOR PORE WATER EXTRACTION

This extraction technique was developed for laboratory use in conjunction with standard sampling techniques. It is most effective when sampling devices which are used preserve the sediment-water interface and the overlying water. The sediment sample to be extracted should be homogenized before the extraction procedure begins.

1.2 PORE WATER SAMPLE EXTRACTION PROCESS AND HANDLING

Sediments used for pore water analysis should be processed to remove the pore water as soon as possible after collection to minimize effects due to chemical changes (ASTM 1994). Extraction of pore water within 24 hours of collection is recommended, but in no case should the sediments be

stored longer than 2 weeks after collection before extraction of pore water. The homogenized sediment sample should be centrifuged at ambient temperature (ASTM 1994), of the sediments at the time of collection, for 30 minutes at a speed of 3,200 gravity units (g) using a refrigerated centrifuge equipped with a swinging bucket type rotor capable of spinning 100 to 1000 mL bottles. A second centrifugation will be performed for 30 minutes at 10,000 g. Tubes or bottles of glass or polycarbonate are recommend in order to minimize adsorption of soluble contaminants on the container walls. The supernatant should be (re-) centrifuged to further remove any suspended particulate matter. A pipette should be used to transfer the supernatant to 1-L amber glass bottle for storage until used. An aerobic environment should be maintained. Pore water samples can be held at 4° C in the dark until testing begins. Pore water samples may also be frozen but should be (re-) centrifuged after thawing to minimize artifactual responses resulting from precipitated suspended particulate matter in the sample (Carr and Chapman 1995).

1.3 DOCUMENTATION

As with any sampling, careful documentation is necessary. Comments relating to starting and ending time of the centrifugal process, composition of centrifuge tubes or bottles, volumes obtained, and salinity of pore water should be recorded in the laboratory notebook.

REFERENCES

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APPENDIX G

STANDARD OPERATING PROCEDURE
FOR
10-DAY WHOLE SEDIMENT TOXICITY TEST USING THE ESTUARINE AMPHIPOD,
Eohaustorius estuarius

APPENDIX G

STANDARD OPERATING PROCEDURE FOR 10-DAY WHOLE SEDIMENT TOXICITY TEST USING THE ESTUARINE AMPHIPOD, *Eohaustorius estuarius*

1.0 APPLICATION

The amphipod sediment bioassay can be used to determine the potential acute impact of whole sediment contaminants on benthic organisms through a short-term exposure. Methods outlined in this standard operating procedure (SOP) are based on ASTM Standard E1367-92, *Standard Guide for Conducting 10-Day Static Sediment Toxicity Tests with Marine and Estuarine Amphipods* (ASTM 1992), and *Methods for Assessing the Toxicity of Sediment-associated Contaminants with Estuarine and Marine Amphipods* (EPA 1994).

2.0 TEST ORGANISMS

The regulations require that benthic bioassays be conducted with filter feeding, depositing feeding, and burrowing species.

2.1 SPECIES SELECTION

This test is designed for the specific requirements of *Eohaustorius estuarius*. *Eohaustorius estuarius* is a free-burrowing amphipod found on protected and semi-protected beaches from the lower intertidal to shallow subtidal waters exclusively on the Pacific coast from British Columbia south to central California (From Environment Canada 1992, as stated by EPA 1994). It is an estuarine species which has been reported from areas where pore water salinity ranges from 1 to 25 parts per thousand (ppt).

Table G-1 summarizes the tolerance limits of this species.

2.2 AGE

All amphipods to be used for testing must be of a uniform age and size. As a guideline, amphipods of 3 to 5 millimeters (mm) in length, or those amphipods retained on a 1.0 mm mesh screen, are commonly used for testing.

2.3 COLLECTION AND SHIPPING

Eohaustorius estuarius is not available commercially, but has routinely been collected for toxicity tests from Beaver Creek near Newport, Oregon, and on the west coast of Vancouver Island, BC, Canada. *Eohaustorius estuarius* is found mainly on protected and semi-protected beaches from mid-water level to shallow subtidal, within the upper 10 cm of sediment along the Pacific coast from British Columbia south to at least central California (EPA 1994). It can also be found on open coasts in beds of freshwater streams flowing into the ocean, and in sand banks in estuaries.

Subtidally *Eohaustorius estuarius* can be collected with a small dredge or grab (e.g., PONAR, Smith-McIntyre, or Van Veen), or by skimming the sediment surface with a long-handled, fine-mesh net (EPA 1994). Intertidal populations can be collected with a shovel. These amphipods can be isolated from the collection site sediment by gentle sieving with a 1.0 mm mesh screen. At least one-third more amphipods should be collected than are necessary.

For shipping of *E. estuarius*, use small plastic containers (about 500 mL) with sealable lids. Fill the containers three-quarters full with sieved collection site sediment (fine sand) and then cover with a 1 cm layer of collection site water (EPA 1994). Limit the number of amphipods to about 100 per container. Once the amphipods have burrowed, pour off the water, but keep the sand moist. Seal the containers and ship for receipt overnight.

Criteria for acceptance of shipped *E. estuarius* is as follows (EPA 1994):

- Exhibition of swimming behavior upon placement in water.
- Full digestive tracts.

- Grayish- or yellowish white in color.
- Mortality should be less than 5 percent.
- Containers should be intact.
- Sediment temperature should be between 4 and 10°C.

The supplier should provide the physical parameters of the collection site, including the temperature and salinity. In addition, collection site sediment should be supplied for acclimation and for use as a control sediment.

2.4 ACCLIMATION

Organisms are held in tanks at least 48 hours before testing in 4- to 8-L volume containers (EPA 1994). The containers should have approximately 2 to 4 cm of sediment which has been sieved with a 0.5 mm mesh screen. Amphipod density should not exceed 1 amphipod/cm^2. Water used to hold *E. estuarius* should be adjusted to 20 ppt at a rate that must not exceed 5 ppt per 24 hours. Overlying water temperature must not be changed by more than 3°C per day and maintained at that temperature for a minimum of 2 days. Fluorescent lights should be used to provide from 500 to 1000 lux at the surface of the sediment in the holding tanks.

Temperature and salinity should be measured daily during adjustment. Thereafter, temperature, pH, dissolved, and salinity should be measured at least at the start and the end of the acclimation period. Once test conditions have been achieved, then the amphipods should be held at these conditions for at least 2 days before testing begins (EPA 1994). Amphipods should not be held for more than 10 days prior to testing.

3.0 CONTROL WATER

Artificial sea water of approximately the same temperature, salinity, and dissolved oxygen content as the water at the sites to be tested, is prepared using ACS reagent grade chemical salts and deionized water. The recipe for synthetic GP-2 sea water was published by the U.S. Environmental Protection Agency (EPA 1988) and is listed in Table G-2.

Once the necessary volume of GP-2 has been prepared, it is aged, with aeration, for 2 weeks before it is used in bioassay testing. If a residue or precipitate is present after aging, the sea water is filtered through 0.45 μm filter, before it is used. Salinity adjustments are made, if necessary, with distilled water (to decrease salinity) or a brine prepared from distilled water and artificial sea salts (to increase salinity). Dissolved oxygen is maintained above 40 percent saturation.

4.0 TEST PARAMETERS

Test parameters for this SOP are as follows (ASTM 1992; EPA 1994):

Test Type:	Whole-sediment toxicity test, static
Temperature:	15°C ± 2°C
Salinity:	20 ppt
Light Quality:	Wide-spectrum fluorescent lights
Illuminance:	500 - 1,000 lux
Photoperiod:	24L:0D
Test Chamber:	1-liter (L) glass beaker or jar with an inner diameter of about 10 centimeters (cm)
Sediment Volume:	175 mL (2 cm)
Overlying Water Volume:	800 mL
Renewal of Overlying Water:	None
Size of Amphipods:	3 to 5 mm
Number of Organisms/Chamber:	20
Number of Replicates:	5
Feeding Regime:	None
Overlying Water Aeration:	Aerate overnight before start of test, and throughout to maintain greater than or less than (≥) 90 percent saturation

Overlying Water Quality Monitoring:	Temperature, dissolved oxygen, salinity, and pH in overlying water daily; ammonia (total and unionized) and sulfides at days 2 and 8 in the overlying water; salinity, ammonia, sulfides, and pH of pore water at beginning and end of test.
Test Duration:	10 days
Endpoints:	Survival and reburial
Test Acceptability:	Minimum survival of 90 percent in controls and satisfaction of performance-based criteria as outlined below.

5.0 SEDIMENT PREPARATION 48 HOURS BEFORE TESTING

The sediment preparation instruction listed below should be followed 48 hours before testing.

- Homogenize the sediment within its storage container.
- Remove an aliquot from the center interior of the control sample container.
- Examine sediment for indigenous organisms and if present, press-sieve the sediment through a 1.0 mm mesh screen.
- Remove any indigenous organisms.
- Collect approximately 250 mL of sediment for each test chamber.
- Hold the sediment at 4° C until the bioassay chambers are prepared.

6.0 SEDIMENT PREPARATION 24 HOURS PRIOR TO TESTING

The following sediment preparation instructions should be followed before test begins.

- Prenumber the bioassay beakers randomly for all treatments including the control.
- Add an aliquot, approximately 175 mL, of sediment to a test chamber to fill the chamber to 2 cm.

- Calculate the net weight of the sediment added to the chamber to assist with the preparation of replicate sediment samples.

- Net weight must be calculated for each test sediment and the control to account for varying sediment densities.

- Settle each sediment in the chambers by gently tapping the glass or by smoothing the sediment surface with a polyethylene spatula.

- Add sea water to fill the beaker to 975 mL. This procedure requires that a polyethylene disk attached to a nylon filament be used during filling so that the sediments are not disturbed.

- Place the disk on top of the sediment while the sea water is added.

- Remove the disk and rinse between replicates.

- Use a different disk for each sediment that is tested.

- Aerate each chamber and allow the sediments to settle overnight.

7.0 INTRODUCTION OF ORGANISMS TO TEST CHAMBERS

The following procedures for introduction of test amphipods to the test chambers is recommended.

- Select a set of 10 to 15 test chambers to be initiated at one time including replicates and controls.

- Sieve *E. estuarius* from holding containers using a 0.5 mm sieve. Enough organisms are sieved to provide approximately one third more amphipods than are needed for one set of test chambers. This will allow for the selection of healthy individuals.

- Transfer organisms using a bulb pipette with a polished 6 mm opening, from the sieve to a sorting tray containing sea water of the holding temperature and salinity.

- Select amphipods from the sorting tray and transfer to dishes containing 150 mL control sea water each. Each dish should contain 20 organisms.

- Sort an additional group of amphipods and preserve with 5 percent sugar, buffered formalin for later measurement. This group represents the initial size of the organisms.

- Add the organisms to each test chamber. The appropriate disk is placed on the water surface while the contents of each dish are gently poured over the disk and into the test chamber. If any amphipods are stuck in the dishes, gently wash them out using control sea water.

- Bring the water level up to 975 mL with control sea water.

- After one hour, replace any organisms that die or do not bury.

8.0 DAILY MONITORING OF THE TESTS

The following parameters should be monitored during the test (EPA 1994).

- All chambers should be checked daily and observations made to assess amphipod behavior such as avoidance.

- Record but do not remove any dead organisms.

- Record the number of organisms which have emerged from the sediments.

- Record abnormal behavior such as amphipods failing to rebury into the sediments.

- Measure and record the following water parameters daily: temperature, salinity, dissolved oxygen, and pH.

- Measure the ammonia (total and unionized) and sulfides concentration in overlying water in each test chamber at test initiation (day 2) and test completion (day 8). In conjunction with ammonia, measure pH and temperature. Measurements of ammonia, pH, salinity, and temperature in sediment pore water should be made at the beginning and end of the test. Pore water should be extracted following the method described in Appendix F.

- The number of amphipods swimming in the water column and trapped in the air-water interface should be noted.

9.0 REFERENCE TOXICANT TESTING

The quality of field collected amphipods should be verified by conducting a reference-toxicity test. Monthly reference-toxicity tests should be conducted on *E. estuarius* cultures using procedures as outlined below (EPA 1994). If reference-toxicity tests are not conducted monthly, a reference toxicant

test is run simultaneously with the sediment test to compare the relative health of the amphipod specimens being used.

This test is run without sediment in clean sea water for 96-hour and an LC50 value can be calculated. ACS Reagent-grade cadmium chloride, $CdCl_2$, is used as a reference toxicant. Test parameters are as follows (EPA 1994):

Test Type:	Water-only
Dilution Series:	Control and at least five test concentrations (0.5 dilution factor)
Toxicant:	Cadmium Chloride ($CdCl_2$)
Temperature:	15°C ± 2°C
Salinity:	20 ppt
Light Quality:	Chambers should be kept in dark
Photoperiod:	24 hours dark
Renewal of Water:	None
Size of Amphipods:	3 to 5 mm
Test Chamber:	1 L glass beaker or jar
Volume of Water:	800 mL
Number of Organisms/Chamber:	20
Replicates:	3
Aeration:	Recommended unless >90 percent saturation can be achieved without aeration
Water Quality:	Temperature daily; salinity, ammonia (total and unionized), sulfides, pH, and dissolved oxygen at beginning and end of test.
Test Duration:	96 hour
Endpoint:	Survival (LC50); reburial (EC50)

Test Acceptability: 90 percent control survival

The reference toxicant tests are conducted following the same method outlined in this SOP with the following exceptions:

- Prepare a concentrated stock solution of cadmium chloride at a concentration of 1 mg/mL using GP-2 control/dilution sea water described in Section 3, which has been adjusted to test salinity (15 ppt ± 2 ppt).
- Refer to Table G-3 for the preparation of the geometric dilution series based on a test volume equal to that of the sea water and test sediment used in the loaded solid-phase test. Five unreplicated concentrations and a control are used in the testing.
- Randomly place prenumbered toxicant treatment containers in growth chamber (15°C ± 2°C) for testing.
- Load toxicant treatments in the growth chambers.
- Allow toxicant treatment containers to equilibrate overnight.

10.0 TEST COMPLETION

The following is performed following 10 days of test exposure.

- Surviving amphipods in the water column can be removed by pipetting.
- Rinse the contents of each exposure chamber through a 0.5 mm sieve.
- Mix the material retained on the screen with the clean sea water and search thoroughly for organisms.
- Gently prod organisms or observe under magnification to determine if the organisms are alive. If they show any response including pleopod twitching, the amphipod is considered to be alive.
- Record the total number of live and dead organisms. Any animal that does not respond to gentle prodding is presumed dead.

11.0 ACCEPTABILITY OF THE TEST

Test acceptability requirements for a 10-day sediment toxicity with *E. estuarius* are listed below (EPA 1994). Performance criteria for testing are as follows:

- The amphipods must from 3 to 5 mm long at the end of the test.
- Average survival in the control sediment must be greater than or equal to 90 percent at the end of the test.

Performance criteria for field collection are as follows:

- Reference-toxicant tests must be performed on each batch of field-collected amphipods used in sediment tests.
- Acclimation rates to test salinity and temperature should not exceed 3°C and 5 ppt in 24 hours.
- Amphipods must exhibit active swimming behavior upon placement in water, have full digestive tracts, and display a grayish or yellowish white color after receipt from the field.

Additional requirements are as follows:

- All organisms must be from the same population.
- Tests should begin within 10 days after receipt of amphipods.
- All test chambers should be identical and should contain the same amount of sediment and overlying water.
- Negative-control sediment must be included in a test.
- The daily mean temperature must be within ±1°C of the desired temperature. The instantaneous temperature must always be within ±3° of the desired temperature.
- The dissolved oxygen must not fall below 60 percent of saturation in any chamber.
- Natural physico-chemical characteristics of the test sediment collected from the field should be within the tolerance limits of the test organisms.

- Treatments must be randomly assigned to individual test chambers.
- Test amphipods must be randomly distributed to test chambers.

12.0 STATISTICAL METHODS

Statistical analysis of the data should follow the guidance provided by ASTM (1992) and EPA (1994).

13.0 POTENTIAL PROBLEM AREAS

The following are potential problem areas that may be encountered during tests.

- Caution should be taken at end of test to recover all organisms. Laboratory personnel should be cautioned to spend at least 10 minutes/replicate in examining sieved material (EPA 1994).

14.0 RECORD KEEPING

The report should document the following information either directly or by reference to available documents (ASTM 1992; EPA 1994).

- Name of test and investigator(s), name and location of laboratory, and dates of start and end of test.
- Source of negative control, reference sediment, and test sediment with the method of collection, handling, shipping, storage, and disposal of sediment.
- Source of overlying water, its chemical characteristics, and a description of any pretreatment.
- Results of any demonstration of the ability of the species to survive, grow, or reproduce in the overlying water.
- Source, history, and age of test organisms.
- Source and date of collection of the test organisms, scientific name, name of person who identified the organisms, and the taxonomic key used. In addition, provide age, life-stage, and means and ranges of weights and lengths. Observed diseases or

unusual appearance, treatment, holding and acclimation procedures should also be recorded.

- Source and composition of food, procedure used to prepare food, feeding method, frequency, and ration.
- Description of the experimental design and test chambers used; the depth and volume of sediment and overlying water in the chambers; lighting; number of replicates and number of test organisms per replicate; date and time test started and ended.
- Records of temperature, salinity, pH, dissolved oxygen, and ammonia tested in overlying water and in pore water.
- Biological endpoints used along with appropriate measurements.
- A table of the biological data for each test including the control(s) in sufficient detail to allow independent statistical analysis.
- Statistical test used and results of analysis of the data.
- Results of reference toxicant tests.
- Information on calibration of equipment and instruments used during the test.
- Copy of the chain-of-custody record, the sample log-in sheet, the original bench sheets for the test organism responses during the sediment test(s), and original signatures and dates of laboratory personnel performing the test(s).
- Summary of general observations made during the test.
- Any deviations from the required procedures and any other relevant information.

REFERENCES

American Society for Testing and Materials (ASTM). 1992. Standard Guide for Conducting 10-Day Static Sediment Toxicity Tests with Marine and Estuarine Amphipods. ASTM 1992 Annual Book of Standards, Vol 11.04, E1367-92, Philadelphia.

U.S. Environmental Protection Agency (EPA). 1988. "Short-Term Methods for Estimating Chronic Toxicity of Effluents and Receiving Water to Marine and Estuarine Organisms." EPA/600/4-87/028.

EPA. 1994. "Methods for Assessing the Toxicity of Sediment-associated Contaminants with Estuarine and Marine Amphipods." EPA/600/R-94/025, June.

TABLE G-1

TOLERANCE LIMITS FOR THE ESTUARINE AMPHIPOD, *Eohaustorius estuarius* (FAMILY HAUSTORIIDAE)

Geographic range	Pacific from British Columbia to central California
Habitat	Free burrowing; protected and semi-protected beaches from lower intertidal to shallow subtidal
Life cycle	Probably annual
Temperature	Approximately 0 to 21° C
Salinity	Oligo-mesohaline; pore water salinity from 1 to 25 ppt
Sediment type	Clean, medium-fine sand with some organic matter
Sediment depth	Upper 10 cm of sediment
Nutrition	Meiofauna predator, but also ingests sedimentary organic matter - deposit feeder

TABLE G-2

SALTS USED IN THE PREPARATION OF GP-2 ARTIFICIAL SEA WATER (30 ppt SALINITY) (EPA 1988)

Compound	Concentration (g/L)
NaCl	21.03
Na_2SO_4	3.52
KCl	0.61
KBr	0.088
$Na_2B_4O_7 \circ 10H_2O$	0.034
$MgCl_2 \circ 6H_2O$	0.02
$CaCl_2 \circ 2H_2O$	1.32
$SrCl_2 \circ 6H_2O$	0.02
$NaHCO_3$	0.17

TABLE G-3

GEOMETRIC DILUTION SERIES FOR REFERENCE TOXICANT TEST

Test Concentration (mg/L)*	Control Volume (mL)	$CdCl_2$ Volume (mL)	Final Volume (mL)
Control	1000	0	1000
0.16	982	16	1000
0.31	969	31	1000
0.63	937	63	1000
1.23	887	123	1000
2.50	750	250	1000
5.00	500	500	1000

* Test concentrations are prepared from a stock solution containing 10 mg/L cadmium chloride ($CdCl_2$).

APPENDIX H

STANDARD OPERATING PROCEDURE
FOR
PREPARATION OF TISSUE FOR ANALYSIS

APPENDIX H

STANDARD OPERATING PROCEDURE FOR PREPARATION OF TISSUE FOR ANALYSIS

1.0 INTRODUCTION

This standard operating procedure provides information on the preparation of invertebrate or fish tissue for both metals and organics analysis. This procedure is based on the recommended protocols for the Puget Sound Estuary Program (PSEP 1986, 1989) and the U.S. Environmental Protection Agency's (EPA) "Fish, Field and Laboratory Method for Evaluating the Biological Integrity of Surface Waters" (Klenim and others 1993).

2.0 SAMPLE PREPARATION FOR ORGANIC CONTAMINANTS IN TISSUE

The following sections detail guidance for sample preparation for organic contaminants in tissue.

2.1 COLLECTION PRECAUTIONS

In the field, sources of tissue contamination include sampling gear, boats and motors, grease from ship winches or cables, engine exhaust, dust, and ice used for cooling. Efforts should be made to minimize handling and to avoid sources of contamination. For example, to avoid contamination from ice, the whole samples should be wrapped in aluminum foil, placed in watertight plastic bags, and immediately cooled in a covered ice chest. Tissue sample collection and preparation requirements are summarized in Table H-1 (PSEP 1989).

2.2 PROCESSING

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 in isopropanol,

and finally rinsed with organic free distilled water. Work surfaces should be cleaned with isopropanol, washed with distilled water and allowed to dry completely.

The removal of biological tissues should be carried out by or under the supervision of an experienced biologist. Tissue should be removed with clean stainless steel or quartz instruments (except for external surfaces). The specimens should come into contact with precleaned glass surfaces only. Polypropylene and polyethylene (plastic) surfaces and implements are a potential source of contamination and should not be used. To control contamination when resecting tissue, technicians should use separate sets of utensils for removing outer tissue and for resecting tissue for analysis.

2.3 PREPARATION OF TISSUE SAMPLES

Special care must be taken to avoid contaminating the selected organism with slime and/or adhering sediment. The proper handling in the preparation of tissue samples to decrease the likelihood of contamination cannot be over emphasized.

The entire organism should be cleaned and placed into storage containers as specified. If more than one specimen of an organism will be used for analysis, these individuals should be placed in the same container together to prevent confusion later in the laboratory.

Because of the low limits of detection for many environmental analyses, clean field and laboratory procedures are especially important. Sample contamination can occur during any stage of collection, handling, storage, or analyses. Potential contaminant sources must be known and steps taken to minimize or eliminate them.

Large sheets of heavy duty aluminum foil should be used to carefully fold and completely wrap the tissue samples. When filling out I.D. labels use pencil or waterproof marker and place the foil wrapped sample in a secured plastic bag.

2.4 STORAGE

Recommended holding times for frozen tissue samples have not been established by EPA, but a maximum 1 year holding time is suggested for organic analytes. This holding time is different than the 6 month holding time suggested for inorganic analytes (except mercury [28 days]). For extended sample storage, precautions should be taken to prevent desiccation. National Institute for Standards and Technology is testing the effects of long-term storage of tissues at temperatures of liquid nitrogen (-120° to -190°C). At a minimum, the samples should be kept frozen at -20°C until extraction. This will slow biological decomposition of the sample and decrease loss of moisture. Liquid associated with the sample when thawed must be maintained as part of the sample because the lipid tends to separate from the tissue. Storage of samples should remain under the control of the sample collector until relinquished to the analytical laboratory.

The whole organism may be frozen and stored if no resection of internal organs will be conducted and the ultimate analysis is whole body. However, if resection of organs is required, these tissues should be removed prior to freezing and can be stored frozen in appropriate individual containers. The tissues may then be ground and homogenized at a later date and refrozen in sample packets for shipment on dry ice to the analytical laboratory(s).

To avoid sample deterioration, it is recommended that all samples be frozen solid prior to shipment. The frozen and logged samples should be wrapped in newspaper to provide additional insulation for the samples which are shipped in well sealed insulated containers with an appropriate quantity of dry ice. The quantity of dry ice should be sufficient to eliminate any defrosting of the samples during the time of priority transport. However, in the event that a delay occurs in transit, these recommendations will provide some assurance that the samples will arrive in usable condition. Under no circumstances should unfrozen tissue be shipped either with or without dry ice because the quality of the sample cannot be assured.

2.5 TISSUE PREPARATION

Organic contaminants are not evenly distributed throughout biological tissue. Therefore, to obtain a homogenous sample, the whole organism must be ground to a homogeneous consistency. This procedure should be carried out by the sample collector on partially thawed samples.

Chop the sample into small pieces, unless the sample is small enough to fit in a hand crank meat grinder (300 gm or less) or a food processor (Hobart Model 8181D or equivalent) (EPA 1990). Then pass the whole sample through a meat grinder. Grinding of biological tissue is easier when the tissue is partially frozen. Chilling the grinder with a few chips of dry ice will reduce the tendency of the tissue to stick to the grinder. Do not freeze the grinder since hard frozen tissue is difficult to force through the chopper plate.

The ground sample is divided into quarters, opposite quarters are mixed by hand with a clean stainless steel spatula and then the two halves are mixed back together. Repeat the mechanical grinding, quartering, and hand mixing two more times. No chunks of tissue should be present at this point as they will not be efficiently extracted. Very small organisms may be homogenized in a high speed blender.

When compositing large individual organisms, individual tissue should be ground separately following the above described procedure. Then take equal amounts from each tissue sample to be composited to provide a total equal to that required for extraction or the total number of split and archived samples required by the study plan.

If the ground tissue is to be re-frozen prior to extraction and analysis, weigh out the exact amount for extraction into a small container. Using a top loading balance, tare a 2 oz. glass jar (or a small sheet of aluminum foil that can be formed into a sealed packet) to 0.0 gm and carefully dispense a 20.0 gm portion of homogenized tissue into the container. Tightly seal the container or foil packet. Repeat with additional containers for duplicates, splits, or archived samples. Lipid material tends to migrate during freezing; therefore, storing a weighed portion ensures extraction of a representative portion of the tissue if the foil or container is completely rinsed with solvent by the analytical chemist.

Whenever a ground sample is to be split between two or more laboratories, the ground sample must also be mixed with reagent grade anhydrous sodium sulfate (previously heated to 400° C to drive off any phthalate esters acquired during storage). To ensure the homogeneity of the sample prior to splitting, transfer 100 gm of ground tissue to a 600 mL beaker. Add 250 gm of anhydrous sodium sulfate and mix thoroughly with a stainless steel spoon or a spatula. There should not be any lumps and the mixture should appear homogeneous. Dispense exactly 70.0 gm of mixture to each lab and note on the package that it contains 20 gm of tissue.

When preparing tissue for volatile analysis, grind it in an area free of volatile organic compounds. The meat grinder or food processor must be heated in an oven for 30 minutes at 105°C after solvent rinsing and then allowed to cool at room temperature. Immediately after grinding the tissue, weigh duplicate 1 gm portions into culture tubes with screw caps. Analyze immediately or store in a freezer.

3.0 SAMPLE PREPARATION FOR METAL CONTAMINANTS IN TISSUE

The following sections detail sample preparation procedures for tissue residue analysis.

3.1 COLLECTION PRECAUTIONS

The major difficulty in trace metal analyses of tissue samples is controlling contamination of the sample after collection. In the field, sources of contamination include sampling gear, grease from winches or cables, engine exhaust, dust, or ice used for cooling. Care must be taken during handling to avoid these and any other possible sources of contamination. For example, during sampling the ship should be positioned such that the engine exhausts do not fall on deck. To avoid contamination from melting ice, the samples should be placed in watertight plastic bags.

Sample resection and subsampling of the organisms should be carried out in a controlled environment (e.g., dust-free room). In most cases, this requires that the organisms be transported on ice to a laboratory rather than being resected in the field. It is recommended that whole organisms not be frozen prior to resection if analyses will be conducted only on selected tissues, because freezing may cause internal organs to rupture and contaminate other tissue. If organisms are eviscerated in the field, the remaining tissue (e.g. muscle) may be wrapped as described above and frozen (PSEP 1986).

Resection is best performed under "clean room" conditions. The "clean room" should have positive pressure and filtered air and also be entirely metal-free and isolated from all samples high in contaminants (e.g., hazardous waste). At a minimum, care should be taken to avoid contamination from dust, instruments, and all materials that may contact the samples. The best equipment to use for trace metal analyses is made of quartz, TFE (tetrafluorethylene), polypropylene, or polyethylene. Stainless steel that is resistant to corrosion may be used if necessary Corrosion-resistant stainless steel is not magnetic, and thus can be distinguished from other stainless steels with a magnet. Stainless steel scalpels have been found not to contaminate mussel samples (Stephenson et al., 1979). However, low concentrations of heavy metals in other biological tissues may be contaminated significantly by any exposure to stainless steel. Quartz utensils are ideal but expensive. To control contamination when resecting tissue, separate sets of utensils should be used for removing outer tissue and for removing tissue for analysis. For bench liners and bottles, borosilicate glass would be preferred over plastic if trace organic analyses are to be performed on the same sample.

Prior to use, utensils and bottles should be thoroughly cleaned with a detergent solution, rinsed with tap water, soaked in acid, and then rinsed with metal-free water. For quartz, TFE, or glass containers, use 1+1 HNO_3, 1+1 HCl, or aqua regia (3 parts conc. HCl = 1 part conc HNO_3) for soaking. For plastic material, use 1+1 HNO_3 or 1+1 HCl. Reliable soaking conditions are 24 hours at 70° C (APHA 1989; 1992). Do not use chromic acid for cleaning any materials. Acids used should be at least reagent grade. For metal parts, clean as stated for glass or plastic, except omit the acid soak step. If trace organic analyses are to be performed on the same samples, final rinsing with methylene chloride is acceptable.

Sample size requirements can vary with tissue type and detection limit requirements. In general, a minimum sample size of 6 g (wet weight) is required for the analysis of all priority pollutant metals. To allow for duplicates, spikes, and required reanalysis, a sample size of 50 g (wet weight) is recommended. Samples can be stored in glass, TFE, or high-strength polyethylene jars.

3.2 PROCESSING

Samples should be frozen after resection and kept at -20° C. Although specific holding times have not been recommended by EPA, a maximum holding time of 6 months for inorganic analytes (except for

mercury samples, which should be held a maximum of 28 days) would be consistent with that for water samples.

When a sample is thawed, the associated liquid should be maintained as a part of the sample. This liquid will contain lipid material. To avoid loss of moisture from the sample, partially thawed samples should be homogenized. Homogenizers used to grind the tissue should have tantalum or titanium parts rather than stainless steel parts. Stainless steel blades used during homogenization have been found to be a source of nickel and chromium contamination. Some trace metal contamination during processing cannot be avoided and it is therefore necessary to determine and control the amount of contamination introduced during processing. Contamination can be monitored by introducing a dry ice blank into the blender and analyzing the chips.

To avoid trace metal contamination during processing the preferred method is to proceed to a chemical digestion process which minimizes or eliminates resection, homogenization, or grinding. Chemical digestion is best limited to specific organ tissues from large fish or to smaller sized whole fish.

3.3 IDENTIFICATION OF COMPOSITE WHOLE ORGANISM SAMPLES

Composite whole organism samples will be made up of three to ten organisms with any deviation in number clearly identified. The limitation on the variance between individuals in each composite will be as previously described. The same field information should be provided as described above for whole body composite samples. The same handling precautions as described above should be followed for either organic or trace metal contaminants.

REFERENCES

American Public Health Association (APHA). 1989. "Standard Methods for Examination of Waste and Wastewater. 17th Ed."

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U.S. Environmental Protection Agency (EPA). 1990. Extraction and Analysis of Organics in Biological Tissue, Method OB 8/90, USEPA, Environmental Services Division, Region IV, Analytical Support Branch, Athens, GA.

TABLE H-1

SUMMARY OF SAMPLE COLLECTION AND PREPARATION QA/QC REQUIREMENTS FOR FISH AND INVERTEBRATE TISSUE (MODIFIED FROM PSEP 1986, 1989)

Variable	Sample Size (a)	Container (b)	Preservation	Maximum Holding Time (c)	Maximum Extract Holding Time
Organic Compounds					
Wholebody Tissues	--	A	Freeze (-18°C)	1 yr	40 days
Semivolatiles	25 g	G, T, A	Freeze (-18°C)	1 yr	40 days
Volatiles	5 g	G, T	Freeze (-18°C)	14 days	--
Trace Metals					
Wholebody Tissues	--	W, P, B	Freeze	6 mo	
All Metals	5 g	P, B	Freeze	6 mo	
(except Hg)	0.2 g	P, B	Freeze	28 days	

Notes:

a Recommended wet weight sample sizes for one laboratory analysis. If additional laboratory analyses are required (that is, replicates), the field sample size should be adjusted accordingly. If specific organs are to be analyzed, more tissue may be required.

b G = glass; A = wrapped in aluminum foil and placed in watertight plastic bags; T = PTFE (Teflon); P = linear polyethylene; B = borosilicate glass; W = watertight plastic bags.

c This is a suggested holding time. No EPA criteria exist for the preservation of this variable.

APPENDIX I

STANDARD OPERATING PROCEDURE
FOR
28-DAY WHOLE SEDIMENT BIOACCUMULATION TEST USING THE MARINE POLYCHAETE, *Nephtys caecoides*

APPENDIX I

STANDARD OPERATING PROCEDURE FOR 28-DAY WHOLE SEDIMENT BIOACCUMULATION TEST USING THE MARINE POLYCHAETE, *Nephtys caecoides*

1.0 APPLICATION

Chronic sublethal sediment bioassays are relevant because bioaccumulation of contaminants from a sediment matrix is a slow process compared to uptake from solution (Dillon and others 1993). Thus, benthic infaunal organisms, such as a polychaete, are typically exposed to a continuous but low level of contamination. This test evaluates the potential for bioaccumulation of sediment-associated contaminants to the tissues of marine polychaetes.

2.0 TEST ORGANISMS

Test species should be in intimate contact with the sediment, ingest sediment, and provide adequate biomass for chemical analysis at test termination.

2.1 SPECIES SELECTION

This test is designed for the specific requirements of *Nephtys caecoides*. This species is a benthic infaunal polychaete living in sandy muds of bays and lagoons along the California coast (Smith and Carlton 1980) and is a detritivore. It is accepted in the California regulatory community as an appropriate test species for sediment evaluation, and a substantial amount of toxicological data for a variety of contaminants already exists for this species (Battelle Marine Sciences Laboratory 1994).

2.2 SPECIES SOURCE

This species may be collected in the bays and lagoons along the California coast and occurs naturally in San Francisco Bay (PRC 1995). A commercial source for *Nephtys caecoides* is Brezina and Associates of Dillon Beach, California, (707)878-2853.

2.3 SIZE

All *N. caecoides* to be used for testing must be of a uniform size.

2.4 ACCLIMATION

Nephtys caecoides should be held in their native sediment under flow through or static renewal conditions. If flow-through conditions are not possible, remove and replace 75 percent of sea water with fresh sea water every other day. Vigorous aeration is necessary to maintain the health of this species (Battelle Marine Sciences Laboratory 1994). The polychaetes to be used for the test should be acclimated to test conditions. Temperature changes should not exceed 2°C per day, and salinity changes not more than 2 ppt per day. The test organisms should be held for at least 2 days before use, but no more than 7 days. The polychaetes should not be fed. The water quality should be measured and recorded daily.

3.0 CONTROL WATER

Natural sea water from an uncontaminated source or artificial sea water should be used as control water. Artificial sea water can be prepared using American Chemical Society (ACS) reagent grade chemical salts and deionized water. The recipe for synthetic GP-2 sea water was published by the U.S. Environmental Protection Agency (EPA 1988) and is listed in Table I-1. Once the necessary volume of GP-2 has been prepared, it is aged, with aeration, for 2 weeks before it is used in bioassay testing. If a residue or precipitate is present after aging, the sea water is filtered through a 0.45 μm filter, before it is used. Salinity adjustments to culture or test water can be made by adding the 30 ppt sea water to the test or culture water.

4.0 TEST PARAMETERS

Test parameters for this SOP are as follows (Battelle Marine Sciences Laboratory 1994):

Test Type:	Static renewal
Test Duration:	28 days
Temperature:	20 ± 1°C
Salinity:	20 ppt ± 2 ppt
Light Quality:	Ambient laboratory
Light Intensity:	50 to 100 ft-c
Photoperiod:	16L/8D
Test Chamber Size:	37 L
Overlying Water Volume:	16.1 L overlying water
Sediment Depth:	3 cm (3.9 L)
Renewal of Test Solutions:	Daily
No. Organisms per Test Chamber:	30
No. Replicate Chambers per Treatment:	5
No. Organisms per Treatment:	150
Feeding Regime:	None
Overlying Water Aeration:	Trickle-flow (< 100 bubbles/min.)
Dilution Water:	Natural seawater or modified GP-2.
Test Treatments:	Site sediment and native sediment
Dilution Series:	Not applicable
Overlying water quality Monitoring:	Temperature, salinity, pH, and dissolved oxygen measured daily; ammonia (total and

	unionized) and sulfides measured at the beginning and end of test and weekly.
Endpoints:	Survival and bioaccumulation
Test Acceptability:	Minimum mean survival in native control sediment is not less than 80 percent; minimum mean survival of 70 percent in any single control sediment; adequate biomass at test completion for detection of target analytes; and performance criteria specifications as outlined in Section 10 below.

5.0 SEDIMENT PREPARATION PRIOR TO TESTING

The following sediment preparation instructions should be followed before tests begin.

5.1 48 HOURS PRIOR TO TESTING

The sediment preparation instruction listed below should be followed 48 hours before testing begins.

- Homogenize the sediment within its storage container.
- Remove an aliquot from the center interior of the storage container.
- Press-sieve the sediment through a 1.0 mm mesh screen.
- Collect approximately 3.9 L of sediment for each test chamber.
- Remove any macrobenthic organisms or debris found in the sediment during sieving.
- Hold the sediments at 4°C until the bioassay chambers are prepared.

6.2 24 HOURS PRIOR TO TESTING

The following steps should be taken to handle the sediment 24 hours prior to initiation of the test:

- Add an aliquot, approximately 3.9 L, of sediment to each test chamber.
- Calculate the net weight of the sediment added to each chamber to assist with the preparation of replicate sediment samples.

- Net weight must be calculated for each test sediment and the control to account for varying sediment densities.

- Settle each sediment in the chambers by gently tapping the glass or by smoothing the sediment surface with a polyethylene spatula.

- Add 16.1 L of test water. This procedure requires that a polyethylene disk attached to a nylon filament be used as a baffle during filling so that the sediments are not disturbed.

- Place the disk on top of the sediment while the test water is added.

- Remove the disk or rinse between replicates.

- Use a different disk for each sediment that is tested.

- Water should be renewed in each test chamber so that every 24 hours where the water is completely changed 5 times.

- Aerate each chamber and allow the sediments to settle overnight.

6.0 INTRODUCTION OF POLYCHAETES TO TEST CHAMBERS

The following procedures for introduction of test polychaetes to the test chambers is recommended:

- Select a set of 10 to 15 test chambers to be initiated at one time.

- Place 10 polychaetes in a baking dish containing 500 mL of filtered sea water at test temperature. Organisms should be handled carefully. Damaged or inactive worms should be avoided.

- Similar-sized polychaetes should be used for the test.

- Add polychaetes to the test chamber by inverting the baking dish and gently pouring the polychaetes into the test container.

- Repeat the procedure so that there will be 30 individuals in each test chamber.

- Sort an additional group of 20 polychaetes and preserve by freezing for later baseline tissue analysis.

- Bring the overlying water level up to a total volume of 20 L with control water.

- Turn over any polychaetes that are not oriented with their ventral side up using a clean 1-mL pipet.

- Observe polychaetes within 2 hours of test initiation. Remove or replace dead or nonburying test organisms. If dead or nonburying polychaetes are greater than 10 percent of the total number of organisms tested, the test should be terminated and initiated again with a new stock of test organisms.

7.0 MONITORING OF THE TESTS

The following parameters should be monitored during the test:

- All chambers should be checked daily and observations made to assess test polychaete behavior such as sediment avoidance, failure to rebury, and dead organisms.

- Overlying water salinity, temperature, pH, and dissolved oxygen are to be measured at least daily.

- Total ammonia should be measured at the beginning and then weekly.

8.0 REFERENCE TOXICANT TESTING

The quality of *N. caecoides* obtained from an outside source should be verified by conducting a reference-toxicity test. The supplier should supply this data or the toxicity laboratory must conduct five reference toxicity tests before starting a sediment test.

Monthly reference-toxicity tests should be conducted on *N. caecoides* cultures using procedures as outlined below. If reference-toxicity tests are not conducted monthly, a reference toxicant test is run simultaneously with the sediment bioaccumulation test, for each new batch of organisms, to compare the relative health of the polychaete specimens being used.

This test is run without sediment in clean water. At a 96-hour exposure, an LC50 value can be calculated. ACS Reagent-grade cadmium chloride ($CdCl_2$), is used as a reference toxicant. Test parameters are as follows (Dillon and others 1993):

Test Type:	Water-only test
Dilution Series:	Control and at least 5 test concentrations (0.5 dilution factor)
Toxicant:	$CdCl_2$
Temperature:	15 ± 2°C
Salinity:	30 ppt ± 2 ppt
Light quality:	Ambient laboratory
Illuminance:	50 to 100 foot-candles
Photoperiod:	16L:8D
Renewal of Water:	None
Size of Organisms:	Same
Test Chamber:	600-mL glass beaker or container to provide area space equal to >21 m^2 per worm (covered with glass or plastic)
Volume of water:	500 mL (minimum)
Number of organisms/chamber:	3 minimum
Number of replicate chambers/treatment:	5 minimum
Feeding:	None
Aeration:	None
Dilution water:	Culture water, natural sea water, or artificial sea water
Overlying Water quality Monitoring:	Temperature, salinity, pH, and dissolved oxygen daily; ammonia (total and unionized) and sulfides at the beginning and end of test.
Test duration:	96 hours
Endpoint:	Survival (LC50)
Test acceptability:	90 percent control survival

9.0 TEST COMPLETION

The following test completion procedures are performed following 28 days of test exposure:

- Prior to test termination, set up two 7-L glass aquaria for use as depuration chambers. Each container should container at least 2 cm of clean, uncontaminated sediment.
- On test day 28, record the water quality parameters on all replicates.
- Gently rinse the contents of each exposure chamber through a 1-mm mesh sieve. Place the recovered polychaetes in a pyrex baking dish filled with 1 L of filtered seawater.
- Gently prod the polychaetes to determine if the organisms are alive. If the organism shows any response, including parapodia twitching, it is considered alive. Any organism that is not moving is presumed dead.
- Record the total number of live and dead polychaetes.
- Transfer the live polychaetes to the depuration chambers.
- Depurate the polychaetes for 48 hours at test conditions.
- Remove fecal material and debris daily. Do not feed the test organisms.
- At the termination of the depuration period, recover the polychaetes by gently passing the sediment through a 1 mm mesh screen.
- Place the recovered polychaetes in a storage container and allocate the tissues for chemical analysis as appropriate. The storage container should be consistent with the appropriate analysis.
- Freeze samples until analyzed.

10.0 ACCEPTABILITY OF THE TEST

Test acceptability requirements for a 28-day sediment toxicity test with *N. caecoides* are listed below. Performance criteria for testing are as follows:

- Size of *N. caecoides* at the start of the test must be the same.

- Average survival of *N. caecoides* in the native control sediment must be greater than or equal to 80 percent and survival in any control sediment replicate must be at least 70 percent at the end of the test.

- Adequate biomass must be present at the test completion for detection of target analytes.

- Temperature, salinity, pH, and dissolved oxygen in the overlying water within a treatment should not vary by more than 50 percent during the test.

Performance-based criteria for culturing *N. caecoides* are as follows:

- All polychaetes should be from the same population and species.

- The laboratory should perform monthly 96-hour water-only reference toxicity tests to assess the sensitivity of culture organisms. If reference toxicity tests are not conducted monthly, the lot of organisms used to start a sediment test must be evaluated using the reference toxicant $CdCl_2$.

- Records should be kept on the frequency of restarting cultures and the age of brood organisms.

Additional requirements are as follows:

- All test polychaetes must be the same size.

- Tests must be started within 2 weeks after collection of the sediment.

- All test chambers should be identical and contain the same amount of sediment and overlying water.

- The daily mean test temperature must be within $\pm$ 2°C of the desired temperature. The instantaneous temperature must always be within $\pm$ 3°C of the desired temperature.

- The dissolved oxygen must not fall below 60 percent of saturation in any chamber.

- Treatments must be randomly assigned to individual test chambers.

- Test polychaetes must be randomly distributed to test chambers.

11.0 STATISTICAL METHODS

Statistical analysis of the data should follow the guidance provided by EPA and the U.S. Army Corps of Engineers for evaluation of dredged material proposed for discharge (EPA/COE 1994).

12.0 POTENTIAL PROBLEMS

The following are potential problems that may be encountered during tests:

- Worms should not be placed in salinities less than 20 ppt.
- Juvenile worms cannot survive total ammonia levels greater that 40 mg/L.

13.0 RECORD KEEPING

The test report should document the following information, either directly or by reference to available documents:

- Name of test and investigator(s), name and location of laboratory, and dates of start and end of test
- Source of reference sediment and test sediment with the methods of collection, handling, shipping, storage, and disposal of sediment
- Source of overlying water, its chemical characteristics, and a description of any pretreatment
- Results of any demonstration of the ability of a species to survive, grow, or reproduce in the overlying water
- Date and location of collection, scientific name, name of person who identified the organisms, and the taxonomic key used. Size of test organisms and any observed diseases or unusual appearance, treatments, holding and acclimation procedures should also be provided
- Description of the experimental design and test chambers used; the depth and volume of sediment and overlying water in the chambers; lighting; number of replicates and umber of test organisms per replicate
- Records of temperature, salinity, pH, and ammonia; dissolved oxygen concentration (as percent saturation); and any aeration used prior to initiating the test and during the conduct of the test

- Biological endpoints used along with appropriate measurements
- A table of the biological data for each test including the control(s) in sufficient detail to allow independent statistical analysis
- Statistical tests used and results of analysis of the data
- Results of reference toxicant tests
- Information on calibration of equipment and instruments used during the test
- Copy of the COC record and sample log-in sheet. Original bench sheets for the test organism responses during the sediment test(s) with original dated signatures of laboratory personnel performing the test(s) should be included
- Summary of general observations made during the test
- Any deviations from the required procedures and any other relevant information

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TABLE I-1

SALTS USED IN THE PREPARATION OF GP-2 ARTIFICIAL SEA WATER (30 ppt SALINITY)

Compound	Concentration (g/L)
$NaCl$	21.03
Na_2SO_4	3.52
KCl	0.61
KBr	0.088
$Na_2B_4O_7 \bullet 10H_2O$	0.034
$MgCl_2 \bullet 6H_2O$	0.02
$CaCl_2 \bullet 2H_2O$	1.32
$SrCl_2 \bullet 6H_2O$	0.02
$NaHCO_3$	0.17

APPENDIX J

STANDARD OPERATING PROCEDURE
FOR
20-DAY WHOLE SEDIMENT SURVIVAL AND GROWTH TEST USING THE MARINE POLYCHAETE, *Neanthes arenaceodentata*

APPENDIX J

STANDARD OPERATING PROCEDURE FOR 20-DAY WHOLE SEDIMENT SURVIVAL AND GROWTH TEST USING THE MARINE POLYCHAETE, *Neanthes arenaceodentata*

1.0 APPLICATION

This toxicity test evaluates the survival and growth as measured by a change in biomass for the polychaete, *Neanthes arenaceodentata*, after 20 days of exposure to test sediments relative to a reference and control sediment. This test method is based on the protocol developed by Battelle/Marine Sciences Laboratory (1994) for the U.S. Army Corps of Engineers (COE) dredge material disposal program in San Francisco Bay and by PTI Environmental Services (PTI) (1990) for toxicity testing of sediments in Puget Sound with modifications suggested by Kendall (1994) and Littleton and Kendall (1995).

2.0 TEST ORGANISMS

Test species should be in intimate contact with the sediment and ingest sediment.

2.1 SPECIES SELECTION

This test is designed for the specific requirements of the polychaete *Nereis (Neanthes) arenaceodentata*, hereafter referred to as *Neanthes arenaceodentata*, which is the Latin name most familiar to toxicologists. This species is a benthic infaunal polychaete widely distributed in shallow marine and estuarine benthic habitats of Europe, the Pacific, and all three coasts of North America (Dillon and others 1993; PTI 1990). This species is a deposit feeder and has been found to be tolerant of a range in grain size (5 to 100 percent sand). This species constructs one or more mucoid tubes in the upper 2 to 3 centimeters (cm) of sediment. This deposit-feeder ingests particles up to 70 micrometers (μm) in diameter with a preference for those around 12 μm (Whitlatch 1980, as cited in Dillon and others 1993).

This species, primarily the Pacific population, is accepted in the regulatory community as an appropriate test species for sediment quality evaluation. A substantial amount of toxicological data for a variety of contaminants already exists for the Pacific population of this species.

2.2 SPECIES SOURCE

Recent evidence suggests that the Pacific and Atlantic populations of *N. arenaceodentata* are dissimilar. Researchers have maintained the species name, but have indicated the source of the brood stock to facilitate latter comparison of results. The Pacific stock has been the one most often tested.

Brood stock of the Pacific population should be obtained from the laboratory of Dr. Donald J. Reish, California State University at Long Beach (310/431-7064). Sufficient notification time should be given to Dr. Reish to ensure that the number of required juveniles will be available for the tests.

2.3 AGE

All juvenile *N. arenaceodentata* to be used for testing must be of a uniform age and weight. Organisms used for testing should be 2 to 3 weeks post-emergence and weigh between 0.5 to 1.0 milligrams (mg) dry weight. For consistency of aging test organisms, initiation of emergence should be that point when feeding juveniles emerge from the egg case. Beginning of feeding can be identified by the presence of food particles in the gut.

2.4 ACCLIMATION

Prior to testing, *N. arenaceodentata* juveniles should be maintained in sediment at 20 °C in 28 parts per thousand (ppt) artificial seawater (Dillon and others 1993; Dillon and others 1995). The photoperiod is 12 hours of light and 12 hours dark. Emergent juveniles (EJ) are raised to adulthood in 38-Liter (L) all-glass aquaria with 100 EJ per aquarium in 30 L of aerated seawater with a 2- to 3-cm layer of fine-grain, uncontaminated marine sediment. The polychaetes are fed twice weekly a slurry of finely ground (less than or equal to 0.50 millimeters [mm]) TetraMin® flakes (100 mg) and alfalfa (50 mg). This feeding regime will not impact water quality in a static-renewal system (Dillon and others 1993; Dillon and others 1995). Every 3 weeks 80 percent of the seawater volume is renewed.

Laboratory cultures maintained at 20 to 22 °C generally require 12 to 17 weeks for completion of the life cycle (Dillon and others 1995).

3.0 CONTROL WATER

Filtered natural seawater from an uncontaminated source or artificial sea water should be used as the control water. Artificial sea water can be prepared using American Chemical Society (ACS) reagent grade chemical salts and American Society for Testing and Materials (ASTM) Type 2 deionized water. The recipe for synthetic GP-2 sea water was published by the U.S. Environmental Protection Agency (EPA 1988) and is listed in Table J-1. Once the necessary volume of GP-2 has been prepared, it is aged, with aeration, for 2 weeks before it is used in bioassay testing. If a residue or precipitate is present after aging, the filtered sea water is filtered through a 0.45-μm filter before it is used.

4.0 TEST PARAMETERS

Test parameters for this SOP are as follows (Battelle/Marine Sciences Laboratory 1994; PTI 1990):

Test Type:	Static renewal
Test Duration:	20 days
Temperature:	20 ± 1°C
Salinity:	28 ± 2 ppt
Light Quality:	Ambient laboratory
Light Intensity:	50 to 100 ft-candles (ft-c)
Photoperiod:	24L/0D
Test Chamber Size:	1 L
Overlying Water Volume:	750 milliliters (mL) total volume
Sediment Depth:	2.0 cm (~210 mL)
Renewal of Test Solutions:	Every third day

Age of Test Organisms:	2 to 3 weeks post emergence or dry weight of 0.5 to 1.0 mg
Number of Organisms per Test Chamber:	5
Number of Replicate Chambers per Treatment:	5
Number of Organisms per Treatment:	25
Feeding Regime:	Feed 8 mg (dry weight) of TetraMarin® in a slurry per worm every other day. Maintain dose regardless of mortality (40 mg per container).
Overlying Water Aeration:	Trickle-flow (less than 100 bubbles per minute)
Dilution Water:	Natural seawater or GP-2 artificial sea water
Test Treatments:	Site sediment, reference sediment, and control sediment
Dilution Series:	Not applicable
Overlying Water Quality Monitoring:	Ammonia (total and unionized), sulfides, salinity, pH, and dissolved oxygen measured at the beginning and end of the test and every third day in at least one replicate. Temperature should be measured daily. Interstitial salinity should be measured at the beginning and end of test.
Endpoints:	Survival, biomass, and growth rate
Test Acceptability:	Minimum mean control survival of 90 percent. Survival in any single sediment replicate should not be less than 80 percent. Performance criteria specifications must be met.

5.0 SEDIMENT PREPARATION PRIOR TO TESTING

The following sediment preparation instructions should be followed before tests begin.

5.1 48 HOURS PRIOR TO TESTING

The sediment preparation steps listed below should be followed 48 hours before testing begins.

- Homogenize the sediment within its storage container.
- Remove a portion from the center interior of the storage container.
- Search the sediment for indigenous organisms.
- If indigenous organisms are present, wet-sieve the sediment through a 0.5-mm mesh screen using a small amount of water.
- Collect approximately 210 mL of sediment for each test chamber.
- Measure the interstitial salinity of the sediment using a refractometer.
- Introduce the sediment into water used for the bioassay.
- Allow the sediment to settle for at least 4 hours.
- Decant the excess water without disturbing the sediments.
- Hold the sediments at 4 °C until the bioassay chambers are prepared.

5.2 24 HOURS PRIOR TO TESTING

The following steps should be taken to handle the sediment 24 hours prior to initiation of the test:

- Add an aliquot, approximately 210 mL, of sediment to each test chamber.
- Calculate the net weight of the sediment added to each chamber to assist with the preparation of replicate sediment samples.
- Net weight must be calculated for each test sediment and the control to account for varying sediment densities.

- Settle each sediment in the chambers by gently tapping the glass or by smoothing the sediment surface with a polyethylene spatula.
- Bring volume of test water to 750 mL. This procedure requires that a polyethylene disk attached to a nylon filament be used as a baffle during filling so that the sediments are not disturbed.
- Place the disk on top of the sediment while the test water is added.
- Remove the disk or rinse between replicates.
- Use a different disk for each sediment that is tested.
- Aerate each chamber and allow the sediments to settle overnight.

6.0 INTRODUCTION OF POLYCHAETES TO TEST CHAMBERS

The following procedures for introduction of test polychaetes to the test chambers is recommended:

- Select a set of 30 test chambers to be initiated at one time.
- Post-emergent polychaetes of 2 to 3 weeks old should be placed in prelabeled, 100-mL holding beakers, five polychaetes per beaker.
- Transfer polychaetes by swirling the water in each container and then pouring the contents into each test chamber.
- Sort an additional group of 20 polychaetes, rinse with ammonium formate solution, placed on a preweighed aluminum pan, and dry at 50 °C to a constant weight over 24 hours. This group represents the initial biomass of the polychaetes.
- Bring the overlying water level up to a total volume of 750 mL with control water.

7.0 MONITORING OF THE TESTS

The following parameters should be monitored during the test.

- All chambers should be checked daily, and abnormal or unusual events should be recorded.

- Overlying water ammonia (total and unionized), hydrogen sulfides, salinity, pH, and dissolved oxygen are to be measured at the beginning and end of the test and every third day in at least one replicate. Temperature should be measured daily. Salinity of the sediment should be measured at the beginning and end of the test.

8.0 REFERENCE TOXICANT TESTING

The sensitivity of *N. arenaceodentata* obtained from an outside source should be verified by conducting a reference-toxicity test on the same batch of polychaetes used in toxicity testing. Either the supplier should supply these data or the toxicity laboratory must conduct five reference toxicity tests before starting a sediment test to establish a range of sensitivity.

Reference-toxicity tests should be conducted on *N. arenaceodentata* cultures using procedures as outlined below (Dillon and others 1993). If reference-toxicity test data are not supplied by the organism supplier, a reference toxicant test is run by the laboratory for each new batch of test organisms or once per month, whichever comes first, to compare the relative sensitivity of the polychaete specimens being used.

The reference toxicity test is run without sediment in clean water. At a 96-hour exposure, an lethal concentration 50 percent (LC50) value can be calculated. ACS reagent-grade cadmium chloride ($CdCl_2$), is used as a reference toxicant. Test parameters are as follows (Dillon and others 1993; Dillon and others 1995):

Test Type:	Water-only test
Dilution Series:	Control and at least 5 test concentrations (0.5 dilution factor)
Toxicant:	$CdCl_2$
Temperature:	20 ± 1°C
Salinity:	28 ± 2 ppt
Light quality:	Ambient laboratory
Illuminance:	50 to 100 ft-c

Photoperiod:	24L:0D
Renewal of Water:	None
Age of Organisms:	2 to 3 weeks or dry weight 0.5 to 1.0 mgs
Test Chamber:	1-L glass beaker
Volume of Water:	750 mL
Number of Organisms per Chamber:	10 minimum
Number of Replicate Chambers per Treatment:	5 minimum
Feeding:	None
Aeration:	None
Dilution Water:	Culture water, natural sea water, or artificial sea water
Water Quality:	Ammonia (total and unionized), sulfides, salinity, pH, and dissolved oxygen at the beginning and end of test. Temperature daily
Test Duration:	96 hours
Endpoint:	Survival (LC50)
Test Acceptability:	90% control survival

9.0 TEST COMPLETION

The following is performed following 20 days of test exposure.

- Rinse the contents of each exposure chamber through a 0.5-mm mesh sieve.
- If necessary, remove worms from tubes.
- Gently prod the polychaetes to determine if the organisms are alive. If they show any response including parapodia twitching, the polychaete is considered alive.

- Record the total number of live and dead polychaetes. Any animal that does not respond to gentle prodding is presumed dead.

- Rinse worms in isotonic 0.9 percent (W:V) ammonium formate or distilled water.

- Dry weight of polychaetes should be determined by pooling all living organisms from a replicate and drying the sample at about 50 °C for 24 hours to a constant weight. The sample is then brought to room temperature in a desiccator and weighed to the nearest 0.01 mg to obtain mean weight per surviving polychaete per replicate.

The response criteria are survival, total biomass, individual biomass (total biomass divided by the number of surviving worms), and growth rate. Growth rate is calculated as follows (Kendall 1994):

$$G = \frac{DW_t - DW_i}{t}$$

where

G	=	estimated individual growth rate (mgs dry weight per day)
DW_t	=	estimated individual dry weight at termination (mgs)
DW_i	=	mean estimated individual dry weight at initiation (mgs)
T	=	exposure time (days)

10.0 ACCEPTABILITY OF THE TEST

Test acceptability requirements for a 20-day sediment toxicity test with *N. arenaceodentata* and test organism suitability are listed below. Performance criteria for testing are as follows:

- *N. arenaceodentata* polychaetes at the start of the test must be between 2 and 3 weeks. Organisms must weigh between 0.5 to 1.0 mgs dry weight.

- Average survival of *N. arenaceodentata* in the control sediment must be greater than or equal to 90 percent at the end of the test.

- The mortality in any sediment replicate should not exceed 20 percent.

- Temperature, salinity, pH, and dissolved oxygen in the overlying water within a treatment should not vary by more than 50 percent during the test.

Performance-based criteria for test organisms are as follows:

- All polychaetes should be from the same population and species.
- The laboratory should perform monthly 96-hour water-only reference-toxicity tests to assess the sensitivity of culture organisms. If supplier does not provide reference toxicant data, then the testing laboratory should conduct a series of five reference toxicity tests. If reference-toxicity tests are not conducted monthly, the lot of organisms used to start a sediment test must be evaluated using the reference toxicant $CdCl_2$.
- Records should be kept on the frequency of restarting cultures and the age of brood organisms.
- Laboratories should record the following water-quality characteristics of the cultures at least weekly and the day before the start of a sediment test: pH, salinity, temperature, and ammonia (total and unionized). Dissolved oxygen and temperature should be measured weekly.

Additional requirements are as follows:

- Tests must be started within 2 to 8 weeks after collection of the sediment.
- All test chambers should be identical and contain the same amount of sediment and overlying water.
- The daily mean test temperature must be within $\pm$ 1°C of the desired temperature. The instantaneous temperature must always be within $\pm$ 3°C of the desired temperature.
- The dissolved oxygen must not fall below 60 percent of saturation or 6.0 milligrams per liter (mg/L) in any chamber.
- Treatments must be randomly assigned to individual test chambers.
- Test polychaetes must be randomly distributed to test chambers.
- Test chambers should be randomly placed in the test area.

11.0 STATISTICAL METHODS

One-way analysis of variance (ANOVA) is used to test the null hypothesis that response in the reference sediment is not statistically different from that in the test sediments. ANOVA is conducted for both survival and estimated individual growth rate. Homogeneity of variances is evaluated with either Bartlett's test or Levene's test using appropriate transformation as needed. Normality is evaluated by plotting residuals or can be evaluated by a computer program. Mean separation may be performed via Tukey's HSD test, Dunnett's, or another appropriate parametric statistical test. Non-parametric statistical tests may be required. All differences can be assumed to be significant if P is less than 0.05.

12.0 POTENTIAL PROBLEM AREAS

The following problem areas may be encountered during tests and should be prepared for by the testing laboratory.

- The brood population (Pacific coast) must be noted with the report so that comparison with data using the same brood stock can be made.
- The number of individuals per test chamber is critical (Dillon and others 1993). Each worm requires a surface area of less than 21 square centimeters. Addition of sediment will allow for the addition of more worms.
- Worms should not be tested in sediments where interstitial salinities are less than 20 ppt.
- Juvenile worms can not survive total ammonia levels greater that 40 mg/L.

13.0 RECORD KEEPING

The report should document the following information either directly or by reference to available documents:

- Name of test and investigator(s), name and location of laboratory, and dates of start and end of test

- Source of reference sediment and test sediment with the method of collection, handling, shipping, storage, and disposal of sediment
- Source of overlying water, chemical characteristics, and a description of any pretreatment
- Source, history, and age of test organisms
- Source, history, and age of brood stock, culture procedure or source and date of collection of the test organisms, scientific name, name of person who identified the organisms, and the taxonomic key used. In addition, age, life-stage, means and ranges of weights and lengths should be provided. Observed diseases or unusual appearance, treatments, holding and acclimation procedures should also be recorded
- Source and composition of food, procedure used to prepare food, feeding methods, frequency, and ration
- Description of the experimental design and test chambers used; the depth and volume of sediment and overlying water in the chambers; lighting; number of replicates and number of test organisms per replicate
- Records of temperature, salinity, pH, and ammonia; dissolved oxygen concentration (as percent saturation), and any aeration used prior to initiating the test and during the conduct of the test
- Biological endpoints used along with appropriate measurements
- A table of the biological data for each test including the control(s) in sufficient detail to allow independent statistical analysis
- Statistical tests used and results of analysis of the data
- Results of reference toxicant tests.
- Information of calibration of equipment and instruments used during the test
- Copy of the chain-of-custody record, the sample log-in sheet and the original bench sheets for the test organism responses during the sediment test(s), with original signatures and dates of laboratory personnel performing the test(s)
- Summary of general observations made during the test
- Any deviations from the required procedures and any other relevant information.

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TABLE J-1

SALTS USED IN THE PREPARATION OF GP-2 ARTIFICIAL SEA WATER (30 ppt SALINITY)

Compound	Concentration (g/L)
$NaCl$	21.03
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KCl	0.61
KBr	0.088
$Na_2B_4O_7 \bullet 10H_2O$	0.034
$MgCl_2 \bullet 6H_2O$	0.02
$CaCl_2 \bullet 2H_2O$	1.32
$SrCl_2 \bullet 6H_2O$	0.02
$NaHCO_3$	0.17

APPENDIX K

STANDARD OPERATING PROCEDURE
FOR
48-HOUR EMBRYO-LARVAL DEVELOPMENT TEST USING THE BLUE MUSSEL,
Mytilus edulis

APPENDIX K

STANDARD OPERATING PROCEDURE FOR 48-HOUR EMBRYO-LARVAL DEVELOPMENT TEST USING THE BLUE MUSSEL, *Mytilus edulis*

1.0 INTRODUCTION

The embryo-larval development test is used to evaluate the toxicity of estuarine and marine waters, pore water, or elutriates. The test uses the blue mussel, *Mytilus edulis*, as described by Chapman and others (1995). The test endpoints are mortality and normal shell development.

The bivalve shell development test will be used to test the elutriate from petroleum-contaminated soils. A dilution series will be implemented for the analysis of the elutriate to calculate an effect concentration (EC)10, EC25, EC50, no observed effect concentration (NOEC), and lowest observed effect concentration (LOEC). These values provide information about the dose response relationship.

2.0 TEST ORGANISMS

The blue or edible mussel, *Mytilus edulis*, is found from the Arctic Ocean to Isla Cedros, Baja California; west coast of South America, Japan, Australia, and North Atlantic (Morris and others 1980). The blue mussel is often found in clusters, attached by byssus to rocks and especially to wharf pilings. It occurs in the low intertidal zone to subtidal to 40 meters depth in bays and sheltered areas. Along the East Coast, this species is the common rocky intertidal species. On the West Coast, this species is typically more abundant in protected bays and estuaries. The blue mussel is usually about 2 inches long but may grow up to 4 inches in length. Spawning occurs in the late fall and winter along the central California coast. Larvae swim freely for about 4 weeks and settle mainly in winter and late spring in the central California area (Morris and others 1980).

Adult *M. edulis* (brood stock) should be obtained from the same source: either commercial harvesters or a chemically uncontaminated area. If the brood stock is obtained from a commercial source, the original collection area should be identified. The mussels are best shipped in damp towels or seaweed

and kept cool (8 °C). To condition mussels to spawn, they must be held from 1 to 8 weeks at 15 to 18 °C. Within 24 hours of collection or purchase, adults should be transported to the test laboratory and placed into flowing seawater similar in character to that from which they were taken.

Upon receipt, any debris should be removed from the shell of the mussels, and the mussels placed in flowing sea water. The adults should be maintained in glass aquaria or fiberglass troughs or tanks. The mussels should be checked daily, and obviously unhealthy individuals should be removed and discarded. Prior to spawning, the animals should be brushed or gently scraped to remove barnacles and other encrusting organisms, thus alleviating problems of egg and sperm contamination, especially through potential barnacle spawning.

The recommended temperature for holding the mussels is 8 °C. Conditioning bivalves to spawning condition usually requires holding the individuals from 1 to 8 weeks at a higher temperature, that is 18 °C for mussels (Chapman and others 1995). The salinity should be maintained at 30 parts per thousand (ppt) for maintenance of the adults.

3.0 CONTROL WATER

Uncontaminated filtered natural sea water or artificial sea water may be used as control water and diluent. Natural sea water should be collected from uncontaminated areas to avoid contamination and should be held at less than or equal to 15 °C for no longer than 2 days before introduction of organisms. Artificial sea water, of approximately the same temperature, salinity, and dissolved oxygen content as the water at the sites to be tested, may be prepared using ACS reagent grade chemical salts and deionized water. The recipe for synthetic GP-2 sea water was published by the U.S. Environmental Protection Agency (EPA 1988) and is listed in Table K-1.

Once the necessary volume of GP-2 has been prepared, it is aged, with aeration, for 2 weeks before it is used in bioassay testing. If a residue or precipitate is present after aging, the sea water is filtered through a 0.45-micrometer (μm) filter before it is used. Salinity adjustments are made, if necessary, with distilled water (to decrease salinity) or brine prepared from distilled water and artificial sea salts (to increase salinity). Dissolved oxygen is maintained above 40 percent saturation.

4.0 TEST PARAMETERS

Test parameters for this SOP are as follows (Chapman and others 1995):

Test Type:	Static nonrenewal
Temperature:	18 °C ± 1 °C
Salinity:	30 ± 2 ppt
Light Quality:	Ambient laboratory light
Light Intensity:	50 to 100 foot-candles (ft-c)
Photoperiod:	16L:8D
Test Chamber:	30 milliliters (mL)
Test Solution Volume:	10 mL
Renewal of Test Solution:	None
Age of Test Organisms:	1.5- to 2.5-hour-old embryos
Number of Organisms/ Chamber:	150 to 300
Number of Replicates:	4
Test Concentrations:	5 and a control
Dilution Series:	0.5
Feeding Regime:	None
Aeration:	None; dissolved oxygen must not fall below 4.0 milligrams per liter (mg/L)
Test Solution Water Quality Monitoring:	Temperature, pH, salinity, and dissolved oxygen at the start of test and daily; ammonia (total and unionized) and sulfides at the beginning and end of test
Test Duration:	48 hours or until complete development up to 54 hours

Endpoints:	Survival and normal shell development
Test Acceptability:	Control survival must be greater than or equal to 50 percent for mussels in control chambers; greater than or equal to 90 percent normal shell development in surviving controls; and must achieve a minimum significant difference of less than 25 percent in any control for survival and normal shell development.
Sample Volume Required:	1 liter (L) per test

5.0 ELUTRIATE PREPARATION

Soils or sediments used for this test will be mixed with uncontaminated control water to obtain an elutriate sample for testing (EPA/COE 1994). The elutriate is prepared by subsampling approximately 1 L of the sediment/soil sample from a homogenous original sample. The sediment/soil and uncontaminated control water are then combined in a sediment-to-water ratio of 1 to 4 on a volume basis at room temperature (22 $\pm$ 2 °C). This is best accomplished by volumetric displacement. After the correct ratio is achieved, the mixture is stirred vigorously for 30 minutes with a mechanical or magnetic stirrer. At 10-minute intervals, the mixture is also stirred manually to ensure complete mixing. After the 30-minute mixing period, the mixture is allowed to settle for 1 hour. The liquid plus the material remaining in suspension after the settling period represents the 100 percent liquid plus suspended particulate phase. The supernatant is then carefully siphoned off, without disturbing the settled material, and immediately used for testing. With some very fine-grained sediments/soils, it may be necessary to centrifuge the supernatant until the suspension is clear enough for the organisms to be visible in the testing chamber.

The recommended test concentrations are 100, 50, 25, 12.5, and 6.35 percent. However, a range-finding test should be conducted to verify these recommended concentrations.

6.0 BIOASSAY PROCEDURE

The bioassay procedure described below is based on Chapman and others (1995) and should be followed if any questions arise. Select at least a dozen bivalves, and place them into a container of 15

°C seawater, and allow them to acclimate. Over a 15- to 20-minute period, increase the temperature, but do not exceed 20 °C. If spawning still does not occur, replace the water with water at the original temperature and, after 15 minutes, gradually increase the temperature again. Spawning can also be induced by injecting the posterior adductor muscle with 0.5 molar (M) potassium chloride (KCl).

Separate spawning individuals into their own glass container with seawater at 15 °C. Early in the spawning process, collect some gametes and examine for sex and quality, such as good sperm motility or vacuolated, small, or abnormally shaped eggs. Sperm and egg suspensions to be used for the test should be passed through a nytex screen of about 75 μm to separate clumps of gametes or extraneous material.

Pooled eggs should be placed into a 1 L beaker with sufficient dilution water to achieve an egg density of about 5,000 to 8,000 eggs per mL in about 800 to 900 mL of water. To achieve an acceptable level of sperm, several egg suspensions of equal density should be fertilized using a range of sperm volumes, for example, 100 mL of egg suspension plus 1, 3, and 10 mL of sperm suspension. This test fertilization should be accomplished within one hour of spawning. Use embryos produced from eggs fertilized with the lowest amount of sperm giving normal embryo development after 1.5 to 2.5 hours after fertilization was induced. Usually, greater than 90 percent of the eggs should be fertilized. The mussels should show a single polar body, or embryos should have advanced to the two-cell stage.

Adjust the embryo suspension to a density of 1,500 to 3,000 per mL. Confirm by counting chamber counts on 1 mL subsamples from a stirred suspension. Add 0.1 mL of the embryo suspension to 10 mL of test solution into each of the randomized test vials.

7.0 DAILY MONITORING OF THE TESTS

The following parameters should be monitored during the test in each test chamber and the control.

- Measure and record the following water parameters just before introduction of test organisms and daily thereafter: temperature, salinity, dissolved oxygen, and pH.
- Measure ammonia (total and unionized) and sulfides at the start and end of the test.

8.0 REFERENCE TOXICANT TESTING

The quality of field collected mussels should be verified by conducting a reference-toxicity test. Monthly reference-toxicity tests should be conducted on *M. edulis* cultures using procedures as outlined below. If reference-toxicity tests are not conducted monthly, a reference toxicant test is run simultaneously with the bivalve test to compare the relative health of the specimens being used. This involves determining 48-hour (or longer) median lethal concentration (LC_{50}) and median effects concentration (EC_{50}) values for mussel larvae exposed to reference toxicants in clean, filtered or ultra violet (UV)-treated sea water under the same general test conditions as the water, pore water, or elutriate bioassays. Such data are necessary to determine the relative sensitivity of the larvae. Bioassays to establish an LC_{50} or an EC_{50} involve four or five logarithmic concentrations and a control. At least one treatment should give a partial response below the LC_{50} and EC_{50} and one above the LC_{50} and EC_{50}. Statistical procedures for LC_{50} and EC_{50} estimates are given in APHA (1985) and ASTM (1989).

This test is run in clean sea water, with a 48-hour exposure, and an LC_{50} value can be calculated. ACS reagent-grade copper chloride ($CuCl_2$) is used as a reference toxicant. Test parameters are as follows:

Test Type:	Water only
Temperature:	18 °C ± 1 °C
Salinity:	30 ± 2 ppt
Light Quality:	Ambient laboratory light
Light Intensity:	50 to 100 ft-c
Photoperiod:	16L:8D
Test Chamber:	30 mL
Test Solution Volume:	10 mL
Renewal of Test Solution:	None
Age of Test Organism:	1.5 to 2.5 hour old embryos

Number of Organisms per Chamber:	150 to 300
Number of Replicates:	4
Test Concentrations:	5 and a control
Dilution Series:	0.5
Feeding:	None
Aeration:	None; dissolved oxygen must not fall below 4.0 mg/L
Test Solution Water Quality:	Temperature, pH, salinity, and dissolved oxygen at the start of test and daily; ammonia (total and unionized) and sulfides at the beginning and end of test
Test Duration:	48 hours or until complete development up to 54 hours
Endpoints:	Survival (LC_{50}) and normal shell development (LC_{50})
Test Acceptability:	Control survival must be greater than or equal to 50 percent for mussels in control vials; greater than or equal to 90 percent normal shell development in surviving controls.
Sample Volume Required:	1 L per test

9.0 TEST COMPLETION

At the end of 48-hours, examine a control test chamber to check for complete development. If development is complete, terminate the test. If development does not appear to be complete, the test should be continued until complete development occurs but not beyond 54 hours total test duration. To terminate the test, add 0.25 mL of concentrated formalin to each test vial. Do not shake the vials. After preservation, observe the larvae with an inverted microscope or by quantitative transfer of all larvae onto a counting chamber and counting using a compound microscope. Carefully count and score all larvae as either normal or abnormal. Embryos and larvae are considered abnormal if they have not yet reached the D-hinge stage.

10.0 ACCEPTABILITY OF THE TEST

Test acceptability requirements for a 48 hour bivalve embryo development bioassay are listed below. Performance criteria for testing are as follows:

- Mussel larvae must be from 1.5 to 2.5 hours old at the start of the test.
- Control survival must be greater than or equal to 50 percent.
- Normal shell development in surviving controls should be greater than or equal to 90 percent.
- A percent minimum significant difference of less than 25 must be achieved relative to the control for survival and for shell development.

Performance criteria for field collection are as follows:

- Reference-toxicant tests must be performed on each batch of field-collected mussels used in the tests.
- Mussels must not have spawned as a result of the collection and transportation.

Additional requirements are as follows:

- All organisms must be from the same population.
- All test chambers should be identical and should contain the same amount of test water.
- The daily mean temperature must be within ± 1 °C of the desired temperature. The instantaneous temperature must always be within ± 3 °C of the desired temperature.
- The dissolved oxygen must not fall below 4.0 mg/L in any chamber.
- Test mussel larvae must be randomly assigned to individual test chambers.
- Test mussels must be randomly distributed to test chambers.

11.0 STATISTICAL METHODS

The data should be tabulated and summarized. The LC_{50}, EC_{50}, no observed effects concentration (NOEC), and lowest observed effects concentration (LOEC) shall be determined for each test endpoint. Statistical comparisons among treatments shall be made using analyses of variance (ANOVA) and Dunnett's one-tailed *t*-test (which controls the experiment wise error rate) on the arcsine square-root-transformed data. An estimate of the elutriate concentration which would cause a 50 percent toxic effect for mortality (LC_{50}) and abnormal development (EC_{50}) shall be calculated using Trimmed Spearman-Karber analysis with Abbott's correction (Hamilton, Russo, and Thurston 1977). The one-way ANOVA followed by Dunnett's Procedure (Dunnett 1955) to compare single treatments to the control shall be used in order to estimate no effect and least effect concentrations (NOEC and LOEC values).

12.0 RECORD KEEPING

The report should document the following information either directly or by reference to available documents.

- Name of test and investigator(s), name and location of laboratory, and dates of start and end of test
- Source of reference sediment/soil and test sediment/soil used for making the elutriate with the method of collection, handling, shipping, storage, and disposal of sediment
- Source of test solution, chemical characteristics, and a description of any pretreatment
- Results of any demonstration of the ability of the species to survive, grow, or reproduce in the test solution
- Source and history of test organisms
- Source and date of collection of the test organisms, scientific name, name of person who identified the organisms, and the taxonomic key used. Observed diseases or unusual appearance, treatment, holding and acclimation procedures should also be recorded
- Source and composition of food, procedure used to prepare food, feeding method, frequency, and ration

- Description of the experimental design and test chambers used; the volume of water in the chambers; lighting; number of replicates and number of test organisms per replicate
- Records of temperature, salinity, pH, dissolved oxygen, sulfides, and ammonia tested in test solution
- Biological endpoints used along with appropriate measurements
- A table of the biological data for each test including the control(s) in sufficient detail to allow independent statistical analysis
- Statistical test used and results of analysis of the data
- Results of reference toxicant tests
- Information on calibration of equipment and instruments used during the test
- Copy of the chain-of-custody record, the sample log-in sheet, the original bench sheets for the test organism responses during the test(s), and including original signatures and dates of laboratory personnel performing the test(s)
- Summary of general observations made during the test
- Any deviations from the required procedures and any other relevant information

13.0 REFERENCES

American Public Health Association (APHA). 1985. "Standard methods for the examination of water and wastewater." 16th edition. Washington, D.C.

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Hamilton, M.A., R.C. Russo, and R.V. Thurston. 1977. "Trimmed Spearman-Karber method for estimating median lethal concentration in toxicity bioassays." *Environmental Science and Technology*, Vol. 11, No. 7, Pages 714 to 719; Correction Vol.12, Page 417 (1978).

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TABLE K-1

SALTS USED IN THE PREPARATION OF GP-2 ARTIFICIAL SEA WATER (30 ppt SALINITY) (EPA, 1988)

Compound	Concentration (g/L)
$NaCl$	21.03
Na_2SO_4	3.52
KCl	0.61
KBr	0.088
$Na_2B_4O_7 \bullet 10H_2O$	0.034
$MgCl_2 \bullet 6H_2O$	0.02
$CaCl_2 \bullet 2H_2O$	1.32
$SrCl_2 \bullet 6H_2O$	0.02
$NaHCO_3$	0.17

APPENDIX L

NAVY RESPONSES TO AGENCY COMMENTS ON THE PHASE II ECOLOGICAL RISK ASSESSMENT DRAFT FINAL QUALITY ASSURANCE PROJECT PLAN

APPENDIX L

NAVY RESPONSES TO AGENCY COMMENTS ON THE PHASE II ECOLOGICAL RISK ASSESSMENT DRAFT FINAL QUALITY ASSURANCE PROJECT PLAN NAVAL STATION TREASURE ISLAND

The following responses were prepared to address the comments provided by the U.S. Environmental Protection Agency (EPA) Region 9, and California Environmental Protection Agency (CalEPA) Department of Toxic Substances Control (DTSC). These agencies submitted comments regarding the "Naval Station Treasure Island (NAVSTA TI) Phase II Ecological Risk Assessment Draft Final Quality Assurance Project Plan (QAPP)". The EPA comments are dated October 15, 1996, and the Cal EPA comments are dated October 18, 1996.

The comments and responses are divided into three sections: (1) comments provided by James Polisini of the Human and Ecological Risk Division at DTSC, (2) comments provided by Bart Simmons of the hazardous materials laboratory at DTSC, and (3) EPA comments.

The page numbers referenced in some comments refer to the draft final version of the QAPP. The page numbers may have changed after the revisions were made to the final version of the QAPP. The section numbers for the document have not changed.

COMMENTS PROVIDED BY JAMES POLISINI, DEPARTMENT OF TOXIC SUBSTANCES CONTROL

1. **Comment:** **Section 2.4, page 11:**
Please specify the '...other measures of bioavailability...' which will be used to assess ecological risk. Bioavailability has been discussed during development of the Phase II ecological risk assessment work plan, but sediment pore water is the only measure of 'bioavailability' currently presented in the work plan.

   Response: The analysis of acid volatile sulfide and simultaneously extracted metals (AVS/SEM) has been added to the QAPP. Analysis of sediment pore water and AVS/SEM are the two measures of bioavailability that will be used.

2. **Comment:** **Section 2.4, page 12:**
How will the Phase II ecological risk assessment be performed for aquatic receptors in the event the San Francisco Bay-specific Low Screening Value (LSV) and the High Screening Value (HSV) are not developed prior to completion of the Treasure Island (TI) investigations? Please include some alternate sediment screening methodology or criteria.

   Response: The San Francisco Bay-specific LSVs and HSVs have not yet been developed. The Navy will use the ambient values released by the Regional Water Quality Control Board (RWQCB 1996, EPA and others 1996) and the effects range values of Long and others (1995) as sediment screening criteria. If both sediment screening criteria for a particular analyte are available, the

highest concentration will be used to evaluate the data. Sample locations with sediment concentrations higher than the screening criteria will be considered for further investigation.

For screening analyte concentrations detected in sediment pore water, the Navy will use the ambient water quality criteria (AWQC) (EPA 1992) instead of the California water quality objectives (RWQCB 1995), which were struck down by court order. Screening criteria will also be taken from the Great Lakes water quality initiative tier II values for chemicals without AWQC values.

3. **Comment:** **Section 2.4, page 12 and Section 3.0, page 16:**
Offshore sediment samples are designated as '1500 feet offshore'. In planning discussions for the Phase II ecological risk assessment these offshore sediment samples were proposed and discussed as transects of sediment sampling consisting of several sediment samples along a transect extending outward from the TI shore to evaluate any trends in sediment concentration. Please amend the text to indicate that these 'offshore' sediment samples will be obtained as part of the transect sediment sampling. Additional discussions may be necessary if a stratified random sampling strategy has replaced the originally-proposed transect sampling to evaluate sediment concentration trends.

Response: The Phase II sampling methodology specifies sampling along transects from nearshore to farshore off of TI to evaluate whether there is a concentration gradient for the contaminants. The placement of sampling locations "1,500 feet offshore" are aligned along a transect and are a part of the transect sampling plan. The text will amended as follows:

Section 2.4, fifth paragraph, third sentence will read as follows: "Samples will be collected along transects, and the farthest offshore sampling location will be about 1,500 feet offshore." Section 3.0, third paragraph, third and fourth sentences will read as follows: "Samples will be collected along transects, and the farthest offshore sampling location will be about 1,500 feet offshore. The sampling locations in the Clipper Cove area were chosen to provide aerial coverage and not to evaluate a concentration gradient."

4. **Comment:** **Section 3.0, page 17:**
The discussion of Type I and Type II statistical error and null and alternative hypotheses incorrectly states the null and alternate hypotheses. There are actually two independent null hypotheses and two alternate hypotheses. One tests whether TI is the source of the sediment contamination and the other tests whether the sediment sampling location is toxic or non-toxic. It is possible for the sediment to be toxic and TI not the source of the contamination as well as for the sediment to be non-toxic but there exist a trend in sediment concentration from the TI shore outwards. Please amend the discussion of null and alternative hypotheses to clearly separate the two hypotheses being evaluated.

Response: The discussion of the null and alternative hypotheses will be changed to reflect the two independent decisions that should be made. Additional discussion of the data quality objectives (DQO) has also been included to document the seven-step process for their development as specified by the EPA QA/G-4 guidance (1994b) (see also Bart Simmons' comment number 1).

5. **Comment:** **Section 3.2, page 18:**
What type of litigation is 'anticipated' in which the TI sediment and pore water may be used? We propose removing this phrase from the QAPP.

Response: While litigation at NAVSTA TI is not necessarily anticipated, it does remain a possibility. The Navy is currently facing possible litigation for releases from underground storage tanks and storm water outfalls. The Navy proposes to keep the original statement.

6. **Comment:** **Section 3.4, page 21:**
We do not agree with the general statement that 'Sediment and tissue samples are not routinely analyzed with duplicates.' Field duplicate samples may be appropriate, and are used, for investigations which require a finer level of discrimination than the risk characterization purposes of the TI Phase II ecological risk assessment. We propose that the sentence be amended to state 'Sediment and tissue samples are not routinely evaluated with field duplicates in CERCLA investigations.'

Response: The sentence will be replaced with "Sediment and tissue samples are not routinely evaluated with field duplicates in CERCLA investigations at NAVSTA TI."

7. **Comment:** **Table 1, page 23, and Appendix K, Section 5.0, page K-4:**
Footnote 'b' of Table 1 refers to 'elutriates' for bioassays on echinoderm and bivalve larvae. The bivalve larvae test also discusses preparation of an elutriate. Elutriates are not a measure of potential ecological threat from in-place sediments. If the elutriate samples are meant to determine the difference in bioassay response between sediment pore water and sediment elutriate that purpose should be clearly stated.

Response: The sediment pore water will be tested using only the echinoderm larvae. The bivalve larvae will only be used to test the soil elutriate that will be tested as part of the plan to develop site-specific petroleum hydrocarbon cleanup values (see Section 7.2, phase II ecological risk assessment work plan [PRC Environmental Management Inc. 1996]).

8. **Comment:** **Table 2, footnote 'g':**
A footnote designated 'g' in Table 2, referring to a 1-to-1 slurry of water, does not appear in the body of the table. Please correct this error.

Response: Footnote "g" applies to the analysis of ammonia in sediments. The footnote will be added to the body of the Table 2.

9. **Comment:** **Section 8.0, page 46:**
Laboratories are certified by the Environmental Laboratory Accreditation Program (ELAP), which is in the California Department of Health Services, not the California Department of Toxic Substances Control.

Response: The association of ELAP will be corrected in the text of the final QAPP.

10. **Comment:** **Tables 11, 12, 13, 14, and 15:**
The proposed quantitation limits and contract-required quantitation limits should be evaluated by the Hazardous Materials Laboratory (HML) to determine if they are appropriate for the individual analytical method. The quantitation limit of 0.026 μg/kg for polycyclic aromatic hydrocarbons (PAH) (Table 12) in sediment, however, is more than sufficient to assess the ecological hazard associated with these compounds in sediment.

Response: Bart Simmons of the HML reviewed the entire draft final QAPP including the proposed quantitation limits. The comments provided by HML are addressed in the following pages.

11. **Comment:** **There appear to be several inconsistencies in the descriptions of the bioassay tests:**

A. Second bullet item, page 69:
In the echinoderm bioassay, it is impossible to have an 'average survival in the control' equal to or greater than 70 percent when the test acceptability criterion is 'Greater than 80 percent normal shell development in the controls' (Test acceptability, page 68). If the test acceptability criterion is 80 percent normal shell development in the surviving echinoderm larvae, please state the Test Acceptability criterion in that manner.

B. Page 68:
In the echinoderm bioassay, the Test Chamber is listed as 20 ml minimum while the Sample Volume Required is listed as 500 ml. If the Sample Volume Required is 500 ml of sediment to produce the required pore water, please list the Sample Volume Required as sediment.

C. Pages 65 and 71:
The amphipod bioassay lists illuminance in units of lux while all other descriptions use foot candles. Please be consistent in the use of illumination units.

D. Page 75:
The echinoderm bioassay lists the actual concentrations of 100 percent,

50 percent, 25 percent, 12.5 percent, and 6.25 percent while the bivalve shell development test lists the dilution factor of 0.5 rather than the actual concentrations. Please be consistent in the description of the dilution series.

E. Page 68:
The echinoderm bioassay lists a Photoperiod of 16 hours light and 8 hours dark (16L:8D) while the reference toxicant testing (Section 8.0, page E-8) lists the Photoperiod as 'None.' The reference toxicant should be tested under the same conditions as the TI samples. Please amend these sections so that they are correct and agree, or provide justification for the difference.

F. Section 8.8.1, page 65:
The amphipod whole sediment test lists a Photoperiod of 24 hours light and 0 hours dark. The amphipod protocol lists a Photoperiod of 24 hours dark for reference toxicant testing (Section 9.0, page G-7). Please amend these sections so that they are correct and agree, or provide justification for the difference.

Response:

A. The test acceptability requirement is greater than 80 percent normal shell development (term used in the EPA guidance [Chapman and others 1995]) in controls and a percent minimum significant difference of less than 25 percent in controls must be achieved. The control survival rate requirement will be changed to read as follows: "Average survival in the control must be greater than or equal to 80 percent at the end of the test."

B. The test chamber volume of 20 ml is required for the completion of one echinoderm bioassay. The sample volume required for the echinoderm toxicity testing described in Section 8.8.2 is the total amount (500 ml) of sediment pore water required for all replicates and dilutions that must be tested. The sediments collected from TI to date have yielded about 500 to 700 ml of sediment pore water for every gallon of sediment collected. The sample volume requirement will be changed to read as follows: "Sample Volume Required: 500 ml of sediment pore water (approximately 1 gallon of sediment is required to produce this volume of sediment pore water)."

C. The illumination units for the amphipod bioassay will be changed to read as follows: "Illuminance: 50 to 100 foot/candles."

D. The test concentrations for the bivalve shell development test will be changed to read as follows: "Test Concentrations: 100 percent, 50 percent, 25 percent, 12.5 percent, and 6.35 percent."

E. The photoperiod for the echinoderm reference toxicant test should be 16 hours of light and 8 hours of dark. The list of test parameters, Section 8.0, Appendix E, will be changed to read as follows: "Photoperiod: 16L/8D."

F. Although the photoperiod requirement seems contradictory, the EPA test manual specifies that the amphipod test be conducted under 24 hours of light and no hours of dark (EPA 1994a, page 69), while the reference toxicant test specifies 24 hours of dark and no light (EPA 1994a, page 51). In addition, the Navy has previously performed test, using these criteria.

12. Comment: **Section 9.4.1, page 86:**
Please indicate the negative control for the pore water bioassays. The discussion of negative controls concentrates on negative controls in sediment bioassays.

Response: A discussion of negative controls for the pore water bioassay will be added to this section. The discussion will be taken from the bioassay standard operating procedures (SOP) in Appendices E, G, and K.

13. Comment: **Section 2.4, page H-3:**
Recommended holding time for frozen tissue is first proposed as '... a maximum of 1 year' and then as '... a maximum holding time of 6 months.' (Section 3.2, page H-6). What will be the maximum holding time for frozen tissues?

Response: The holding time for organic analytes in frozen tissue samples is 1 year, while the holding time for metal analytes, except mercury, is 6 months (EPA 1995a). The holding times for mercury is 28 days. No changes to the QAPP are necessary.

14. Comment: **There is no discussion of the initial amphipod bioassay of sediments and the final amphipod bioassay of selected sediments to assess the effect of the extended sediment holding time. There is, also, no discussion of the initial echinoderm larvae test of pore water and the final echinoderm test of pore water to evaluate the effect of freezing the extracted pore water. Both of these demonstrations were agreed to in the discussion of the Phase II ecological risk assessment. Please include these demonstrations in the discussion of the pore water and sediment bioassays.**

Response: If pore water is frozen, the Navy will conduct toxicity testing to assess the effect of the extended storage conditions for both the sediments and sediment pore water toxicity testing as specified in the phase II ecological risk assessment work plan (PRC 1996). A new section will be added as follows:

9.4.3 Assessment of Extended Storage Time

Because of the nature of this project the sediment and sediment pore water samples used for toxicity testing may be stored for a period of 8 weeks until the chemistry results have been received from the analytical laboratory. If extended storage of the samples is required, split samples from five sampling locations will be used to assess the effect of the extended holding time for both sediments and sediment pore water, as stated in the work plan (see Section 7.1.2.3). The split samples will be

prepared by extracting a double volume of pore water. The chemical and biological tests will immediately be performed using half of the split pore water sample. The other half of the split pore water sample will be frozen and analyzed at the end of the 8-week holding period.

COMMENTS PROVIDED BY BART SIMMONS, HAXARDOUS MATERIALS LABORATORY, DEPARTMENT OF TOXIC SUBSTANCES CONTROL

1. **Comment:** **Page 15, Section 3.0 discussed the Quality Objectives and Criteria for Measurement Data (DQO). This process does not fully follow the Guidance for Planning for Data Collection in Support of Environmental Decision Making Using the Data Quality Objectives Process, EPA QA/G-4, Interim Final. The Guidance document has a seven step process to establish Data Quality Objectives. The QAPP should be revised to conform with the DQO process.**

   Response: Additional discussions of the DQOs will be included to document the seven-step process for their development, as specified by the EPA QA/G-4 guidance (1994b).

2. **Comment:** **Page 11, Section 2.4, Project Narrative and page 16, Section 3.0, Quality Objectives and Criteria for Measurement Data.**

   The QAPP stated that the whole sediment total chemistry values will be compared to the San Francisco Bay specific LSVs/HSVs and the contaminant results in the pore water will be compared to San Francisco Bay water quality objectives (RWQCB 1995) or the federal AWQC (EPA 1994e). These standards or action levels (San Francisco Bay LSVs/HSVs, RWQCB 1995, federal AWQC, are referenced to Section 7.1 of the EA WP and is not provided.

   These standards or actions levels used for decision making should be listed in the QAPP. The proposed analytical methods together with the contract required detection limit (CRDL), quantitation limits or detection limits should be reviewed to ensure that they can achieve the listed standards or action levels of the San Francisco Bay or the federal.

   Response: The LSVs/HSVs referenced in the QAPP are not currently available and were therefore not provided. Since the LSVs/HSVs are still not available for use, different screening criteria (as discussed in the response to James Polisini's comment number 2) were selected for use and will be listed in the revised QAPP. The CRDLs have been reviewed, and the analytical procedures have been modified, if necessary, so that the chemicals of potential concern can be detected at the required levels.

3. **Comment:** **Page 18, the reference Phase II EA WP (PRC 1996), which discussed the rationale for selection of sampling locations, number of samples to be**

collected and the methods for collecting samples, is not available for review. The sampling locations, number of samples and methods of collecting samples should be reviewed to ensure that they would provide the necessary information to meet the project needs.

Response: A draft, draft final, and final Phase II ecological risk assessment work plan were reviewed by DTSC, RWQCB, and EPA. Comments on the draft final document were received by the Navy in December 1995. Based on the comments received, a final Phase II ecological risk assessment work plan was prepared and submitted on April 10, 1996 (PRC 1996).

COMMENTS PROVIDED BY THE EPA

The subject Quality Assurance Project Plan (QAPP), prepared by PRC Environmental Management, Inc., and dated June 28, 1996, was reviewed. Review of this document was based on the documents "EPA Requirements for Quality Assurance Project Plans for Environmental Data Operations," May 1994 (QA/R-5), "Guidance for the Data Quality Objectives Process," September 1994 (QA/G-4), "Methods for Assessing the Toxicity of Sediment-associated Contaminants with Estuarine and Marine Amphipods," June 1994 ((EPA/600/R-94/025), and "Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms," August 1995 (EPA/600/R-95/136).

It is recommended that the QAPP not be approved until the information requested has been provided and the discrepancies between bioassay procedures presented in the body of the QAPP and SOPs included in the appendices have been addressed.

Major Concerns

1. **Comment:** **Analytical Methods. The SOPs for all bioassays include analysis of the test water or overlying water for pH, ammonia, sulfides, dissolved oxygen, salinity and temperature during testing, but the methods for salinity and temperature are not included in the tables of analyses to be measured. These should be included, along with pH, ammonia and any other analyte that may be measured using methods different from those used in chemical analysis.**

   Response: The analytical methods for pH, ammonia, sulfides, dissolved oxygen, salinity, and temperature will be included in the tables of analyses and quantitation limits. Precision and accuracy objectives for these analyses will not be reviewed since the methods are used to monitor the test conditions.

2. **Comment:** **Control Water. The source of natural sea water should be included in the discussion of natural and artificial sea water.**

   Response: The actual source of the control water (whether natural or artificial) will be identified by the toxicity testing laboratory when the specific testing

laboratory has been identified. The requirements for the control water are specified in the Section 9.4.1 of the QAPP.

3. **Comment:** **Section 3.4.5: Comparability. It is stated that the data quality indicators of precision, accuracy, and completeness are listed in Appendix A. Completeness requirements have not been included in the appendix tables. This omission should be addressed.**

Response: The completeness goal for the project is described in Section 3.4.4. Only the data quality indicators for precision and accuracy are listed in Appendix A. This will be stated in the final QAPP.

4. **Comment:** **Section 6.0: Calibration Procedures and Frequency, Table 6. Field Equipment Calibration. In addition to daily calibration in air, the dissolved oxygen meter should be calibrated by a Winkler titration on a regular schedule.**

Response: The calibration of the dissolved oxygen meter using a Winkler titration will be performed before the equipment is sent to the field. This requirement will be added to Section 6.0 and Table 6.

5. **Comment:** **Section 8.8: Bioassay Protocols. The general statement is made that any bioassay will be repeated if more than 10% of the control animals die. In the specific protocols, the requirement for control animal survival ranges from 90% (worms) to 50% (bivalve larvae). The general statement should be changed to refer to the different test requirements. Specific requirements listed in the protocol sections should be consistent with those listed in the appendices.**

Response: Section 8.8 will be changed to read as follows:

> The complete bioassay series must be repeated if more than 10 to 50 percent (depending on the respective toxicity test requirements as presented in Sections 8.8.1, 8.8.2, 8.8.3, and 8.8.4) of the control animals die or show evidence of sublethal effects.

6. **Comment:** **Section 8.8.2: Echinoderm Development Test. There are several issues that need to be addressed in this section:**

A. The test parameters include a dilution series. A dilution series is not generally run with a pore water bioassay. If it is intended that a dilution series be run, except for the reference toxicant, some explanation should be included in the discussion of the test. Test concentrations have also been included in the list of test parameters in Appendix E. The discussion should include this list as well.

B. A brine control is listed among the test concentrations, but there is no discussion of the use of brine in the text. The appropriate use of brine should be discussed in this section. In addition, the salting up protocol

that will be used to meet the test requirement of 34 ppt should be included in the appendices.

C. Test acceptability is stated to be "greater than 80% shell development," but the echinoderm larvae do not develop shells. The endpoint for this test is development to the pluteus larvae stage; this is discussed in Appendix E. The references to the endpoint should be corrected throughout this section.

D. All references to the organisms during the test should include the word Larvae, so that it in clear that it is the embryo life stage that is being used in the test. The conditions for test acceptability, therefore, should state that the echinoderm larvae should be less than one hour old at test initiation, and that the larvae must be randomly distributed.

Response: Section 8.8.2: Echinoderm Development Test

A. The echinoderm development test will be used to test both the sediment pore water and the elutriate from petroleum-contaminated soils. A dilution series was implemented for the analysis of the elutriate to calculate an effect concentration (EC)10, EC25, EC50, no observed effect concentration (NOEC), and lowest observed effect concentration (LOEC). These values provide information about the dose response relationship. Tests of single concentrations, providing pass/fail information, are used during the analysis of the sediment pore water, in which case the test concentration requirement is not applicable. This information will be included in the QAPP.

B. Brine will not be used to increase the salinity of the sediment pore water test solution; therefore, reference to a brine control will be deleted. As discussed in Section 8.8.2 and Section 4.0 of Appendix E, the test parameters will be changed as follows: "Test Concentrations: five concentrations for each site and control water."

C. The Navy realizes that the endpoint is the pluteus stage and that echinoderm larvae do not develop shells. The wording for the test acceptability requirements follows the exact wording specified by EPA (Chapman and others 1995, page 359). The reference to "shell" will be deleted from the test acceptability item in the list of test parameters in Section 8.8.2 and Section 4.0 of Appendix E.

D. The test acceptability requirements in Section 8.8.2 will be changed to read as follows: "The age of the echinoderm larvae must be less than 1 hour at the start of the test." The last bullet of the same section will be changed to read as follows: "Test echinoderm larvae must be randomly distributed to test chambers."

The appropriate bullets in Section 10.0 of Appendix E will also be changed as indicated previously.

7. **Comment:** **Section 8.8.3: Polychaete Whole Sediment Test. Several issues should be addressed in this section.**

A. The genus/species of the organism to be used should be included in the title of the test.

B. Both "test solutions" and "overlying water" are listed among the test parameters. Since it is the sediment that is being tested, it would be clearer if all references to "test solution" were changed to "overlying water."

C. Among the performance based criteria for test organisms is the discussion of reference toxicant tests to determine organism condition. It is stated that reference toxicant data will either be obtained from the organism supplier or from a laboratory data base of at least five tests, as well as monthly tests performed using the same species. In the introduction to the bioassay section (Section 8.8) and in Section 9.4.2 (Positive Controls), it is stated that reference toxicants will be run with every test. The apparent discrepancy between using external data, monthly data generated in house or concurrent reference toxicants tests for determination of organism condition should be resolved.

Response: Section 8.8.3: Polychaete Whole Sediment Test

A. The first sentence under Section 8.8.3 will be changed to read as follows: "Test parameters for *Neanthes arenaceodentata* are as follows (Battelle Marine Sciences Laboratory 1994; PTI 1990)."

B. The heading "Test Solution Volume" specified in Section 8.8.3 and Section 4.0 of Appendix J will be changed to "Overlying Water Volume."

C. The testing laboratory will have a reference toxicant history for each source of test organisms or have a control chart from the supplier. During the time that testing will be conducted with the respective organism, the testing laboratory will either conduct a reference toxicant test with each new batch of test organisms or once a month, whichever comes first. The wording will be changed as follows:

- No changes to Section 8.8
- The second bullet of performance based criteria, Section 8.8.3, will read as follows: "Reference toxicant tests must be conducted on each batch of test organisms or one per month."
- The following will replace the first paragraph of Section 9.4.2:

  All bioassays will be conducted using well established positive (toxic) controls. Reference toxicants are used to provide information on mortalities or increased sensitivity that may occur as a result of

disease, changes in tolerance sensitivity, or loading density. The laboratory must have a reference toxicant control chart for each source of the test organism, which may be obtained from the organism supplier. In addition, the testing laboratory must conduct reference toxicity tests for each new batch of test organisms or one per month, whichever comes first."

8. **Comment:** **Section 8.8.4: Bivalve Shell Development Test. Several issues should be addressed in this section.**

A. The genus/species of the organism to be used should be included in the title of the test.

B. The test parameters indicate that a dilution series is to be run with the elutriate. As this is an in-place sediment, rather than an introduced contaminated material, it is not clear why a dilution series is required. The rationale for using dilutions should be presented in this section.

C. The test parameters include "overlying water," listing the measurements to be taken in this medium. The bivalve test does not include overlying water; there is no sediment in the test chambers. The reference to overlying water should be changed to "test solution."

D. Please refer to Comment 6D. This is also relevant to Appendix K: Standard Operating Procedure for 48-Hour Embryo-Larval Development Test Using the Blue Mussel Mytilus edulis, Section 10.0, Acceptability of the Test.

Response: Section 8.8.4: Bivalve Shell Development Test

A. The first sentence of Section 8.8.4 will be changed as follows: "Test parameters for *Mytilus edulis* are as follows (Chapman and others 1995)."

B. The bivalve shell development test is being used to test the elutriate from petroleum-contaminated soils as part of the procedure to develop cleanup goals for petroleum-contaminated soils (see Section 7.2 of the phase II ecological risk assessment work plan [PRC 1996]). A dilution series is used to calculate an EC10, EC25, EC50, NOEC, and LOEC and will be used to develop a cleanup goal.

C. The heading "Overlying Water Quality" in the test parameters section of Section 8.8.4 will be changed to "Test Solution Quality."

D. In Section 10.0 of Appendix K, the following bullets will be changed. The first bullet will read "Mussel larvae must be from 1.5 to 2.5 hours old at the start of the test." The last bullet will read "Test mussel larvae must be randomly distributed to test chambers."

9. **Comment:** **Section 9.4: Bioassay Quality Control. This section should include discussion of preparation and use of a brine control.**

Response: The reference to brine controls has been deleted; see response to EPA comment number 6B.

10. **Comment:** **Appendix E: Standard Operating Procedure for 72-Hour Development Abnormality Toxicity Test using the Echinoderm Strongylocentrotus purpuratus. Several issues need to be addressed.**

A. Section 2.0: Test Organisms and Section 4.0: Test Parameters. It is stated that the biological criterion of test acceptability is no more than 10% abnormal development in the control. Test acceptability is listed in the test procedures of Section 8.8.2 and in the Test Parameters section (4.0) of the appendix as 80% normal development. This discrepancy needs to be addressed.

B. Section 5.0: Sediment Preparation and Pore Water Extraction Before Testing. The information in this section contradicts the information provided in Table 5 (QAPP Section 4.0). In this section, sediments from which pore water is to be extracted may be held for 14 days; in the QAPP table, the pore water may be held up to 14 days, with no indication of sediment holding time. This issue should be expanded and all references to sediment and pore water holding times made consistent.

C. Section 6.0: Bioassay Procedure. It is stated that although the best way to determine the concentration of organisms in the test chambers would be to subsample, the presence of sediment in the chambers prevents the achievement of adequate mixing for representative sampling. Following the procedure for preparing pore water, there should be no or very little sediment in the test chambers. This discussion should be changed to reflect the procedures being followed in this test.

D. The test parameter of number of organisms/chamber should be changed to 25/ml. Test acceptability, 90% control survival, does not coincide with test acceptability with test solutions. This discrepancy needs to be addressed.

E. Section 10.0: Acceptability of the Test. Please refer to Comment 6D.

Response: Appendix E:

A. The last sentence of the last paragraph of Section 2.0 of Appendix E will be changed as follows: "The biological criterion of acceptability is that the larvae, spawned by adults in the dilution water, must not incur more than 20 percent abnormal development."

B. Sediment pore water will be extracted from sediments within 48 hours of sample collection. While this is much shorter than the 14 day holding time

specified by the guidelines (ASTM 1994), a shorter holding time was deemed necessary for the TI project. The extracted pore water will be immediately frozen and then stored for up to 8 weeks, if necessary, before the toxicity tests are performed. If the pore water is not frozen the chemical and biological analyses will meet the holding times specified in Tables 3 and 5 of the QAPP. The chemical analytical holding time will start on the day the pore water is extracted.

Footnote "h," Table 5, will be changed to read as follows: "Pore water will be extracted from sediment within 48 hours after collection. The pore water will be prepared and analyzed within the holding time appropriate for each analysis."

C. The last four sentences of the next to the last paragraph of Section 6.0 of Appendix E will be deleted.

D. In Section 8.0, the test parameter for number of organisms per chamber will be changed to read as follows: "Number of Organisms/Chamber: 25/mL". The test acceptability requirements will be changed to read as follows: "Test Acceptability: 80 percent control survival."

E. In Section 10.0, these bullets will be changed. The first bullet will read "The age of the echinoderm larvae must be less than 1 hour at the start of the test." The last bullet will read "Test echinoderm larvae must be randomly distributed to test chambers."

11. Comment: **Appendix F: Standard Operating Procedure for Extraction of Pore Water, Section 1.2. Pore Water Sample Extraction Process and Handling. It is recommended that the pore water be extracted at 2,500 g for 30 minutes, in order that the method be comparable to that used to develop the extensive pore water database developed for the Bay Toxics Cleanup Program (Regional Water Quality Control Board 1996).**

Response: The Navy proposes using the following centrifugation speed for the extraction of pore water. An initial centrifugation will be performed for 30 minutes at a speed of 3,200 g. A second centrifugation will also be performed for 30 minutes at a speed of 10,000 g. These speeds will allow the results to be compared to the other Naval facilities that have used this process. This change will also be added to Appendix E.

Other Concerns

1. Comment: **Section 4.0: Sampling Procedures, Table 1. Analytical and Toxicological Testing Parameters. From the presentation of the bioassay protocols in later sections, it appears that the bivalve test will be run with elutriate and the echinoderm test with pore water. In Table 1, the bivalve test is included among the sediment tests and the echinoderm test is included among the elutriate tests. These discrepancies need to be resolved.**

Response: The echinoderm development test will be used to test both the sediment pore water and the soil elutriate used to develop petroleum cleanup goals. The bivalve development test will be used to test the soil elutriate only. Table 1 will be corrected.

2. **Comment:** **Section 4.0: Sampling Procedures, Table 5. Sample Container, Holding Time, and Preservative Requirements for Sediment, Pore Water and Elutriate Bioassays, Item h. A reference should be provided for the information concerning the holding time for sediment from which pore water is to be extracted.**

Response: The holding time listed in Table 5 for the extraction of sediment pore water will be changed from 56 days to "48 hours/14 days." Footnote "h" will be changed to read as follows: "Pore water will be extracted from sediment within 48 hours after collection. The sediment pore water will be analyzed within 14 days of sample collection, unless the pore water is kept frozen."

3. **Comment:** **Appendix F: Standard Operating Procedure for Extraction of Pore Water, Section 1.2. Pore Water Sample Extraction Process and Handling. The "g" after the number indicating the speed at which the centrifuge is to be run refers to "gravity," not "gram," as is indicated in the text.**

The water that has been separated from the sediment by centrifugation is referred to as the "filtrate." This should be changed to "supernatant."

Response: The definition of "g" and the use of the term "supernatant" will be changed in the final QAPP.

4. **Comment:** **Appendix G: Standard Operating Procedure for 10-day Whole Sediment Toxicity Test Using the Estuarine Amphipod *Eohaustorias estuarius*, Section 10.0 Test Completion and Appendix J: Standard Operating Procedure for 20-Day Whole Sediment Survival and Growth Test Using the Marine Polychaete *Neanthes arenaceodentata*, Section 9.0 Test Completion. The statement, made in both sections, that "Any animal that is not living is presumed dead" is a tautology. It should be changed to read "Any animal that does not respond is presumed dead."**

Response: The section will be changed to read as follows: "Any organism that does not respond is presumed dead."

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