NAVAL FACILITIES ENGINEERING SERVICE CENTER
Port Hueneme, California 93043-4370

User's Guide
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GUIDE FOR PLANNING AND CONDUCTING SEDIMENT PORE WATER TOXICITY IDENTIFICATION EVALUATIONS (TIE) TO DETERMINE CAUSES OF ACUTE TOXIDITY AT NAVY AQUATIC SITES

Prepared by

Science Applications International
Corporation
Newport, Rhode Island 02840

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EXECUTIVE SUMMARY

This document provides guidance for Remedial Project Managers (RPMs) and their contractors to plan and conduct Toxicity Identification Evaluation (TIE) studies that will aid in characterizing and managing toxic freshwater and marine sediments associated with Naval facilities. TIEs are used to identify cause and affect relationships between toxicity observed in sediment toxicity tests and factors that have contributed to the observed effects. When properly executed, they help identify classes of stressors that cause toxicity to aquatic life, facilitating evaluation of the need for remediation, and when required, the development of appropriate, cost-effective remedial alternatives. An introduction to the utility of TIEs, directed towards RPM needs, is available as a complement to this guide (SAIC 2002a; http://p2ashore.nfesc.navy.mil/cgi-bin/project_descriptions/cfpr_results.cfm?PROJECT_ID=174).

This guide provides a general but comprehensive approach for planning and conducting TIEs, along with examples to illustrate many of the features of successful TIE studies. Organizationally, it follows a manual-style approach to facilitate planning and executing a TIE study. Sections 1 and 2 provide overview information to assist RPMs in determining if a TIE is appropriate for their site, and provide guidance through the planning process. Sections 3 and 4 focus on the technical aspects of conducting a TIE, and are thus presented from the perspective of a contractor implementing the TIE. Section 5 provides supplemental information including ranges of costs of TIEs, a description of the technical Task Package appended to assist first-time practitioners, a brief review of frequently-asked questions and answers, and general conclusions regarding the utility of TIEs for Navy sites.

In preparing this guide, TIE guidelines provided by the U.S. EPA (1992; 1996) were combined with new approaches developed by SAIC to resolve factors that drive acute toxicity in the bulk sediment assays. Since results from bulk sediment testing are frequently a key component in the weight-of-evidence used to assess the need for remedial actions at aquatic sites adjacent to Naval Facilities, this guide will facilitate the standardization of an approach to determine the greatest elements of ecological risk at those sites. This document provides remedial project managers and technical support contractors involved in site remediation planning with TIE guidance that emphasizes the approach as well as procedures, and provides examples to facilitate the actual conduct of TIE studies.

Results from TIE studies can be used during various phases in the progression of site characterizations conducted to determine risks from contaminants. TIE findings are best used in interpreting the results of Ecological Risk Assessments (ERA) to establish the causes of observed adverse effects on aquatic receptors. They can also serve to support or adjust analyte-specific Preliminary Remediation Goals (PRGs) that are used to develop the remedial alternatives evaluation in the Feasibility Study (FS). Ultimately, they provide a stronger technical basis for meeting the requirements of the National Contingency Plan (NCP) and the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Because adverse effects resulting from tests with many Navy site sediments may be resulting principally from non-contaminant stressors such as ammonia, TIEs can also provide strong evidence for cases where the Navy is not culpable for observed effects.

This guide emphasizes an over-all approach, with the need for site-specific method modifications to relate directly to the needs of individual Installation Restoration (IR) programs. It provides practical recommendations for designing and implementing TIE studies that can be used to evaluate the effectiveness of various strategies for reduction of identified risks, and to develop an appropriate context for Applicable or Relevant and Appropriate Requirements (ARAR) compliance.

This approach has been adopted by Engineering Field Activity Northeast (EFANE) and Naval Facilities Engineering Command, and has been demonstrated at several sites within USEPA Region 1. Additional information on the approach is available from the EFANE environmental risk assessment team.

TABLE OF CONTENTS

Page

LIST OF FIGURES VI

LIST OF TABLES VI

LIST OF APPENDICES VII

LIST OF ACRONYMS VIII

LIST OF ACRONYMS (CONTINUED) IX

1.0. INTRODUCTION 1-1
- 1.1. Background 1-1
- 1.2. Utility of TIEs for Evaluating and Managing Risks at Navy Aquatic Sites 1-2
- 1.3. Status of the TIE Methodology 1-3
- 1.4. Objective 1-6
- 1.5. Summary of Navy TIE Case Studies 1-6
- 1.6. Organization of this Guide 1-7

2.0. DESIGNING A TIE INVESTIGATION 2-1
- 2.1. Determine Applicability for TIE 2-1
  - 2.1.1. Assessment of Observed Toxicity 2-1
  - 2.1.2. Assesment of Toxicity Potential from Existing Chemistry Data 2-3
  - 2.1.3. Assessment of TIE Applicability—Case Study Example 2-7
- 2.2. Define TIE Study Objectives 2-8
- 2.3. Select Station Locations 2-9
- 2.4. Select TIE Test Organisms 2-10
- 2.5. Develop Work Plan for Team Review 2-11

3.0. FIELD AND LABORATORY ACTIVITIES FOR TOXICITY IDENTIFICATION EVALUATIONS 3-1
- 3.1. Field Sampling 3-1
  - 3.1.1. Planning Logistics 3-1
  - 3.1.2. Sediment Collection and On-site Handling 3-2
- 3.2. Toxicity Screening 3-3
  - 3.2.1. Bulk Sediment Testing Procedures 3-3
  - 3.2.2. Optional Screening with Pore Water Tests 3-3
- 3.3. Pore Water Extraction 3-4
  - 3.3.1. Selecting Sediment Samples for Pore Water TIE Test 3-4
  - 3.3.2. Determining Pore Water Volume Requirements 3-4
  - 3.3.3. Selecting an Extraction Method 3-5
  - 3.3.4. Maintaining Integrity of Pore Water Samples 3-7
- 3.4. Chemical Characterization of Sediments and Pore Waters 3-8
- 3.5. TIE Manipulation Approach 3-9
  - 3.5.1. Sequential Pore Water Treatments 3-9
  - 3.5.2. Independent Treatments 3-12
  - 3.5.3. Chemical Analysis of Treated Pore Waters 3-14
- 3.6. TIE Toxicity Testing 3-15

3.6.1. U.S. EPA Guidance for Freshwater and Saltwater Testing 3-15
3.6.2. Special Considerations for TIE Testing 3-15
3.6.3. Include a Sample Spiked with at least one CoC 3-19
3.6.4. Conduct a Pre-test 3-19

4.0. INTERPRETING THE TIE RESULTS 4-1
4.1. Toxicity Characterization 4-1
4.1.1. Evaluate Bulk Sediment vs. Pore Water Results 4-1
4.1.2. Synthesize Responses to TIE Treatments 4-2
4.1.3. Dealing with Ambiguous Data 4-7
4.2. Characterizing Chemistry Data for TIE Samples 4-8
4.3. Evaluating the TIE study in a Risk Assessment Framework 4-9
4.4. Spatial and Temporal Considerations 4-11

5.0. SUPPLEMENTAL INFORMATION AND CONCLUSIONS 5-1
5.1. Cost Information 5-1
5.2. Appended TIE Task Package 5-2
5.3. Frequently Asked Questions 5-2
5.4. Conclusions 5-4

6.0. REFERENCES 6-1

LIST OF FIGURES

Figure 1-1. Flow diagram showing fractionation sequence of Toxicity Identification Evaluation (TIE) treatments. 1-5

LIST OF TABLES

Table 2-1. Site evaluation criteria for TIE applicability: Example application of TIE site selection criteria to candidate sites. 2-7
Table 3-1. Comparison of pore water extraction methods. 3-5
Table 3-2. Estimation of the number of organisms required for TIE testing. 3-16
Table 4-1. Sediment vs. pore water test results for Indian Head samples. 4-2
Table 4-2. Sea urchin data from pore water TIE conducted with Hunters Point samples. 4-3
Table 4-3. Summary of cumulative toxicity removed by TIE treatments in the Hunters Point study with sea urchins. 4-6
Table 4-4. Synthesis of findings from the Indian Head TIE with amphipods (*Hyalella)* and fish (*Pimephales)*. 4-9
Table 5-1. Example costs for conducting a TIE with sediments. 5-1

LIST OF APPENDICES

Appendix A. Sediment and Pore Water Benchmarks used in Calculating Hazard Quotients for a TIE Study

A-1. Selection of benchmarks used in calculating sediment Hazard Quotients for a TIE study.
A-2. Water quality criteria and estimated benchmarks used in calculating Hazard Quotients for a TIE study.

Appendix B. TIE Task Package Including SAIC TIE Standard Operating Procedures

B-1. General lists of supplies required for conducting TIE study tasks.
B-1.1. Supplies and equipment required for field sample collection.
B-1.2. Supplies and equipment required for pore water extraction via syringe or centrifuge method.
B-1.3. Supplies and equipment required for sample manipulation.
B-1.4. Supplies and equipment required for animal testing.
B-1.5. Supplies and equipment required for in-house analyses of sample water quality parameters.

B-2. SAIC Standard Operating Procedures (SOPs) for conducting major TIE tasks.

TIE-100 GENERAL TIE PROCEDURES
TIE-200 TIE SOPS FOR SAMPLE COLLECTION
TIE-210 Sediment Sampling
TIE-220 Pore Water Extraction- Syringe
TIE-230 Pore Water Extraction- Centrifuge
TIE-300 TIE SOPS FOR SAMPLE MANIPULATION
TIE-310 Hypersaline Brine Preparation and Use
TIE-400 TIE SOPS FOR TIE MANIPULATION
TIE-410 Untreated Sample Preparation
TIE-420 Sodium Thiosulfate Manipulation
TIE-430 EDTA Chelation
TIE-440 Sample Filtration
TIE-450 Solid Phase Extraction Using C_{18}
TIE-460 Solid Phase Extraction Using OASIS®
TIE-470 Low pH Manipulation
TIE-480 High pH Manipulation
TIE-490 Ammonia Removal Using Zeolite
TIE-4100 Ammonia Removal Using *Ulva*
TIE-500 TIE SOPS FOR TOXICITY TESTING
TIE-510 Sea Urchin Sperm Cell Test
TIE-520 Mysid Survival Test

LIST OF ACRONYMS

ARAR	Applicable or Relevant and Appropriate Requirements
AVS	Acid Volatile Sulfide
BTAG	Biological and Technical Assistance Group
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
CoC	Chemical of Concern
CoPC	Chemical of Potential Concern
EDTA	Etheylenediaminetetraacetic acid
EFANE	Engineering Field Activity Northeast
ELU	Elutriate
EPA	Environmental Protection Agency
EqP	Equilibrium Partitioning
ERL	Effects-Range Low
ERM	Effects-Range Median
ERA	Ecological Risk Assessment
FA	Freshwater Acute
FC	Freshwater Chronic
FS	Feasibility Study
GC/MS	Gas Chromatography/ Mass Spectroscopy
HMW	High Molecular Weight
HPLC	High Pressure Liquid Chromatography
HQ	Hazard Quotient
IR	Installation Restoration
K_{oc}	Organic carbon partition coefficient
K_{ow}	Octanol-water partition coefficient
NA	Not Available
NAVFAC	Naval Facilities Engineering Command
NFESC	Naval Facilities Engineering Service Center
NOAA	National Oceanic and Atmospheric Administration
NSWC	Naval Surface Warfare Center
PAH	Polycyclic Aromatic Hydrocarbon
PCB	Polychlorinated Biphenyl
PEL	Probable Effect Level
PRG	Preliminary Remediation Goal
PW	Pore water
RAOs	Remedial Action Objectives
RPM	Remedial Project Manager
SA	Saltwater Acute
SAIC	Science Applications International Corporation
SC	Saltwater Chronic
SEM	Simultaneously Extractable Metals
SPE	Solid Phase Extraction

LIST OF ACRONYMS (CONTINUED)

SWDIV	Southwest Division
STS	Sodium thiosulfate
TIE	Toxicity Identification Evaluation
TRE	Toxicity Reduction Evaluation
TOC	Total Organic Carbon
UET	Upper Effect Threshold
VS	Validation Study
WQC	Water Quality Criteria
WQSV	Water Quality Screening Value

1.0. INTRODUCTION

This document was developed to serve as a guide for the conduct of Toxicity Identification Evaluation (TIE) studies of toxic sediments at Naval facilities. TIEs are used in aquatic risk assessments as an additional line of evidence in sediment toxicity characterizations. This guide will assist Navy Remedial Project Manager (RPMs) and their contractors in planning and conducting effective TIEs that will help identify classes of stressors that cause toxicity at Navy aquatic sites, facilitating the development of appropriate, cost-effective remedial alternatives. Following a careful study design process developed to meet site-specific goals, TIEs for sediments generally involve a suite of tests with sediment extracts (i.e., pore waters). Pore waters are manipulated through multiple procedures that remove the toxicity of individual toxicant classes (e.g., metals, organics, or ammonia) from sediment pore waters. Associated reductions in toxicity are used to characterize causative factors. When properly executed, TIEs identify cause and affect relationships between toxicity observed in sediment toxicity tests and factors that have contributed to the observed effects.

TIEs are particularly useful where site-specific cleanup criteria are needed to address risks, and where remedial options include the removal or treatment of specific contaminants. In these cases, TIEs will reduce uncertainty regarding which of the identified risk factors are contributing adverse effects that drive the remedial process. A general introduction to the utility, scope and applications of TIEs to characterize sediment toxicity at Navy sites is available in a white paper at http://p2ashore.nfesc.navy.mil/cgi-bin/project_descriptions/cfpr_results.cfm?PROJECT_ID=174.

1.1. BACKGROUND

From the initiation of Installation Restoration (IR) studies to the completion of Feasibility Studies, ecological risk evaluations at contaminated Navy aquatic sites are used to determine if and how remediation should be conducted. Remedial plans for reduction of ecological risks often rely upon correlations between bulk sediment toxicity and sediment screening values that are not intended to serve as regulatory limits (e.g., NOAA screening values; NOAA 1999). While these data do serve as flags for potential actionable risks, they are not direct evidence that the suspected Chemicals of Concern (CoCs) have actually caused toxicity. Normally, there is a substantial degree of uncertainty in the link between cause and effect, particularly when confounding factors (e.g., ammonia) are involved in the toxic response. Without knowledge of causative factors driving toxicity, cleanup goals may be set to satisfy overly conservative or inappropriate assumptions. Consequently, decisions to conduct time-consuming and costly remedial actions can be errant, and depending on the nature of the action may do little to remediate the principal risks at the site.

The selection of appropriate cleanup goals can potentially be greatly improved by identifying the risk-causing chemicals through TIE studies. While Remedial Action Objectives (RAOs) and Preliminary Remediation Goals (PRGs) must comply with applicable standards, validation or modification of clean up requirements may be based on site-specific findings, including TIE results. TIEs can serve to support or adjust PRGs that are used to develop the remedial alternatives evaluation in the Feasibility Study (FS). Ultimately, TIEs can contribute to the

technical basis for plans to meet the requirements of the National Contingency Plan (NCP) and the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Because adverse effects resulting from tests with many Navy site sediments may be resulting principally from non-contaminant stressors such as ammonia, TIEs can also provide strong evidence for cases where the Navy is not culpable for observed effects.

The regulatory acceptance of TIE results for pioneer programs at Navy sites has been high. For example, EPA granted a finding of "No Further Action" for the Naval Submarine Base-New London, CT at an Engineering Field Activity (EFA) Northeast IR site (Goss Cove), supported by evidence presented from a TIE study (SAIC 1999). The study revealed that ammonia (a ubiquitous non-CoC sediment constituent) was responsible for toxicity rather than conventional sediment contaminants (i.e., PAHs, metals), saving approximately $2M in remediation costs (Navy RPM News 1999). Another sediment TIE study that was recently co-sponsored by EFA Northwest and Naval Facilities Engineering Service Center (NFESC) demonstrated that at a contaminated site in Puget Sound, risks to aquatic life were not due to ordnance compounds (Carr and Nipper 2000). Organics, metals and ammonia were associated with toxicity at the site, avoiding the misplacement of $9 million for ordnance cleanup by the U.S. Navy (NFESC 2001).

The Naval Facilities Engineering Command (NAVFAC), in response to the need for specific guidance to apply TIEs to Navy sites, sponsored a cooperative effort with Engineering Field Activity Northeast (EFANE) and its contractor, Science Applications International Corporation (SAIC) to develop this guide document. The intent of this guidance is to present a synthesis of existing TIE methodologies and applications as well as to incorporate recent TIE developments that have enhanced the overall utility and regulatory acceptance of TIEs as part of contaminated sediment investigations. As a functional step in preparing materials for this guide, TIE studies were conducted as part of two sediment investigations, one located at the Indian Head Naval Surface Warfare Center in Maryland and the other at the former Hunters Point Shipyard in California. These studies serve to demonstrate TIE testing and data interpretation procedures that will effectively contribute to contaminant-associated ecological risks characterization and management efforts.

1.2. UTILITY OF TIES FOR EVALUATING AND MANAGING RISKS AT NAVY AQUATIC SITES

This document offers a general understanding of how TIEs can be used as a bridge between risk assessments and the planning of appropriate remedial actions. As demonstrated by the Goss Cove study, the TIE provided sufficient evidence that ammonia was the primary contributor to site risks to support a finding of "No Further Action" at this EFANE IR site (Navy RPM News 1999). The demonstration TIE conducted with Indian Head sediments provided evidence that silver, identified as the principal Chemical of Concern (CoC) in a previous RI report, was a relatively minor source of toxicity compared with ammonia and other metals. It also revealed that sources of contamination were mixed and variable within a limited spatial scale. This is important information that will contribute to the development of remedial options. The Hunters Point TIE Demonstration contributed to determining which areas may be degraded due to ammonia effects, and where contaminants caused sediment toxicity, the same contaminants were associated with toxicity at reference locations, rather than attributable specifically to the Shipyard.

Careful evaluation of existing data for each of these sites preceded the decision to conduct TIE tests. Well-defined objectives and study designs contributed largely to the successful outcome of each TIE study. It is important to use the approaches presented in Section 2.1 of this guide to identify the sites where TIE studies are most likely to resolve issues associated with remedial planning. Good candidate sites for TIEs are those where site-specific cleanup criteria are needed to address risks, and where remedial options include the removal or treatment of specific contaminants. In these cases, TIE findings may serve to preempt time-consuming and costly remedial actions that would ultimately fail to reduce risk. While TIEs provide evidence for the causes of toxicity, results should always be evaluated through multiple lines of evidence, and ultimately, in the context of a comprehensive assessment of risk. The TIE resolves sources of acute risks to aquatic organisms, while the more comprehensive assessment should address multiple exposure pathways and associated uncertainties.

1.3. STATUS OF THE TIE METHODOLOGY

Existing EPA TIE methodology. EPA has published general guidance for freshwater and saltwater TIEs (Ankley et al. 1992; U.S. EPA 1996), providing general background and reference information for prospective TIE studies addressing contaminated sediments, and includes detailed descriptions of the TIE procedures and some discussion of potential applications. The level of detail presented in these guides requires that the user have a working knowledge of toxicity testing procedures that were developed for effluent water samples (U.S.EPA 1991). Both of the EPA documents provide useful interpretative guidance and examples for specific TIE results. However, they generally do not provide an overall perspective regarding the TIE study design required to address project-specific goals, or a context for larger sediment investigations where communication of study findings to the non-scientist is as important as the technical execution.

The state of TIE science is evolving along with applications that demonstrate the utility of findings, but the new developments and refined methods are not easily accessible to potential users such as Navy RPMs and support staff conducting sediment investigations. Revised standard methods follow limited EPA publication cycles. As a result, the adoption of TIE testing as a standard tool in sediment investigations on Navy sites has been impeded.

To date, the standardized approach for conducting TIEs involves three phases: Phase I "characterizes" the general classes of contaminants (e.g., metals vs. PAHs) through a set of independent sample manipulation and toxicity testing; Phase II "identifies" individual contaminants using a combination of analytical chemistry, toxicity testing and in some cases, serial TIE manipulations and testing; and Phase III "confirms" physical/chemical properties of the suspected toxicants through additional re-testing including procedures such as back addition of the chemical to sample material. The approach presented in this document is consistent with the elements of each Phase, while favoring a default method that includes synoptic measures of chemistry with each TIE study (combines Phase I and II), and defers Phase III and other additional TIE methods to be applied only when initial results are ambiguous. On a case-by-case basis, each TIE study should be designed to anticipate and maximize the cost effectiveness of obtaining the requisite information. An approach that is generally applicable, and that provides a

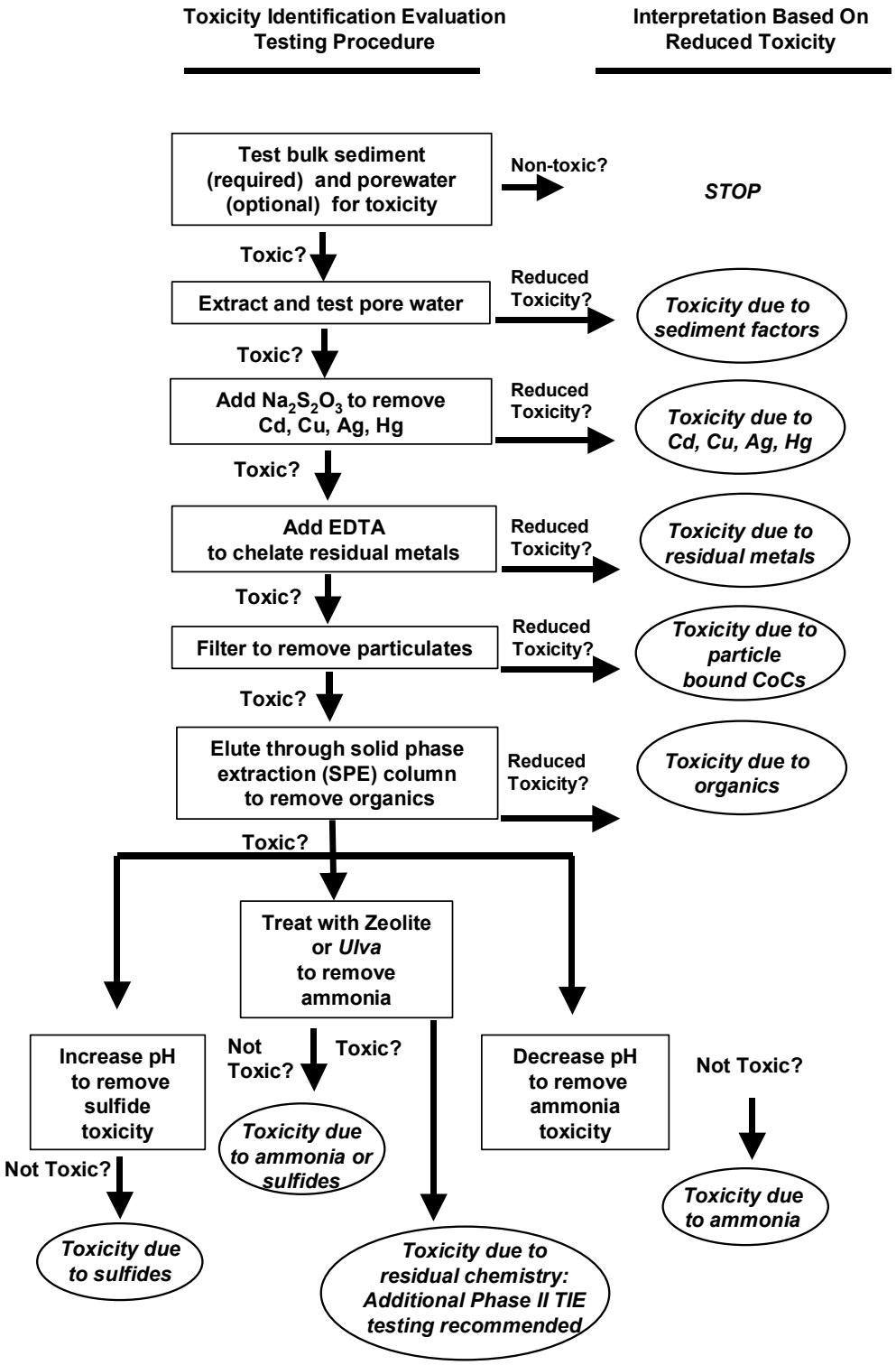
sufficient technical basis for regulatory decisions at many sites, is described in this document for Navy applications.

Proposed Navy TIE methodology. The TIE procedures presented in this guide provide a line of evidence to characterize the causes of sediment toxicity for Navy aquatic sites. Developed largely as a way to apply EPA methods, the principal aspect of the Navy's approach that sets it apart from EPA protocols is that sequential testing is an integral part of the proposed standard TIE test series (Figure 1-1). In the Navy procedure the goal is to fully resolve all sources of toxicity in a sample, such that at some point in the sequence, a completely non-toxic sample is obtained. The TIE treatments are largely the same as EPA procedures, but in contrast to the Phase I EPA general method (where samples are run in parallel), sample manipulations are consecutive (i.e., in sequence), such that the sources of toxicity are potentially less with each step.

The nuances of parallel vs. sequential testing will be discussed further within this document. However, some of the key differences that favor a sequential approach are related to "masking"; and "additivity." Masking occurs when the ability to discern the effect of removing one toxic fraction is impeded by remaining sources of toxicity. In sequential testing, the probability of masking is reduced with each step in the sequence because toxicants addressed by prior treatments are no longer present in the sample. Admittedly, masking can still occur in the early steps of the sequence; but these concerns can be addressed by subsequent testing where the specific steps are reversed.

The principle of additivity relates to a simplifying assumption that the total toxicity in a sample cannot exceed the sum of its parts. In traditional TIEs, the sum of reduction in toxicity from all parallel treatments could be $\geq$ 100%, suggesting either that there is error in the estimate of toxicity reduction from a given treatment, or that there are potential synergistic interactions of contaminant mixtures that have been expressed because of the treatment. In the former case, uncertainty is raised about the accuracy of the result; in the latter case, it is the uncertainty about the reality of complex (and ultimately, mysterious) chemical interactions. In either case, the parallel TIE procedures yield interpretations that lack confidence as to whether all sources of toxicity have been accounted for (i.e., sum of toxicity reductions = 100%). As discussed above, the goal of the sequential TIEs is to render the sample non-toxic, with identification of each class of toxicant as it is eliminated or sequestered from the sample. The two approaches are essentially identical when toxicity is attributable to a single class. However, experience has shown that a complex variety of contaminant mixtures are often found at aquatic sites, and accordingly, the uncertainty involved in the interpretation of parallel TIE results has been high. This uncertainty has limited the application of TIE technology as a standard line of evidence for assessments of ecological risk from contaminated sediments. In fact, the challenge posed by these difficulties led to the development of the sequential methods and interpretive procedures presented in the remainder of this document. This guide also addresses integration of TIE findings as a line-of-evidence for risk assessments and for use in remedial planning.

Figure 1-1. Flow diagram showing fractionation sequence of Toxicity Identification Evaluation (TIE) treatments.



1.4. OBJECTIVE

The specific objective of this document is to provide a guide for planning and conducting TIEs at Navy sites that have been identified for remedial investigations or where preliminary risk assessments suggest adverse effects to the aquatic environment. The methods presented generally incorporate EPA's methods for specific TIE manipulation steps, and reflect EPA guidance for interpreting results (Ankley et al. 1992; U.S. EPA 1996). This document intends to extend that guidance by providing recommendations for developing effective TIE study designs. It also includes a step-by-step sequential methodology that should facilitate organization of TIE studies. By following illustrations of site evaluations, study design, field and laboratory activities, data analysis and interpretation, and ultimately, integration into a risk assessment framework, the user is introduced to most of the critical features that determine success or failure of a TIE study. Most of the illustrations are from the two Navy demonstration sites chosen as case studies. While these examples have relevance to a wide array of Navy sediment site applications, they may not apply to every Navy sediment site where sediment contamination has been identified as a risk issue.

1.5. SUMMARY OF NAVY TIE CASE STUDIES

The TIE Demonstration Project involved two Navy sites: 1) Indian Head Naval Surface Warfare Center (NSWC) on a tributary of the Potomac River in Maryland, and 2) the former Hunters Point Naval Shipyard in San Francisco Bay, California. The sites were chosen using two principal criteria: 1) A clear need to resolve uncertainties regarding chemicals causing toxicity to gain regulatory acceptance of site management decisions, and 2) representation of unique issues associated with assessments of sediment toxicity. The Indian Head study area includes a small stream and its associated tidal, fresh receiving water, Mattawoman Creek. The Hunters Point site is classified as a saltwater site, with a mean salinity of approximately 20 ppt. The site selection process and criteria used to select these sites are described in further detail in Section 2.1.3.

For Indian Head, objectives for the TIE were established to meet the needs of the IR support team (EFA Chesapeake IR staff and contractors), the Activity Team (Indian Head NSWC staff) and the Regulatory Team (Region III Biological Technical Assistance Group (BTAG)) efforts to address contamination associated with a landfill at NSWC. Silver concentrations measured in sediments of the small stream adjacent to Olsen Road Landfill were above the action level, and had been identified by the BTAG as the CoC for aquatic receptors. Previous bulk sediment toxicity testing results indicated that each of thirteen representative sediment samples were toxic (Tetra Tech NUS 1999a). Ammonia was implicated as a confounding factor contributing to observed toxicity, but other contaminants had not been conclusively excluded as toxic factors (Tetra Tech NUS 1999b). Hence, for the TIE demonstration, six sediment samples from the small stream were chosen to resolve uncertainty regarding the sources of observed toxicity. A preliminary Risk Assessment Study had been proposed for the Mattawoman Creek receiving water to better characterize the nature and extent of all aquatic contamination from NSWC. To contribute to improved planning for that study, an additional nine sediment samples in the TIE

study were selected to represent an area of Mattawoman Creek adjacent to an Organics Plant and Scrap Yard at NSWC and a nearby burn pit area.
For Hunters Point, the Navy Southwest Division (SWDIV) of NAVFAC was in the process of completing a Validation Study (VS) for Parcel F covering the offshore area at Hunters Point. The VS was called for because of uncertainties regarding environmental risks in the study area referred to as the "Low-Volume Footprint" subsection of the Hunters Point Shipyard. Screening levels for copper, chromium, lead, zinc and polychlorinated biphenyls (PCBs) were exceeded. Nevertheless, toxicity was most strongly correlated to total ammonia, and therefore the need for remedial action within the "Low-Volume Footprint" was uncertain. Hence, in conjunction with the VS, the TIE team coordinated with SWDIV to obtain extra volumes of sediment for eleven of the 59 proposed sampling stations, including the reference station, to provide split samples for the TIE demonstration. The intent was to determine the relative degrees of toxicity attributable to site-specific contaminants and ammonia.

While both Demonstration Case Studies are used as examples in this Guide, the reader is referred to the site reports for each individual document (SAIC 2001; 2002) to gain a better understanding of the site-specific elements involved in TIE study design, as well as TIE procedures and the interpretive process. Reports from these case studies can be found at (*insert URL when documents are posted on NFESC web site*).

1.6. ORGANIZATION OF THIS GUIDE

With the intent to follow the functional and chronological order of an effective TIE study, this Guide has been organized into six principle sections: Section 2.0, Designing a TIE Investigation; Section 3.0, Field & Laboratory Activities for Sediment TIEs; Section 4.0, Interpreting the TIE Results; Section 5.0, Conclusion and Recommendations; Section 6.0, Appended TIE Task Package; and Section 7.0, References. Section 2.0 provides a procedure for reviewing existing data to determine the utility of a TIE study (Section 2.1), defining study objectives (Section 2.2), choosing station locations (Section 2.3), selecting test species (Section 2.4), and developing a work plan (Section 2.5). Section 3.0 provides an overview of field sampling considerations (Section 3.1), and reviews procedures for toxicity screening tests (Section 3.2), TIE manipulation procedures (Section 3.3) and TIE toxicity tests (Section 3.4). Section 4.0 includes recommended approaches for interpreting the TIE test results (Section 4.1), synthesizing exposure characterization information using chemical and physical sample measurements (Section 4.2), applying the TIE findings within a weight of evidence linking effects with potential causality (Section 4.3), and evaluating spatial and temporal uncertainty (Section 4.4). Section 5.0 of the Guide provides a summary and discussion, including the value of TIEs to RPMs (Section 5.1), recommendations for future TIE research (Section 5.2), and a list of "Frequently-asked" questions and answers (Section 5.3). Section 5.0 contains information regarding TIE costs (Section 5.1), a brief description of the appended TIE Task Package developed as a workbook for TIE studies with many of the protocols and worksheets that can be used to facilitate the completion of an effective study(Section 5.2), Frequently Asked Questions regarding TIEs (Section 5.3) and conclusions (Section 5.4). Finally, references cited in this Guide are presented in Section 6.0.

2.0. DESIGNING A TIE INVESTIGATION

A successful TIE study will often require a high degree of collaborative effort between members of the site team, including the RPM, technical representatives and contractor performing the TIE. Understanding the roles of each member of a site team will facilitate identification of relevant data, and hence this coordination should begin during the design phase of the planning process. For instance, where the TIE is coordinated with an ongoing investigation, it may be that much of the synthesis and analysis of existing data has been completed. In reviewing these findings, the person responsible for conducting the TIE should seek interpretive guidance from those who completed the data analysis, but should also be familiar with the form and completeness of the existing data.

If it is determined that a TIE is appropriate for the site, the specific objectives of the TIE should be developed in the context of other site investigative efforts and all parties involved should agree upon these study objectives. In this Section, procedures for reviewing existing data to determine the applicability of a TIE to address the study uncertainties (Section 2.1), defining study objectives (Section 2.2), choosing station locations (Section 2.3), and selecting test species (Section 2.4) are provided. Finally, the various elements of a work plan that describe how each phase of the study will be integrated and coordinated are discussed (Section 2.5). Also included here are a range of approximate costs for conducting the field collections, pore water extractions, sample manipulations, laboratory testing and data analysis for a TIE study, depending on the degree of cost sharing that can be achieved through coordination with other site investigations.

2.1. DETERMINE APPLICABILITY FOR TIE

The first step in designing a TIE study is to evaluate the existing data to see if a TIE is appropriate. Each case is unique, but generally, the best candidate sites for TIE testing include sites where previous toxicity has been observed, chemical concentrations are at potentially toxic levels, and projected remedial costs are high given existing uncertainty about what chemicals or confounding factors are contributing to toxicity.

Because the intent of a TIE study is to resolve the nature of sediment toxicity, data on bulk sediment toxicity should be demonstrated and evaluated prior to conducting a TIE (Section 2.1.1). An evaluation of the chemical concentration data with regard to the potential sources and magnitude of toxicity may be available or may need to be reevaluated (Section 2.1.2). Included here are the evaluations of other types of data (e.g., organic carbon, ammonia and sulfide concentrations) that result in environmental modification of toxicity (higher or lower) from that which would be otherwise predicted by the absolute contaminant measurements. The goal is to establish potential links between a point source of contamination and observed adverse effects that may be resolved by the TIE study.

2.1.1. Assessment of Observed Toxicity

Magnitude of toxicity. In reviewing existing sediment toxicity data, a principal factor is the degree of toxicity exhibited. EPA guidance recommends that TIEs are most useful if potency is

at least two toxic units (U.S. EPA 1996). The number of toxic units is defined as the sample concentration divided by the LC_{50} (concentration at which 50% mortality is observed). Hence, two toxic units is a concentration that is twice the LC_{50} concentration for a given toxicant. Thus for pore waters with two toxic units, 50% mortality would occur in a pore water sample at the 50% dilution level. While it is possible to conduct successful TIEs on samples with less toxicity, higher uncertainty is to be expected. For example, if only 25% mortality is observed in a bulk sediment test, this provides little capacity to observe reductions in toxicity due to TIE treatments, particularly if toxicity is due to more than one toxic fraction. At the 25% level, effects are approaching statistical detection limits (e.g., *Ampelisca*, 20% mortality; Thursby et al. 1997). Another reason to consider the degree of observed toxicity is to determine the number of serial dilutions to include in the TIE design. Dilutions may only be useful where high levels of toxicity have been observed in undiluted samples. If, for instance, a bulk sediment test results in 90% mortality, then a series including 100%; 50%; 25%; and 10% samples may be advisable. In contrast, results with less than 50% mortality may indicate fewer dilutions are appropriate. Pore water screening tests are often useful in establishing the appropriate dilution series for a TIE, particularly when 100% mortality occurs in a bulk sediment test

Confounding factors contributing to toxicity. In evaluating bulk sediment toxicity data it is also important to review the specifications of the test methods employed. Some bulk sediment testing protocols include daily water purges prior to testing, in order to reduce ammonia to non-toxic levels. In such cases, if it is known that sufficient ammonia has been removed to render it non-toxic, then the TIE study plan need not include ammonia removal steps. In other cases, ammonia accumulation in sediments prior to testing may be a function of holding time, and replication of these test conditions may or may not be desirable. Other toxicant effects may also vary with storage conditions and holding times (U.S. EPA 2001a; ASTM 2000; Environment Canada 1994). If the principal objective is to attribute likely causes of toxicity observed in previous tests, TIE study methods should replicate the methods used previously (e.g., purging or not; maintaining the same holding times, and adhering to the same seasonal schedule). However, if the objective is to best evaluate sources of risk at the site, other methods may be preferable (e.g, minimize holding time).

Species sensitivity. Another factor to consider in reviewing existing toxicity data is species sensitivity. Depending on the status of the site investigation (i.e., Ecological Risk Assessment or Feasibility Study) existing data may be limited to one set of bulk sediment test results, multiple species, or even pore water or elutriate (suspended sediment mixture) testing. If proposed studies (aside from the TIE) are to include specific species chosen to represent a particular degree or type of ecological response, then it is often important to use the same species in the TIE. Aside from ecological representation, it may be useful to choose two species that exhibit differential degrees of sensitivity to know toxicants. For instance, the sea urchin (*Arbacia punctulata*) larval development test is generally sensitive to cationic metals, but less sensitive than other standard test species such as inland silversides (*Menidia beryllina*) to certain organic contaminants (Bay et al. 1993) such that both species might be included to address both metals and organics as sources of toxicity.

Spatial and temporal considerations. Finally, in reviewing existing data, it is also important to consider how well spatial and temporal scales of concern have been addressed for the current

study objectives. Characterizations of existing data may demonstrate a range of contaminant loads with variability between and within areas. Some sources of contamination may be shared between areas while others may represent more spatially limited areas and/or 'hot spot' concerns. Concentrations of non-CoCs that may cause toxicity (e.g, ammonia and sulfides) may also vary, particularly with depth but also potentially with season. Hence, it is important to consider the need to include multiple strata in the sampling design for the TIE study. In addition, if the data are greater than a few years old, or if the study area is in a particularly dynamic zone for sediment flux and transport, then a baseline survey to determine the current degree of bulk sediment toxicity, and/or spatial distribution of toxicity may be warranted.

2.1.2. Assesment of Toxicity Potential from Existing Chemistry Data

As with the toxicity data, an assessment of all available and relevant chemistry data from the site is part of the planning process for a potential TIE study. Following a standard risk-based approach, the importance of the chemical concentration data is assessed using benchmarks appropriate for each media. Here, Hazard Quotients (HQs) are calculated as measured chemical concentrations divided by appropriate effects-based benchmarks. If the resulting quotient is greater than one then toxicity is possible, while a quotient less than one indicates toxicity is unlikely.

The ability of HQs to be a useful predictor of potential toxicity hinges on the appropriate selection and use of benchmarks. For TIE planning, benchmarks representing acute toxicity thresholds are selected because the TIE tests themselves will detect only acute effects due to the short-term nature of the tests. A list of appropriate benchmarks available at the time this Guide was completed is presented in Appendix A ; more appropriate benchmarks may become available, and should be evaluated for applicability to the site being evaluated. While HQ derivation based on standard matrix-specific (i.e., sediment or water) benchmarks should be applied as a starting point in the evaluation process, ancillary analyses using species-specific benchmarks should also be conducted if sufficient data exists, to provide as complete an understanding as possible of the factors that may be contributing to toxicity. Guidance on identifying these scenarios is presented below.

Sediment benchmark exceedences. Sediment contaminant benchmarks are numerical chemistry values derived from a large database of measured adverse effects associated with environmental samples (as opposed to laboratory spikes). They represent a variety of endpoints (e.g., toxicity, decreased benthic diversity) and inherently reflect the cumulative response to the co-occurrence of multiple contaminants. The interpretation of the chemistry data relative to benchmarks involves the HQ approach discussed above. To simplify interpretation, calculated HQs can be summed and additionally classified according to the likelihood of toxicity occurring based on the magnitude of the benchmark exceedence. For instance, a moderate likelihood of toxicity might be associated with an HQ less than five (HQs 1-5), while a high probability is likely associated with HQs between 5 and 20; and an even higher probability where HQs are >20. The breakpoints will depend upon the CoCs involved, the range of exceedences and the risk assessor's degree of confidence in the reliability of benchmark values for predicting acute toxicity.

Sediment-based HQ summations have been shown to correlate with sediment toxicity and are thus useful in deciding whether a TIE would be applicable at the site. However, the data sets supporting these chemical-specific benchmarks have originated from highly contaminated sites where co-contaminants are also at very elevated levels. Accordingly, the resulting chemical-specific benchmarks do not strictly represent a single contaminant but rather the effects of multiple stressors. As a result, predictions of adverse effects in some cases (e.g., concentrations of multiple CoCs exceeding the NOAA Effects Range Median concentration) may actually reflect responses to long-term "chronic" exposures, rather than short-term acute exposures. As a result, these sediment HQs may suggest toxicity in samples that may prove non-toxic in the TIE, or otherwise contradict the TIE findings. This scenario is particularly likely for contaminants that are not normally acutely toxic in bulk sediment tests, such as high molecular weight (HMW) PAHs. With these uncertainties in mind, it is important to evaluate other measures of potential toxicity, as discussed in the following sections.

Divalent metals bioavailability. Simultaneously Extractable Metal:Acid Volatile Sulfide (SEM:AVS) measurements are conducted on sediments to assess the bioavailability and hence toxicity of divalent metals. In this method, the amount of metal liberated from the sample during extraction is measured, and at the same time, the quantity of sulfide released from the sediment is also measured. Sulfides are a common constituent of organic-rich sediments that will bind divalent metals in direct proportion to their respective molar concentrations (Hansen et al. 1996). Divalent metals are not bioavailable when SEM is less than AVS (on a molar basis). Hence, whenever SEM and AVS data are available, they may be used to qualify the potential for divalent metal toxicity.

The difference approach (SEM-AVS) is preferred as it most accurately represents available SEM concentrations. More traditionally, a ratio approach (SEM/AVS) tends to misrepresent available concentrations of SEM at low AVS concentrations. The EPA National Sediment Quality Inventory has adopted the difference approach; an SEM-AVS value of 5 μM/g dry wt is recommended as a screening value for identification of bedded sediments of concern with regard to potential divalent metal effects on aquatic biota (U.S. EPA 1997).

Until recently SEM:AVS analyses were not typically included in sediment chemistry measurements, hence the evaluation of historical sediment data for potential divalent metals toxicity is problematic. Where SEM and AVS data are not available, the concentration of SEM may be roughly estimated to be equal to the corresponding bulk sediment concentration due to similarity in the chemical extraction methods for SEM and typical bulk sediment metals analysis (both are weak acid digestion methods). Iron concentration in bulk sediment may be used as an indicator of AVS binding capacity. This is because the principal form of AVS is generally iron monosulfide (FeS), although the more stable pyrite form (FeS_2) might also be present. Generally, when molar concentrations of iron are low, AVS concentrations are also low. This deviation from recommended measurement of SEM-AVS introduces an added level of uncertainty. SEM and AVS measurements should be included in all TIE studies, as part of the weight of evidence that may contribute to better understanding of metal bioavailability. While a preponderance of studies have found that the relationship between SEM and AVS is generally a good predictor of toxicity, it must be recognized that, like other assessment tools, it is not an absolute predictor of metal toxicity. O'Day, et al. (2000) recently demonstrated adverse effects

of San Francisco Bay sediments and pore waters to amphipod and sea urchins, where AVS was measured in excess of SEM. That study also found that most of the available AVS was represented by dissolved pore water sulfides that were not associated with iron.

Pore water benchmark exceedences. Similar to the bulk sediment benchmark comparisons, pore water chemistry data are used for comparison with water quality benchmarks to assess the potential for toxicity of the sample. The advantage of pore water benchmarks over sediment benchmarks is that they tend to reflect location-specific sediment characteristics (e.g., low Total Organic Carbon (TOC) or low AVS increasing the proportion of contaminants available in pore water). Appropriate benchmarks must be chosen based on the nature and objectives of the study. U.S. EPA Water Quality Criteria Freshwater or Saltwater Acute Values (WQC-FA; WQC-SA) are generally appropriate. In the absence of a water-derived benchmark, pore water benchmarks for organics can be derived from sediment benchmarks using the Equilibrium Partitioning (EqP) model approach of DiToro et al. (1992) as follows:

$$1)\ C_p = C_s/(f_{oc} * K_{oc})$$

In the above equation, organic chemical pore water concentrations (C_p, µg/L) are calculated from the corresponding sediment concentration (C_s; µg/kg) based on the fraction of organic carbon (f_{OC}) in the site sediment (f_{oc} = %TOC/100) and the organic carbon/water partitioning coefficient (K_{OC}) for the CoPC. Values for K_{oc} are determined from the relationship developed by the U.S. EPA (Karickhoff et al. 1989):

$$2)\ \log_{10}K_{oc} = 0.00028 + 0.983*\log_{10}K_{ow}$$

where K_{ow} = the octanol/water partition coefficient. In this process, it is assumed that the resultant value provides a level of protection equivalent to other water quality based benchmarks. These derived values are sediment benchmarks transformed into water-equivalent benchmarks using the EqP model and a default value of 1% sediment TOC concentration. When the sediment-based benchmarks are applied to the site sediment, the benchmark is adjusted site-specifically, (multiplying by the measured TOC in the sample). It is noted that these estimated benchmarks tend to be overly conservative and in many cases they are several orders of magnitude lower than published WQC benchmarks (based on lowest observed effect level) when both are available for comparison.

If concentrations of chemicals measured directly in pore water are available, these should be used to generate pore water HQs. For pore water TIEs it is generally cost-effective to directly measure cationic metals (e.g., using Inductively Coupled Plasma Mass Spectrometry-EPA Method 200.8). However, the cost of measuring organics in pore water are higher, and therefore concentrations predicted using the EqP model described above are generally recommended. This is advantageous, because large volume requirements and the expense of direct analysis often prohibit measurement of organic contaminants. As with sediment HQs, pore water concentrations are divided by the pore water benchmarks to calculate Hazard Quotients (HQs). These HQs are used to assess the potential for pore water chemicals to cause toxicity.

Non-CoC benchmark exceedences. Ammonia is recognized as a source of potential toxicity in bulk sediment tests. Standard methods for conducting these tests cite ammonia tolerance limits for individual species because it is known to confound the interpretation of toxicity with regard to CoCs in sediments (U.S. EPA 1994; 2001b). Where ammonia data are available, they can be used to evaluate the likelihood that 'confounding factors' have contributed to toxicity. Where tolerance limit (benchmark) exceedences are found, TIEs are most likely to be useful in determining whether non-CoC sources contributed to toxicity. In order to evaluate the relative contributions of ammonia, the hazard quotient approach has been applied using both total and un-ionized concentrations. As with pore water contaminants, the U.S. EPA Water Quality Criteria values may also be used as benchmarks to calculate HQs.

Hydrogen sulfide is another potential contributor to toxicity in pore waters that is often overlooked. In a review focusing on sediment toxicity, Wang and Chapman (1999) provide a comprehensive summary of the available data concerning sulfide toxicity to benthic invertebrates and report 96-hr acute LC_{50} values ranging from 0.02-1.1 mg/L total sulfides. As with ammonia, total sulfides or calculations of the more toxic unionized H_2S may be applied to derive Hazard Quotients to quantitatively assess potential sulfide toxicity.

Species-specific benchmark exceedences. Whenever possible, it is desirable to use species-specific benchmarks to derive chemistry HQs that are directly applicable to the species used in a TIE test. For many CoCs and ammonia, these values may be available in the literature. Application of literature values should always follow careful review of specific study conditions and chemical and physical factors describing the sample matrices. Studies that represent water or sediment conditions that vary substantially from those in the existing sediment/pore water data should not be used in the assessment. EPA's on-line searchable database, AQUIRE is one useful source of citations to review for species-specific values.

Summary. The HQ approach is useful in evaluating a number of environmental conditions that may influence toxicity, and accordingly is particularly useful in determining suitability of a site for TIE. Benchmarks are available for sediment and water, both marine and freshwater, enabling calculation of HQs for a variety of matrices. Benchmarks are also available to assess the bioavailability of divalent metals and potential for effects from non-CoCs (e.g., ammonia). In summarizing the above discussion, a site may be considered appropriate for a TIE study if the observed toxicity to aquatic organisms is associated with one or more of the following lines of evidence:

- Bulk sediment concentrations that exceed benchmarks for probable effects (e.g., NOAA Effects Range Median (ER-M) concentrations; NOAA Probable Effect Levels (PELs) and Upper Effect Threshold (UET) concentrations (NOAA 1999);
- The difference between divalent metal concentrations (SEM)) and acid volatile sulfides (AVS) is greater than EPA criteria;
- Measured or predicted pore water concentrations are above acute WQC;
- Toxicity due to non-CoC sources (e.g., NH_4^+) is likely confounding the elucidation of CoC contributions to toxicity; and
- Contaminants other than the measured CoCs are likely to exist and may be contributing to or account for the observed toxicity.

2.1.3. Assessment of TIE Applicability—Case Study Example

Along with an evaluation of study-specific objectives, results from the assessment of existing toxicity data and chemical exposure, as described in Section 2.1.2, provide the information needed to determine whether a TIE is recommended. In some cases, data gaps may be identified and resolved, prior to making this determination.

Table 2-1 presents a matrix that was used to determine the suitability of TIE studies for each of the three sites considered in the demonstration project. The Indian Head and Hunters Point study areas were selected because sufficient data existed to establish a high likelihood that toxicity would be observed in sediments collected for a TIE study, and that the data presented considerable uncertainty regarding the principle sources of observed toxicity. In the case of Hunters Point, another advantage was that the TIE study could be coordinated with a planned field study. In contrast, the Quantico site was not deemed to be a good candidate for TIE studies because sediment toxicity had not been clearly established. Additionally, HQs for contaminants at this site indicated that risks to aquatic receptors were more likely due to bioaccumulative chemicals; these effects are not addressed through the current TIE methods.

Table 2-1. Site evaluation criteria for TIE applicability: Example application of TIE site

	Candidate Site		
Criteria	Indian Head, MD	Hunters Point, CA	Quantico Embayment, MD
Acutely toxic sediments?	Yes	Yes	UNK
Sediment CoCs above benchmarks?	Yes	Yes	Yes
Pore water CoCs above benchmarks?	UNK	Yes	UNK
Field survey planned?	No	Yes	No
Variety of stressors present?	Yes (silver and other cationic metals; ordnance compounds and other organics)	Yes (cationic metals, organics)	Yes (pesticides, PCBs)
Confounding factors present?	Yes (ammonia)	Yes (ammonia)	Yes (low D.O.)

UNK = Unknown; site data not available.

selection criteria to candidate sites.

Integrating the TIE into an ongoing study (as opposed to after the traditional RI study is completed) provides a stronger framework for interpretation of data from the TIE and other

assessments in the study and ultimately improves the technical weight-of-evidence for assessing various remediation options. Each case is unique, but the sites where projected remedial costs are high and the factors contributing to toxicity are uncertain are generally the best candidates for TIE testing. Conversely, if the contaminated area of concern is small, if minimal toxicity has been observed, or if there is a clear link between a point source of contamination and observed adverse effects, a TIE may not be warranted.

2.2. DEFINE TIE STUDY OBJECTIVES

Once it has been determined that a TIE will contribute substantially to the technical basis for site assessment and/or remedial planning, the specific goals of the study must be resolved. The specific experimental approach, including station location selection and the choice of test species should be determined based on study objectives. Some examples of specific objectives to be addressed through TIE studies include:

1. Determine what classes of contaminants were associated with the effects observed in bulk sediment testing at a specific set of stations in a previous study.
2. Determine if cationic metals such as copper, cadmium, nickel, lead, zinc or silver are contributing to observed toxicity.
3. Determine if organic contaminants such as PAHs, PCBs or pesticides are contributing to observed toxicity.
4. Determine whether natural sediment factors such as ammonia and/or hydrogen sulfide are principally responsible for observed toxicity.
5. Evaluate whether there are conditions in the sediments that have reduced the potential for effects associated with metals and/or organic contaminants.
6. Determine if sources of toxicity other than cationic metals, non-polar organics, ammonia and hydrogen sulfide may be contributing to toxicity (e.g., ionic imbalance).

Objectives that are not generally addressed through a typical TIE study are exemplified in the following hypothetical questions:

- Which specific metal(s) and/or non-polar organic compound(s) with high HQs contributed the most to observed toxicity? In this case, development of a TIE protocol specific to the metal and/or organic contaminants should be considered.
- What is the degree of variability in toxicant sources across several (e.g., 30) stations with variable HQs and variable sediment types? Nature and extent studies should address site variability, while the TIE should focus on selected sediments dominated by representative mixtures of suspected toxicants found at the site, including samples with differing sediment characteristics that may mediate toxicity.
- Why was the degree of toxicity observed in the bulk sediment test different from toxicity observed in pore water tests? In this case, sediment handling and extraction studies, as well as sediment and pore water chemical analyses are suggested. Adams et al. (2001) provides a review of important factors governing interpretation of results from studies involving both bulk sediment and pore water testing, and anyone planning a pore water TIE study should be familiar with this document. In addition, EPA is currently in the process of developing TIEs that involve treatments of whole sediments rather than pore

waters. While these methods are still in the developmental stage, they may offer new opportunities for additional testing, particularly where sediment toxicity and pore water toxicity are dissimilar.

2.3. SELECT STATION LOCATIONS

Generally, sampling locations should be chosen to represent areas with the greatest potential for toxicity. The number of samples tested should be dictated by the study objectives and spatial variability within the site, as well as practical considerations such as laboratory space and availability of test organisms. The initial samples, selected for collection from the field site, are tested for bulk sediment toxicity, with the intent that a subset of the most toxic will be further tested using TIE procedures. The selection of a subset of samples is completed primarily because it would not be cost effective to complete TIE procedures on samples for which no bulk sediment toxicity was observed or for samples which are spatially representative of each other (i.e., are potentially influenced by the same contaminant/s).

Typically, the sample selection is based on data collected from previous site investigations. Selected samples should also adequately represent the potential spatial variability to be considered in the context of the study objectives. For instance, if the primary objective is to determine the contaminant type(s) and other sediment characteristics that governed toxicity in previous bulk sediment testing, then the number and distribution of TIE stations should represent all of the major risk variables identified in the evaluation of existing data. Sampling depth should match the expected exposure of organisms in the field, but with consideration for the dynamics of the system to be surveyed. For example, if the site is prone to storm events or currents that disturb sediments to extensive depths, increasing sampling depth might be necessary to adequately represent likely potential risk factors. If the TIE study has been integrated with another site investigation, the sediment samples can be collected as part of that investigation, and be made available for TIE testing. If sediment is not available or if volumes of available samples are insufficient to conduct screening and possibly fractionation, additional sampling will have to be conducted for the TIE (discussed in Section 3.1).

Sites assessed under this TIE Demonstration Project provide valuable examples of evidence to consider in selecting sampling locations for a TIE. In the case of Hunters Point, samples were available from a large number of stations (59) for consideration for the TIE, as the study was performed after a recent large site investigation (SAIC, 2002b). These stations had already been chosen to represent the higher concentrations of the range of CoCs at the site as well as a broad range of ammonia concentrations, so, for the TIE investigation, the eleven samples chosen for the TIE analysis were chosen largely to represented various sources of inputs and depth profiles. The first two samples, consisting of surface (0-5 cm; HP-1) and subsurface (5-10 cm; HP-2) sediments collected at the same station, were selected because of copper, zinc and lead concentrations that exceeded ERL values. The subsurface sample was selected due to anticipated changes in sediment characteristics at depth. Samples HP-3 and HP-4 were selected to address a single hot spot with four target CoCs exceeding ERM levels and copper, zinc and lead concentrations above Effects-Range Low (ERL) values, respectively. Samples HP-5 to 10 were selected to represent toxic sediment with mixtures of contaminants that exceed ERL values, and also with consistent Effects-Range Median (ERM) exceedences for zinc. Sample HP-5 and

HP-6 represented surface and lower depth horizons for a station that is located near a landfill. More details on the sample selection process and TIE analysis can be found in the Hunter's Point TIE Final Work Plan (SAIC 2002b).

Like Hunters Point, the Indian Head station selection process was facilitated with data from a recently conducted field sampling program that supported the Remedial Investigation (SAIC 2001). Based on the chemistry and toxicity data generated, 15 samples were chosen for bulk sediment screening tests. All of the stations chosen had been found to be toxic in previous toxicity tests, or if they had not been tested in bulk sediment tests, they had measured CoC values that were higher than those measured in toxic samples. The secondary factors used in selecting the subset of eleven stations that were chosen for the Indian Head TIE study included spatial representativeness (e.g., with regard to potential habitat types and point sources), and also to characterize samples with similar toxicity but differing distributions of contaminants. Ultimately, in the Indian Head study all samples were confirmed to be toxic in bulk sediment tests, and sample selection invoked best professional judgment to address the study-specific objectives. Details on the sample selection process and TIE analysis can be found in the Indian Head TIE Work Plan (SAIC 2000).

2.4. SELECT TIE TEST ORGANISMS

The choice of test species for the pore water TIE should be appropriate for the site and study objectives and must also be amenable to TIE testing protocols. A species used in the historic and/or previous bulk sediment tests (e.g., amphipods) is often a good choice. A second species may also be chosen to be consistent with past studies completed at the site, but it is often more important to use a second test species that is expected to be responsive to the prevailing chemical exposure concentrations. The advantage in testing two or more species is that results will reflect the relative sensitivities of the respective organisms, providing additional information to deduce cause and affect relationships. Where trends in relative toxicity to pore water samples follow the same pattern as those differences observed in single toxicant tests, this could be used to support TIE findings for each individual species. For instance, when one test species is more responsive to the ammonia reduction step than the other test species, and the first species also exhibits greater effects from pore water prior to TIE treatments, then known ammonia toxicity values can be used to support the conclusion that ammonia is a major factor controlling toxicity.

For freshwater sites the amphipod, *Hyallela azteca*, the daphnids, *Ceriodaphnia dubia* and *Daphnia spp*, the fathead minnow, *Pimephales promelas*, the oligochaete, *Lumbriculus variagatus* and the midge, *Chironomus tentans,* are all amenable to pore water TIEs. For saltwater sites, the three amphipod species (e.g., *Ampelisca, Leptocheirus, and Rhepoxynius*) frequently used in bulk sediment tests are appropriate. In addition, the sea urchin (e.g., *Arbacia punctulata*) or bivalve (e.g., *Mytilus sp.*) larval development test or a larval fish test (e.g., *Cyprinidon variagatus* or *Menidia spp.*) may be suitable. Tests with sea urchins, bivalves, larval fish or mysids (e.g., *Americamysis bahia*) are especially recommended because they have highly developed testing and culture protocols that have been used in National Pollutant Discharge Elimination System (NPDES) permit program, as well as known sensitivities for many typical contaminants with published effect values available through literature review. Additional suitable species are listed in the EPA manual for marine TIEs (U.S. EPA 1996).

In all cases, the species must be amenable to small volume testing in 10-20 ml exposure chambers. In some cases, standard tests may need to be modified (e.g., use of younger organisms) to accommodate the small volume requirement. Methods for reduced volume testing for many species are outlined in the EPA TIE guidance documents, although not all methods had been thoroughly tested or refined for small volume TIEs at the time of publication. Another consideration is the availability of enough individual organisms to fulfill the requirements of the experimental design (e.g., 5000 neonates; see Section 3.6.2). Because the approach for conducting TIE studies requires a larger number of organisms than is required for routine toxicity testing, it is important to be confident that the culture laboratory is experienced in production and transport of the requisite numbers of organisms, and that organisms will be available in the season in which testing will be conducted. Accordingly, it is important to discuss availability of preferred test organisms with one or more reputable culture facilities to ensure that sufficient quantities will be available.

Finally, ecological and/or economic relevance may be important factors in species selection. It is often preferable to choose species that would be exposed to sediment and pore water. These so-called benthically-associated species are more directly relevant to the Ecological Risk Assessment than exposures of species and life stages that are wholly pelagic or planktonic (e.g., striped bass, zooplankton). In some cases, remediation may be focused on the protection of a particular species with economic value (e.g., winter flounder). In these cases, special seasonal limitations may apply with regard to availability of test animals (seasonality will be an important consideration in the spawning cycle of all species, and consultation with test organism suppliers should be included in the early stages of planning). Non-standard test species may also require unique test conditions (e.g., high or low temperatures), and arrangements for these contingencies should also be addressed as early as possible in the planning process.

2.5. DEVELOP WORK PLAN FOR TEAM REVIEW

After study objectives have been defined, sample locations selected, and test organisms chosen, a work plan should be prepared to document these key components of a TIE study. If sediments need to be collected for the TIE, the work plan should propose field sampling and laboratory analysis procedures (discussed in Section 3.0). The work plan should also propose a schedule of field and laboratory activities. Guidance in the development of project plans that integrate study objectives and design and data quality objectives with quality assurance plans can be found in the EPA Quality Assurance Document EPA, QA/R-5 (U.S. EPA 2001c).

As TIE study plans characteristically involve the review of multiple historic studies and incorporation of data from ongoing studies, the work plan should identify the relevant available datasets (e.g., sediment chemical concentration data) as well as the sources for and recipients requiring each dataset. Identification of these parties is especially critical when the contractor performing the TIE did not perform previous risk assessment studies or is not performing all of the ongoing studies for the site. Effective information exchange is important to ensure that the TIE addresses site investigation objectives, and conversely, to ensure that those conducting the TIE investigations have all relevant site information available for assistance in interpreting TIE results. In cases where the analysis of data collected under other investigations has yet to be

performed, quality assurance and data analysis should be coordinated to ensure compatibility between the site investigation and TIE study approaches.

To establish the requisite agreement regarding study objectives and a workable plan to meet those objectives, the individuals that will perform each of the Work Plan components described above as well as those individuals who will apply results from the TIE study should be involved in the planning process. If this is accomplished successfully, the work plan should serve as documentation and as a reference for coordinating TIE study activities. It is critical to provide specific schedules for each set of study activities, and in some cases, it is advisable to build in contingencies for extenuating circumstances (e.g., weather days). Costing (see Section 5.1) for each component of the study should also be addressed up front to assure that the plan reflects priorities and available resources. Each member of the Study Team should review the plan before it is finalized, and supply input particularly when it appears that there are inconsistencies between plans and objectives, or if there are apparent or potential scheduling conflicts. Prior to study initiation, the final Work Plan should be reviewed and accepted by all parties.

3.0. FIELD AND LABORATORY ACTIVITIES FOR TOXICITY IDENTIFICATION EVALUATIONS

The sequence and logistics associated with conducting field and laboratory activities for a TIE study are described below, along with references to more detailed procedures and requisite protocols. For every study, specific QA/QC and safety plans should be developed and/or approved by the principal investigator of the study. Generally, following guidance provided by the EPA (U.S. EPA 1995) for preparing QA/QC plans for sediment sampling and analysis ensures that all appropriate objectives are addressed. As part of a QA plan, Chain of Custody forms (as in TIE-100, Appendix B-2) should be used to document sample transfer from field collection through final analysis.

3.1. FIELD SAMPLING

In general, the tasks associated with field efforts for TIE studies are similar to those for any study that involves sediment collection for exposure or effect characterizations. While the major elements of field activities for sediment TIE tests are emphasized below, the field effort should be consistent with standard methods that have been published as aids for sediment collections for environmental monitoring. Users of this Guide are referred to two documents that contain detailed guidance for collection and handling of sediment: *U.S. EPA's Methods for Collection, Storage and Manipulation of Sediments for Chemical and Toxicological Analyses: Technical Guide* (2001a), and Environment Canada's *Guidance Document on Collection and Preparation of Sediments for Physicochemical Characterization and Biological Testing* (1994). These references include recommendations for every aspect of sediment sampling typically involved in sediment assessment studies, including vessel(s) and equipment to use, sampling methods and shipping procedures, and discussions of sampling requirements that may vary with study objectives.

3.1.1. Planning Logistics

Objective. The success of a TIE study field sampling effort requires not only a sampling strategy that addresses the goals of the study, but also a logistics plan that provides for effective execution of the strategy.

Procedures. To decrease the potential for project delays, a field sampling schedule should be developed that allows for appropriate weather contingencies and that accommodates the schedules of the toxicity testing laboratory and analytical chemistry laboratory that will be receiving the samples. Routes of access to the site, including vessel launching/docking facilities, access to shoreline sampling locations and parking for a sample transport vehicle should be identified. Access to these areas may require permits or passes depending on the nature of the site. Once site access is obtained, a preliminary visit to the site will determine if sampling locations are best accessed by vessel or by wading from shore, and help verify that the substrate at each station is appropriate for the TIE (i.e. not rocky) and that adequate sample volumes can be collected.

After establishing a schedule and site access procedures, supplies and equipment needed for sampling should be procured. A list of supplies and minor equipment typically required for collecting TIE samples is presented in Appendix B-1-1. After procuring supplies, the field team

should verify the proper operation of the necessary sampling equipment, which typically includes a survey vessel, shallow-water sample collection tools (e.g., sediment scoops, waders, etc.), deep-water sample collection devices (e.g., sediment grabs, scoops) and navigation system. Typically, a vessel-mounted differentially-corrected Global Positioning System (DGPS) is the most practical navigation system for surveys of this type, as these devices are accurate (± 3 m) and fairly easy to operate. A hand-held DGPS may be used instead, and may be necessary if nearshore sampling locations will be reached on foot. The geographic coordinates of each sampling location should be uploaded to the DGPS (or associated navigation software) at the time of sampling.

3.1.2. Sediment Collection and On-site Handling

Objective. The manner in which sediments are collected, stored, characterized, and manipulated can greatly influence the results of any sediment quality evaluation. Thus, standard procedures, such as those presented in U.S. EPA (2001a), should be followed when collecting and handling sediment samples. Ultimately, the applicability of the TIE as a line of evidence for assessment of risk and management of sediments requires that sampling for the TIE study is consistent with known and accepted methods and meets the specific data quality objectives established for the study.

Procedures. Upon arrival at the sampling site, the location of sampling stations should be determined using accurate navigation equipment such as a differentially corrected Global Positioning System (DGPS). Station identification may be facilitated if station markers (such as stakes in shallow water or buoys in deep water) were installed during previous sampling efforts. At each station, after completing any synoptic water sampling that might be required for associated studies, the sediment collection device (grab, scoop, etc.) is used to fill one five-gallon bucket with site sediment following the procedures in TIE Standard Operating Procedure (SOP) TIE-210 (Appendix B-2). This quantity is typically sufficient to meet the volume requirements for toxicity screening, pore water extraction and chemical and physical analyses. Wherever possible, sampling events should be planned to coincide with related studies. This provides the most robust and comparable data sets for evaluation and may have the cost-effective advantage of shared resources (e.g., vessels and vessel support).

Sediment Collection for the Indian Head TIE

- Sample vessel was provide by NSWC On-Site Office, alleviating complicated vessel logistics arrangements.
- Stakes marked sampling locations from previous study; locations were confirmed with DGPS.
- Relocation of one station was required because coarse sand/pebble prevented grab sampling.
- Another station was relocated to represent a more depositional area.
- Two sediment sampling devices were employed: a Ponar grab for deeper water stations and scoops for shallow water stations.

Highlights of the sampling approach and site-specific challenges experienced during the Indian Head TIE are noted in the adjacent text box.

3.2. TOXICITY SCREENING

The objective of the station selection process is to locate sediments with relatively high levels of toxicity. However, a lack of historical site chemistry data, a change in environmental conditions, and other factors may result in the collection of non-toxic samples. Accordingly, a bulk sediment toxicity test (e.g., a 10-day test) is performed as the first step of the TIE process to identify any non-toxic samples so that they may be eliminated from the TIE (see Figure 1-1). Conducting a TIE on non-toxic samples is not useful, as the objective of a sediment TIE is to provide evidence of the factors driving observed toxicity.

Bulk sediment tests are the principal means used to evaluate potential adverse effects of contaminated sediments. Those tests evaluate the effects of sediment-associated contaminants on aquatic organisms, which are driven by the presence of chemical contaminants and influenced by bioavailability factors. If bulk sediment toxicity is marginal (e.g., < 50% mortality is observed), and/or the species chosen for the pore water TIE are not known to be sensitive to the CoPCs, then follow-up pore water screening tests with the TIE test species may be an important step to establish the degree of toxicity expected from each samples.

3.2.1. Bulk Sediment Testing Procedures

Collected sediment samples are homogenized and subsampled for bulk sediment toxicity testing. Testing should be conducted following standardized procedures. Uniform sediment toxicity testing procedures for a variety of test species have been published (U.S. EPA 1994; 2001b). The use of standard procedures not only increases data accuracy and precision and facilitates test replication, but also increases the efficiency of the regulatory process by defining the comparative value of test results.

A sediment toxicity test procedure should be selected for the bulk sediment test based on the appropriateness of the test species to the site samples being evaluated. If pre-testing indicates toxicity with a particular species, that species should be considered for inclusion in the TIE. A species that is compatible with site environmental conditions (e.g., salinity, temperature) should be selected. Other useful criteria include representation of endemic populations, and/or sensitivity to suspected contaminants. Availably of the test species selected during the season in which testing is planned should also be confirmed. It is prudent to select a back-up species should the preferred species become unavailable.

3.2.2. Optional Screening with Pore Water Tests

Objective. Prior to initiating the TIE sequence, which begins with baseline pore water toxicity testing, it is recommended that samples identified as toxic by the bulk sediment test be subject to a pore water screening test (see Figure 1-1). The screening requires much less pore water than the actual TIE test (~40-60 mL vs. ~2 L; see Section 3.3.2) and does not need to be conducted with the same "batch" of pore water used for TIE treatment. While the pore water screen is optional, it enhances efficiency of the TIE by: 1) identifying pore waters that may be non-toxic despite toxicity of the corresponding bulk sediment (i.e., because of sediment-specific factors), saving the expense of conducting a TIE on a non-toxic sample; and 2) providing the opportunity

to limit the dilution series to be tested in the TIE in cases where higher-dilution samples would be non-toxic. For instance, if results of screening tests conducted with undiluted pore waters indicated less than a 50% effect relative to the control response, then the TIE could be conducted with only full-strength and 50% dilution samples. Conversely, if the screening test results indicated greater than 50% effect, a series of three or four dilutions (e.g., 10%, 25%, 50% and 100%) is recommended. When bulk sediment testing results in complete mortality, a dilution series screening test may serve to optimize dilutions for the TIE.

Procedures. Screening tests should employ at least one of the species that will be used in the TIE study. At a minimum, the species expected to be more sensitive to the toxicants or confounding factors associated with the pore water should be tested. Screening procedures should follow the procedures to be used in testing TIE manipulations; SOPs for several commonly-used species are presented in TIE-500 (Appendix B-2). To obtain samples for the test, pore water must be extracted from the collected sediment following the procedures in Section 3.3.

3.3. PORE WATER EXTRACTION

The principal decision points for pore water extraction are 1) What samples to extract; 2) How much pore water to extract; 3) How to accomplish the extraction; and 4) How to handle and store the extracted pore water. Factors used to determine appropriate choices for each selection process are described below.

3.3.1. Selecting Sediment Samples for Pore Water TIE Test

Through a review of bulk sediment screening test results and/or results from pore water screening tests, the most toxic sediment samples should generally be selected for pore water extraction. When all samples are considerably toxic (e.g., at least 50 % effect relative to control), other selection criteria such as obtaining samples that represent a range of potential contaminant sources may be used as discriminators.

Pore waters are extracted from the chosen bulk sediment samples following one of the methods described below. The sediments should be re-homogenized and subsampled for chemical analyses prior to extraction, as described in Section 3.4.

3.3.2. Determining Pore Water Volume Requirements

To run a TIE as diagramed in Figure 1-1, a rule of thumb is to extract 2 liters of pore water per bulk sediment sample. This should be sufficient to conduct a full series of TIE manipulations with two species, and up to three dilutions, using the low-volume exposure methods described in Section 2.4. The actual volume of pore water to collect will vary on a study-specific basis, and will depend on the extraction methods, the number of dilutions required, and the species selected for testing.

3.3.3. Selecting an Extraction Method

When working with pore water samples, it is important to consider potential changes in the bioavailability of toxicants during collection, extraction, or storage. Pore waters used in TIE testing will always be, to some degree, operationally defined by the method of pore water extraction chosen and any other additional treatments that may be required to provide suitable conditions for testing. While several methods have been described for extracting pore water from bulk sediments (Adams et al. 2001; Environment Canada 1994; U.S. EPA 2001a), there are three methods that are most amenable to collection of the quantities needed for pore water TIEs: (1) the syringe method (Winger and Lassier 1991); (2) centrifugation; and (3) pressurization extraction, as described in Table 3-1.

Table 3-1. Comparison of pore water extraction methods.

Extraction Method	Advantages[1]	Disadvantages[1]	Example variability in PW characteristics[2]	
			Toxic Units	Copper Hazard Quotient[3]
Syringe	• Ease of operation; requires inexpensive equipment • Suitable for a range of grain sizes • Can generate large volumes of pore water	• Potential sorption of metals and organics on air stone • Slow, labor-intensive process to collect required volume, especially in fine sediments due to air stone clogging • Collection of overlying water may occur with in situ collection • Degassing of pore water may occur	13	194
Centrifuge (low and high speed)	• Ease of operation • Duration and speed of centrifugation can be varied to optimize extraction • Time-efficient collection of required volume • Can generate large volumes of pore water • Effective with fine-medium grained sediments	• Requires expensive equipment (e.g., refrigerated centrifuge with 1 L bucket capacity) • Labor-intensive (e.g., sediment loading) • Potential sorption of organics into centrifuge bucket/tube • Cells may lyse during centrifugation • Not effective with sandy sediments	18 (low speed); 5.60 (high speed)	611 (low speed); 78 (high speed)
Pressurization	• Can be used with highly bioturbated sediments as does not lyse cells • Can generate large volumes of pore water • Suitable for a wide range of sediment grain sizes	• Potential loss of organics on filter • Changes in dissolved gasses may occur	<2	22.8

1 – SETAC 2001.
2 - values are for unfiltered pore water extracted from freshwater sediments; (U.S. EPA 1991).
3 – calculated using Water Quality Criteria-Freshwater Acute value of 18 µg/L.

Syringe method. The syringe method, developed by Winger and Lassier (1991), is a simple and relatively inexpensive option for collecting pore water. This method employs a large-volume syringe (e.g., 50 ml) to draw a vacuum on an air stone filter embedded in the sediment, effectively withdrawing interstitial water from the sediment. Briefly, the air stone, which is connected to the syringe via plastic tubing, is inserted into the sediment in the collection bucket. Then, a vacuum is applied by drawing the syringe plunger and bracing it in the extended position with an appropriately-sized spacer. Resulting pore water is collected in the syringe body. Specific procedures are outlined in TIE-220 (Appendix B-2). To expedite collection, several syringe systems can be placed in a five-gallon sample bucket simultaneously.

The primary advantage of the syringe method is that it is generally adaptable to all sediment types, and does not require sophisticated equipment. However, the time required to obtain sufficient volumes from each sample is variable, and depends on the sediment matrix. Extraction from sediments with high percentages of fines may take several days.

As with all suction methods, drawbacks include potential loss of equilibration between the interstitial water and the solids, oxidation of toxicants and frequent filter clogging. The syringe method does not appear to alter sample ammonia concentrations, as ammonia concentrations in pore waters collected by syringe were similar to those collected with *in situ* peepers (Sarda and Burton 1995). The syringe method is labor-intensive (systems need to be frequently reset in order to collect the requisite one to two liters of pore water) and time consuming.

Centrifugation. Centrifugation is probably the most efficient and reproducible method for pore water extraction, and is recommended if organics are the primary contaminants of concern (Adams et al. 2001). This method, which is based on centrifuge extraction procedures for routine toxicity testing of interstitial waters, involves placing bulk sediments in a large-capacity centrifuge (e.g., a bucket-style centrifuge with 1 L capacity per sample) and centrifuging them at low speed (e.g., 7,400 x g) for 15-30 minutes (ASTM 2000; Environment Canada 1994). Specific procedures are outlined in TIE-230 (Appendix B-2). In some cases, subsequent high-speed centrifugation (e.g., 10,000 x g) may be necessary or desirable, particularly if the selected test species have low tolerance to suspended particles. It is usually preferable to minimize the volume centrifuged at high speed by performing low speed centrifugation first because the high-speed centrifugation is generally more volume limited.

ASTM (2000) recommends that the temperature for centrifugation should reflect the ambient temperature of collection to insure that the equilibrium between particles and interstitial water is not altered. In some cases, a temperature of 4 °C may be used instead, to minimize temperature-mediated chemical and biological processes (Environment Canada 1994).

Limitations of centrifugation include difficulty in separating interstitial water from sediments that are predominately coarse sand (U.S. EPA 2001a; Adams et al. 2001), and limited availability of high capacity centrifuges due to their high cost. In addition, disposable centrifuge sleeves are a relatively large project-specific expense.

Pressurization. The pressurization method involves applying low-pressure force to bulk sediment using a mechanical press or squeezing device. The pressurization method is best suited to specialized situations, such as the collection of pore waters to correspond with depth profiles from core samples (Bender, et al. 1987). The greatest disadvantage of the method is the absence of standardization. Because filtration is an integral part of the pressurization method, the characteristics of the filters and filtering apparatus should be carefully considered. Numerous studies have shown that filters reduce toxicity and contaminant concentrations by retaining contaminant-associated particles and also by contaminant sorption onto the filter matrix (Bray et al. 1973; Troup et al. 1974; Sasson-Brickson and Burton 1991 Schults et al. 1992).

In selecting an extraction method, it should be noted that both the concentrations and toxicity of pore water constituents present are known to vary with the extraction method (Adams et al. 2001; ASTM 2000; U.S. EPA 1996). An example of this variability, in terms of toxic units and copper hazard quotients in extracted pore waters, is provided in the Table 3-1. While an extraction method is often chosen for practical reasons (e.g., availability of apparatus), baseline toxicity and TIE toxicity reductions should be interpreted in the context of what is known regarding extraction processes associated with the chosen method. As is the case with all protocols used in laboratory toxicity testing, pore water testing represents potential exposure risks to a subset of the aquatic community, and the extraction process further defines the type of exposure that is characterized by the test.

3.3.4. Maintaining Integrity of Pore Water Samples

Pore water characteristics are affected by holding times and storage conditions and by the treatment of sediments prior to pore water extraction. Limits for effective holding times for sediments and pore waters are governed by sediment type and contaminant characteristics (ASTM 2000; U.S. EPA 2001a). Chemical changes (e.g., oxidation, sorption, volatilization) occur with sample storage and preparations steps required for both bulk sediment and pore water testing. In order to minimize possible confounding factors resulting from chemical changes after sampling, sediment storage should be as short as possible (i.e., less than two weeks) and preferably less than 24 hours. Intact sediment samples and extracted pore water should be stored in the dark at 4 °C, and with minimum headspace in sample containers with low absorptive capacity for metals and organic compounds (Adams, et al. 2001). Flushing headspace with nitrogen is the best way to minimize oxidative processes. After pore water extraction, TIE manipulations should begin as soon as possible (<24 hrs). Where practicality precludes immediate testing after sample processing, freezing pore water for subsequent testing may be appropriate (Carr and Chapman 1995), but freezing adds a layer of uncertainty, particularly when interpreting results relative to bulk sediment toxicity.

3.4. CHEMICAL CHARACTERIZATION OF SEDIMENTS AND PORE WATERS

Rationale. As an integral part of all sediment TIE studies, the sediments and pore waters that undergo chemical analyses should be both spatially and temporally collocated with samples chosen for TIE testing. Ideally, chemical evaluations are performed on a subset of the same sample batches processed for TIE testing (e.g., homogenized sediments and extracted pore waters). If evaluations were performed on a separate batch of samples, inter-batch variations in storage and handling could alter chemical constituents, and hence make the correlation of chemical concentrations to the degree and form of toxicity observed more difficult.

Re-homogenization of the sediments for pore water extraction and chemistry sub-sampling should occur at the end of the 10-day bulk sediment testing. Samples for sediment and pore water chemistry should be collected at the same time, because the principal objective is to relate the chemistry of both media to results from TIE tests with pore waters. Any deviations from QA/QC criteria for each type of analysis should be considered in evaluation of the data, and should also be noted in the study report.

Sediment procedures. After homogenization, sediments are sub-sampled into clean glass bottles for chemical and physical analyses. If sent to an off-site analytical laboratory, samples should be air-freighted on ice for overnight delivery.

It is recommended that sediments be analyzed for metals, SEM:AVS, PAH, PCB and pesticide contaminants generally following the methods outlined in the NOAA Status and Trends Program (NOAA 1998). The multi-elemental techniques recommended by NOAA provide sensitive results with a high degree of accuracy and precision. Sediment TOC (e.g., EPA Method SW9060), percent moisture (e.g., ASTM Method D2260), and grain size analysis (e.g. ASTM Method D422) should also be measured. Grain size should be summarized into three size fractions: gravel, sand, and fines (silt + clay).

Pore water procedures. Generally, pore water characterization of the study samples is limited to determination of metal concentrations (all metal CoPCs, and generally including at least Cu^{2+}, Ni^{2+}, Pb^{2+}, Cd^{2+} and Zn^{2+}, and also recommend Al^{2+}, Mn^{2+}) and water quality measurements. Concentrations of organics are usually not measured due to the high volume requirements and high cost of the analysis, but can be estimated from sediment concentrations using the methods described in Section 2 for evaluation of existing data. Water quality measurements should always include alkalinity and hardness (for freshwater sites), salinity (for marine sites), total ammonia, total sulfides and pH. These measurements should be performed using recognized instrumentation and methods, as well as laboratory-specific SOPs. Dissolved Organic Carbon (DOC) and TOC in pore water (e.g., EPA Method SW9060) may also be measured as indicators of bioavailability of toxicants.

Using the above water quality results, the more toxic form of total ammonia (un-ionized ammonia) and total sulfides (hydrogen sulfide) should be estimated. Dissociation constants and representative exposure conditions (pH, temperature and salinity) are input into algorithms published by Hampson (1977) and Millero et al. (1988) to calculate un-ionized ammonia and hydrogen sulfide, respectively.

For chemical analysis, a separate sub-sample is collected from the extracted pore water sample. Volumes of 50 ml's for metal analysis, 100 ml for each organic carbon measurement, and 20-50 ml for sulfide analysis are generally sufficient. For metals analyses, sub-samples are preserved with 10% nitric acid. Sub-samples for TOC analyses are preserved with 10% sulfuric acid, while sub-samples for DOC analyses are not treated. The metals and TOC/DOC samples are shipped in clean polyethylene containers. Sub-samples for sulfide analysis are fixed immediately with zinc acetate and may be measured using colorimetric or titrametric methods (American Public Health Association 1995).

3.5. TIE MANIPULATION APPROACH

As illustrated in Figure 1-1, the general TIE method involves four sequential manipulations followed by three independent treatments. The principle of the sequential approach, which begins with the most specific treatments and ends with the most general, is that deductive logic can be applied to resolve the factors governing toxicity. For sediment pore water constituents, sodium thiosulfate (STS) and ethylenediaminetetraacetic acid (EDTA) act quite specifically on certain groups of common heavy metal contaminants. By treating the metals first, and then applying filtration and solid phase extraction (SPE) to remove organic contaminants, reductions in toxicity following each individual treatment can be more directly associated with specific toxicant groups than they could be if the treatments were conducted in parallel.

By applying the independent *Ulva* or zeolite treatment, and associated pH adjustments at the end of the sequential treatments, the role of ammonia as a contributor to toxicity can be more clearly discerned. This is because these final treatments could also remove metals and organics to varying degrees. Therefore their application as final treatment removes this interpretive complexity from the process. Similarly, pH adjustments can affect the toxicity of multiple potential contaminants, including certain metals and potentially toxic organic compounds. The elimination of toxicity due to these groups prior to the pH treatments facilitates the direct association between pH changes and commensurate changes in the relative toxicity of both ammonia and sulfides due to ionic shift.

The actual TIE procedure begins by sub-sampling untreated pore waters that will serve as baseline exposures for comparison of toxicity observed in all subsequent treatments. Then, following each TIE treatment, additional sub-samples are also collected for TIE toxicity testing. Individual treatment methods are summarized below. In order to organize and document the sequential manipulation and sampling of each pore water for TIE testing purposes, a sample tracking system should be implemented (e.g., TIE-100, Appendix B-2). The objective of each treatment step is described below, along with an overview of the SOPs for each manipulation (TIE-400, Appendix B-2).

3.5.1. Sequential Pore Water Treatments

1. *Establish baseline toxicity with untreated pore water*. For this step, sub-samples of untreated pore water are tested to assess toxicity relative to TIE-manipulated sub-samples. Even if pore water tests were performed during toxicity screening (Section 3.2), new baseline

samples should still be collected and tested to correspond temporally with the manipulated treatments for each sample.

2. *Reduce metals concentrations with STS and EDTA.* Two treatments are conducted in sequence to reduce bioavailability of metals, specifically by rendering them unavailable for direct uptake into cell tissues. First is the addition of STS ($Na_2S_2O_3$) and second is the addition of EDTA. Reduction in toxicity of the sample after either or both treatments indicates the presence of metals in toxic concentrations.

While treatments with these additives have generally proved effective for common metal contaminants, their affinities for various metals and metal complexes, and consequent effectiveness in reducing toxicity may be a function of multiple factors. Mediating factors may include water hardness, mixed metal interactions, the toxic load of the sample (i.e., are concentrations just above toxic thresholds or much higher?) and the absolute concentrations of metals present versus concentration of the additive agent. Treatment levels recommended below are based on EPA Guidance (1996). These recommended treatments have been optimized for general applicability for a broad suite of potential metal toxicants, and to preclude toxicity of the additives to test species. While prescribed additions of STS and EDTA effectively reduce toxicity of individual metals and metal mixtures, in some cases residual metal toxicity can occur. Hockett and Mount (1996) tested the effect of varying the additive concentration (for both STS and EDTA) to constant concentrations of individual metals and demonstrated considerable variability in toxicity reduction. In the present TIE procedures, spiked samples are tested to ensure that the selected STS/EDTA concentrations are sufficient to remove the potential site-related metals.

A. *Reduce cationic metals and oxidants with STS*: Sodium thiosulfate addition is performed as the first metals reduction step because it is generally effective with a smaller subset of metal contaminants relative to EDTA. It is reported by EPA to be most effective in reducing toxicity due to Cd^{2+}, Cu^{2+}, Ag^{1+} and Hg^{2+} (with lesser affinity for Ni^{2+}, Zn^{2+}, Pb^{2+} and Mn^{2+} (U.S. EPA 1996)). Reduction in toxicity of the sample after STS treatment indicates the above metals are present in toxic concentrations. Sodium thiosulfate is added at the rate of 50 mg/L with no apparent effects on test species (U.S. EPA 1991; 1996).

Considerations. STS is more efficient than EDTA in reducing toxicity due to silver (Ag^{+}) and selenium (Se[VI]) (Hockett and Mount 1996). The STS treatment was originally developed for effluent TIEs, where its reducing capacity removed the toxicity of oxidants such as chlorine, ozone, chlorine dioxide, mono and dichloramines, bromine, iodine and manganous ions. In sediment pore waters, cationic metals are more prevalent because the water-based oxidants tend to be less stable and are not sequestered to large degrees in most sediments. The reducing capacity of STS may extend to some electrophilic organic chemicals (e.g., carbamate pesticides such as carbofuran), and where these are CoCs, it may be advisable to develop a separate TIE treatment to address them. Also, where oxidants (e.g., bromine) are present, the capacity of STS to reduce metal ions may be reduced (Shubauer-Berrigan et al. 1993).

B. Chelate cationic metals with EDTA: This reducing agent chelates divalent cationic metals (i.e., Al^{2+}, Ba^{2+}, Fe^{2+}, Mn^{2+}, Sr^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, Cd^{2+}, Co^{2+}, and Zn^{2+}) (Schubauer-Berigan et al. 1993; Ankley et al. 1992). Reduction in toxicity of the sample after EDTA treatment indicates that members of the above listed group of metals are present in toxic concentrations. If reduction in toxicity does not occur with STS, but does occur with EDTA addition, there are two potential explanations. One possibility is that the metals causing toxicity are amongst the group that is less reactive with STS (Ni^{2+}, Zn^{2+}, Pb^{2+} and Mn^{2+}) and the other is that the magnitude of toxicity was high enough that the addition of both reducing agents was required to affect toxicity. Generally, a fully or partially toxic response following the sequential EDTA treatment indicates that something other than divalent cationic metallic compounds is a major contributor to sediment toxicity. In other words, either metals are not toxic, or alternatively, if the samples remain fully toxic (i.e., no normal response is observed), other toxic agents may be masking the reductions in toxicity associated with metals. EDTA is added at the rate of 60 mg/L with no apparent effects on test species. According to the marine TIE guide (1996) this could potentially chelate 26 mg of divalent metal per liter.

Considerations. It should be noted that EDTA preferentially binds with divalent cationic metals such as copper, mercury, cadmium, zinc, nickel, lead and manganese. The degree to which the metal is chelated by the EDTA may be proportionate to its stability constant[1]; the higher the stability constant, the more effectively the metal will be chelated by the EDTA in solution (Hockett and Mount 1996).

3. *Extract particulate-associated contaminants with filtration.* Because filtration may remove metals and organics, the placement of the filtration step after the treatments for metals (STS and EDTA) reduces ambiguity of interpretations associated with filtration effects. Filtration is operationally defined by filter type and the filtration procedure used. To assure the removal of all suspended particles that could clog or compromise the integrity of the SPE column used in the following procedure, pore water is generally filtered with 0.45 μm membrane filter (e.g., polyvinylidene fluoride to minimize sorption of organics). Toxicity tests conducted on the pre- and post-filtered fraction permit elucidation of potential toxicity associated with large colloids or particulates in the pore water. Filtration has not been found to affect the concentrations of pore water ammonia. Filters used in this step should be retained for any subsequent analyses that may be helpful in resolving any reduction in toxicity that may occur due to filtration.

4. *Extract organics with a Solid Phase Extraction (SPE) column*. In this step, filtered pore water samples are eluted through a SPE column (traditionally, a C_{18} column, or alternatively, Waters, Oasis® column) to remove organic compounds (Waters 2001). According to general recommended manufacturer's procedures, the pore water is eluted through the column at a rate of 10 ml/min. For each pore water sample, the column is exchanged after 500 ml is eluted. The column should be monitored visually, and if its capacity appears to be exhausted

[1] Stability constant refers to the equilibrium reaction of a metal cation and a ligand (i.e., thiosulfate or EDTA) to form a chelating, mononuclear complex. The absolute stability constant is expressed by the product of the concentrations of the reactants. The apparent stability constant allows for the non-ideality of the system because of the combination of the ligand with other complexing agents in the solution.

prior to elution of 500 ml, more frequent exchanges should occur. Equally, if existing data indicate high loads of dissolved organic contaminants, the elution capacity may be less than 500 ml. Always consult guidelines for 'breakthrough' provided by the manufacturer of the column, and use conservative estimates; for some samples it may be advisable to change the column after eluting 100 ml. A reduction in toxicity response to the extraction treatment indicates the potential role of organic compounds as a contributor to the toxicity of pore waters.

Considerations. Traditional use of C_{18} columns has been effective in removing non-polar organic contaminants such as benzenes, PCBs and PAHs (Ho et al. 1997), but using C_{18} columns to remove more polar organics including many pesticides may be less effective (Waters 2001). Predictions that energetic contaminants in pore waters collected during the Indian Head TIE demonstration would not be effectively removed by C_{18} elution (Waters, unpublished) prompted pioneering of the use of Oasis® SPE columns to remove organic contaminants in pore water TIEs. The Oasis® column design includes both hyrophilic and lipophilic sorbents, allowing removal of many polar organics in addition to the non-polar organics that are sorbed by C_{18}. Other advantages of the Oasis® column SPE relative to the traditional C_{18} column are the lack of pH sensitivity in extraction efficiency and increased surface area, allowing more extraction sites per gram of column packing material. In trial TIEs the Oasis® product performed efficiently in removing toxicity in one sample spiked with fluoranthene and another spiked with the energetic compound, nitrobenzene. Each spiked sample also included copper, and TIE metal chelation steps (sodium thiosulfate and EDTA additions) preceded filtration and SPE steps to assure that the sequential TIE manipulations did not compromise the expected reduction in toxicity. While the trials indicated that Oasis® columns might effectively replace $C_{18,}$ in the Hunters Point TIE, the Oasis® elution increased toxicity in some samples. This indicates that with certain pore water matrices, Oasis® packing materials were either toxic or affect toxicity. Collaborative studies with Waters are planned to improve organic extractions for TIEs using SPEs.

3.5.2. Independent Treatments

<u>*Remove Ammonia with Zeolite (freshwater).*</u> For freshwater samples, a zeolite cation exchange material (e.g., Ammonex® by Argent Chemicals; SIR-600® by ResinTech) is added to remove ammonia (Ankley et al. 1990; Besser et al. 1998). A reduction in sample toxicity is indicative of ammonia as a contributor to pore water toxicity in the precursor sample.

Considerations. Zeolites come in many forms, and the effectiveness of treatment for removal of ammonia toxicity may be dependent on the form and composition of the zeolite used. Crystal structure and chemical composition are both important variables. Particle density, cation selectivity, molecular pore size, and strength are some of the properties that can differ with zeolite types and sources. The effectiveness of zeolites in removing ammonia depends on exchange with three other major cations: potassium (K^+), calcium (Ca^+), and sodium (Na^+). Other elements such as magnesium (Mg^+) may also be present in the mineral matrix. Exchange sites on a particular zeolite may contain nearly all K^+, nearly all Na^+, some C^+ or Mg^+, or a combination of these. It is important to take these differences into account when assessing which zeolite to use. Clinoptilolite, in ground or granular form is the zeolite generally applied for

ammonia removal in aquatic systems. Within the types of clinoptilolite, some have high exchange capacity for cationic metals as well as ammonia (Olin and Bricka 1998), and some appear to be more specific to ammonia (Besser et al. 1998). In TIE applications the overall ionic composition of the pore water, including hardness factors, are likely to contribute to its variable performance.

Remove Ammonia with Ulva (saltwater). For saltwater samples, treatment with the green seaweed (*Ulva lactuca*) has been demonstrated to be more effective than zeolite in removing ammonia. *Ulva* is a cosmopolitan macroalgae, and is generally found in estuarine lagoons, often floating on mudflats. It inhabits the upper to mid-intertidal, and in some locations may be found up to the subtidal zone and is associated with nutrient-enriched conditions. For TIE purposes, the algae is generally collected on the day prior to test treatments and held in aerated seawater at 15°C. Batches of *Ulva* to be added to each pore water sample are prepared by weighing out 1g of *Ulva* per 15 ml sample. Whenever possible, whole leaves of *Ulva* should be added to each sample. The pre-weighed batches may be held together with skewer sticks and stored in seawater until addition. After addition, the samples are incubated for 5 hours at 15°C (Ho et al. 1997; 1999).

Considerations. The pertinent literature has established that *Ulva* can be used to remove toxic concentrations of ammonia from pore waters as an effective TIE treatment, but that some associated effects are possible. Ho et al. (1999) found that *Ulva* treatments reduced ammonia concentrations to below the threshold for toxicity in most marine species, and that metals and especially organics were also entrained by *Ulva,* but with a slower rate of uptake. Findings of Lapota and Grovhoug (2001) in studies with combinations of ammonia, copper and zinc were similar. The latter study used *Strogylocentrotus purpuratus,* a common toxicity test species that is hypersensitive to ammonia, and found that low levels of ammonia (5 mg/L) could be removed to non-toxic levels (< 1 mg/L). Although *Ulva* is clearly effective in removing significant amounts of ammonia, it must be recognized that the ammonia assimilation capacity of *Ulva* over a reasonable TIE treatment time (e.g., five hours) is limited. Ho et. al. (1999) found that even after eight hours of treatment, ammonia removal in seawater spiked with 80 mg/L ammonia was variable, with residual concentrations ranging from non-detectable to >30 mg/L. Thus, when sensitive species such as *S. purpuratus* are used in TIE tests, it is likely that ammonia toxicity may be reduced but not eliminated. Such was the case with several of the Hunters Point Demonstration pore waters. Another potential complication is that *Ulva* may, in some cases, produce toxic exudates, particularly in association with low oxygen concentrations (Johnson and Welsh 1985). However, this phenomenon has not been demonstrated in TIE studies to date.

Manipulate Ammonia and Sulfide with graduated pH. As noted above, methods to remove ammonia, while generally effective, may provide inconclusive evidence to deduce ammonia toxicity. The manipulation of pH to shift ammonia ionic balance and related toxicity is a useful practice to provide additional evidence of the role of ammonia in causing toxicity, as well as discriminate between ammonia and hydrogen sulfide as potential toxicants (Ankley et al. 1992; U.S. EPA 1996). Low pH changes the ionic balance to promote higher concentrations of toxic hydrogen sulfide, while high pH favors a balance where ammonia is more toxic. Accordingly, if sample toxicity increases with increased pH, ammonia is the suspected toxicant. If sample toxicity increases with decreased sample pH hydrogen sulfide is suspected.

Considerations. Numerous methodologies have been developed in efforts to manipulate and maintain target pHs, including HCl addition, CO_2 addition and buffer control methods (Ankley et al. 1992) conducted with and without chambers sealed from the atmosphere (U.S. EPA 1996). Buffers are effective for freshwater samples but have limited utility in saltwater samples. It is important to review the pH tolerance limits of each test species, and assure that suitable conditions are maintained. It is also important to consider that large shifts in pH (e.g., 0.5 units) in the absence of a gradual period of acclimation may result in lethal or sublethal stress to some test organisms. It is advisable to provide some acclimation for at least 24 hours before test initiation for prospective high and low pH treatments. If this is not feasible or practical, pre-testing responses of the test organisms to altered pH may be conducted to determine individual species tolerance limits. In some cases (e.g., where baseline pore water pHs are high or low), one pH manipulation may be sufficient to establish pH-related toxicity factors.

A. *Increase Hydrogen Sulfide with Low pH.* The low pH manipulation may be achieved by adding the buffer 3-N-Morpholino propanesulfonic acid (MOPS; Sigma Chemicals) to result in a 0.4M solution in the 100% pore water samples. The final pH depends on the buffering capacity of the individual pore waters, but generally remains relatively stable (within 0.2 units) during the test (Ankley et al. 1992). This treatment is generally less effective in saltwater samples. Dilute hydrochloric acid (e.g. 1N) additions may be preferable in saltwater, but daily or twice-daily adjustments may be required due to the high ionic strength and strong buffering capacity.

B. *Increase Unionized Ammonia with High pH.* The high pH treatment is produced by adding 1N sodium hydroxide (NaOH) to 100% pore water. As with low pH, these adjustments are generally less stable in saltwater samples, and frequent adjustments may be required to maintain stability to within a few tenths. In freshwater, NaOH itself may be toxic, and therefore repeated NaOH adjustments may not be feasible in pore waters that continually drift to lower pHs.

3.5.3. Chemical Analysis of Treated Pore Waters

Various chemical analyses can be conducted in association with TIE manipulations, particularly with regard to assessing removal efficiency for organics, ammonia and sulfides. Organics can be measured as extracts from both filter papers and SPE columns using High Pressure Liquid Chromatography (HPLC) and/or a Gas Chromatography/Mass Spectrometry system. This provides more direct evidence that analytes present in the toxic sample were not present in a less toxic sample (a confirmatory step that may be useful in determining site-specific thresholds for toxicity). Similarly, ammonia and sulfides can be measured before and after the zeolite or *Ulva* treatments. In contrast, TIE treatments for metals involve binding agents that sequester rather than physically remove the metal from the pore water, and therefore routine confirmatory analyses are not available. In cases where high metal concentrations in porewater samples contribute to uncertainty regarding the effectiveness of STS and EDTA treatments, it may be useful to use ion-specific electrodes or methods under development such as resin-impregnated gel samplers to measure residual concentrations of bioavailable metals (Shine et al., 2002). While these methods may provide additional information for interpretation of TIE results, they are not generally accepted as accurate quantitative methods. Metal analyses may also be

performed on samples retained by filters and in filtrate because the fraction of dissolved metal is not altered by STS or EDTA treatments. It is desirable to quantify the partitioning between particulate and dissolved fractions of total recoverable metal, because dissolved metal generally yields a stronger correlation with observed toxicity (U.S. EPA, 1999).

3.6. TIE TOXICITY TESTING

Selection of TIE toxicity test species and pre-TIE screening tests have been described above. This section briefly describes the information provided in U.S. EPA guidance documents, a summary of factors that may require additional attention in adapting standard test methods to pore water TIE study designs, and finally, the importance of including spiked samples as an integral component of every TIE study. It is often useful to use spiked samples as a pre-test to "shake down" the proposed TIE manipulation series and test protocols, i.e., to confirm that the treatments are effective in removing target chemicals and that test organisms are sensitive to target CoCs and or critical confounding factors (e.g., ammonia).

3.6.1. U.S. EPA Guidance for Freshwater and Saltwater Testing

EPA documents (Ankley et al. 1992 and U.S. EPA 1996) provide general guidance on a range of topics, from conducting toxicity tests with routine test species to recommending additives for use in TIE manipulations (e.g. STS and EDTA). Each document provides different types of information that may be useful in designing successful pore water TIEs, and reflects the information available at the time of publication and/or factors that were considered particularly important for each type of testing. For instance, the freshwater document (1992) provides useful summaries of the different sensitivities of individual species to metals and ammonia while the saltwater document (1996) provides a list of pH tolerance ranges for recommended test species by geography (Atlantic and Gulf Coast and Pacific Coast). The saltwater document provides an Appendix of test conditions and test acceptability criteria for each recommended species.

3.6.2. Special Considerations for TIE Testing

In refining methods for toxicity tests, each study design should include the following elements:

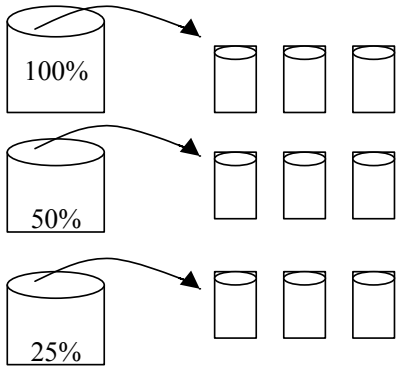
- An appropriate replication and dilution series;
- Monitoring procedures for small volume exposures;
- Appropriate feeding regimes;
- Reasonable test durations for non-renewal tests; and
- Realistic and meaningful test acceptability criteria.

Selecting replicate and dilution series. Although many of the methods used in standard tests such Whole Effluent Toxicity (WET) tests developed for NPDES effluent monitoring are applicable to TIEs, the TIE tests differ from WET tests in that they do not need to provide the statistical power necessary to determine the presence or absence of toxicity. With this in mind, the broader objectives of the multi-metric TIE design (and the attendant large number of organisms required to test each sample under multiple treatment conditions) can generally be

accommodated by limiting the number of replicates to three, and also limiting the number of dilutions representing each treatment to three (in addition to the 100%, undiluted treatment). Under this strategy, using five animals per replicate, each pore water sample requires approximately 500 test organisms for each species tested. Table 3-2 provides an example calculation of the suggested number of animals that would be used in a TIE study conducted with ten sediments. The factors included are: 1) animals per replicate; 2) number of replicates; 3) dilutions per treatment; 4) number of treatments; 5) number of pore water samples and 6) a separate allocation for control water testing with each TIE treatment. Particularly when the principal sources of toxicity are likely to be from multiple sources, this broad-based design balances the need for replication to estimate uncertainty with the need to evaluate all of the TIE-treated pore waters.

Table 3-2. Estimation of the number of organisms required for TIE testing.

	Number of Test Animals					
	Animals	Replicates	Dilutions	TIE Treatments	Samples	Total Animals
Field Samples	5	3	3	8	10	3600
Control	5	3	1	8	1	120
					Subtotal	3720



Optional Additonal Animals		
Water Quality Rep.	Reserve Organisms (e.g.,10%)	Total Animals
1200	480	1680
40	-	40
	Subtotal	1720
	Grand Total	5440

It is generally recommended that a dilution series be incorporated in the test design (see Section 2.1.1). Individual dilution results are particularly useful when a very toxic mixture consists of contaminants and/or confounding factors that do not contribute equally to toxicity. Here, the objective is to have one dilution that represents just enough potency to cause a high level effect (e.g., near 100% mortality in the untreated sample). Then, sequential treatments would result in progressively less effects. For instance, if toxicity due to metals occurs in a 10% dilution and toxicity from both metals and ammonia occurs in the 100% sample, then metal binding treatments might only reduce toxicity in the 10% dilution due to the fully lethal presence

of ammonia in the 100% sample. Such was the case in the Hunters Point TIE study. In another scenario, metals and ammonia might be present in equitably toxic proportions, and hence the dilution series would facilitate the observations of partial reductions in toxicity. An example of a work sheet to facilitate planning and preparation of dilutions is provided in Appendix B (SOP Attachment XI).

In addition to the standard test replicates, a replicate solely devoted to water quality monitoring should also be included in the test design. The water quality replicate is necessary because monitoring equipment such as pH and D.O. electrodes may stress test organisms when placed in the low volume test chambers. Test organisms expected to affect exposure conditions should be included in the water quality replicate. This replicate should not be used to assess biological effects as water quality measurement apparatus may possibly damage or otherwise stress the organisms.

Test chamber monitoring. Representative test chambers should be monitored daily, particularly because the tests are conducted without renewal and changes may occur due to evaporation or bacteria associated with mortality. Dead organisms should be removed at least daily. Salinity and hardness changes may be corrected, when they occur, through addition of deionized water.

Feeding regimes. Where feeding is required for some TIE test species, it should be limited to a maintenance ration. The addition of food introduces the potential for sorption of toxicants (i.e. reduction in exposure concentrations), and also may contribute high oxygen demand in small-volume exposures. In some cases, the advantages of longer exposures must be balanced against the need for feeding. Many newly-hatched fish larvae do not require feeding for 48 hours or more. Other species, such as mysids and daphnids do require feeding if exposures are in excess of 24 hours. Knowledge of the uptake properties and persistence of the expected toxicants, as well as the kinetics of responses of various test organisms can be applied to optimize individual test designs.

Low dissolved oxygen. The chemical oxygen demand of the sample, combined with the oxygen consumption of test organisms may decrease D.O. concentrations below those required for survival. In the Demonstration Study with Hunters Point pore waters, extremely low D.O. condition occurred. In this case, each of the pore water samples was pre-treated with pure medical grade oxygen immediately prior to TIE testing. Ultimately, in several Hunters Point samples this additional treatment was insufficient to avoid low oxygen-related mortality in the fish species tested, while the sea urchin larval development test was not compromised by the low D.O. Where high oxygen demand is to be expected (based on screening tests or historic data), it is always advisable to include hypoxia tolerant specie(s) in the TIE design. Regarding the addition of pure oxygen, the degree to which oxygen reactions might reduce toxicity by converting metals (O'Day et al. 2000) and ammonia to less toxic forms, cannot be readily determined within the scope of a standard TIE study.

Test duration. As mentioned above, reasonable test durations must be selected with consideration for the tolerance of the test species for small volume exposure and/or reduced feeding rations, as well as the stability of the test solutions in the absence of renewal. Some tests such as echinoderm fertilization tests (1 hr exposure) and the bivalve and echinoderm embryo-larval tests (48 or 72 hrs) have defined exposure durations that are required to achieve the desired developmental endpoint. For others, such as embryo or larval fish tests, and acute tests with mysids and daphnids, test durations can be selected with consideration for the tolerance of the species to the small volume conditions as well as the expected stability of the non-renewed pore waters. The potency of the samples is an additional consideration. Where strong effect signals are expressed within 24 hours, it may not be necessary to continue the exposure of a given species for longer durations. However, where minimal toxicity is observed, longer exposures may be required to exhibit toxic effects.

Test acceptability criteria. As reflected in EPA's 1996 guidance for saltwater TIEs, test acceptability criteria for responses of organisms to control water may be more relaxed than they would be in a test designed to establish whether or not a sample is toxic. Because toxicity has already been established for the TIE test samples, and because in many cases modifications to standard test designs (e.g., use of younger test specimens) may result in lower control

performance, experience has shown that useful results may often be obtained with lower control standards, for example, 80% survival rather than 90%. It is advisable to evaluate the variance between replicate responses for each species, for each individual TIE study, to determine what percent difference between treatment responses should be considered significant. EPA's recent Guidance and Recommendations for Whole Effluent Toxicity (WET) Testing document (U.S. EPA 2000a) includes specific technical guidance on nominal significant differences, confidence intervals, and concentration-response relationships, as well as new guidance on dilution series selection, dilution water, and suggestions (with additional technical clarification) regarding WET test methods published by EPA and incorporated by reference into regulations. While this guidance was prepared for application to effluent samples, it serves as a useful reference for TIE tests as well.

3.6.3. Include a Sample Spiked with at least one CoC

The spiked sample, which is prepared by adding a know toxic concentration of a CoC to control water, represents a positive control that can be used to evaluate the efficacy of the TIE manipulation procedures. Generally, the spike should include a metal, an organic compound, and an ammonia or sulfide. The choice of spiking constituents should reflect the anticipated contributors to toxicity. Furthermore, it may be useful to add ammonia and/or hydrogen sulfide to the sample after SPE manipulation so that reduction in spiked metal and/or organic toxicity is not masked. It is important to set spiked concentrations such that toxicity is expected from each analyte, if possible, and that the sequential removal (e.g., first the metal and then the organic) would each result in partial removal of toxicity.

3.6.4. Conduct a Pre-test

Conducting a preliminary test with spiked water to evaluate the viability of the expermental design is recommended. A spike test may provide the opportunity to collect data to establish or confirm the sensitivity of a test organism to a particular CoC. The test may also allow the refinement of protocols, for instance the capacity of an SPE column to remove a known quantity of an organic contaminant without exceeding the column's capacity. It also serves as a trial run to finalize logistical considerations such as labor hour requirements.

At the conclusion of the field and laboratory phase of the TIE study, the first step is to conduct a preliminary evaluation of the outcome relative to the work plan requirements to determine if the technical objectives were successfully accomplished. At this point it is important to highlight any elements of the test that require special consideration in the data interpretation phase. For example, some samples may have required different preparations than others. In the case of the Hunters Point TIE study, several pore water samples had to be treated with pure oxygen prior to testing to prevent mortality from low D.O. concentration. Hence, results from D.O. monitoring during the test were reviewed to determine influence on toxicity results. As many procedural factors may influence test outcome, it is highly recommended that the study manager use a review form to assure the completion of each requisite data sheet, and to determine the degree of achievement of the study Data Quality Objectives. In Appendix B to this guide, the first example SOP (SOP 100) provides a Check List that might be used for review purposes.

4.0. INTERPRETING THE TIE RESULTS

This Section addresses the main components of interpretation of the study data, including Toxicity Characterization (bulk sediment and pore water), Exposure Characterization (potential chemical and physical causes of effects) and finally, the Weight-of-Evidence assessment process.

4.1. TOXICITY CHARACTERIZATION

Toxicity test results should be evaluated through a step-by-step process. The main purpose of the TIE is to further characterize biological effects associated with toxicity, and in keeping with the study design, the first step involves interpretation of bulk sediment and pore water results. The next step is to synthesize the collective results from the entire TIE manipulated samples to determine whether signals for specific classes of contaminants/confounding factors are apparent. The existing guidance for interpretation of TIE test results is limited, and continuing to evolve. Some suggested approaches are presented below, but each TIE data set should be interpreted within the context of the individual study design, and no blanket rules should be applied.

4.1.1. Evaluate Bulk Sediment vs. Pore Water Results

Evaluation of results from bulk sediment tests and untreated pore water tests is relatively straightforward. Generally, results for each of the sample stations relative to control responses are analyzed to determine statistically significant effects. Statistical differences (e.g., ANOVA followed by post hoc analysis such as Dunnett's Test) can be based on variance in the full data set (pooling stations, treatments and dilutions), or by using Student's T tests to evaluate each station and dilution as unique samples.

Qualitatively, the toxicity results are also evaluated to determine whether bulk sediment tests and pore water tests have yielded results that are similar. As an illustration, Table 4-1 shows both agreement and differences between the response of *Hyalella* to bulk sediment and pore water exposures from the Indian Head study. In samples IH-10 and IH-12 toxicity was exhibited in bulk sediment exposures but not pore water. This variation may have been due to the filtration associated with the syringe extraction method used.

The species used in bulk sediment testing is not always used for TIE testing, although it is generally recommended, at least when bulk sediment toxicity is high. Testing with different species precludes direct comparison of results. When TIE study objectives, for whatever reason, favor the use of species other than those used in bulk sediment testing, analysis of the relative sensitivities of the bulk sediment and TIE species should be conducted by deriving species-specific hazard quotients for CoPCs and other potential stressors (see Section 2.1.2, above and Section 4.2.3, below).

Table 4-1. Sediment vs. pore water test results for Indian Head samples.

Sample Identification	Percent Survival	
	Bulk Sediment Test Mean (SD)	Porewater Test Mean (CI)[1]
Lab Control	79% (±15%)	100%
IH-10	1% (±4%) *	93% (±12%)
IH-11	8% (±18%) *	27% (±43%) *
IH-12	0% (±0%) *	93% (±12%)
IH-13	24% (±19%) *	66% (±19%) *
IH-14	18% (±22%) *	Not tested
IH-15	0% (±0%) *	0% *

* Statistically different (a = 0.05) compared to the Control data.

[1] CI = Confidence interval based on bootstrap analysis of replicate data.

In addition to known differences between the tolerance limits of the species tested, in some cases the longer exposure duration of the bulk sediment test may account for greater observed toxicity in bulk sediment tests than in shorter duration pore water tests. But in many cases bulk sediment tests result in less observed toxicity than pore water tests. This may occur when: 1) Chemical exposure is a mix of pore water and overlying water due to species-specific behavior; and 2) Potential toxicants in sediments are retained in particulate or other complex forms, reducing bioavailability relative to extracted pore water. By design, TIE studies with extracted pore water often represent a worst-case scenario for exposure in the field. Ultimately, evaluation of TIE results requires some interpretation regarding the ecological risks associated with detected signals. Specific considerations may include concordance of test results, as well as the relative sensitivity of the species and life stage used in the TIE.

4.1.2. Synthesize Responses to TIE Treatments

The interpretation of TIE toxicity responses is based on both the observed magnitude of toxicity in the treated sample and the relative change in toxicity from the previous samples in the TIE sequence. Because no single approach for evaluating TIE results can comprehensively synthesize all of the potentially useful information derived from the tests, using multiple assessment methods is recommended. The suggested approaches described below include an ordered synthesis of raw data, derivation of standard statistical endpoints, and finally, a technique that combines all TIE treatment effects across the dilution range to assign a magnitude value to the signals associated with each toxicant class.

Review raw data. To begin the TIE data review process, it is always important to work first with a summary of the raw data, including responses to each dilution of each sample, by TIE treatment. Table 4-2 provides an illustration of this type of preliminary synthesis, as it was

prepared for the Hunters Point study. Highlighting is used to identify apparent changes in toxicity with each TIE treatment.

Table 4-2. Sea urchin data from pore water TIE conducted with Hunters Point samples.

	TIE Treatment Result (% normal development)[1]					
		Metals		Particulates	Organics	NH_4
Station-dilution	Untreated	STS	EDTA	Filtered	Oasis	*Ulva*
HPSPIKE - 10	**0**	**8**	**58**	**26**	**19**	55
HPSPIKE - 25	**0**	**0**	**43**	**15**	**6**	**47**
HPSPIKE - 50	**0**	**0**	**6**	**4**	**0**	**51**
HPSPIKE - 100	**0**	**0**	**0**	**1**	**0**	60
HP1 - 10	**12**	**47**	63	**57**	**33**	**50**
HP1 - 25	**0**	**23**	**30**	**27**	**12**	**43**
HP1 - 50	**0**	**0**	**0**	**0**	**0**	**48**
HP1 - 100	**0**	**0**	**0**	**0**	**0**	55
HP2 - 10	**10**	**29**	**57**	**56**	**30**	**53**
HP2 - 25	**4**	**18**	**28**	**18**	**4**	**41**
HP2 - 50	**0**	**0**	**0**	**0**	**0**	**44**
HP2 - 100	**0**	**0**	**0**	**0**	**0**	**55**
HP3 - 10	**2**	**6**	**28**	**0**	**0**	**53**
HP3 - 25	**0**	**0**	**0**	**0**	**0**	**45**
HP3 - 50	**0**	**0**	**0**	**0**	**0**	**45**
HP3 - 100	**0**	**0**	**0**	**0**	**0**	**53**
HP4 - 10	**21**	**64**	**56**	**58**	**37**	54
HP4 - 25	**0**	**13**	**14**	**6**	**6**	58
HP4 - 50	**0**	**0**	**0**	**0**	**0**	49
HP4 - 100	**0**	**0**	**0**	**0**	**0**	**28**
HP5 - 10	**31**	**68**	**32**	**59**	**24**	56
HP5 - 25	**0**	**0**	**4**	**0**	**1**	**50**
HP5 - 50	**0**	**0**	**0**	**0**	**0**	**36**
HP5 - 100	**0**	**0**	**0**	**0**	**0**	**51**
HP6 - 10	**4**	**50**	**48**	60	**37**	48
HP6 - 25	**13**	**57**	**44**	59	**32**	**36**
HP6 - 50	**3**	**33**	**31**	**22**	**21**	52
HP6 - 100	**0**	**0**	**14**	**0**	**2**	63
HP7 - 10	**0**	**39**	**36**	NP	**31**	**43**
HP7 - 25	**0**	**0**	**2**	NP	**2**	**31**
HP7 - 50	**0**	**0**	**0**	NP	**0**	**29**
HP7 - 100	**0**	**0**	**0**	NP	**0**	**39**
HP8 - 10	**0**	**0**	**1**	**0**	**0**	**41**
HP8 - 25	**0**	**0**	**0**	**0**	**0**	**37**
HP8 - 50	**0**	**0**	**0**	NP	**0**	**40**
HP8 - 100	**0**	**0**	**0**	NP	**0**	**31**
HP9 - 10	**2**	**51**	**36**	**43**	**33**	**43**
HP9 - 25	**0**	**0**	**3**	**0**	**0**	**42**
HP9 - 50	**0**	**0**	**0**	**0**	**0**	**43**
HP9 - 100	**0**	**0**	**0**	**0**	**0**	**43**
HP10 - 10	**0**	**1**	**4**	**0**	**0**	**31**
HP10 - 25	**0**	**0**	**0**	**0**	**0**	**18**
HP10 - 50	**0**	**0**	**0**	**0**	**0**	**35**
HP10 - 100	**0**	**0**	**0**	**0**	**0**	**3**
HPREF - 10	**3**	**44**	**35**	62	**29**	49
HPREF - 25	**14**	**34**	**35**	**49**	**20**	**42**
HPREF - 50	**2**	**9**	**2**	**6**	**4**	**37**
HPREF - 100	**0**	**0**	**0**	**0**	**0**	**41**
PC-100	59	78	67	66	58	67

NP = no pore water available.

1 - Shaded values indicate improvement (greater than or equal to 5%) relative to previous treatment(s).

Bold values are statistically less than (a=0.05) than performance control per Dunnett's test.

By reviewing results from the entire study site as a whole, patterns of response may become evident. For example, at the top of Table 4-2, the spiked sample becomes less toxic after EDTA treatment, but only in the dilute samples. The same pattern is expressed for several of the site samples. The highlighting also shows that *Ulva* treatment was the most effective of all treatments in removing toxicity.

This data set also provides a good illustration of the range of effects that may be observed across a dilution series. Dilutions often provide a means to segregate the effects of multiple toxicant classes. This reduces the 'masking effect' by reducing the over-all toxicity of multiple toxicant classes, allowing observations of effective treatments in the earlier TIE steps. For example, it can be seen from the Hunters Point data that the STS and EDTA treatments affected greater change in toxicity in the more dilute samples. In this case, results suggest that while reduction in metal toxicity may have occurred in all of the samples, the toxicity due to metals was only apparent when the remaining toxic constituent, ammonia, was below its effect threshold. This demonstrates that if the more dilute samples were not included in the TIE, the metal signal would have been missed.

Derive Lethal Concentration (LC) endpoints. Although the 'big picture' can sometimes be seen best by reviewing the data as a whole, generally it is also useful to reduce the results to a more standard presentation form. Typically, calculations of 'Point Estimate' values such as the LC_{50} are used to synthesize dose-response relationships observed over a dilution series (U.S. EPA 2000a). The LC_{50}, for instance, is the pore water percentage that would result in 50% mortality. The 'LC' convention in aquatic toxicology was established as a useful expression of the relative toxicities of different toxicants to a particular species, with lower LC values representing greater toxicity. For TIE data, lethal or other effect concentrations ('LC' or 'EC' values) may also be used to represent changes in the relative toxicity of a sample following each of the TIE treatments. Commercial statistical packages (e.g., Toxcalc® (Tidepool Software); Toxstat® (West, Inc.)) are available to readily perform these calculations, including any specific endpoint that may be useful (e.g., LC_{50} or LC_{20}). Choices are available for parametric (e.g., probit, logit) and non-parametric (e.g., linear interpolation or Spearman Karber) estimation techniques that also provide confidence intervals, in some cases. These values do provide a useful tool for simplification of TIE findings, particularly where one or more TIE treatments have dramatically reduced toxicity. Also, because the values represent a standard 'language' used in aquatic toxicity testing, they facilitate interpretation to a broad audience.

In general, however, TIE data are not as well suited to Point Estimate analysis as standard toxicity tests, due to their multivariate design. In TIE tests the dilution series is generally the same for all TIE treatments while the underlying gradient of chemical exposure concentrations is unknown. Due to these circumstances, a highly toxic sample tested prior to treatment may be fully toxic in all dilutions, partially toxic after one or more treatments, and non-toxic at some point in the sequential manipulation process. In contrast, tests designed specifically for 'LC' or 'EC' analysis, will have a dilution series that is optimized (e.g., through range-finder tests) to result in partial effects in intermediate dilutions, with no effect and complete effects at either end of the dilution series (U.S. EPA 2000a). The TIE design does not allow for this type of optimization, because the effect range changes from one treatment to the next, but the dilution

series is constant. Furthermore, the testing design used to establish an LC or EC value (e.g., a five concentration series) is generally more than can be accommodated in a full TIE investigation (e.g., eight treatments of ten site samples plus a control sample and a reference sample).

While the LC_{50} represents the most common and easily recognized Point Estimate for controlled study designs, one way to make the most of a less-than-optimal data is to choose an endpoint that lends the greatest 'responsiveness' across the most treatments. In some cases, where small reductions in toxicity occur over many treatments or dilutions, the LC_{75} might represent these changes the most clearly. In the Indian Head TIE study, the LC_{20} was chosen as a sensitive signal for changes in toxicity from one treatment to the next; many observed effects were < 50%, and the LC_{50} could not be calculated. It is generally advisable to choose an endpoint that is not lower than the statistical resolution of the test (frequently LC_{20}- LC_{30}). The recent EPA guidance for statistical analyses of Whole Effluent Test results provides a full treatment of this issue (U.S. EPA 2000a).

Cumulative expression of toxicity reduction. Another approach for synthesizing the effects of individual TIE treatments that captures all of the reductions in toxicity that may occur over multiple dilutions is one that presents the treatment results as an overall percentage of the total reduction in toxicity that has occurred. For example, a 15% reduction in toxicity in each of three dilutions would be equivalent to 45% reduction in one dilution. This approach has the advantage of providing a measure that fully reflects all of the signals that may be presented by the TIE. It incorporates small reductions in toxicity that can occur over multiple dilutions and quantifies a pattern that is clearly observed in the raw data analysis, but that may be obscured by the Point Estimate approach. Table 4-3 provides an illustration of this type of analysis using Hunters Point data. For the spiked sample, the table shows that of all of the toxicity that was observed in the untreated sample in all dilutions, 79% percent of those effects were eliminated through the TIE process. For the toxicity that was removed by the TIE treatment of the spiked sample, only 4% of that reduction was due to STS, while 46% occurred following EDTA treatment, and the remainder was removed by the *Ulva* treatment.

While the cumulative approach, as described above, will accurately reflect the TIE laboratory findings, it must be recognized that the fractions of toxicity that are accounted for do not necessarily represent the greatest sources of toxicity. Those sources that are successfully identified within the context of the TIE study design favor the treatments that are performed last. For example, Table 4-3 shows that the greatest reductions in toxicity, for most samples, occurs as a result of the *Ulva* treatment to remove ammonia, while the STS and EDTA treatment responses do signal effects associated with metals. Theoretically, the reason that greater fractions of toxicity were accounted for by the *Ulva* treatment could either be: 1) Ammonia contributed the most to the toxicity in the samples, or 2) After the STS and EDTA treatments, ammonia was still present at concentrations that caused severe effects, and removal of metal toxicity (however much it might contribute to toxicity in the absence of ammonia) had a limited effect on the over-all response. Here, the data suggest that metals are potentially present at potencies that are equivalent or greater than the ammonia toxicity, such that reductions in toxicity due to STS and EDTA additions are evident in the more dilute samples. Reviewing the raw data in Table 4-2, this pattern can be seen in the Hunters Point data.

Table 4-3. Summary of cumulative toxicity removed by TIE treatments in the Hunters Point study with sea urchins.

Station	Total Toxicity Removed by TIE[1]	Reduction in Toxicity due to each TIE Treatment (as % of total effects observed in untreated sample)[2]				Treatments that Removed Toxicity, in order of Relative Effectiveness[3]
		STS	EDTA	Filtraton	*Ulva*	
HP-SPIKE	79	4	46	0	51	*Ulva* = EDTA
HP-1	72	29	11	0	63	*Ulva* > STS > EDTA
HP-2	70	17	19	0	63	*Ulva* > EDTA = STS
HP-3	73	2	10	0	87	*Ulva* > EDTA
HP-4	68	29	1	0	72	*Ulva* > STS
HP-5	68	20	2	0	82	*Ulva* > STS
HP-6	72	62	7	6	38	STS > *Ulva* > EDTA = Filtration
HP-7	53	18	1	0	71	*Ulva* > STS
HP-8	56	0	0	0	99	*Ulva*
HP-9	64	23	1	0	74	*Ulva* > STS
HP-10	32	0	1	0	95	*Ulva*
HP-REF	60	35	1	16	44	*Ulva* = STS > Filtration

[1] % of Normal development that was restored by the cumulative TIE treatment in all 4 dilutions as a % of the *Ulva* control response (67%).

[2] % of Normal development that was restored by each individual TIE treatment in all 4 dilutions, expressed as a percent of the total toxicity removed (e.g. for HP-spike, 4% of the 79% overall improvement was due to STS treatment).
Zero values are reported when no reduction in effect was observed relative to previous treatment.
Oasis SPE is not included because no reductions in toxicity were associated with this treatment.

[3] ">" = 10% or higher difference in toxicity reduction; treatments resulting in <5% reduction not listed.

4.1.3. Dealing with Ambiguous Data

The Hunters Point examples provided in Section 4.1.2 illustrate that TIE studies can provide an important line of evidence relating causes and effects for interpreting sediment toxicity. Even when the outcome of a TIE leaves some questions or ambiguous responses, it generally reduces the field of uncertainties associated with toxic responses. Furthermore, there are steps that can be taken to reduce the degree of uncertainty associated with TIE results. Some of the more common 'ambiguous' outcomes are addressed below.

'Masking' effects. One of the most common causes of ambiguity in TIE responses is the 'masking effect', as exhibited by ammonia in the Hunters Point samples. The sequential testing approach reduces this problem, particularly where ammonia is not a source of toxicity. For instance, if treatments for metals and organics sequentially remove toxicity, resulting in a non-toxic sample, then ammonia can be eliminated from the list of principal source of toxicity. However, the process of elimination is less effective for organic contaminants, and even less so for metals, when ammonia is a principal source of toxicity. The dilution series very often provide the evidence needed to resolve the presence of mixed toxicants. In some cases an appropriate dilution series can be identified using pore water screening tests to assure that partial effects occur. Alternatively, follow-on tests may be conducted, reversing the order of the TIE sequence to remove the 'masking' constituents first. This was done with a limited number of Hunters Point samples, and the follow-on study did demonstrate that some toxicity remained after ammonia removal, and that STS and EDTA removed most of the residual toxicity. However, it is important to recall that in the recommended sequence the last steps are the least specific. For instance, *Ulva* and zeolite are the least specific, as they remove metals and organics in addition to ammonia to varying degrees, depending on the specific characteristics of the mixtures being tested. When those manipulations are conducted first, their non-specificity complicates interpretation of the results.

Enhanced toxicity. In some cases, it may appear that a particular TIE treatment increases toxicity. This also occurred in the Hunters Point study, where the SPE Oasis® treatment resulted in lower normal responses than the previous treatment. This is incongruous with the experimental design (except in the case of pH adjustments that may increase or decrease toxicity depending on the constituent toxicants), and should be addressed through further study. If this occurs, water quality data for each treatment should also be reviewed, because treatments may result in changes that effect toxicity (e.g., change in pH). Alternatively, matrix interactions (e.g., competition for binding sites) may favor the binding of less toxic constituents, resulting in the release of the more toxic components. This could potentially occur with metals as well as organics. In these cases, the more plausible interactions should be tested through TIE treatments of spiked mixtures that include suspected competitor constituents, as well as individual chemical spiked samples.

Non-toxicant interactions. Another type of situation where results may be ambiguous is where water quality conditions in the sample, aside from those that are intentionally manipulated, may contribute to effects. This occurred in the Hunters Point samples, where some samples had oxygen concentrations low enough to result in mortality of the fish species tested, and in other samples toxicant effects may have been enhanced by the low D.O. Enhanced toxicity can occur

with all classes of toxicants, and it is therefore very important to review available information regarding lethal and sub-lethal stresses to the organisms used in TIE testing.

A common source of stress may be ionic imbalance, having contributed to toxicity in both effluent and sediment TIEs (Ho and Caudle 1997; Adams et al. 2001). In a recent TIE with marine sediments from the Calcasieu River in Louisiana, it was found that a high concentration of calcium was the most likely source of toxicity in at least one pore water sample (SAIC 2002c). Because specific TIE treatments have not been developed to address problems of ion imbalance (it is difficult to selectively remove major ions such as calcium) this problem is normally only considered when the traditional TIE treatments fail to remove toxicity. Where known stressors have contributed to observed effects, it is important to track these co-factors to account for potential effects that could erroneously be attributed as TIE treatment effects. Changes in D.O. and pH, particularly without acclimation, are perhaps the most common co-contributors to changes in toxicity in TIE tests.

Performance controls. Finally, in some cases control samples that have been subjected to TIE manipulation treatments do not fully meet the data quality objectives that were developed for the study. In this event, additional judgment must be applied in deciding to continue to consider the data, at least qualitatively, or to reject the data entirely. Where controls perform at a slightly sub-optimal level (e.g., 70+% survival), then it is generally appropriate to include the findings of the TIE in the evaluation of the study data. Particularly when the TIE signal magnitude is large (e.g., response changes from 0% to 70% survival), the result generally should be interpreted as a treatment effect. A simple rule for discriminating useful data from invalid data is that potential treatment-related effects should be larger than the range of control responses.

4.2. CHARACTERIZING CHEMISTRY DATA FOR TIE SAMPLES

The analysis of potential stressors in the TIE samples with respect to the observed toxicity in TIE treatments is a key step in determining the sediment constituents responsible for toxicity. Recall that the intent of the Hazard Quotient (HQ) approach is to discern potential toxicant effects that might be observable in the short-term TIE exposures. Hazard Quotients for each of the analytical measurements taken on split sediment and pore water samples generated for the TIE study should be derived using the same methods as those described in Section 2.1.2. Long-term (chronic) effects are beyond the scope of the TIE investigation and can only be adequately addressed through a full Ecological Risk Assessment.

To complete the acute HQ assessment, chemistry data from each of the selected stations is evaluated for toxicity potential based on one or more of the following characteristics:

- Bulk sediment concentrations that exceed benchmarks for potential/probable effects;
- Divalent metal concentrations (simultaneously extracted metal (SEM)) that enhance potential for divalent metal (Cu, Cd, Pb, Ni, Zn and sometimes Ag);
- Pore water benchmark exceedences, to reflect location-specific bioavailability;
- Species-specific benchmarks for CoPC sources and other potential toxicants (e.g., NH_4^+) that confound the elucidation of CoPC contributions to toxicity;
- Spatial variation that might reflect novel environmental conditions or CoPC distributions that may represent gradients in chemical availability.

4.3. EVALUATING THE TIE STUDY IN A RISK ASSESSMENT FRAMEWORK

In TIE studies, it is always true that the sum of the acquired data is worth more than the individual components. Within an aquatic risk assessment, specific lines of evidence are used to support each of the risk-characterizing components (e.g., bulk sediment toxicity, TIE results and HQs) evaluated individually, and then in concert. When the independent assessments agree, then the identification of specific CoCs or confounding factors as potential sources of ambient toxicity are strongly supported. When the three lines of evidence are discordant, then the individual implications of each must be reviewed with consideration for the possible reasons for discrepancies.

Establishing agreement of TIE responses and chemical HQs. Evaluating agreement between TIE results and HQs requires that the synthesis of results from each TIE manipulation be considered using each of the approaches discussed in Section 4.1.2 (raw data review, point estimate analysis, and cumulative effects from each manipulation). If any of the approaches implicate a prescribed group of toxicants (e.g., metals, organics or ammonia), then the analysis turns to a review of HQs for members of those groups. For example, in the Indian Head Study, EDTA removed the observed toxicity in *Hyalella* in three samples, but in a fourth sample, zeolite removed the toxicity (Table 4-4). All samples had high metal HQs; three for manganese and one for zinc. While the HQ for total ammonia was approximately equivalent in all samples, the sample that exhibited the zeolite signal had an anomalously high pH, driving the more toxic unionized ammonia to higher levels than in the other samples. Where the zeolite effect occurred, a reasonable interpretation is that ammonia may have potentially 'masked' reduction in toxicity due to manganese.

Table 4-4. Synthesis of findings from the Indian Head TIE with amphipods (*Hyalella)* and fish (*Pimephales).*

Indian Head Sample	Largest TIE Signal		Highest Hazard Quotient		Summary of Pore water TIE Results
	Hyalella	*Pimephales*	*Hyalella*	*Pimephales*	
IH- 6	EDTA	Residual toxicity	Mn = 4.5	Mn = 19	EDTA signal and HQ for manganese are consistent.
IH-8	Zeolite	Residual toxicity	Mn = 5.7	Mn = 24	Zeolite and low pH signals consistent with ammonia toxicity. Manganese toxicity may have been masked by ammonia toxicity.
IH-11	EDTA	Residual toxicity	Mn = 5.5	Mn = 23	EDTA signal and HQ for manganese are consistent.
IH- 15	EDTA	EDTA	Zn = 343	Zn = 208	EDTA signal and HQ for zinc are consistent.

Differential responses between species. Using species-specific HQs, the next line of evidence to review is the responses of each TIE species to reveal potential sensitivity-related differences. One useful approach is to evaluate the relative toxicity of untreated pore water to the two species tested relative to the species-specific HQs. If the species with the highest HQs is also the species most affected by a given sample, then the relative species sensitivity analysis provides strong evidence for the toxicity of those compounds with the highest HQs. If species-specific reductions in toxicity in response to the TIE are consistent with the relative HQs for each species and each toxicant class, then the bridge between probable cause (HQ assessment) and effect (TIE assessment) greatly reduces uncertainty in associated risk-driven management decision processes.

The Indian Head study results in Table 4-4 present a good example for interpreting the different sensitivities of the TIE species tested. The TIE with *Hyalella* provided evidence of metal removal, with species-specific HQs for manganese and zinc that were concordant with the TIE responses. For *Pimephales*, the TIE response was consistent with zinc toxicity in IH-15, but the STS and EDTA treatments were not sufficient to remove most of the observed toxicity in the samples that were most likely toxic due to manganese. The relatively higher species-specific HQ for *Pimephales* exposure to manganese in these samples supports a reasonable hypothesis that the post STS and EDTA samples retained enough free manganese to be toxic to *Pimephales* but not *Hyalella*.

Agreement with other study results. An additional aspect of the 'Weight of Evidence' approach is to evaluate TIE results as compared with prior or concurrent study findings, including other assessment endpoints, broader sample database, spatial/temporal distribution of chemistry and toxicity. Methodologies for specific 'weighting' of the findings of each study should be developed to address objectives that are site and program specific. Two applicable approaches to using TIE and other pore water data are: the Sediment Quality Triad (SQT) approach (Chapman et al. 1997) and the PRG development process (SAIC 2001). In the SQT approach the three assessment endpoints are: 1) chemical HQs; 2) toxicity; and 3) benthic community structure, measured on the same sediment. Adams et al. (2001) recommend that pore water testing should be used in conjunction with bulk sediment testing as part of the triad, particularly because pore water tests often represent species more sensitive than those used in standard bulk sediment tests. Using both pore water and bulk sediment test results provides a more balanced weight-of evidence than either test type alone in contributing to the triad assessment.

The PRG method constitutes another approach to use the correspondence between sediment chemistry and biological effects to determine sediment concentrations that discriminate conditions of minimal, uncertain, and major biological effects. It is a data exercise used to select the important CoCs and the concentrations of these CoCs that will protect aquatic biota from site-specific chemical risks. The selected CoCs are the "Limiting" CoCs in that these analytes have been found to be associated with the greatest site-specific toxic effects; remedial goals may be set to reduce chemical exposure to below the limiting CoC PRG concentrations in order to protect from adverse effects. Where TIE and PRG results agree, the evidence relating site-specific contaminants and contaminant classes to observed adverse effects is strong. What the TIE accomplishes to complement PRG analysis is direct cause and effect evidence, not only for CoCs, but also for confounding factors such as ammonia. Both procedures were recently used in

an evaluation of the Calcasieu Estuary Superfund Site to determine load limits for that water body (SAIC 2002c). In this case, the TIE and PRG were concordant in the evidence they provided for both metals and organic as CoCs. Findings from these biology-based evaluations, in conjunction with estimations of source loads, historic loads and transport and fate models provide the best basis for management plans to protect or mitigate benthic habitats.

4.4. SPATIAL AND TEMPORAL CONSIDERATIONS

TIE results generally provide a limited spatial representation of the site, relative to other biotic and abiotic measures, and also represent a single event, a snapshot in time of the potentially time-variable site conditions. Variance in spatial and temporal distributions of the chemical, biotic, and abiotic stressors is a principal source of uncertainty in Ecological Risk Assessments that must be addressed with appropriate sampling designs. Interpreting TIE results with regard to these uncertainties may be accomplished in the context provided by more comprehensive evaluations such as the sediment Triad studies or PRG analyses. For instance, if the TIE study may conclude that HQs and TIE signals were all consistent with a particular organic contaminant, or class such as pesticides, it is then prudent to review the degree to which the study stations represent the nature and extent of contamination and toxicity in the entire study area, and over time, as available data allow.

The evaluation of TIE results should conform to stated study and data quality objectives. For example, in a "hot spot" study, the TIE data should be evaluated to determine if they demonstrate consistency with expectations for the targeted hot spot, or a different level of spatial variability. If the hotspot actually represents consistent levels of toxicity across a spatial domain, the TIE should help determine if different toxicant classes are causing this toxicity. There are cases where spatial variability is high within the limited TIE study sample locations, such as the Indian Head TIE study, where samples collected within 100 feet of each other yielded different results. In such cases, the TIE findings may suggest that investigation of contaminant sources needs to be resolved in future risk evaluations. Results from the TIE study may also be interpreted with respect to reference site responses. If toxicity is attributable to the same toxicants in both the study area and the off-site 'reference area', the TIE strengthens the case for non-site sources. If HQs and TIE responses are different from those found in previous studies, it is important to consider the respective likelihood of both long and short-term variability. If the study site is a depositional area, historic loads may be covered with different sediments than those represented in recent or historic studies. Alternatively, a summer study may yield different results from a winter study due to changing depth profiles of the sediment redox potential, and/or different rates of biological activity may produce varying levels of ammonia in the sediment. Another important temporal consideration involves changes that may occur in stored sediments, even when holding time requirements have been met. Hunters Point samples, for instance, had high ammonia variability within a one or two month holding cycle, that may not have represented realistic in situ variability (Battelle and Neptune 1999). When expressed in the appropriate spatial and temporal framework, TIE signals that indicate toxicity due to specific classes of toxicants improve risk characterizations, serving as a valid and efficient way to link cause and effect. In cases where the degree of spatial and temporal variability is poorly understood, it is important to acknowledge this uncertainty in applying a site-wide interpretation of TIE results.

5.0. SUPPLEMENTAL INFORMATION AND CONCLUSIONS

Sections 1 through 4 of this guide are intended to provide the reader with a practical approach to conducting TIEs on toxic contaminated sediments. This section provides some supplemental material including a summary of the range of itemized costs that may be associated with conducting TIEs, a summary of the TIE Task Package included to assist in the actual implementation of the approach presented above, and some frequently asked questions, and finally, some general conclusions.

5.1. COST INFORMATION

Costs for conducting a TIE are variable, dependent on the degree of coordination with other studies, the number of toxic samples that are identified for testing, the number and type of toxicity tests to be performed, and various other factors that will become apparent throughout the remainder of this document. Table 5-1 provides examples of low- and high-end costs that might be associated with TIE studies conducted using the approaches presented in the following sections.

Table 5-1. Example costs for conducting a TIE with sediment pore waters.

Activity	Low End Costs; single inexpensive toxicity test (no field work or chemistry)	High End Costs (with field work and chemistry)
Study Design and Work Plan Preparation	$500	$1,300
Field Sampling	None- covered by other site studies	$2,500
TIE Preparation and Testing		
Bulk sediment testing	None- covered by other site studies	$750 - $1000
Pore water screening (extraction and test; optional)	$200	$500
Pore water extraction	$100 syringe	$200 (high speed centrifugation)
TIE manipulations	$1,000	$1,000
Toxicity testing	$200	$2,000
Chemical analyses		
Bulk sediment (e.g., metals, organics, TOC, SEM, AVS)	None- covered by other site studies	$1,500
Pore water metals[1]	$130	$130
Data Presentation		
Synthesis and analysis	$400	$1,200
Report preparation	$400	$1,200
Per Sample Total Costs	$2,750 (1 sed.)	$12,030
Total Costs[2]	$2,750 (1 sed.)	$164,450[3]

[1] Metals analysis only for pore water; organics predicted using partitioning model (see Section 3.4).

[2] Assume 15 samples for all but TIE preparation and testing, where 10 samples are asssumed.

[3] Where field activities and chemistry costs are not incurred, high-end cost estimate would be $104,450.

5.2. APPENDED TIE TASK PACKAGE

A TIE Task Package, Appendix B of this document, was developed as a workbook for TIE studies. It must be emphasized that these materials are provided to serve as a starting point for TIE study preparation. In every study, and for every site and every laboratory, SOPs should be generated that directly address the objectives of the study, and the logistics associated with that particular study. The TIE Task Package provides examples of the types of supplies, information, and logistical progression that may be required.

The first section of Appendix B (B-1) provides lists of supplies that may be required for each of the five steps of the field and laboratory phases of a TIE study. The second section of Appendix B (B-2) provides generic SOPs that can be used to guide the study participants through the field and laboratory activities associated with a TIE. The first SOP in Section B-2 provides general procedures that guide TIE execution and management, including data sheets for various TIE steps. The following sections present SOPs for Sample Collection, Sample Manipulations, TIE Manipulations, and Toxicity Testing, including many example protocols and worksheets that can be used to facilitate the completion of an effective study. SOPs should also be generated for reporting and analyzing data, but these have not been presented here because their preparation is very study and facility specific, and should be developed by the project team responsible for those tasks. Generic protocols for such activities are available in U.S. EPA guidance for Quality Assurance and Project Plans (U.S. EPA 2001c).

5.3. FREQUENTLY ASKED QUESTIONS

What is the value of a TIE study to an RPM? The risk-based process that the Navy uses to determine the need for clean-up, and what the remedial goals should be normally applies independent exposure and effects characterization, and an application of default benchmarks. TIEs can demonstrate that conclusions based on benchmark analyses alone can be wrong. There is a substantial degree of uncertainty in the link between cause and effect, particularly when confounding factors (e.g., ammonia) are involved in the toxic response. Without knowledge of causative factors driving toxicity, cleanup goals may be set to satisfy overly conservative or inappropriate assumptions. Consequently, in the absence of TIEs, decisions to conduct time-consuming and costly remedial actions can be errant, and the results may do little to remediate the principal risks at the site.

How do TIE findings relate to CoCs identified during the risk assessment? The CoCs and associated concentrations which constitute the PRGs should be risk-based, i.e., reflective of the results of the risk assessment with respect to the selection of those CoCs that "limit" remediation. Like a nutrient that controls the growth of a plant because of limited supply, "limiting" CoCs are those analytes that control the extent of risk because of high concentrations and/or enhanced exposure. While a risk assessment may identify dozens of chemicals that exceed threshold toxicity benchmarks, typically the chemicals having the greatest exceedences determine the level of risk. If a TIE study demonstrates that the CoCs previously defined as "limiting" to aquatic life actually represent lower acute risks than another contaminant or confounding factor, this information should be used in evaluating applicability of RAOs.

Are TIEs appropriate for all sites? Navy facilities often have various chemical contamination issues related to landfills, shipyards, firing ranges and numerous other sources. As part of remedial investigations, surveys have typically been conducted to determine the type and extent of CoCs in offshore sediment and biota, including assessment of associated risks. Where bulk sediment tests have demonstrated toxicity, this may be due to site-related contaminants, or to naturally occurring conditions such as elevated ammonia and/or sulfides, or low dissolved oxygen. TIEs are broadly applicable to a wide variety of these sites and data types, particularly where actionable risk is identified for acute effects on aquatic organisms. Each case is unique, but the sites where projected remedial costs are high and the factors contributing to toxicity are uncertain are generally the best candidates for TIE testing. Conversely, if the contaminated area of concern is small, if minimal toxicity has been observed, or if there is a clear link between a point source of contamination and observed adverse effects, a TIE may not be warranted. Existing toxicity data and chemical characterizations should be evaluated in making the determination of TIE applicability. Bulk sediment toxicity should be demonstrated prior to the development of plans for pore water TIE testing, because the intent of the TIE tests is to resolve the nature of sediment toxicity.

What site-related chemical risks does the TIE capture? An underlying assumption of the "limiting" CoC approach is that remediation of a chemical causing the highest risk will lead to reduction of lesser risks caused by other CoCs. CoCs that are evaluated are assumed to adequately represent the risks posed by all site-related CoCs. This assumption would be flawed if there existed novel chemicals at high concentrations that have not yet been detected or are present in a form that is more bioavailable than had been previously measured. TIE results can serve as a test for the "limiting" CoC approach. One such example would be if the "limiting" CoC was an organic compound/complex, but most toxicity was removed by the metal chelation step. In that case factors controlling site-specific bioavailability should be evaluated, and potential revisions would be warranted to address remedial goals for metals. In another case where benchmarks for organics were marginally exceeded and bulk sediment testing indicated toxicity, a TIE could demonstrate that ammonia was the principal source of acute toxicity. If ammonia is the toxicity source, remediating on the basis of reducing risks caused by organic contaminants might do more harm than good for the benthic community.

Can TIE findings be used to support regulatory decisions (e.g., regarding ARARS)? The process presented herein is designed to support the evaluation of remedial alternatives in accordance with the requirements of the National Contingency Plan (NCP) and Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) guidance. As discussed above, it is assumed that if the need for a TIE exists, then investigations have been performed *a priori* that indicated elevated ecological risks due to site-related chemical exposure. Under the Navy's Installation Restoration (IR) Program, these findings would result in the preparation of an FS for the site, the purpose of which is to outline options for remedial actions to address the chemicals causing risk. TIEs are generally most useful after completion of a preliminary risk assessment, and preferably before the FS is completed. However, if uncertainty regarding the source(s) of toxicity remains during the FS, then a TIE may serve as a "better late than never" option.

5.4. CONCLUSIONS

The Navy TIE Demonstration studies illustrate how TIEs are effective in revealing the causes for sediment toxicity and thereby strengthen the technical basis for evaluating and refining ecological risk assessments. By providing a direct link demonstrating cause and effect, TIEs compliment the use of sediment benchmark hazard quotients that serve as more general indicators of potential risk. TIEs also capture site-specific factors that drive risk, and in this sense, they are a good compliment to the Navy's approach for deriving PRGs. This document provides a detailed approach for designing and conducting TIE studies to resolve uncertainties associated with bulk sediment toxicity. Sequential reduction in toxicity is used to attribute the characteristics of the sediment that affect their toxicity.

TIE studies have demonstrated significant utility to provide evidence of what classes of chemicals are and are not causing the most toxicity in sediments that are the subject of Remedial Investigations. While it is important to recognize that TIE methods will continue to improve as research efforts proceed, it is just as important to use the present methods to their greatest advantage. Evidence provided by TIEs has reduced and will continue to greatly reduce uncertainty involved in risk management decisions that determine the appropriate expenditures of funds for remediation of sediments based on ecological risks. TIEs also increase the probability that targeted remediation of specific classes of contaminants, when required, will actually be effective in reducing ecological risks.

6.0. REFERENCES

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Appendix A.
Sediment and pore water benchmarks used in calculating Hazard Quotients for a TIE study.

Appendix A-1. Selection of benchmarks used in calculating sediment Hazard Quotients for a TIE study.

		Marine Sediment						Selected Marine[1]		Freshwater
Class	Analyte	ER-M	PEL	AET-H	AET-L	SQAL	EPA	BM	Source	(UET)
MET	Aluminum									
MET	Antimony				9.3			9.3	AET-L	
MET	Arsenic	70	42	700	57			70	ER-M	17
MET	Cadmium	9.6	4.2	9.6	5.1			9.6	ER-M	3.0
MET	Chromium	370	160	270	260			370	ER-M	95
MET	Cobalt				10			10	AET-L	
MET	Copper	270	108	1300	390			270	ER-M	86
MET	Lead	218	112	660	450			218	ER-M	127
MET	Manganese				260			260	AET-L	1100
MET	Mercury	0.7						0.7	ER-M	
MET	Nickel	52	43					52	ER-M	43
MET	Selenium				1.0			1.0	AET-L	
MET	Silver	3.7	1.7	6.1	6.1			3.7	ER-M	4.5
BT	TBT				3.4			3.4	AET-L	
MET	Zinc	410	271	1600	410			410	ER-M	520
MET	SEM-AVS						5.0	5	EPA	
PAH	2-Methylnaphthalene	670	201	1900	670			670	ER-M	
PAH	Acenaphthene	500	89	2000	500	1300		500	ER-M	290
PAH	Acenaphthylene	640	128	1300	1300			640	ER-M	160
PAH	Anthracene	1100	245	13000	960			1100	ER-M	260
PAH	Benzo(a)anthracene	1600	693	5100	1600			1600	ER-M	500
PAH	Benzo[a]pyrene	1600	763	3600	1600			1600	ER-M	700
PAH	Benzo[b]fluoranthene			9900	3600			9900	AET-H	
PAH	Benzo[ghi]perylene			2600	720			2600	AET-H	300
PAH	Benzo[k]fluoranthene			9900	3600			9900	AET-H	13400
PAH	Biphenyl					110000		110000	SQAL	
PAH	Chrysene	2800	846	9200	2800			2800	ER-M	800
PAH	Dibenz[a,h]anthracene	260	135	970	230			260	ER-M	100
PAH	Fluoranthene	5100	1494	30000	2500	6200		5100	ER-M	1500
PAH	Fluorene	540	144	3600	540	540		540	ER-M	300
PAH	Indeno[1,2,3-cd]pyrene			2600	690			2600	AET-L	330
PAH	Naphthalene	2100	391	2700	2100	470		2100	ER-M	600
PAH	Phenanthrene	1500	544	6900	1500	1800		1500	ER-M	800
PAH	Pyrene	2600	1398	16000	3300	97000		2600	ER-M	1000
PAH	Total LMW (L) PAHs	3160	1442	24000	5200			3160	ER-M	5300
PAH	Total HMW (H) PAHs	9600	6676	69000	17000			9600	ER-M	6500
PAH	Total PAHs	44792	16770					44792	ER-M	12000
PCB	Total PCBs	180	189	3100	1000			180	ER-M	26
PST	2,4'-DDD	27	7.8	43	16			27	ER-M	
PST	2,4'-DDE	27	374	15	9.0			27	ER-M	
PST	2,4'-DDT	27	4.8	34	34			27	ER-M	
PST	4,4'-DDD	27	7.8	43	16			27	ER-M	60
PST	4,4'-DDE	27	374	15	9.0			27	ER-M	50
PST	4,4'-DDT	27	4.8	34	34			27	ER-M	50
PST	Aldrin									40
PST	alpha-BHC		1.0					1.0	PEL	
PST	alpha-Chlordane		4.8					4.8	PEL	
PST	beta-BHC		1.0					1.0	PEL	
PST	delta-BHC		1.0					1.0	PEL	
PST	Dieldrin		4.3					4.3	PEL	300
PST	Endosulfan I					290		290	SQAL	
PST	Endosulfan II					14		14	SQAL	
PST	Endrin					42		42	SQAL	500
PST	gamma-BHC		1.0							9.0
PST	gamma-Chlordane		4.8					4.8	PEL	
PST	Heptachlor				0.3			0.3	AET-L	10
PST	Heptachlor epoxide									30
PST	Hexachlorobenzene									100
PST	Methoxyclor					190		190	SQAL	
PST	Mirex									800
PST	Toxaphene									
PST	2,4-Dinitrotoluene									
PST	Nitrobenzene									

1- Benchmarks were selected in the following order of priority:

Marine Sediment: 1) ER-M; 2) PEL; 3) AET-H/L; 4) SQAL; 5) EPA.

Units: Metals = µg/g; PCBs, Pesticides (PST), PAHs = ng/g; AVS, SEM= µM/g.

LMW PAH = sum of 7 2-ring & 3-ring PAHs included in NOAA ER-L/ER-M benchmarks (Long et al. 1995); (methylnaphthalene, acenaphthene, acenaphthylene, anthracene, fluorene, naphthalene, phenanthrene).

HMW PAH = sum of 6 4-ring and 5-ring PAHs included in NOAA ER-L/ER-M benchmarks (Long et al. 1995); (benzo(a)anthracene, benzo(a)pyrene, chrysene, dibenz(a,h)anthracene, fluoranthene, pyrene).

Total PAHs - sum of LMW & HMW PAHs; Total PCBs - Sum of individual PCB congeners x 2.

ER-M = NOAA Effects Range-Median (Long et al. 1995 in U.S. EPA 1997).

PEL = Threshold Effects Levels (FDEP 1994 in U.S. EPA 1997).

AET-L/H = Apparent Effects Threshold Low/High (Barrick et al. 1988 in U.S. EPA 1997).

SQAL = EPA Sediment Quality Advisory Levels, based on 1% TOC (U.S. EPA 1997).

EPA = EPA SEM-AVS sediment quality screening value, µM/g dry weight (U.S. EPA 1997).

Appendix A-2. Water Quality Criteria and estimated benchmarks used in calculating pore water Hazard Quotients for a TIE study.

		Water Quality Criteria-Acute[1,2]		Selected Sediment[3]				Koc	Estimated Pore Water[1,4]	
Class	Analyte	Marine	Freshwater	Marine		Freshwater			Marine	Freshwater
MET	Aluminum		750							
MET	Antimony			9.3	AET-L					
MET	Arsenic	69	360	70	ER-M	17	UET			
MET	Cadmium	42	3.9	9.6	ER-M	3.0	UET			
MET	Chromium	1100	16	370	ER-M	95	UET			
MET	Cobalt			10	AET-L					
MET	Copper	4.8	18	270	ER-M	86	UET			
MET	Lead	210	83	218	ER-M	127	UET			
MET	Manganese		1000	260	AET-L	1100	UET			
MET	Mercury			0.7	ER-M					
MET	Nickel	74	1400	52	ER-M	43	UET			
MET	Selenium			1.0	AET-L					
MET	Silver	1.9	4.1	3.7	ER-M	4.5	UET			
BT	TBT			3.4	AET-L					
MET	Zinc	90	120	410	ER-M	520	UET			
MET	SEM-AVS			5	EPA					
PAH	2-Methylnaphthalene	300		670	ER-M			8.0E+3	8.4	
PAH	Acenaphthene		1700	500	ER-M	290	UET	7.1E+3	7.0	4.1
PAH	Acenaphthylene	300		640	ER-M	160	UET	9.6E+3	6.7	1.7
PAH	Anthracene	300		1100	ER-M	260	UET	3.0E+4	3.7	0.9
PAH	Benzo(a)anthracene	300		1600	ER-M	500	UET	4.0E+5	0.4	0.1
PAH	Benzo[a]pyrene	300		1600	ER-M	700	UET	1.0E+6	0.2	6.9E-2
PAH	Benzo[b]fluoranthene	300		9900	AET-H			1.2E+6	0.8	
PAH	Benzo[ghi]perylene	300		2600	AET-H	300	UET	3.9E+6	6.7E-2	7.8E-3
PAH	Benzo[k]fluoranthene			9900	AET-H	13400	UET	1.2E+6	0.8	1.1
PAH	Biphenyl			110000	SQAL			7.8E+3	1410	
PAH	Chrysene	300		2800	ER-M	800	UET	4.0E+5	0.7	0.2
PAH	Dibenz[a,h]anthracene	300		260	ER-M	100	UET	3.8E+6	6.9E-3	2.7E-3
PAH	Fluoranthene		3980	5100	ER-M	1500	UET	1.1E+5	4.7	1.4
PAH	Fluorene	300		540	ER-M	300	UET	1.4E+4	3.9	2.2
PAH	Indeno[1,2,3-cd]pyrene	300		2600	AET-L	330	UET	3.4E+6	7.5E-2	9.6E-3
PAH	Naphthalene		2300	2100	ER-M	600	UET	2.0E+3	104	30
PAH	Phenanthrene		30	1500	ER-M	800	UET	3.0E+4	5.0	2.7
PAH	Pyrene	300		2600	ER-M	1000	UET	1.1E+5	2.5	0.9
PAH	Total LMW (L) PAHs	300		3160	ER-M	5300	UET			
PAH	Total HMW (H) PAHs	300		9600	ER-M	6500	UET			
PAH	Total PAHs	300		44792	ER-M	12000	UET	7.6E+4	59	16
PCB	Total PCBs		2	180	ER-M	26	UET	2.7E+6	6.7E-3	9.7E-4
PST	2,4'-DDD			27	ER-M			9.9E+5	2.7E-3	
PST	2,4'-DDE			27	ER-M			4.4E+6	6.1E-4	
PST	2,4'-DDT			27	ER-M			4.4E+6	6.1E-4	
PST	4,4'-DDD		0.6	27	ER-M	60	UET	9.9E+5	2.7E-3	6.0E-3
PST	4,4'-DDE		1050	27	ER-M	50	UET	4.4E+6	6.1E-4	1.1E-3
PST	4,4'-DDT		1.1	27	ER-M	50	UET	4.4E+6	6.1E-4	1.1E-3
PST	Aldrin		3.0			40	UET	2.5E+6		1.6E-3
PST	alpha-BHC			1.0	PEL			5.4E+3	1.9E-2	
PST	alpha-Chlordane			4.8	PEL			2.5E+6	2.0E-4	
PST	beta-BHC			1.0	PEL			5.6E+3	1.8E-2	
PST	delta-BHC			1.0	PEL			5.5E+3	1.8E-2	
PST	Dieldrin	0.71	2.5	4.3	PEL	300	UET	1.9E+5	2.3E-3	0.2
PST	Endosulfan I		0.2	290	SQAL					
PST	Endosulfan II			14	SQAL			1.1E+4	0.1	
PST	Endrin	3.7E-2	0.2	42	SQAL	500	UET	9.4E+4	4.5E-2	0.5
PST	gamma-BHC	0.2	2.0			9.0	UET	4.6E+3		0.2
PST	gamma-Chlordane			4.8	PEL			1.6E+6	2.9E-4	
PST	Heptachlor	5.3E-2	0.5	0.3	AET-L	10	UET	2.5E+6	1.2E-5	4.1E-4
PST	Heptachlor epoxide		0.5			30	UET	2.5E+6		1.2E-3
PST	Hexachlorobenzene		6.0			100	UET	6.2E+5		1.6E-2
PST	Methoxychlor			190	SQAL					
PST	Mirex					800	UET	5.9E+6		1.4E-2
PST	Toxaphene		7.3E-2							
PST	2,4-Dinitrotoluene		330							
PST	Nitrobenzene		27000							
AMM	Ammonia[5]	0.23								

1- Benchmarks (units = µg/L (mg/L for AMM)).
2 - WQC-SA = saltwater acute (U.S. EPA 1999 [metals, dieldrin, endrin, heptachlor], NOAA 1999);
WQC-SA reported for Chromium VI; WQC-FA = freshwater acute (NOAA 1999);
3 - See Table 2.2-1 for sediment benchmark selection process and definitions.
4 - Estimated from sediment benchmark using equilibrium partitioning relationship (Di Toro*et al.,* 1992),
where estimated = sed. BM/(Koc*0.01).
LMW PAH = sum of 7 2-ring & 3-ring PAHs included in NOAA ER-L/ER-M benchmarks (Long et al. 1995);
(methylnaphthalene, acenaphthene, acenaphthylene, anthracene, fluorene, naphthalene, phenanthrene).
HMW PAH = sum of 6 4-ring and 5-ring PAHs included in NOAA ER-L/ER-M benchmarks (Long et al. 1995);
(benzo(a)anthracene, benzo(a)pyrene, chrysene, dibenz(a,h)anthracene, fluoranthene, pyrene).
Total PAHs - sum of LMW & HMW PAHs; Total PCBs - Sum of individual PCB congeners x 2.
5 - Marine WQC is for un-ionized ammonia; pH-dependent freshwater WQC for total ammonia can be calculated
as WQC-FA= [0.275/(1 + 10 7.204-pH)] + [39.0/(1 + 10 pH-7.204)], (US EPA, 1999).

Appendix B.
TIE task package including SAIC TIE Standard Operating Procedures.

Appendix B-1.
General lists of supplies required for conducting TIE study tasks.

- **Note: consult SAIC TIE Standard Operating Procedures for additional supplies required.**

Appendix Table B-1.1. Supplies and equipment required for field sample collection.

Item	Required Quantity	Comment
5 gal. Plastic Buckets	1 per station	
5 gal. Bucket Lids	Same as bucket qty	
Tyvek suits	as needed	Need dependent on sampling location
Nitrile Gloves	as needed	Need dependent on sampling location
Squirt Bottles	as needed	Need dependent on sampling location
10% Nitric Acid	as needed	Need dependent on sampling location
Methanol	as needed	Need dependent on sampling location
Alconox	as needed	Need dependent on sampling location
Grab sampler	as needed	Need dependent on sampling location
Line for hauling grab	as needed	Need dependent on sampling location
Plastic Sampling Scoops	as needed	Need dependent on sampling location
Trash Cans (for coolers)	as needed	Need dependent on sampling location
Ice	as needed	Need dependent on sampling location
Paper Towels	as needed	Need dependent on sampling location
Trash Bags	as needed	Need dependent on sampling location
Sharpie Markers	as needed	Need dependent on sampling location
Electrical Tape	as needed	Need dependent on sampling location
Clear Tape	as needed	Need dependent on sampling location
Duct Tape	as needed	Need dependent on sampling location

Appendix Table B-1.2. Supplies and equipment required for pore water extraction via syringe or centrifuge method.

Item	Application	Required Quantity	Comment
B+D Brand 60 cc slip tip syringes	Syringe Method	Dependent on volume of sample needed.	Calculate qty. of syringes needed to collect required sample volume in time available, assume worst-case senario (high clay content in sediment samples) rate of 1 syringe to collect 5 mL sample/hour (rule of thumb: 6-8 syringe setups per sample).
Mist-Air Stones, medium	Syringe Method	Dependent on volume of sample needed.	1 stone for each syringe required, plus spare stones to replace clogged stones.
Silastic Tubing, 0.125 x 0.250	Syringe Method	1.5' per syringe setup	
1-5 mL pipet tips	Syringe Method	1 per syringe setup	
Plastic spoons - to make holes for stones to sit in when sediments are hard or sandy	Syringe Method	as needed	
Centrifuge: low speed, 1 L bucket style, with sleeves	Centrifuge Method	1 sleeve per sample	takes 8-10 hrs. total to collect 10 samples using low and high speed centrifuges
Centrifuge: high speed, ~500 mL capacity, with tubes	Centrifuge Method	1 tube per sample	used to centrifuge supernatant from low speed centrifuge
HDPE bottles (PW collection).	Syringe and Centrifuge Method	1 per sample	Bottle volume dependent on volume of pore water required. Decontaminated used bottles may be substituted for new.
Nitrile Gloves	Syringe and Centrifuge Method	as needed	
Paper Towels	Syringe and Centrifuge Method	as needed	
Bucket opener tool	Syringe and Centrifuge Method	as needed	
Cooler (transport pore waters to lab)	Syringe and Centrifuge Method	as needed	
Garbage bags	Syringe and Centrifuge Method	as needed	
Electrical tape	Syringe and Centrifuge Method	as needed	
Labeling tape	Syringe and Centrifuge Method	as needed	
Sharpie Markers	Syringe and Centrifuge Method	as needed	
Safety Glasses	Syringe and Centrifuge Method	as needed	

Appendix Table B-1.3. Supplies and equipment required for sample manipulation.

Item	Application	Required Quantity	Comment
General Purpose Filters	particulate analysis	Plan on 20 per sample (filtering + zeolite)	
SPE (SEP-PAK Classic C-18 columns or Oasis)	organic analysis	1 for every liter of water to be processed.	
Methanol, ACS/HPLC certified	organic analysis	Needed for C-18 step - 25 mL/1000 mL of sample, plus 25mL, and for decontamination through C-18 step.	
1 micron filters	organic analysis		check filter description for most appropriate (note hydrophobic best for organic or hydrophylic best for metals)
filter apparatus for 1 micron filters	organic analysis		e.g. Millipore
Zeolite	ammonia analysis	estimate about 5 kg per project.	e.g. Ammonex; note different zeolites have varying capacities to strip cations and organics as well as
Ulva	ammonia analysis		saltwater; requires collection or purchase from biological supply company
Sodium Thiosulfate	metals analysis	3 g per project	
EDTA	metals analysis	3 g per project	
Alconox	General Supplies	as needed	freshwater
10% Nitric Acid Solution	General Supplies	Sufficient qty. for decontamination through all steps	
MOPS	General Supplies	1 pouch per project	freshwater
Chemicals for Spiked	General Supplies	dependant on project	
1M NaOH, ACS certified	General Supplies	for pH manipulations	
1N HCL, ACS certified	General Supplies	for pH manipulations	
Disposable 5 mL pipettes	General Supplies	as needed	
Disposable 1 mL pipettes	General Supplies	as needed	
Latex or Nitrile Gloves	General Supplies	as needed	
HDPE bottles for sub-samples	General Supplies	8 per sample	Bottle volume should match sample volume to eliminate headspace.
plastic petri dishes for filter storage	General Supplies	2 per sample	
Labeling Tape	General Supplies	as needed	
Safety Glasses	General Supplies	as needed	

Appendix Table B-1.4. Supplies and equipment required for animal testing.

Item	Application	Required Quantity	Comment
Scintilation Vials (20 mL)	Required Supplies		glass preferred unless metal is a CoPC; if glass is used, must pre-rinse to remove borosilicate
Animials	Required Supplies	15 per sample per treatment for each species	
Disposable 5 mL pipettes	Required Supplies	min of 10 per sample	
Return Address Lables (Laser)	Required Supplies	Used to label vials	

Appendix Table B-1.5. Supplies and equipment required for in-house analyses of sample water quality parameters.

Item	Application	Required Quantity	Comment
Ammonia test kit/meter	ammonia measurement	1	e.g. colorimetric or titrametric test kit or ion-specific electrode
Hardness test kit/meter	hardness measurement	1	e.g. colorimetric or titrametric test kit
Alkalinity test kit/meter	alkalinity	1	e.g. colorimetric or titrametric test kit
Sulfides test kit/meter	sulfide measurement	1	e.g. colorimetric or titrametric test kit
thermometer		1	
refractometer/salinometer		1	
pH meter		1	
DO meter		1	

Appendix B-2.
SAIC Standard Operating Procedures (SOPs) for conducting major TIE tasks.

SCIENCE APPLICATIONS INTERNATIONAL CORPORATION
TIE STANDARD OPERATING PROCEDURE

Table of Contents

(Revision number and date following each title)

TIE-100 GENERAL TIE PROCEDURES 0 3/25/02

TIE-200 TIE SOPS FOR SAMPLE COLLECTION.

TIE-210 Sediment Sampling 3 3/25/02

TIE-220 Pore Water Extraction- Syringe 0 3/25/02

TIE-230 Pore Water Extraction- Centrifuge 0 3/25/02

TIE-300 TIE SOPS FOR SAMPLE MANIPULATION.

TIE-310 Hypersaline Brine Preparation and Use 0 3/25/02

TIE-400 TIE SOPS FOR TIE MANIPULATION.

TIE-410 Untreated Sample Preparation 0 3/25/02

TIE-420 Sodium Thiosulfate Manipulation 0 3/25/02

TIE-430 EDTA Chelation 0 3/25/02

TIE-440 Sample Filtration 0 3/25/02

TIE-450 Solid Phase Extraction Using C_{18} 0 3/25/02

TIE-460 Solid Phase Extraction Using OASIS® 0 3/25/02

TIE-470 Low pH Manipulation 0 3/25/02

TIE-480 High pH Manipulation 0 3/25/02

TIE-490 Ammonia Removal Using Zeolite 0 3/25/02

TIE-4100 Ammonia Removal Using *Ulva* 0 3/25/02

TIE-500 TIE SOPS FOR TOXICITY TESTING.

TIE-510 Sea Urchin Sperm Cell Test 0 3/25/02

TIE-520 Mysid Survival Test 0 3/25/02

1.0 PURPOSE

The purpose of this SOP is to establish general procedures and practices to be implemented in the specific TIE procedures that follow.

2.0 SCOPE

This general procedure and the following specific procedures have been developed to establish methods for conducting a Toxicity Identification Study of an aquatic site. Procedures are presented for the various phases of a typical study, including sample collection, sample preparation via manipulation, TIE manipulation of the prepared sample and finally toxicity testing of the individual manipulated samples.

3.0 REFERENCES

The TIE procedures are based on the following references. In some cases, SAIC has modified methods described in these references to accommodate the specific requirements of the TIE design.

3.1 U.S. EPA (United States Environmental Protection Agency). 1991. Sediment toxicity identification evaluation: Phase I (characterization), Phase II (identification) and Phase III (confirmation) modifications of effluent procedures. EPA-600/6-91/007. Environmental Research Laboratory, Duluth, MN.

3.2 U.S. EPA (United States Environmental Protection Agency). 1996. Marine Toxicity Identification Evaluation (TIE), Phase I Guidance Document. EPA/600/R-096/054. U.S. EPA Office of Research and Development, Washington, DC.

4.0 POINTS OF CONTACT

Questions regarding the TIE SOPs should be addressed to:

Sherry Poucher
Greg Tracey
Science Applications International Corporation
221 Third Street
Newport, RI 02840
401 847.4210

5.0 RESPONSIBILITIES

The roles of key personnel involved in conducting a TIE study are defined below.

5.1 QUALITY ASSURANCE/QUALITY CONTROL (QA/QC) OFFICER

The QA/QC Officer is responsible for:

5.1.1 Approving Standard Operating Procedures (SOPs) and
5.1.2 Verifying that SOPs are being implemented.

5.2 HEALTH AND SAFETY (H&S) OFFICER

The H&S Officer is responsible for ensuring that appropriate program, company and contractual H&S policies and procedures are in effect and verifying enforcement of same by line management.

5.3 PROGRAM OR PROJECT MANAGER

The Program or Project Manager is responsible for:

5.3.1 Ensuring that all personnel are properly trained;
5.3.2 Ensuring that this and all appropriate procedures are followed; and
5.3.3 Verifying that the appropriate training records are submitted.

5.4 FIELD MANAGER

The Field Manager is responsible for:

5.4.1 Ensuring that all personnel perform their assigned duties in accordance with this procedure when it is applicable;
5.4.2 Ensuring compliance with the sampling and analysis plan (SAP); and
5.4.3 Overall management of field activities.

6.0 SAFETY

6.1 A Safety Plan should be developed prior to conducting a TIE study. The plan should include safety protocols for the specific laboratory and field tasks to be conducted during the study, and list any potential site-specific hazards. Field samples collected during a TIE study may be contaminated with various constituents depending on the nature of the study site. Necessary precautions, in the form of protective clothing, equipment, etc., should be listed in the Safety Plan and used where risks to health may exist.

6.2 The program policy should maintain an effective program to control employee exposure to chemical, radiological, and physical stress that is

consistent with U. S. Department of Energy (DOE) and Occupational Safety and Health Administration (OSHA) established standards and requirements.

7.0 GENERAL

7.1 Any deviation from specified requirements will be justified to and authorized by the Project Manager and/or the relevant Program Manager.

7.2 Deviations from requirements are sufficiently documented to allow re-creation of the modified process.

7.3 Refer to the site- or project-specific H&S Plan for relevant H&S requirements.

7.4 Refer to the project/task-specific SAP for relevant sampling and analysis requirements.

7.5 SAIC and subcontractor personnel who use this procedure must provide documented evidence of having been trained on the procedure to the Program or Project Manager for transmittal to the Central Records Facility (CRF).

7.6 Inclement weather, such as rain, snow, or impending lightning storms, will be avoided when conducting field sampling activities.

8.0 PROCEDURES

The recommended sequence for performing TIE manipulation procedures is presented in Attachment I.

9.0 RECORDS

Most of the TIE procedures require measuring and recording certain test parameters and results, as well as documentation that major TIE tasks (e.g. field sampling, sample manipulation, toxicity testing) are completed. The following attachments, consisting of quality control forms and data and calculation sheets, are designed to facilitate and document preparation for and execution of a TIE study. Measurements taken during the performance of specific TIE procedures should be recorded on the appropriate data sheet. Completed data sheets should be stored in a project binder until data contained on the sheets can be entered into project files.

10.0 ATTACHMENTS

The following table lists attachments included with this SOP. The table should be used as a checklist to document completion of each datasheet, so that the completeness of the final TIE data package may be verified.

	Attachment	Qty Required	Date Com-pleted	Initials
☐	Attachment I - Recommended sequence for conducting TIE manipulations and associated functional treatment name of each manipulation.	n/r	n/r	n/r
☐	Attachment II – Example Chain of Custody (CoC) form used to document transfer of TIE samples.	1 for each transfer of sample batch		
☐	Attachment III - Sample ID key used to assign Lab Sample IDs to field samples to facilitate laboratory tasks.	1 per study		
☐	Attachment IV - Bottle labeling used to identify individual TIE treatment and replicate samples.	sufficient qty to label all test samples		
☐	Attachment V- Example calculation sheet used to determine water volumes required for TIE sample replicates.	1 for each test		
☐	Attachment VI – Sample tracking sheet used to document completion of each sample processing step.	1 per study		
☐	Attachment VII - Data sheet used to record test water quality parameters.	1 for each species per test		
☐	Attachment VIII - Data sheet for recording organisms counted at specified timepoints.	1 for each species per test		
☐	Attachment IX - Data sheet for recording test kit parameters used to determine alkalinity of TIE samples.	1 for each test		
☐	Attachment I - Data sheet for recording test kit parameters used to determine total hardness of TIE samples.	1 for each test		
☐	Attachment XI - Data sheet for recording test kit parameters used to determine ammonia concentration of TIE samples.	1 for each test		
☐	Attachment XII - Example calculation sheet used to determine concentration of hydrogen sulfide in TIE samples.	1 for each test		
☐	Attachment XIII - Example calculation sheet used to determine concentration of un-ionized ammonia in TIE samples.	1 for each test		

n/r = not required.

Attachment I
Recommended sequence for conducting TIE manipulations and associated functional treatment name of each manipulation.

Procedural manipulation name	**Functional treatment name**
Untreated	Baseline condition
Sodium thiosulfate (STS; $Na_2S_2O_3$)	Cationic metals and oxidants reduction
Ethylenediamine tetra-acetic acid (EDTA)	Cationic metals chelation
Filtration	Particulate fraction extraction
Solid phase extraction (SPE) column	Organic fraction extraction
Low pH	Ammonia/sulfide ionic shift
High pH	Ammonia/sulfide ionic shift
Zeolite/*Ulva*	Ammonia removal

Attachment II

Example Chain of Custody (CoC) form used to document transfer of TIE samples.

Science Applications International Corporation

Chain of Custody Record

Science Applications International Corporation/ 221 Third Street/ Admiral's Gate/ Newport RI 02840 phone (401)847-4210 fax (401)849-9786

Project:	Client Name and Contact:

Sample No.	Containers No.	Containers Type	Collection Date	Collection Time	Sample Description	Requested Parameters
Total :						

Packed/Released By	Date	Time	*Received By*	Date	Time	Remarks:
Signature:			Signature:			
Printed Name:			Printed Name:			
Released By	Date	Time	*Received By*	Date	Time	
Signature:			Signature:			
Printed Name:			Printed Name:			

Final Destination:	Contact Name and Phone Number:	Shipping Method:
		Page _____ of _____

Attachment III

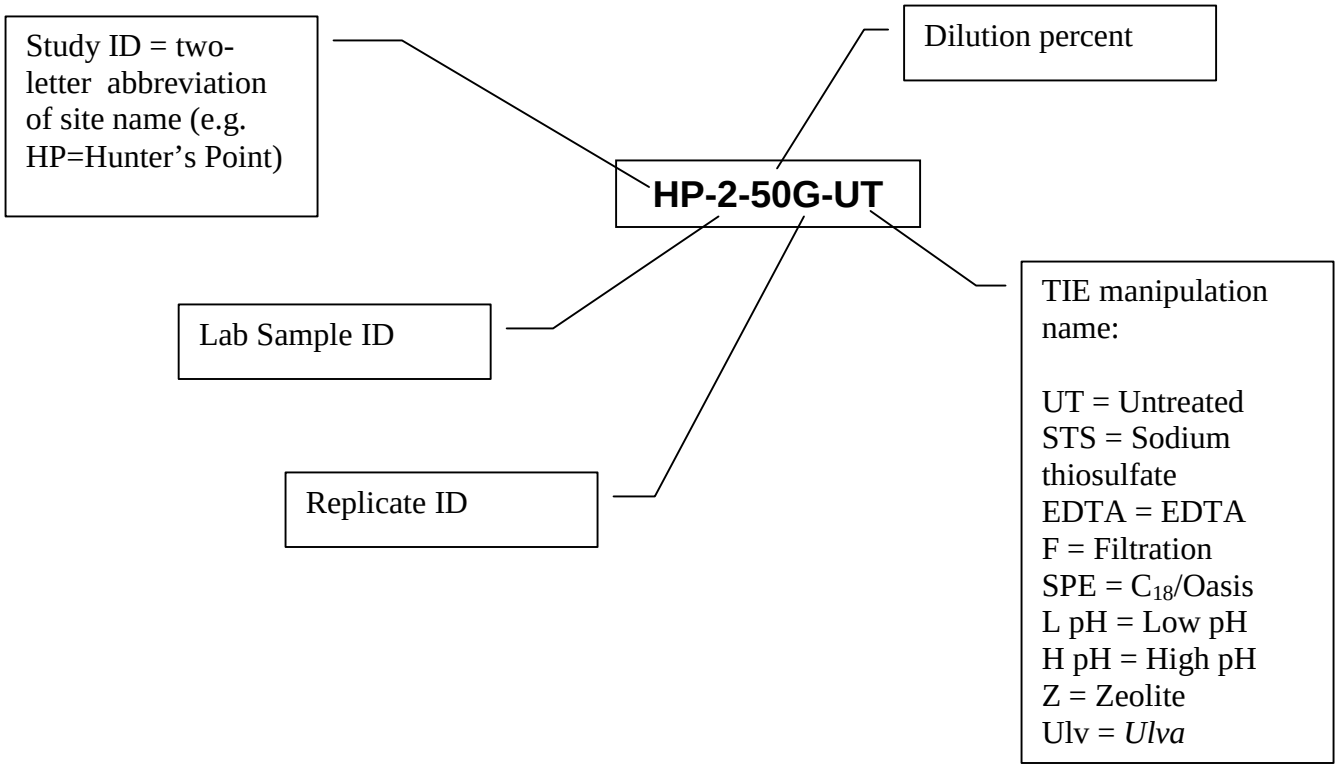
Sample ID key used to assign Lab Sample IDs to field samples to facilitate laboratory tasks.

Sample ID Key

Field Sample ID	Lab Sample ID[1]	Sample Description
n/r	(Study ID)-Control	Performance Control
n/r	(Study ID)-Spike	Spiked Sample
	(Study ID)-1	
	(Study ID)-2	
	(Study ID)-3	
	(Study ID)-4	
	(Study ID)-5	
	(Study ID)-6	
	(Study ID)-7	
	(Study ID)-8	
	(Study ID)-9	
	(Study ID)-10	
	(Study ID)-REF	Reference Station

1 - (Study ID) = two-letter abbreviation of the study site name.

Attachment IV
Bottle labeling used to identify individual TIE treatment and replicate samples.



Attachment V
Sample tracking sheet used to document completion of each sample processing step.

TIE Sample Tracking Sheet

Description: Using one tracking sheet for each TIE sample, document the completion of each processing step.

Lab Sample ID: _____

Tech. Initials	Processing Step Description	Record this Parameter:	Value (w/ units)	Date	Start Time	End Time
	Record time and date of sediment sample collection.	n/r	n/r		n/r	
	Samples delivered to lab for bulk sed. toxicity testing.	n/r	n/r		n/r	
	Sample homogenized and sub-sampled for sediment chemistry.	n/r	n/r		n/r	
	Begin pore water collection.	No. airstones set				n/r
	Record sediment type.	Sed. type			n/r	n/r
	Stop pore water collection.	Vol. collected (estimate)			n/r	
	Measure sample temp., DO, pH, hardness, alkalinity and ammonia. Record results on appropriate data sheet(s).	see data sheet(s)	n/r		n/r	
	Sub-sample for lab analysis of metals and/or organics.	n/r	n/r		n/r	
	Record samples on COC, package, and FedEx to lab. Place copy of COC in project binder.	n/r	n/r		n/r	
	Treat sample with ***Sodium thiosulfate*** (STS) and let stand for 1 h.	Vol. of sample to manipulate				
	Record volume of ***STS*** added.	Vol. of STS added		n/r	n/r	n/r

n/r = not required

Lab Sample ID: _____

Tech. Initials	Processing Step Description	Record this Parameter:	Value (w/ units)	Date	Start Time	End Time
	Sub-sample and distribute into ***STS*** vial set, per the Replicates and Dilutions sheet.	n/r	n/r		n/r	
	Treat sample with ***EDTA*** and let sit for 3h.	Vol. of sample to manipulate			n/r	
	Record volume of ***EDTA*** solution added.	Vol. EDTA added		n/r	n/r	n/r
	Sub-sample and distribute into ***EDTA*** vial set, per the Replicates and Dilutions sheet.	n/r	n/r		n/r	
	Sample ***Filtered*** with filtering setup (e.g. Millipore). Save filters.	No. filters used				
	Sub-sample into 6 oz bottle, ~185 mL (no head-space) for later distribution to ***Filtered*** vial set. Label and Refrigerate.	n/r	n/r		n/r	
	Measure sample pH.	pH			n/r	
	Process sample through ***SPE*** column. Max of 1000 ml of sample volume per column. Record sample pH.	pH				
	Adjust pH back to pre-Oasis or C18 level with HCL and/or NaOH. Record final pH.	final pH			n/r	
	Sub-sample into a 6 oz bottle, ~185 mL (no head-space) for later distribution to ***SPE*** vial set. Label and refrigerate.	n/r	n/r		n/r	
	END of sequential manipulations.	n/r	n/r	n/r	n/r	n/r
	Manipulate a 185 mL sub-sample with MOPS for low pH.	starting pH			n/r	
	Record volume of MOPS added.	Vol. MOPS added		n/r	n/r	n/r
	Record ending pH.	ending pH		n/r	n/r	n/r
	Distribute into ***low pH*** vial set per the Replicates and Dilutions sheet.	n/r	n/r		n/r	

n/r = not required

Lab Sample ID: _____

Tech. Initials	Processing Step Description	Record this Parameter:	Value (w/ units)	Date	Start Time	End Time
	Manipulate a 185 mL sub-sample for ***high pH*** .	starting pH			n/r	
	Record ending pH.	ending pH		n/r	n/r	n/r
	Distribute into ***high pH*** vial set per the Replicates and Dilutions sheet.	n/r	n/r		n/r	
	Treat remaining sample with ***zeolite/Ulva*** (~4 h). Record ending ammonia reading.	ending ammonia				
	Sub-sample and distribute into **Z** vial set per the Water Volume Calculation Worksheet.	n/r	n/r		n/r	
	Distribute ***Untreated*** sub-sample into appropriate vials.	n/r	n/r		n/r	
	Distribute ***Filtered*** sub-sample into appropriate vials.	n/r	n/r		n/r	
	Distribute ***SPE*** sub-sample into appropriate vials.	n/r	n/r		n/r	

n/r = not required

Attachment VI
Data sheet used to record test water quality parameters.

Water Quality Data Sheet

Parameter: ☐ Temp. ☐ DO ☐ pH ☐ Salinity **Unit:_____**					
Project: Date: Time:					
Technician Name:					
Organism: Timepoint:					
Treatment:					
		Replicate			
Sample ID	**Dilution**	**A**	**B**	**C**	**Comment**
(Study ID)-Control	100				
(Study ID)-Spike	10				
(Study ID)-Spike	25				
(Study ID)-Spike	50				
(Study ID)-Spike	100				
(Study ID)-1	10				
(Study ID)-1	25				
(Study ID)-1	50				
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(Study ID)-9	100				
(Study ID)-10	10				
(Study ID)-10	25				
(Study ID)-10	50				
(Study ID)-10	100				
(Study ID)-REF	10				
(Study ID)-REF	25				
(Study ID)-REF	50				
(Study ID)-REF	100				

Attachment VII
Data sheet for recording organisms counted at specified timepoints.

Test Organism Counts Data Sheet

Date:		Time:			
Technician Initials:					
Organism:		Timepoint:			
Treatment:					
		Replicate			
Sample ID	**Dilution**	**A**	**B**	**C**	**Comment**
(Study ID)-Control	100				
(Study ID)-Spike	10				
(Study ID)-Spike	25				
(Study ID)-Spike	50				
(Study ID)-Spike	100				
(Study ID)-1	10				
(Study ID)-1	25				
(Study ID)-1	50				
(Study ID)-1	100				
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(Study ID)-10	10				
(Study ID)-10	25				
(Study ID)-10	50				
(Study ID)-10	100				
(Study ID)-REF	10				
(Study ID)-REF	25				
(Study ID)-REF	50				
(Study ID)-REF	100				

Attachment VIII
Data sheet for recording test kit parameters used to determine alkalinity of TIE samples.

Alkalinity Data Sheet

Description: Data sheet designed for use with Hach. Co. alkalinity test kit (model AL-AP MG-L).

Date: Time:
Technician Initials:

Sample ID	High or Low Range Test Used?	Sample Volume Used	Volume Sulfuric Acid Standard Added	Multiplication Factor	Alkalinity (phenolphthalein alkalinity as $CaCO_3$)	Volume Sulfuric Acid Standard Added	Total Volume Sulfuric Acid Standard Added from both steps	Multiplication Factor	Alkalinity (methyl orange alkalinity as $CaCO_3$)
		(mL)	*(drops)*		*(mg/L)*	*(drops)*	*(drops)*		*(mg/L)*

COMMENTS:

Attachment IX
Data sheet for recording test kit parameters used to determine total hardness of TIE samples.

Total Hardness Data Sheet

Description: Data sheet designed for use with Hach. Co. total hardness test kit (model #5-EP MG-L).

Date: Time:
Technician Initials

Sample ID	Sample Volume Used *(mL)*	Volume EDTA Titrant Added *(drops)*	Multiplication Factor	Hardness (as $CaCO_3$) *(mg/L)*	Initials of Analyst

COMMENT:

Attachment X
Data sheet for recording test kit parameters used to determine ammonia concentration of TIE samples.

Ammonia Data Sheet

Description: Data sheet designed for use with Hach. Co. ammonia-nitrogen pocket colorimeter (silicylate method).

Date: Time:
Technician Initials

Sample ID	Sample Volume Used *(mL)*	Volume of DI *(mL)*	Instrument Readout	Multiplication Factor	Final Result

COMMENTS:

Attachment XI
Example calculation sheet used to determine water volumes required for TIE sample replicates.

Water Volume Calculation Sheet

Description: Example calculation of sample and dilution water volumes to combine (bold) to yield volume needed for each dilution (shaded). Example replicate scheme: Species 1 = Replicates A, B and C; Species 2 = Replicates E, F, G. Water quality/spare = Replicate D (fill with sample last).

Replicates A, B and C

Prepare	20	mL per dilution.
Desired Dilution (%)	Volume of sample to add (mL)[1]	Volume of dilution/ control water to add (mL)[2]
10	**2**	**18**
25	**5**	**15**
50	**10**	**10**
100	**20**	**0**
Total mL	**37**	**43**

1 - Volume of sample (mL) = (desired dilution (%)/100) x 20 mL per dilution.
2 - Volume of dilution/control water (mL) = ((100-desired dilution (%))/100) x 20 mL per dilution.

Replicates D, E, F and G

Prepare	10	mL per dilution.
Desired Dilution (%)	Volume of sample to add (mL)	Volume of dilution/ control water to add (mL)
10	**1**	**9**
25	**2.5**	**7.5**
50	**5**	**5**
100	**10**	**0**
Total mL	**18.5**	**21.5**

Attachment XII
Example calculation sheet used to determine concentration of hydrogen sulfide in TIE samples.

Hydrogen Sulfide Calculation Sheet

Description: Example worksheet used to calculate concentration of hydrogen sulfide (bold) using measured water quality parameters (shaded).

Sample ID	Total Sulfide (M)	Temp (C)	Salinity (ppt)	pH	Temp (K)	I	A	B	A'	pfm	[H]	pK_1	pK_1'	K_1'	H_2S M	H_2S mg/L[1]
(Study ID)-Control					273.16	0.00	-0.108	0.0095	0.49	0	1	7.36	7.36	4.32E-08	0.00E+00	**0.0**
(Study ID)-Spike					273.16	0.00	-0.108	0.0095	0.49	0	1	7.36	7.36	4.32E-08	0.00E+00	**0.0**
(Study ID)-1					273.16	0.00	-0.108	0.0095	0.49	0	1	7.36	7.36	4.32E-08	0.00E+00	**0.0**
(Study ID)-2					273.16	0.00	-0.108	0.0095	0.49	0	1	7.36	7.36	4.32E-08	0.00E+00	**0.0**
(Study ID)-3					273.16	0.00	-0.108	0.0095	0.49	0	1	7.36	7.36	4.32E-08	0.00E+00	**0.0**
(Study ID)-4					273.16	0.00	-0.108	0.0095	0.49	0	1	7.36	7.36	4.32E-08	0.00E+00	**0.0**
(Study ID)-5					273.16	0.00	-0.108	0.0095	0.49	0	1	7.36	7.36	4.32E-08	0.00E+00	**0.0**
(Study ID)-6					273.16	0.00	-0.108	0.0095	0.49	0	1	7.36	7.36	4.32E-08	0.00E+00	**0.0**
(Study ID)-7					273.16	0.00	-0.108	0.0095	0.49	0	1	7.36	7.36	4.32E-08	0.00E+00	**0.0**
(Study ID)-8					273.16	0.00	-0.108	0.0095	0.49	0	1	7.36	7.36	4.32E-08	0.00E+00	**0.0**
(Study ID)-9					273.16	0.00	-0.108	0.0095	0.49	0	1	7.36	7.36	4.32E-08	0.00E+00	**0.0**
(Study ID)-10					273.16	0.00	-0.108	0.0095	0.49	0	1	7.36	7.36	4.32E-08	0.00E+00	**0.0**
(Study ID)-REF					273.16	0.00	-0.108	0.0095	0.49	0	1	7.36	7.36	4.32E-08	0.00E+00	**0.0**

1 - The calculation of H2S, is based equilibrium of the un-ionized form with the bisulfide ion (HS-) and H+ in water. A conditional dissociation constant of 7.0, is generally modified by pH, temperature and salinity factors, as follows:

for temperature pK1 (T) = 32.55 + 1519.44/T - 15.672 log10T + 0.02722T,

where T = temperature (^{0}C); ^{0}K = T + 273.15.

Then pK1' = pK1 + AS2 + BS,

where A = - 0.2391 + 35.685/T, B = 0.0109 - 0.3776/T and S = Salinity in mg/Kg,

and $[H_2S]$ = Total Sulfide/ (1 + (K1'/[H]))

where [H] = 10 -pH + pfm and pfm = -A[(I2/1 + I2) -3I].

I = 0.7 at 35 mg/Kg salinity; pfm = 0.12 ; [H] = 10 -7.5 + 0.12 = 4.169 x 10-8

at pH = 7.5, 20 ^{0}C, pK1' = 7.0485 - 0.694 + 0.336 = 6.691 K1' = 10-691 = 1.23 x 10-7

100/1+2.95= 25.3

Attachment XIII
Example calculation sheet used to determine concentration of un-ionized ammonia in TIE samples.

Un-ionized Ammonia Calculation Sheet

Description: Example worksheet used to calculate concentration of un-ionized ammonia (bold) at a pressure of 1 atm using measured water quality parameters (shaded) .

Sample ID	Total Ammonia (mg/L)	Temp (C)	Salinity (ppt)	pH	Temp (K)	I	I Rounded	pK	Un-ionized Ammonia (mg/L)
(Study ID)-Control					273.2	0.50	1	9.26	**0.0**
(Study ID)-Spike					273.2	0.50	1	9.26	**0.0**
(Study ID)-1					273.2	0.50	1	9.26	**0.0**
(Study ID)-2					273.2	0.50	1	9.26	**0.0**
(Study ID)-3					273.2	0.50	1	9.26	**0.0**
(Study ID)-4					273.2	0.50	1	9.26	**0.0**
(Study ID)-5					273.2	0.50	1	9.26	**0.0**
(Study ID)-6					273.2	0.50	1	9.26	**0.0**
(Study ID)-7					273.2	0.50	1	9.26	**0.0**
(Study ID)-8					273.2	0.50	1	9.26	**0.0**
(Study ID)-9					273.2	0.50	1	9.26	**0.0**
(Study ID)-10					273.2	0.50	1	9.26	**0.0**
(Study ID)-REF					273.2	0.50	1	9.26	**0.0**

1 - Un-ionized ammonia = Total ammonia/(1+10(pK+0.0324(298-Temp.(K))+0.0415(1/Temp.(K))-pH)); (Hampson 1977).

TIE-200 TIE SOPs for sample collection.

1.0 PURPOSE

This procedure establishes protocols for the collection of intertidal and subtidal surface sediments for extraction of TIE pore water samples and subsequent chemical and toxicological analysis.

2.0 SCOPE

This procedure applies to the collection and handling of sediment samples for the purpose of conducting a TIE study. Methods for extraction of pore waters from the sediments and subsequent sample manipulation for the purpose of the TIE are discussed in following SOPs.

3.0 REFERENCES

Sediment collection methods described below have been adopted from the NOAA Status and Trends Program wherein sediments are sampled at predesignated sites, following which samples are composited to provide an integrated site specimen (Lauenstein and Yound, 1986; Launstein et al., 1986).

3.1 Lauenstein, G.G., Schantz, M.M., Wise, S.A., and Zeisler, R. 1986. Specimen Banking in the National Status and Trends Program: Development of protocols and first year results. In Oceans '86 Conference Record, Vol. 2., Washington, DC: Marine Technology Society. pp. 586-590.

3.2 Lauenstein, G.G., Yound, D.R. 1986. National Status and Trends Program for Marine Environmental Quality Benthic Surveillance Project: Cycle III Field Manual. NOAA Technical Memorandum NOS OMAN 28. Costal and Estuarine Assessment Branch, NOS/NOAA. Rockville, MD. 26pp.

4.0 PROCEDURES

4.1 EQUIPMENT AND SUPPLIES

The materials and equipment required or available for sediment sampling may vary depending on the approach used for sampling. For subtidal sampling, a boat is required. The investigator should determine the deployment capabilities of the boat to be used and the equipment available prior to planning the sampling event. The Smith-MacIntyre and or Van Veen grab may be deployed from a vessel with suitable winches, and an A-Frame

or Davit arrangement. The ponar grab is small enough to be deployed and retrieved by hand and is therefore suitable for use in small boats that lack hydraulic winches (though a winch is helpful).

Other supplies include:

- pre-cleaned Polyethylene scoops
- Smith-MacIntyre, VanVeen (0.1 m2) or Ponar grab sampler
- Stand - for the grab
- Lead weights - 4 triangular lead weights for Smith-Mac (no weights for the ponar)
- Cocking bar for Smith-Mac
- Hard hats
- Face shield or other eye protection
- Respirator - if conditions dictate
- Standard safety equipment (ie-first aid kit)
- 1/2 - 3/4 line for the Ponar grab if a wire winch is not available
- Tub - into which to dump sediment
- Teflon scoop
- Buckets (2-3) - for rinsing
- Cleansing materials for between station decontamination
- Cable cutter
- Cable crimping tools
- Electrical tape
- Duct tape
- Tools - screw drivers, wrenches, hammer, other
- Sample containers - gallon jars, cleaned and acid stripped
- Coolers with ice, or other means of cold storage
- Waterproof sample notebook
- Waterproof markers
- Suitable protective clothing (from weather as well as contaminants)
- Foul weather gear
- Boots
- Exposure suits (orange worksuits) for winter sampling
- Gloves - appropriate gloves for suspected conditions
- Compass - handheld for taking sitings at sample sites
- Line and twine
- Twine/pocket knife
- Navigation equipment
- Chain-of-Custody form
- Sample containers for analytical subsamples
- Labels for sample containers
- Labels for subsample containers
- indelible marker

Whenever possible, back-up equipment should be carried on board.

4.2 METHODS OVERVIEW

In general, the collection protocol consists of three stages: sediment collection, sediment processing, and sample packing and shipping. The division of the protocol into stages issued as an aid in organizing and simplifying the collection procedures. All information regarding the sample preparation is recorded on the Sampling Data form adopted from NOAA NS&T Program.

Stage I. Sediment Collection- will occur from shore or aboard the vessel under conditions of limited control; sufficient sample volume to meet all analytical requirements is obtained as a composite sample.

Stage II. Sediment Processing- will occur upon the return to shore, in a controlled environment using precleaned sample tools/containers; samples are properly composited, sub-sampled into laboratory bottle ware, sealed and labeled.

Stage III. Sample Packing and Shipping- Performed in a controlled environment, shipped on blue ice in shipping containers provided by laboratory.

4.3 SEDIMENT COLLECTION (STAGE 1)

Sample location

A composite sediment samples is required from each of the stations designated for sediment collection. Sediment will be collected from four grabs or cores within a 20 m radius of the designated station. A modified Van Veen-type grab sampler or a piston core sampler is used to collect the samples of sediment. The required volume of sediment typically needed to meet multiple testing needs (e.g., bulk chemistry, SEM:AVS, sediment toxicity, grain size, TOC, porewater extraction) is five gallons.

Sediment Collection (Stage I).

A. Deploy pre-cleaned Van Veen Grab using manufacturers recommended procedures. Inspect grab upon retrieval for sample quality. An adequate sample will be half to nearly full with sediment and will retain water. Samples which drain water to dryness are subject to washout of fines and are to be rejected.

B. Subsample sediment to required depth (e.g. 0-10 cm) as specified in project work plan. Immediately transfer the sample to a pre-cleaned polycarbonate bucket;

place lid on bucket between grabs. Depending on requirements for analyses, 1-gallon containers of 5 gallon buckets may be used.

C. Repeat procedure until sufficient sample volume has been obtained to form a composite for the station.

D. After grab sampling at a station has been completed, sample labels with the site ID, station ID, sample type, date of collection, and collector name are completed and affixed to the side and top of bucket. Information is logged on the sample log sheet (attached).

E. The combined sediment samples are stored cool (on ice) until returning to shore. (Eight hours maximum storage on ice.)

F. Care must be taken to wash the grab with seawater between each station and to clean the sampling scoop by: soap and water wash, water rinse, distilled water rinse, methanol rinse, and a final distilled water rinse. Rinsate blanks are taken as necessary. Rinsate water is collected in waste container for disposal on shore.

Sediment Processing (Stage II).

A. Inspect sample container for integrity and confirm legibility of labeling.

B. Review Chain of Custody form. Any deviations or modifications from the sampling protocol must be noted on the sample data form (e.g., labeling discrepancies, sample volume).

C. Assemble and label appropriate sampling containers for various analytical subsamples (generally provided or specified by the individual analysis laboratories). Typical containers include bulk organics and metals chemistry (2), SEM:AVS (1), sediment toxicity (1), grain size/ TOC (1), and sediment sample for pore water extraction(1). Refer to project work plan.

D. Composite sample is homogenized and subsampled into individual containers. A power drill with pre-cleaned stainless mixer can be used to homogenize sample.

E. Using a precleaned, polycarbonate sample scoop of appropriate size (50 to 600 ml volume), remove sediment from the composite and apportion into prelabeled, precleaned container.

F. Tighten container lids and seal with electrical tape. Place each sample into separate ziploc bags and place in coolers. Add sufficient blue ice to coolers.

G. Generate chain-of-custody sheets for each laboratory receiving samples. Original CoCs accompany samples; the field sample custodian keeps.a copy of the CoC form.

H. Proceed with the packing and shipping procedure.

Sample Packaging and Shipment (Stage III).

A. Frozen or chilled samples will be repacked with fresh dry ice in an insulated dry ice shipping containers and shipped by overnight express to analytical laboratories as necessary. Since dry ice has a limited capacity to keep samples cold over extended periods of time, samples are not to be shipped on Friday or before holidays.

B. Notify the receiving analytical laboratory personnel by telephone after the specimens are shipped. Require fax copy return of signed Chain of Custody form.

1.0 PURPOSE

This procedure describes the methods used to extract pore water from sediments using a syringe. Pore waters may also be extracted using a centrifuged-based method; the particular extraction method should be selected based on the study design and availability of equipment.

2.0 SCOPE

This procedure establishes methods for pore water extraction using a vacuum-operated system following methods described in Winger and Lasier (1991). Actual volumes needed for analyses and biological testing and holding requirements are task and study specific.

3.0 REFERENCES

Winger, PV and PJ Lasier. 1991. A Vacuum-Operated Pore-Water Extractor for Estuarine and Freshwater Sediments. Arch. Environ. Contam. Toxicol. 21, 321-324.

4.0 PROCEDURES

4.1 EQUIPMENT AND SUPPLIES

- Sediment (4 L yields 500 - 1500 mL pore water)
- Fused-glass air stone (Jungle Laboratories, Cibolo, TX 210-658-3503)
- Hose clamp
- Medical grade silastic tubing (3/16" I.D.)
- 30 or 60 cc polypropylene syringe with catheter tip
- Piece of wood cut to fit between the end of the syringe and the lip of the plunger
- 0.45 μm filter unit (optional as required)
- Centrifuge and centrifuge tubes (optional as required)
- Sample container
- Deionized water in a squirt bottle

4.2 METHODS

1. Label the syringes and sample containers with data transcribed from the sample container.

2. Use tubing to attach fused-glass air stone to the syringe.

3. Insert air stone 8 to 10 cm into the sediment sample.

4. Secure the tubing to container with elastic bands.

5. Attach hose clamp to the tubing.

6. Create a vacuum by loosening the hose clamp retracting the plunger of the syringe.

7. Brace the plunger in the retracted position by inserting a piece of wood between the end of the syringe and the lip of the plunger.

8. Collect 2 to 5 ml of pore-water.

9. Maintain vacuum by tightening hose clamp.

10. Remove the brace.

11. Remove the syringe.

12. Discard turbid pore-water from the initial sample.

13. Rinse syringe and plunger with deionized water from squirt bottle.

14. Reassemble and reattach syringe to tubing.

15. Reestablish the brace.

16. Loosen the hose clamp.

17. Fill the syringe. Minutes required for collection vary depending on particle size of the sediments (i.e., 11 -100,000 mL/hr). For fine-grained sediments, 12 - 18 hours may be required. For sandy sediments (i.e., particle size > 140 μm), less than 5 minutes are required. Store sample in at 4 ^{o}C in the dark during extraction.

18. Remove the airline tubing from the syringe when the syringe is full.

19. Dispense the pore water from the syringe into the pre-labeled sample container. Multiple collections from either > one syringe per sediment sample or > one setting of one syringe per sample require compositing.

20. Store sample at 4 ^{o}C in the dark for further analyses, filtration, or centrifugation as required for analyses.

21. Sediments are discarded in waste barrels for disposal. Barrels are sampled and analyzed to establish proper disposal procedures. Disposal procedures are performed in accordance with local, state, and federal disposal regulations.

1.0 PURPOSE

This SOP describes a procedure for extracting pore water (interstitial water) from sediments using a centrifuge. Pore waters may also be extracted using a syringe-based method; the particular extraction method should be selected based on the study design and availability of equipment.

2.0 SCOPE

This procedure includes extraction methods for low-speed and optional high-speed centrifugation. High-speed centrifugation may be necessary depending on study requirements (e.g. if the selected test species have low tolerance to suspended particles). Sediment pore water extraction yields samples that may be used in toxicological or chemical contaminant evaluations of the pore water. This method employs centrifugation to remove pore water from the interstitial spaces of the sediment by gravitational force, without filtration. This procedure can also be employed to clarify sediment elutriates for toxicological or chemical contaminant assessment.

3.0 REFERENCES

Environment Canada. 1994. Guidance document on collection and preparation of sediments for physicochemical characterization and biological testing. Environmental Protection Series. Report EPS 1/RM/29, December 1994, 132 pp.

U.S. Environmental Protection Agency. 1995. QA/QC Guidance for Sampling and analysis of sediments, water, and tissues for dredged material evaluations (chemical evaluations). EPA 832-B-95-002. Office of Water, Washington, D.C.

Diamond, Jerome, Allen Burton, and John Scott. 2001. Methods for Collecting, Storing, and Manipulating Sediments and Interstitial Water Samples for Chemical and Toxicological Analyses. Draft, submitted to Water Environment Federation.

4.0 PROCEDURES

4.1 EQUIPMENT AND SUPPLIES

- Centrifuge, refrigerated, 6 L capacity, 7400 g
- Centrifuge, refrigerated, 3 L capacity, 10,000 g

- Rotor head
- Centrifuge bottles, 1 L or 0.5 L
- Sample bottles and labels
- Deionized water

4.2 PREPARATION

Sediments used for pore water analysis are to be processed to remove the pore water as soon as possible after collection to minimize effects due to chemical changes. Extraction of pore water within 24 hours of collection is recommended, but in no case should the sediments be stored longer than two weeks from collection before extraction.

4.3 LOW SPEED EXTRACTION

1. Homogenize the collected sediment samples and place in 1-L HDPE or polycarbonate centrifuge bottles.
2. Centrifuge samples for 15-30 minutes at 7400 xg (5200 rpm) using a refrigerated centrifuge.
3. Carefully decent the supernatant (pore water) into appropriate containers for storage. Store pore water samples at $4^{o}C$ in the dark until used for testing.

4.4 HIGH SPEED EXTRACTION

1. Depending on the study requirements, extracted pore water may be re-centrifuged for 30 minutes at 10, 000 xg to further remove suspended particulate material.
2. The supernatant (pore water) is carefully decanted into appropriate containers for storage. The samples should be stored at $4^{o}C$ in the dark until used for testing.

5.0 ATTACHMENTS

Attachment I – Centrifuge volume log.

Attachment I
Centrifuge volume log.

Project:	Date:	Initials:
Centrifuge/speed:	sediment per bottle (g):	No. bottles:
Lab Sample ID	**Centrifuge Start/End Time**	**Volume generated (mL) per centrifuge bottle**

TIE-300 TIE SOPs for sample manipulation.

1.0 PURPOSE

This procedure describes the preparation and use of hypersaline brine (HSB).

2.0 SCOPE

The HSB yielded by this procedure is intended for use in manipulating pore water samples in preparation for the TIE manipulations and subsequent toxicity tests.

3.0 REFERENCES

U.S. Environmental Protection Agency. 1988. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. EPA Office of Research and Development EPA/600/4-87/028 (May 1988).

4.0 PROCEDURES

4.1 EQUIPMENT AND SUPPLIES FOR BRINE PREPARATION

- Container for making HSB--the ideal container for making brine from natural seawater is one that (1) has a high surface-to-volume ratio, (2) is made of a non-corrosive material, and (3) is easily cleaned (fiberglass containers are ideal).
- Uncontaminated source of natural seawater.
- Heat source--used to accelerate evaporation of brine.
- Aerator--used to accelerate evaporation of brine.
- Portable containers for storage of brine--carboys, Cubitainers®, or equivalent.
- Teflon-lined pump--for collecting brine and dispensing into storage containers.
- Filter apparatus with 10um filter for incoming seawater and 1um filter for brine.
- Refractometer--for monitoring the salinity of seawater and brine.
- Thermometer--for monitoring the temperature of brine.

4.2 EQUIPMENT AND SUPPLIES FOR BRINE USE

- Water purification unit—for generation of deionized water or equivalent.
- Graduated cylinders--assorted sizes, for measuring volumes of HSB, deionized water, and effluent or receiving water.

- Beakers--assorted sizes, for mixing HSB, deionized water, and effluent or receiving water.

4.3 METHODS FOR BRINE PREPARATION

1. Before adding seawater to the brine generator, thoroughly clean the generator, aeration supply tube, heater, and any other materials that will be in direct contact with the brine. A good quality biodegradable detergent should be used followed by several (at least three) thorough deionized water rinses.

2. High quality (and preferably high salinity) seawater should be filtered to at least 10 um before placing into the brine generator. Water should be collected on an incoming tide to minimize the possibility of contamination.

3. The temperature of the seawater is increased slowly to 40°C. The water should be aerated to prevent temperature stratification and to increase water evaporation. The brine should be checked daily (depending on volume being generated) to ensure that the salinity does not exceed 100o/oo and that the temperature does not exceed 40°C. Additional seawater may be added to the brine to obtain the volume of brine required.

4. After the desired salinity is attained (100o/oo), the brine should be filtered a second time through a 1 um filter and poured directly into portable containers, such as 20-L (5 gal) Cubitainers® or polycarbonate water cooler jugs. The containers should be capped and labeled with the date the brine was generated and its salinity. Containers of brine should be stored in the dark and maintained at room temperature until used. When stored in this manner, HSB may be held for prolonged periods without apparent degradation.

5. If a source of prepared HSB is already available, test solutions can be made by following the directions below. Thoroughly mix together the deionized water and brine before adding the effluent.

4.4 METHODS FOR BRINE USE

1. Divide the salinity of the hypersaline brine by the expected test salinity to determine the proportion of deionized water to brine. For example, if the salinity of the brine is 100 ^{o}/oo and the test is to be conducted at 20 ^{o}/oo, 100 ^{o}/oo divided by 20 ^{o}/oo = 5.0. The proportion of brine is 1 part in 5 (one part brine to four parts deionized water).

2. To make 1 L of seawater at 20 ^{o}/oo salinity from a hypersaline brine of 100 ^{o}/oo, divide 1 L (1000 mL) by 5.0. The result, 200 mL, is the quantity of brine needed to make 1 L of seawater. The difference, 800 mL, is the quantity of deionized water required.

4.5 TROUBLESHOOTING

Special care should be used to prevent any toxic materials from coming in contact with the seawater being used to generate the brine. If a heater is immersed directly into the seawater, ensure that the heater materials do not corrode or leach any substances that would contaminate the brine. One successful method used is a thermostatically controlled heat exchanger made from fiberglass. If aeration is used, use only oil-free air compressors to prevent contamination.

TIE-400 TIE SOPs for TIE manipulation.

SCIENCE APPLICATIONS INTERNATIONAL CORPORATION
TIE STANDARD OPERATING PROCEDURE

1.0 PURPOSE

This procedure establishes protocols for preparing pore water to serve as the untreated TIE manipulation for baseline toxicity testing.

2.0 SCOPE

This procedure applies to pore water samples extracted from collected sediments via syringe or centrifuge extraction methods.

3.0 REFERENCES

Procedures are based on references cited in general procedure TIE-100.

4.0 PROCEDURES

4.3 EQUIPMENT AND SUPPLIES

- Sub-sample bottles for TIE manipulation
- Lab sub-sample bottles
- Lab COC forms
- Scintillation vials

4.4 DECONTAMINATION PROCEDURES

Wash all glassware to be used during this procedure by following the rinse steps listed below:

1. Alconox
2. Tap water rinse
3. DI rinse
4. 10% Nitric Acid rinse
5. DI rinse
6. Methanol rinse
7. DI rinse x3

4.5 MANIPULATION PROCEDURES

1. Fill 1 scintillation vial with sample. Label and refrigerate for later baseline testing of water quality.
2. Fill, preserve, and label any subsamples needed for laboratory testing of chemical components. Record on COC and ship to lab.

3. Fill a 6 oz bottle (~185 mL), leaving no headspace, with the sample. Label and refrigerate until ready to distribute to vials.

1.0 PURPOSE

The purpose of this procedure is to prepare the cationic metals and oxidants reduction treatment.

2.0 SCOPE

Sodium thiosulfate ($Na_2S_2O_3$) will be used to reduce oxidants such as chlorine, ozone, chlorine dioxide, mono and dichloramines, bromine, iodine, manganous ions, and some electrophilic organic chemicals and to remove cationic metals including Cd^{2+}, Cu^{2+}, Ag^{1+}, and Hg^{2+} in the pore water samples. Reduced toxicity or a non-toxic response will indicate oxidants or cationic metals as contributors to toxicity.

3.0 REFERENCES

Procedures based on references cited in general procedure TIE-100.

4.0 PROCEDURES

4.1 EQUIPMENT AND SUPPLIES

- Stock solution: 2.35g Sodium Thiosufate* $5H_2O$/100 mL Deionized water (DI). This = 15g Sodium Thiosufate/L, or 94.9 mM
- Graduated cylinders
- Pipette
- Sub-sample bottles
- Alconox
- a supply of Deionized water (DI)
- 10% Nitric Acid

4.2 DECONTAMINATION PROCEDURES

Wash all equipment and glassware to be used in this procedure by following these rinse steps:

1. Alconox
2. Tap water rinse
3. DI rinse
4. 10% Nitric Acid rinse
5. DI rinse, 3x

4.3 MANIPULATION STEPS

1. Measure the volume of sample in a graduated cylinder and record.
2. For every mL of sample you have, add 3.4 µL of stock solution to it.
   *((mL sample) * (3.4 µL))/1000 = mL of Sodium Thiosulfate to add*
3. Cap and shake sample to mix.
4. Let sit for 1 hour.
5. Sub-sample into 6 oz (~185mL) bottles leaving no headspace. Label and refrigerate until ready to distribute to vials.

1.0 PURPOSE

The purpose of this procedure is to prepare the cationic metals chelation treatment.

2.0 SCOPE

Samples will be subjected to EDTA chelation to remove divalent cationic metals (*i.e.,* Al^{2+}, Ba^{2+}, Fe^{2+}, Mn^{2+}, Sr^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, Cd^{2+}, Co^{2+}, and Zn^{2+}). A non-toxic response or a partial reduction in toxicity indicates metals as a toxic component of the pore water. A fully or partially toxic response indicates that something other than divalent cationic metallic compounds is a contributor to sediment toxicity.

3.0 REFERENCES

Procedures based on references cited in general procedure TIE-100.

4.0 PROCEDURES

4.1 EQUIPMENT AND SUPPLIES

- Stock solution: 2.78g EDTA*$2H_2O$/100 mL Deionized water (DI).
- This = 25g EDTA/L or 74.4 mM
- Graduated cylinders
- Pipette
- Sub-sample bottles
- Alconox
- a supply of Deionized water (DI)
- 10% Nitric Acid

4.2 DECONTAMINATION PROCEDURES

Wash all equipment to be used during this manipulation by following these rinse steps:

1. Alconox
2. Tap water rinse
3. DI rinse
4. 10% Nitric Acid rinse
5. DI rinse, 3x

4.3 MANIPULATION PROCEDURE

1. Measure the volume of sample in a graduated cylinder and record.
2. For every mL of sample you have, add 2.4 µL of stock solution to it.
   *((mL sample) * (2.4 µL))/1000 = mL of Sodium EDTA to add*
3. Cap and shake sample to mix.
4. Let sit for 3 hour
5. Sub-sample into 6 oz (~185mL) bottles leaving no headspace. Label and refrigerate until ready to distribute to vials.

1.0 PURPOSE

This procedure is conducted to extract the particulate fraction from TIE samples for the purpose of reducing toxicity associated with this fraction.

2.0 SCOPE

This procedure establishes sample filtration methods for preparation of the particulate fraction extraction treatment.

3.0 REFERENCES

Procedures based on references cited in general procedure TIE-100.

4.0 PROCEDURES

4.1 EQUIPMENT AND SUPPLIES

- Filtration flask(s)
- Millipore filtering setup(s)
- 47mm, 0.45µm, general purpose filter *(e.g. Fisher Catalog #09-719-2E) or Durapore hydrophobic*
- Vacuum pump(s) with tubing
- Petri Dishes
- Sub-sampling bottles
- Alconox
- 10% Nitric Acid
- Methanol
- Deionized Water (DI)

4.2 DECONTAMINATION PROCEDURES

Wash all flasks and filtering apparatus pieces to be used in this procedure by following these rinse steps:

1. Alconox
2. Tap water rinse
3. DI rinse
4. 10% Nitric Acid rinse
5. DI rinse
6. Methanol rinse
7. DI rinse x3

4.6 MANIPULATION PROCEDURE

1. Setup the filtering apparatus, and attach to a vacuum pump.
2. Place a filter onto the frittered support using a pair of forceps.
3. Turn on pump, and slowly pour sample into filter setup.
4. When the process slows to a drip, meaning the filter is full, continue to apply the vacuum till the filter is dry.
5. Turn off the pump, and remove the filter using forceps. Place upright into a labeled plastic petri dish.
6. Continue this until the entire sample has been filtered. All filters used can be placed in the same petri dish.
7. Rinse the original sample container 3x with DI, and then pour the processed sample back into it.
8. Sub-sample into a 6 oz bottle (~185 ml) leaving no headspace. Label and refrigerate until ready to distribute to vials.
9. *Unless samples are very silty, takes about 30 min per sample.*

1.0 PURPOSE

The purpose of this procedure is to prepare the organic fraction extraction treatment by passing the sample through a C_{18} column.

2.0 SCOPE

Pore water samples will be subjected to C_{18} extraction to remove organic compounds and metals that are relatively non-polar. A non-toxic response in these exposures will indicate the potential role of organic compounds as the sole contributor to toxicity of pore waters. A fully toxic response will indicate that organic compounds are not responsible for observed pore water toxicity. A partial reduction in toxicity would define a joint toxic action by organic compounds and other factors.

3.0 REFERENCES

Procedures based on references cited in general procedure TIE-100.

4.0 PROCEDURES

4.1 EQUIPMENT AND SUPPLIES

- Peristaltic pump and tubing
- C_{18} columns
- Deionized water (DI)
- Methanol

4.2 PUMP PREPARATION

1. Connect tubing to pump, and set rate for 10 mL/min.

4.3 COLUMN PREPARATION

1. Pump 25 mL DI water through the tubing.
2. Pump 25 mL Methanol through the tubing.
3. Attach an C_{18} column. Attach the shorter of the two ends to the tubing.
4. Pump 25 mL Methanol through the tubing and column.
5. Pump 25 mL DI through the tubing and column. Stop the pump with the tubing still filled with DI so the column does not dry out.

4.4 SAMPLE PROCESSING

1. Check and record sample pH.
2. Pour sample into 1000 mL, pre-washed flasks.
3. Rinse out sample container with DI, Methanol 1x, DI 3x, and place under the C_{18} column to collect the processed sample.
4. Place one flask under the other end, and pump no more than 1000 mL of sample through the column. Keep an eye on the discoloration that will occur in the column. If that discoloration advances past ½ of the column length, replace it (see following step).
5. Once 1000 mL of sample has been pumped through, remove the column, and repeat the column prep part of this procedure. The tubing only needs to be re-preped between samples.
6. Repeat proceeding step until entire sample has been processed.
7. Recheck sample pH, and adjust using 1N HCL and/or 1N NaOH back to the pre-C_{18} filtered value.
8. Sub-sample into 6 oz (~185mL) bottles leaving no headspace. Label and refrigerate until ready to distribute to vials.

4.5 TIME REQUIREMENTS

- Column prep: 10 – 15 min
- 100 mL can be processed in 10 min
- 1h 50 min required to fully prep and process 1000 mL
- *Time varies accordingly to pore water composition:
  - High amounts of organics = longer time
  - Low amounts of organics = shorter time

SCIENCE APPLICATIONS INTERNATIONAL CORPORATION
TIE STANDARD OPERATING PROCEDURE

1.0 PURPOSE

The purpose of this procedure is to prepare the organic fraction extraction treatment by passing the sample through an Oasis® brand cartridge filter.

2.0 SCOPE

Pore water samples will be subjected to Oasis (HLB) extraction to remove organic compounds and metals that are relatively non-polar. A non-toxic response in these exposures will indicate the potential role of organic compounds as the sole contributor to toxicity of pore waters. A fully toxic response will indicate that organic compounds are not responsible for observed pore water toxicity. A partial reduction in toxicity would define a joint toxic action by organic compounds and other factors.

3.0 REFERENCES

Procedures based on references cited in general procedure TIE-100.

4.0 PROCEDURES

4.1 EQUIPMENT AND SUPPLIES

- Peristaltic pump and tubing
- Oasis® (HLB) columns
- Deionized water (DI)
- Methanol

4.2 MANIPULATION STEPS

1. Connect tubing to pump, and set rate for 10 mL/min.

4.3 COLUMN PREP

1. Pump 25 mL DI water through the tubing.
2. Pump 25 mL Methanol through the tubing.
3. Attach an Oasis column. Attach the shorter of the two ends to the tubing.
4. Pump 25 mL Methanol through the tubing and column.
5. Pump 25 mL DI through the tubing and column. Stop the pump with the tubing still filled with DI so the column does not dry out.

4.4 SAMPLE PROCESSING

1. Check and record sample pH.
2. Pour sample into 1000 mL, pre-washed flasks.
3. Rinse out sample container with DI, Methanol 1x, DI 3x, and place under the Oasis column to collect the processed sample.
4. Place one flask under the other end, and pump no more than 1000 mL of sample through the column. Keep an eye on the discoloration that will occur in the column. If that discoloration advances past ½ of the column length, replace it (see following step).
5. Once 1000 mL of sample has been pumped through, remove the column, and repeat the column prep part of this procedure. The tubing only needs to be re-preped between samples.
6. Repeat proceeding step until entire sample has been processed.
7. Recheck sample pH, and adjust using 1N HCL and/or 1N NaOH back to the pre-Oasis filtered value.
8. Sub-sample into 6 oz (~185mL) bottles leaving no headspace. Label and refrigerate until ready to distribute to vials.

4.5 TIME REQUIREMENTS

- Column prep: 10 – 15 min
- 100 mL can be processed in 10 min
- 1h 50 min required to fully prep and process 1000 mL
- *Time varies accordingly to pore water composition:
  - High amounts of organics = longer time
  - Low amounts of organics = shorter time

1.0 PURPOSE

The purpose of this procedure is to prepare the Low pH manipulation by using pH adjustment to induce ammonia and sulfide ionic shift.

2.0 SCOPE

In this procedure, sample pH is manipulated to determine if pH dependent toxicants such as speciated metals, ammonia, hydrogen sulfide, cyanide and some ionizable organic compounds (*e.g.*, pentachlorphenol) are responsible for observed toxicity. For instance, if sample toxicity increases with increasing pH, toxicants such as ammonia are suspected. Conversely, if sample toxicity increases with decreasing sample pH, toxicants such as hydrogen sulfide are suspected. Typical pH adjustments include 1.5 pH units above and below ambient pH (e.g., pH 6 and pH 9, for ambient pH = 7.5 ; or pH 6 and pH 7 for ambient pH 8).

3.0 REFERENCES

Procedures based on references cited in general procedure TIE-100.

4.0 PROCEDURES

4.1 EQUIPMENT AND SUPPLIES

- MOPS buffer
- disposable plastic drinking cups
- a supply of deionized water (DI)

4.2 PREPARATION OF MANIPULATED SAMPLE

1. Prepare stock solution by adding 1 pouch of MOPS to 250 mL of DI water. This results in a 0.4M (400 mM) solution.
2. Sub-sample 185 mL of sample into a labeled, DI rinsed disposable plastic cup.
   The volume of sample used here is the amount needed to fill the all the vials at this step.
3. Measure the pH, and record.
4. Add 8.325 mL of the stock solution to the cup and stir.
   This volume represents 45 µL of stock solution per mL of sample to be manipulated.

5. Measure and record the pH value.
6. Distribute sample into vials per the *Water Volume Calculation* sheet.

4.3 PREPARATION OF PERFORMANCE CONTROL AND DILUTION WATER

1. Sub-sample 2470 mL from the original sample into a separate container.
   This volume represents the total volume needed to fill PC vials and perform dilutions on the remaining 11 samples.
2. Measure and record the pH.
3. Add 111.15 mL of stock solution and mix.
   This volume represents 45 µL of stock solution per mL of sample to be manipulated.
4. Measure and record the pH.
5. Distribute sample into vials per the *Water Volume Calculation* sheet.

1.0 PURPOSE

The purpose of this procedure is to prepare the High pH manipulation by using pH adjustment to induce ammonia and sulfide ionic shift.

2.0 SCOPE

In this procedure, sample pH is manipulated to determine if pH dependent toxicants such as speciated metals, ammonia, hydrogen sulfide, cyanide and some ionizable organic compounds (*e.g.*, pentachlorphenol) are responsible for observed toxicity. For instance, if sample toxicity increases with increasing pH, toxicants such as ammonia are suspected. Conversely, if sample toxicity increases with decreasing sample pH, toxicants such as hydrogen sulfide are suspected. Typical pH adjustments include 1.5 pH units above and below ambient pH (e.g., pH 6 and pH 9, for ambient pH = 7.5; or pH 6 and pH 7 for ambient pH 8).

3.0 REFERENCES

Procedures based on references cited in general procedure TIE-100.

4.0 PROCEDURES

4.1 EQUIPMENT AND SUPPLIES

- 1 M NaOH
- pH meter

4.2 METHOD

1. Add 1 M NaOH in 10 µL aliquots to TIE sample; after each addition, swirl sample and measure pH.
2. Continue to add NaOH and monitor pH until target pH (e.g. 8.8) is obtained. Target pH should generally be 0.1-0.3 higher than conditions desired for testing (e.g. 8.5). Note that saltwater samples generally incur greater drift than freshwater samples.
3. Measure pH immediately prior to testing.
4. Develop a schedule to adjust pH as required in order to keep within desired range of species being tested.
5. Sub-sample bottles for TIE manipulation

4.3 ALTERNATE METHOD

- Conduct exposures in lidded boxes with wicked KOH solution.

1.0 PURPOSE

The purpose of this procedure is to prepare the ammonia removal treatment by using zeolite to remove ammonia from freshwater TIE samples.

2.0 SCOPE

This document describes the procedures used to treat aquatic samples with zeolite. Treatments to manipulate pH may be conducted independently and simultaneously prior to zeolite treatment. Actual volumes needed for analyses and biological testing and holding requirements are task-specific and require consultation with the Project Manager.

3.0 REFERENCES

Procedures based on references cited in general procedure TIE-100.

4.0 PROCEDURES

4.1 EQUIPMENT AND SUPPLIES

- Large plastic disposable cups
- Zeolite (e.g. Ammonex; Argent Chemicals)
- Millipore filter setup
- General purpose filter paper (same used in sample filtration step)
- Alconox
- Deionized water (DI)

4.2 DECONTAMINATION PROCEDURES

Wash all glassware and containers to be used in this manipulation by following these rinse steps:

1. Alconox
2. Tap water rinse
3. DI rinse, 3x
   All metals and organics should be removed at this time, so there is no need for rinses with Methanol or 10% nitric.

4.3 MANIPULATION PROCEDURES

1. Pour sample into a DI rinsed disposable plastic drinking cup.

2. Make sure that you have a minimum volume equal to half of the amount that you will need to fill the vials. The zeolite in this step absorbs at least half of the manipulated volume.
3. Slowly add zeolite to the cup until it reaches just below the water line. Allow sample to sit for a few moments, and then top off such that there is no standing water and the entire sample is in contact with the zeolite.
4. Allow sample to sit for a minimum of 4 hours.
5. Put together the Millipore setup.
6. After 4 hours, place a gloved hand over the cup opening, and pour off the water into the Millipore setup and filter.
7. Use 1 mL of sample to measure the ammonia content, which should be below 1 mg/L. Record on datasheet. If ammonia is to high, return to original zeolite and allow sample to sit again.
8. Fill a 6 oz bottle (~185 mL), with no headspace. Label and refrigerate for later distribution to vials. If enough sample remains, fill a scintillation vial as well for confirmatory post treatment ammonia measurement.

1.0 PURPOSE

The purpose of this procedure is to prepare the ammonia removal treatment by using the seaweed *Ulva lactuca* to remove ammonia from saltwater TIE samples.

2.0 SCOPE

This document describes the procedures used to treat aquatic samples with *Ulva*. Treatments to manipulate pH may be conducted independently and simultaneously prior to Ulva treatment. Actual volumes needed for analyses and biological testing and holding requirements are task-specific and require consultation with the Project Manager.

3.0 REFERENCES

Procedures based on methods described in:

Pelletier, M.C., Ho, K.T., Cantwell, M., Kuhn-Hines, A., Jayaraman, S. and Burgess, R.M. 2001. Use of *Ulva lactuca* to Idenfity Ammonai Toxicity in Marine and Estuarine Sediments. Environ. Toxicol. Chem., 20:12, pp. 2852-2859.

4.0 PROCEDURES

4.1 EQUIPMENT AND SUPPLIES

- 250 mL beakers
- *Ulva*
- Glass stirring rod
- Alconox
- Deionized water (DI)

4.2 DECONTAMINATION PROCEDURES

Wash all glassware and containers to be used in this manipulation by following these rinse steps:

1. Alconox
2. Tap water rinse
3. DI rinse, 3x

   All metals and organics should be removed at this time, so there is no need for rinses with Methanol or 10% nitric.

4.3 MANIPULATION PROCEDURES

1. Pour sample into a DI rinsed 250 mL beaker.
2. Dry pieces of *Ulva* by gently patting with a paper towel.
3. Using a scale, weigh out 13 g (or approximately 0.6 mg/L) of *Ulva* to be placed in the sample
4. Add *Ulva* to sample, making sure that all *Ulva* is completely submerged within the sample.
5. Allow to sit for a minimum of 4 hours.
6. After 4 hours, carefully remove the sample from the beaker, placing it back into its respective (Nalgene®) 6 oz bottle making sure not to remove any *Ulva*.
7. Use 1 mL of sample to measure the ammonia content, which should be below 1 mg/L. Record on datasheet.
8. Fill a 6 oz bottle (~185 mL), with no headspace. Label and refrigerate for later distribution to vials. If enough samples remain, fill a scintillation vial as well for confirmatory post treatment ammonia measurement.

TIE-500 TIE SOPs for toxicity testing.

1.0 PURPOSE

This procedure outlines methods for conducting a sea urchin (*Arbacia punctulata*) sperm cell fertilization test. This toxicity test is commonly utilized to assess the effectiveness of TIE manipulations in reducing/removing toxicity. control.

2.0 SCOPE

The purpose of the sperm cell toxicity test is to determine the concentration of a test substance that reduces fertilization of exposed gametes relative to that of the This method measures the toxicity of TIE-manipulated samples to the gametes of the sea urchin, *Arbacia punctulata*, during a 1 h and 20 min exposure.

3.0 REFERENCES

Dunnett, C.W. 1955. A multiple comparisons procedure for comparing several treatments with a control. JASA 50:1096-1101.

Hamilton, M.A., R.C. Russo, and R.V. Thurston. 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol. 11(7):714-719.

US EPA. 1988. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. Weber, C.I., et al (eds). EPA Office of Research and Development EPA-600/4-87/028 (May 1988).

4.0 PROCEDURES

4.1 EQUIPMENT AND SUPPLIES

- Facilities for holding and acclimating test organisms.
- Laboratory sea urchin culture unit -- See SOP on Culture. To test effluent or receiving water toxicity, sufficient eggs and sperm must be available.
- Environmental chamber or equivalent facility with temperature control ($20+1^{O}C$) for controlling temperature during exposure.
- Water purification system -- Millipore Super-Q, Deionized water (DI) or equivalent.
- Balance -- Analytical, capable of accurately weighing to 0.000l g.
- Reference weights, Class S -- for checking performance of balance.
- Air pump -- for supplying air.

- Air lines, and air stones -- for aerating water-containing adults.
- Vacuum suction device -- for washing eggs.
- pH and DO meters -- for routine physical and chemical measurements. Unless the test is being conducted to specifically measure the effect of one of these two parameters, portable, field-grade instruments are acceptable.
- Transformer, 10-12 Volt, with steel electrodes -- for stimulating release of eggs and sperm.
- Centrifuge, bench-top, slant-head, variable speed -- for washing eggs.
- Fume hood -- to protect the analyst from formaldehyde fumes.
- Dissecting microscope -- for counting diluted egg stock.
- Compound microscope -- for examining and counting sperm cells and fertilized eggs.
- Sedgwick-Rafter counting chamber -- for counting egg stock.
- Hemacytometer, Neubauer -- for counting sperm.
- Count register, 2-place -- for recording sperm and egg counts.
- Refractometer -- for determining salinity.
- Thermometers, glass or electronic, laboratory grade -- for measuring water temperatures.
- Thermometers, bulb-thermograph or electronic-chart type -- for continuously recording temperature.
- Ice bucket, covered -- for maintaining live sperm.
- Centrifuge tubes, conical, 15 mL -- for washing eggs.
- Cylindrical glass vessel, 8-cm diameter -- for maintaining dispersed egg suspension.
- Beakers -- six Class A, borosilicate glass or non-toxic plasticware, 1000 mL for making test solutions.
- Glass dishes, flat bottomed, 20-cm diameter -- to hold adults during gamete collection.
- Wash bottles -- for deionized water, for rinsing small glassware and instrument electrodes and probes.
- Volumetric flasks and graduated cylinders -- Class A, borosilicate glass or non-toxic plastic labware, l0-l000 mL for making test solutions.
- Syringes, 1-mL, and 10-mL, with 18-gauge, blunt-tipped needles (tips cut off) -- for collecting sperm and eggs.
- Pipets, volumetric -- Class A, 1-100 mL.
- Pipets, automatic -- adjustable, 1-100 mL.
- Pipets, serological -- 1-10 mL, graduated.
- Pipet bulbs and fillers -- PROPIPETR, or equivalent.
- Tape, colored -- for labelling tubes.
- Markers, waterproof -- for marking containers, etc.
- Sea Urchins (approximately 12 of each sex).
- Scintillation vials, 20 mL, disposable -- to prepare test concentrations.
- Parafilm -- to cover tubes and vessels containing test materials.
- Gloves, lab coat, disposable -- for personal protection from contamination.
- Safety glasses

- Data sheets (one set per test) -- for data recording (Figure 1).
- Acetic acid, 10%, reagent grade, in sea water -- for preparing killed sperm dilutions.
- Formalin, 10% in seawater -- for preserving eggs.
- pH buffers 4, 7, and l0 (or as per instructions of instrument manufacturer) for standards and calibration check.
- Membranes and filling solutions for dissolved oxygen probe or reagents for modified Winkler analysis.
- Laboratory quality assurance samples and standards for the above methods.
- Reagent water -- defined as distilled or deionized water that does not contain substances that are toxic to the test organisms.
- Effluent, surface water, and dilution water.
- Saline test and dilution water -- The salinity of the test water must be 30 ^{O}/oo. The salinity should vary by no more than + 2 ^{O}/oo among the replicates.

4.2 SAMPLE PREPARATION PROCEDURES

1. Samples are used directly as collected when sample salinity is between 28 and 32 ppt. If samples do not require salinity adjustment, natural seawater is used in all washing and diluting steps and as control water. Local water from an uncontaminated area may be used as an additional control.

2. If salinity adjustment is required, prepare 3 L of control water at 30 ^{O}/oo using hypersaline brine (see TIE SOP on Preparation of Brine). This water is used in all washing and diluting steps and as control water in the test. Natural sea water and uncontaminated local waters may be used as additional controls.

3. Effluent/receiving water samples are adjusted to salinity of 30 ^{O}/oo using hypersaline brine as necessary.

4. The selection of the effluent test concentrations should be based on the objectives of the study. A dilution factor of 0.5 is used with this procedure, starting with a high concentration of 70% effluent (for freshwater effluents). If the effluent is known or suspected to be highly toxic, a lower range of effluent concentrations should be used.

5. Three replicates are prepared for each test concentration, using 5 mL of solution in disposable liquid scintillation vials. A 50% (0.5) concentration series can be prepared by serially diluting test concentrations with control water.

6. All test samples are equilibrated at 20 + 1^{O}C before addition of sperm.

4.3 COLLECTION AND PREPARATION OF GAMETES FOR TEST

1. Select four females and place in shallow bowls, barely covering the shell with seawater. Stimulate the release of eggs by touching the test with electrodes from the transformer. Collect about 3 mL of eggs from each female using a 10 cc syringe with a blunted needle. Remove the needle from the syringe before adding the eggs to a 15 mL conical centrifuge tube. Pool the eggs. The egg stock may be held at room temperature for several hours before use. Note: The egg suspension may be prepared during the l-h sperm exposure.

2. Select four males and place in shallow bowls, barely covering the animals with seawater. Stimulate the release of sperm by touching the shell with steel electrodes connected to a 10 - 12 V transformer (about 30 seconds each time). Collect the sperm (about 0.25 mL) from each male, using a l mL disposable syringe fitted with an 18-gauge, blunt-tipped needle. Maintain the syringe containing pooled sperm sample on ice. The sperm must be used in a toxicity test within 1 h of collection.

3. Using control water, dilute the pooled sperm sample to a concentration of about 5 X l07 sperm/mL (SPM). Estimate the sperm concentration as described below:

a. Make a sperm dilutions of 1:50, 1:100, 1:200, and 1:400, using 30O/oo seawater, as follows:

1. Add 400 uL of collected sperm to 20 mL of sea water in Vial A. Cap Vial A and mix by inversion.

2. Add 10 mL of sperm suspension from Vial A to 10 mL of seawater in Vial B. Cap Vial B and mix by inversion.

3. Add 10 mL of sperm suspension from Vial B to 10 mL of seawater in Vial C. Cap Vial C and mix by inversion.

4. Add 10 mL of sperm suspension from Vial C to 10 mL of seawater in Vial D. Cap Vial D and mix by inversion.

5. Discard 10 mL from Vial D. (The volume of all suspensions is 10 mL).

b. Make a 1:2000 killed sperm suspension and determine the SPM.

1. Add 10 mL 10% acetic acid in seawater to Vial C. Cap Vial C and mix by inversion.

2. Add 1 mL of killed sperm from Vial C to 4 mL of seawater in Vial E. Mix by gentle pipetting with a 4-mL pipetter.

3. Add sperm from Vial E to both sides of the Neubauer hemacytometer. Let the sperm settle 15 min.

4. Count the number of sperm in the central 400 squares on both sides of the hemacytometer using a compound microscope (400X). Average the counts from the two sides.

5. SPM in Vial E = 104 x average count.

c. Calculate the SPM in all other suspensions using the SPM in Vial E above:

SPM in Vial A = 40 x SPM in Vial E
SPM in Vial B = 20 x SPM in Vial E
SPM in Vial D = 5 x SPM in Vial E
SPM in original sperm sample = 2000 x SPM in Vial E

d. Dilute the sperm suspension with a sperm concentration greater than 5 x 107 SPM to 5 x 107 SPM.

Actual SPM/(5 x 107) = dilution factor (DF)

[(DF) x 10] - 10 = mL of seawater to add to vial.

e. Confirm the sperm count by sampling from the test stock. Add 0.1 mL of test stock to 9.9 mL of 10% acetic acid in seawater, and count with the hemacytometer. The count should average 50 + 5.

4. Wash the pooled eggs three times using control water with gentle centrifugation (500xg or lowest possible setting) for 3 min using a tabletop centrifuge. If the wash water becomes red, the eggs have lysed and must be discarded.

a. Dilute the egg stock, using control water, to 2000 + 200 eggs/mL.

1. Remove the final wash water and transfer (by filling the centrifuge tube with control water and repeatedly inverting to resuspend the eggs) the washed eggs to a beaker containing a small volume (about 50 mL) of control water. Add more control water to bring the eggs to a volume of 200 mL ("egg stock").

2. Mix the egg stock using gentle aeration. Cut the point from a pipet tip, then transfer l mL of eggs from the egg stock to a vial

containing 9 mL of control water. (This vial contains an egg suspension diluted l:l0 from egg stock).

3. Mix the contents of the vial using gentle pipetting. Cut the point from a pipet tip, then transfer l mL of eggs from the vial to a Sedgwick-Rafter counting chamber. Count all eggs in the chamber using a dissecting microscope ("egg count").

4. Calculate the concentration of eggs in the stock. Eggs/mL = l0x (egg count). Dilute the egg stock to 2000 eggs mL by the formula below.

b. If the egg count is equal to or greater than 200:

(egg count) - 200 = volume (mL) of control water to add to egg stock

c. If the egg count is less than 200, allow the eggs to settle and remove enough control water to concentrate the eggs to greater than 200, repeat the count, and dilute the egg stock as above.

NOTE: It requires 18 mL of a egg stock solution for each test with a control and five exposure concentrations (three replicates).

d. Transfer 1 mL of the diluted egg stock to a vial containing 9 mL of control water. Mix well, then transfer 1 mL from the vial to a Sedgwick-Rafter counting chamber. Count all eggs using a dissecting microscope. Confirm that the final egg count = 200 + 20 per mL.

4.4 TEST START PROCEDURES

1. Within 1 h of collection add 100 uL of appropriately diluted sperm to each test vial. Record the time of sperm addition.

2. Incubate all test vials at 20 + 1OC for 1 h.

3. Mix the diluted egg suspension (2000 eggs/mL), using gentle bubbling. Add 1 mL of diluted egg suspension to each test vial using a wide mouth pipet tip. Incubate 20 min at 20 + 1^{O}C.

4.5 TEST TERMINATION PROCEDURES

1. Terminate the test and preserve the samples by adding 2 mL of l0% formalin in seawater to each vial.

2. Vials may be evaluated immediately or capped and stored for as long as one week before being evaluated.

3. To determine fertilization, transfer about 1 mL eggs from the bottom of a test vial to a Sedgwick-Rafter counting chamber. Observe the eggs using a compound microscope (100 X). Count about 100 eggs/sample. Record the number counted and the number unfertilized. Fertilization is indicated by the presence of a fertilization membrane surrounding the egg. Adjustment of the microscope to obtain proper contrast may be required to observe the fertilization membrane.

NOTE: Because samples are fixed in formalin, a ventilation hood is set-up surrounding the microscope to protect the analyst from prolonged exposure to formaldehyde fumes.

5.0 ACCEPTABILITY OF TEST RESULTS

The sperm:egg ratio routinely employed should result in fertilization of at least 50% of the eggs in the control chambers.

6.0 TROUBLESHOOTING

Toxic substances may be introduced by contaminants in dilution water, glassware, sample hardware, and testing equipment.

7.0 STATISTICAL ANALYSIS AND DATA USAGE

1. Tabulate and summarize the data. Calculate the percent of unfertilized eggs for each replicate.

2. The endpoints of toxicity tests using the sea urchin are based on the reduction in percent of eggs fertilized. An estimate of the effluent concentration which would cause a 50% reduction in egg fertilization (EC50) is calculated using Trimmed Spearman-Karber analysis (Hamilton, Russo, and Thurston, 1977). Dunnett's Procedure (Dunnett, 1955) is used to estimate no effect and least effect concentrations (NOEC and LOEC values).

3. Data are used along with other toxicity tests in assessing the toxicity of an effluent or receiving water.

1.0 PURPOSE

This procedure describes methods for conducting survival tests with the mysid, *Americamysis bahia*. These toxicity tests are commonly utilized to assess the effectiveness of TIE manipulations in reducing/removing toxicity.

2.0 SCOPE

This procedure describes the preparation of test samples, protocols for conducting the test, and usage and evaluation of data generated from the test.

3.0 REFERENCES

Dunnett, C.W. 1955. A multiple comparisons procedure for comparing several treatments with a control. JASA 50:1096-1101.

US EPA. 1988. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. EPA Office of Research and Development EPA-600/4-87/028 (May 1988).

4.0 PROCEDURES

4.1 EQUIPMENT AND SUPPLIES

- neonate mysids (<48 h old) -- a minimum of 240, obtainable from laboratory cultures or from a commercial supplier (see SOP on Culture).
- Facilities for holding and acclimating test organisms -- See SOP on Culture.
- *Artemia* nauplii, newly hatched -- see SOP on Culture.
- Environmental chamber or equivalent facility with temperature control (26 - 27^{O}C).
- Water purification system -- Millipore Super-Q, deionized water or equivalent.
- Balance -- capable of accurately weighing to 0.00000l g.
- Reference weights, Class S -- for checking performance of balance. Reference weights should bracket the expected weights of the weighing boats and weighing boats plus organisms.
- Drying oven -- 60^{O}C, for drying organisms.
- Desiccator -- for holding dried organisms.
- Air pump -- for supplying air.
- Air lines and air stones -- for aerating cultures, brood chambers, and holding tanks, and supplying air to test solutions with low DO.
- pH and DO meters -- for routine physical and chemical measurements.

- Labels for exposure vials.
- Standard or micro-Winkler apparatus -- for determining DO and verifying DO meter accuracy.
- Dissecting microscope (350-400X magnification) -- for examining organisms in the test vessels to determine their sex and to check for the presence of eggs in the oviducts of females.
- Light box -- for illuminating organisms during examination.
- Refractometer -- for determining salinity.
- Thermometers, glass or electronic, laboratory grade -- for measuring water temperatures.
- Thermometers, bulb-thermograph or electronic-chart type -- for continuously recording temperature.
- Test vessels -- 20 mL borosilicate glass scintillation vials. 1176 test vessels are required for each test (3 replicates for each of 12 samples, plus a control) for the eight TIE treatments. Vials must be soaked overnight in control/dilution water and then rinsed in deionized water before use.
- Beakers or flasks -- six, borosilicate glass or non-toxic plasticware, 2000 mL for making test solutions.
- Wash bottles -- containing deionized water and clean seawater, for washing organisms from containers and for rinsing all glassware and instrument electrodes and probes.
- Volumetric flasks and graduated cylinders -- Class A, borosilicate glass or non-toxic plastic labware, 50-2000 mL, for making test solutions.
- Separatory funnels, 2-L -- Two-four for culturing *Artemia*.
- Pipets, volumetric -- Class A, l-l00 mL.
- Pipets, automatic -- adjustable, l-l00 mL.
- Pipets, serological -- l-l0 mL, graduated.
- Pipet bulbs and fillers – PROPIPET®, or equivalent.
- Droppers, and glass tubing with fire polished edges, 4 mm ID -- for transferring organisms.
- NITEX ® mesh sieves (l50 um and 1000 um) -- for concentrating organisms.
- Depression glass slides or depression spot plates -- two, for observing organisms.
- Data sheets (one set per test) -- for data recording (Figure 1).
- Buffers, pH 4, 7, and l0 (or as per instructions of instrument manufacturer) – for standards and calibration check.
- Reagent water -- defined as distilled or deionized water that does not contain substances which are toxic to the test organisms.
- Pore water spike water and dilution water.

4.2 TEST SOLUTION PREPARATION PROCEDURE

1. Surface water toxicity is determined with samples used directly as collected when the sample salinity is 20 to 30 ppt. If salinity adjustment is necessary, this is accomplished using hypersaline brine (see SOP on Brine) and a brine + deionized

water control of the same salinity must be tested. Natural seawater and/or uncontaminated local water may be tested as additional controls.

2. The selection of the pore water test concentrations should be based on the objectives of the study. One of two dilution factors, approximately 0.3 or 0.5, is commonly used. If the effluent is known or suspected to be highly toxic, a lower range of effluent concentrations should be used, beginning at about 10%. If high mortality is observed during the first 1 or 2 h of the test, additional lower effluent concentrations can be added.

3. The test should begin as soon as possible after sample collection, preferably within 24 h. If the persistence of the sample toxicity is not known, the maximum holding time should not exceed 36 h for off-site toxicity studies. Samples should be stored at 4^{O}C at all times prior to use.

4.3 TEST INITIATION PROCEDURE

1. About 48 hr prior to beginning the test, set up the *Artemia* culture so that nauplii will be available on the day the test begins (see SOP on Culture).

2. Increase the temperature of the water bath, room, or incubator to the required test temperature (26 - 27^{O}C).

3. About 1 h before test initiation, the temperature of the sample should be adjusted to the test temperature (26 - 27^{O}C) and maintained at that temperature while the dilutions are being made. Effluent dilutions should be prepared for all replicates in each treatment in one flask to ensure low variability among the replicates. The salinity and temperature of each dilution should be checked prior to addition to test chambers.

4. Label the test vials with prepared labels that identify the Sample ID, sample concentration, replicate number and TIE treatment.

5. Distribute sample to all vials according to the *Water Volume Calculation* worksheet.

6. Randomly place five 7-day-old animals (one at a time) in each test vial of each treatment using a large bore (4 mm ID) pipette. It is easier to capture the animals if the volume of water in the dish holding the test animals is reduced and the dish is placed on a light table. It is recommended that the transfer pipette be rinsed frequently because mysids tend to adhere to the inside surface.

8. At a minimum, the following measurements should be made in at least one replicate in the control and the high and low test concentrations at the beginning of the test: temperature, dissolved oxygen, pH, and salinity. Measurements of these parameters are recorded on a water quality data sheet. It is generally preferable to

use a replicate created solely for water quality measurements to take initial and during-test measurements. Final measurements should be taken in both the water quality replicate and one animal exposure replicate. If total mortality is observed in any treatment, water quality measurements should also be taken on at lest one replicate of that treatment.

4.4 TEST CONDITION MONITORING PROCEDURE

1. The light quality and intensity under ambient laboratory conditions are generally adequate. Light intensity of 10-20 uE/m^2/s, or 50 to 100 foot candles (ft.c), with a 16 h light and 8 h dark cycle and a 30 min phase-in/out period is recommended.

2. It is critical that the test water temperature be maintained at 26 - 27^{O}C. It is recommended that the test water temperature be continuously recorded. If a water bath is used to maintain the test temperature, the water depth surrounding the test cups should be at least 2.5 cm deep.

3. The test salinity should be in the range of 20 ^{O}/oo to 30 ^{O}/oo and should not vary by more than $\pm$ 2 ^{O}/oo among the test vials on a given day. If effluent and receiving water tests are conducted concurrently, the salinities of these tests should be similar. If salinity reaches 35 ^{O}/oo in any sample, DI water should be added by dropper to reduce salinity by 2 ^{O}/oo.

4. Dissolved oxygen in test vials should not fall below 60% of saturation. If initial DO is between 30 and 60% saturation, samples should be gently aerated prior to testing. If one solution is aerated then all the treatments and the controls must also be aerated. If DO is less than 30%, oxygenation prior to testing may be necessary to prevent DO-related mortality during testing.

5. During the test, the mysids in each test vial should be fed *Artemia* nauplii less than 24-h old at the rate of 50 nauplii per mysid per day.

4.5 DAILY TEST MONITORING PROCEDURE

1. The number of live mysids in each vial is counted and recorded each day. Dead animals and excess food should be removed with a pipette before the new test solutions are added.

2. DO, pH, temperature, and salinity should be measured in two replicates in the control and each test concentration before renewing the test medium each day. Temperature should be monitored in test vials while they are still in the water bath.

5.0 TROUBLESHOOTING

1. Toxic substances may be introduced by contaminants on glassware and testing equipment. Be sure that all equipment is thoroughly cleaned and/or soaked in clean seawater before use in a test.

2. Excess *Artemia* nauplii in exposure vials during the test may sequester metals and other toxic substances, and lower the DO. Care should be taken to avoid overfeeding mysids during the test.

3. Rooms or incubators with high volume ventilation should be used with caution because the volatilization of the test solutions and evaporation of dilution water may cause wide fluctuations in salinity. Covering the test vials with clear polyethylene plastic may help prevent volatilization and evaporation of the test solutions.

6.0 STATISTICAL ANALYSIS AND DATA USAGE

1. Tabulate and summarize the data.

2. The end points of the mysid test are based on the adverse effects on survival. Least effect and no effect concentrations (LOECs and NOECs), for survival are obtained using one-way analysis of variance (ANOVA) followed by Dunnett's Procedure for comparing treatments to a control (Dunnett, 1955).

3. Point estimates of toxic concentrations (e.g. IC_{20},; IC_{50}) and accompanying test statistics may also be generated using specialty software (e.g. Toxcalc® by Tidepool Scientific).