

User's Guide and Technical Resource Document: Evaluation of Sediment Toxicity Tests for Biomonitoring Programs

HEALTH AND ENVIRONMENTAL SCIENCES DEPARTMENT

API PUBLICATION NUMBER 4607



American Petroleum Institute
1220 L Street, Northwest
Washington, D.C. 20005

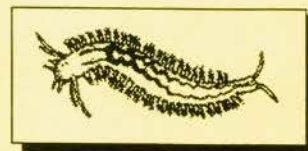
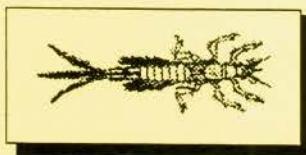
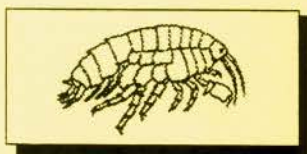


Documents

Document Sharing Hub



**User's Guide:
Evaluation of Sediment
Toxicity Tests for
Biomonitoring Programs**



ABSTRACT

Sediment toxicity test methods are available for marine, estuarine, and freshwater sediments and organisms. The methods can be used for a variety of purposes: for example, assessment of existing environmental conditions, monitoring changes with time, or for NPDES permit compliance. Use of inappropriate test methods or species for a given purpose can impact the toxicity results and their interpretation. This User's Guide has been prepared to assist personnel at petroleum industry facilities (refineries, marketing terminals, and production locations) in understanding sediment toxicity testing and in the selection of test methods and species which are appropriate for their needs. The general aspects of sediment toxicity testing are summarized along with technical requirements and appropriate conditions for each test type. Test methods are evaluated for their reliability, ecological relevance, exposure relevance, availability, interferences, and ability to discriminate toxicants. A companion report (Technical Resource Document) has been prepared to provide detailed technical background information on the methods.

User's Guide and Technical Resource Document: Evaluation of Sediment Toxicity Tests for Biomonitoring Programs

HEALTH AND ENVIRONMENTAL
SCIENCES DEPARTMENT

API PUBLICATION NUMBER 4607

PREPARED BY:
PTI ENVIRONMENTAL SERVICES
15375 SE 30TH PLACE, SUITE 250
BELLVUE, WASHINGTON 98007

OCTOBER 1994



**American
Petroleum
Institute**



Documents

Document Sharing Hub



One of the most significant long-term trends affecting the future vitality of the petroleum industry is the public's concerns about the environment. Recognizing this trend, API member companies have developed a positive, forward looking strategy called STEP: Strategies for Today's Environmental Partnership. This program aims to address public concerns by improving our industry's environmental, health and safety performance; documenting performance improvements; and communicating them to the public. The foundation of STEP is the API Environmental Mission and Guiding Environmental Principles.

API ENVIRONMENTAL MISSION AND GUIDING ENVIRONMENTAL PRINCIPLES

The members of the American Petroleum Institute are dedicated to continuous efforts to improve the compatibility of our operations with the environment while economically developing energy resources and supplying high quality products and services to consumers. The members recognize the importance of efficiently meeting society's needs and our responsibility to work with the public, the government, and others to develop and to use natural resources in an environmentally sound manner while protecting the health and safety of our employees and the public. To meet these responsibilities, API members pledge to manage our businesses according to these principles:

- To recognize and to respond to community concerns about our raw materials, products and operations.
- To operate our plants and facilities, and to handle our raw materials and products in a manner that protects the environment, and the safety and health of our employees and the public.
- To make safety, health and environmental considerations a priority in our planning, and our development of new products and processes.
- To advise promptly, appropriate officials, employees, customers and the public of information on significant industry-related safety, health and environmental hazards, and to recommend protective measures.
- To counsel customers, transporters and others in the safe use, transportation and disposal of our raw materials, products and waste materials.
- To economically develop and produce natural resources and to conserve those resources by using energy efficiently.
- To extend knowledge by conducting or supporting research on the safety, health and environmental effects of our raw materials, products, processes and waste materials.
- To commit to reduce overall emission and waste generation.
- To work with others to resolve problems created by handling and disposal of hazardous substances from our operations.
- To participate with government and others in creating responsible laws, regulations and standards to safeguard the community, workplace and environment.
- To promote these principles and practices by sharing experiences and offering assistance to others who produce, handle, use, transport or dispose of similar raw materials, petroleum products and wastes.

FOREWORD

API PUBLICATIONS NECESSARILY ADDRESS PROBLEMS OF A GENERAL NATURE. WITH RESPECT TO PARTICULAR CIRCUMSTANCES, LOCAL, STATE, AND FEDERAL LAWS AND REGULATIONS SHOULD BE REVIEWED.

API IS NOT UNDERTAKING TO MEET THE DUTIES OF EMPLOYERS, MANUFACTURERS, OR SUPPLIERS TO WARN AND PROPERLY TRAIN AND EQUIP THEIR EMPLOYEES, AND OTHERS EXPOSED, CONCERNING HEALTH AND SAFETY RISKS AND PRECAUTIONS, NOR UNDERTAKING THEIR OBLIGATIONS UNDER LOCAL, STATE, OR FEDERAL LAWS.

NOTHING CONTAINED IN ANY API PUBLICATION IS TO BE CONSTRUED AS GRANTING ANY RIGHT, BY IMPLICATION OR OTHERWISE, FOR THE MANUFACTURE, SALE, OR USE OF ANY METHOD, APPARATUS, OR PRODUCT COVERED BY LETTERS PATENT. NEITHER SHOULD ANYTHING CONTAINED IN THE PUBLICATION BE CONSTRUED AS INSURING ANYONE AGAINST LIABILITY FOR INFRINGEMENT OF LETTERS PATENT.

ACKNOWLEDGMENTS

THE FOLLOWING PEOPLE ARE RECOGNIZED FOR THEIR CONTRIBUTIONS OF TIME AND EXPERTISE DURING THIS STUDY AND IN THE PREPARATION OF THIS REPORT:

API STAFF CONTACT

Alexis E. Steen, Health and Environmental Sciences Department

MEMBERS OF THE BIOMONITORING TASK FORCE

Philip B. Dorn, Ph.D., Shell Development Company

Jerry F. Hall, Ph.D., Texaco Research

W. Raymon Arnold, Ph.D., Exxon Biomedical Sciences, Inc.

Shiv Baloo, Amoco Oil Company

Janis M. Farmer, BP America Research & Development

William R. Gala, Ph.D., Chevron Research & Technology Company

Michael C. Harrass, Ph.D., Amoco Corporation

Michael E. Tucker, Occidental Chemical Company

Thomas C. Ginn was the Project Manager for PTI Environmental Services (PTI). Robert A. Pastorok and Jane E. Sexton of PTI coordinated technical tasks. Authors that contributed to this report were Robert A. Pastorok, Jane E. Sexton, D. Scott Becker, and Chip W. Hogue of PTI. Alexis E. Steen of API and Jerry F. Hall of Texaco Research contributed to the final authorship of the executive summary and the introduction to the report. James Meeker of PTI assisted in the literature review and compilation of information on individual toxicity methods. The API Biomonitoring Task Force provided constructive review comments on the scope of the study and on several drafts of the report.

CONTENTS

	<u>Page</u>
INTRODUCTION	1
EVALUATION OF SEDIMENT TOXICITY TESTS	2
KEY TEST CHARACTERISTICS	2
Habitat Type	2
Exposure Medium	2
Endpoint Type	3
EVALUATION CRITERIA	4
EVALUATION RESULTS	4
SITE-SPECIFIC SELECTION OF SEDIMENT TOXICITY TESTS	12
DEFINITION OF SELECTION CRITERIA	12
OVERVIEW OF TEST SELECTION PROCESS	12
Site Characteristics	12
Regulatory Requirements	12
Selection of Evaluation Tables	15
Biotic Group and Geographic Zone	15
Identify Highest Rank Tests	15
Compare Selected Test(s) with Regulatory Requirements	15
APPLICATION OF SEDIMENT TOXICITY TESTS	16
METHODS AND QUALITY ASSURANCE ISSUES	16
SAMPLING ISSUES	17
Sample Location	17
Sample Collection	17
Sample Handling	17
Sample Preservation	17
Sample Documentation	18
DATA ANALYSIS ISSUES	18
GLOSSARY	21
REFERENCES AND OTHER KEY LITERATURE	25

LIST OF FIGURES AND TABLES

	<u>Page</u>
Figure 1. Approach to selection of sediment toxicity tests for a specific site	14
Table 1. Evaluation of sediment toxicity tests—marine lethal	5
Table 2. Evaluation of sediment toxicity tests—marine sublethal	6
Table 3. Evaluation of sediment toxicity tests—estuarine lethal	7
Table 4. Evaluation of sediment toxicity tests—estuarine sublethal	8
Table 5. Evaluation of sediment toxicity tests—freshwater lethal	9
Table 6. Evaluation of sediment toxicity tests—freshwater sublethal	10
Table 7. Selection of sediment toxicity tests	13



INTRODUCTION

Reliable toxicity tests are currently available for testing of marine, estuarine, and freshwater sediments as part of biomonitoring programs for wastewater discharges. Sediment toxicity tests provide an integrated measure of the effects of sediment contamination that eliminates much of the uncertainty associated with predicting toxicity from sediment chemistry alone. When combined with surveys of animals living in the sediments, sediment toxicity tests can be used to assess existing conditions, rank sites for cleanup priority, and monitor changes in contaminant effects with time (Chapman *et al.* 1992). However, the use of inappropriate test methods or species and the failure to consider physical and chemical factors that can affect the results of the tests may diminish the value of biological toxicity testing (Burton 1991; Hill *et al.* 1993).

The purpose of this User's Guide is to provide information that will enable environmental personnel at petroleum facilities to select sediment toxicity tests and test methods that are scientifically valid and appropriate for a specific site. For those readers who are unfamiliar with sediment toxicity testing, this User's Guide explains general aspects of sediment toxicity testing and how to use available technical information. This document also outlines the technical requirements and appropriate conditions for using different sediment toxicity test methods. A companion document, *Evaluation of Sediment Toxicity Tests for Biomonitoring Programs* (PTI, 1994) hereafter referred to as the Technical Resource Document, has been prepared to provide technical background on the test methods and the detailed rationale for the evaluations presented here. The Technical Resource Document is intended to be used as a reference tool for the test selection process and also as an information resource to support negotiations with agencies concerning the appropriateness of any recommended tests.

Sediment toxicity tests anticipated for future use in biomonitoring programs for National Pollutant Discharge Elimination System (NPDES) permit compliance are addressed in the Technical Resource Document and in this User's Guide. These docu-

ments were developed for use by petroleum industry operations (refineries, marketing terminals, and production facilities) that have discharges to surface waters. However, the Technical Resource Document and this User's Guide contain information that is applicable to other industries and could be used by any wastewater discharger.

The term *sediment toxicity test*, as used here, refers to any laboratory method that measures the adverse biological response of a group of organisms to a sample of test sediment. Some sediment toxicity tests measure lethal effects by determining the number of organisms that are killed during the exposure period. Other tests measure sublethal effects such as developmental abnormalities in juvenile stages, inhibition of reproduction, or reduced growth. Sediment toxicity tests are used in many biomonitoring programs because they integrate the effects of multiple chemicals and can be used in conjunction with chemical measurements and surveys of sediment-dwelling organisms to establish cause-effect relationships. Sediment toxicity tests are also the primary tool for any toxicity identification and evaluation program. Sediment toxicity tests are available for many different species and various life stages of some species. For example, they may be conducted on embryos, larvae, and juveniles of various fish species, as well as embryos and juveniles of invertebrates such as clams, oysters, and sea urchins. Sediment toxicity tests can also be conducted with microscopic algae and bacteria, submerged aquatic plants (e.g., water hyacinth), and wetland plants (e.g., marsh grass).

The next section presents an evaluation of available sediment toxicity tests, including descriptions of habitat type, sediment test systems, and biological endpoints. The following section provides a procedure for selection of tests at a specific site. Finally, brief summaries of sampling and data analysis issues are presented in a section on application of sediment toxicity tests.

Selected terms in this User's Guide are defined in the Glossary.

EVALUATION OF SEDIMENT TOXICITY TESTS

The available test methods were classified by type of habitat (marine, estuarine, and freshwater) to which each method applies and the general endpoint type (lethal or sublethal) specified for each test. This classification scheme resulted in the following six major categories of tests:

- Marine lethal
- Marine sublethal
- Estuarine lethal
- Estuarine sublethal
- Freshwater lethal
- Freshwater sublethal.

Appendix A of the Technical Resource Document presents test classification tables that contain the following information on each test: 1) organisms, including the broad biotic group, scientific name, and life stage of the species used in the test; 2) exposure medium (whole sediment, interstitial water, sediment elutriate, or sediment extract); 3) exposure duration; and 4) primary literature references for test methods. Each test was assigned a number to allow users of the Technical Resource Document to track a given test through the various evaluation tables. In many cases, several of the specified tests were actually variations of a single test method and were assigned the same test number.

KEY TEST CHARACTERISTICS

The key characteristics used to classify sediment toxicity tests are described below (see the *Test Screening Approach* section of the Technical Resource Document for details).

Habitat Type

The primary characteristic that distinguishes marine, estuarine, and freshwater habitat types is water salinity. Salinity strongly influences the distributions of most of the test organisms. In some cases, test organisms are tolerant of both marine and estuarine conditions or both estuarine and freshwater conditions. However, few test organisms tolerate both marine and freshwater conditions. For purposes of this study, habitat categories were defined as follows:

- Marine (≥ 28 ppt)
- Estuarine (> 0.5 ppt and < 28 ppt)
- Freshwater (≤ 0.5 ppt).

Because the division between habitat categories is an artificial distinction, use of a particular habitat designation for a test in this report should not necessarily preclude the application of a test to sediments in other habitats. For example, some tests that are classified as marine tests may be applied to high salinity estuarine sediments, and in some cases, adjusting the salinity of a sediment sample to allow the use of a particular test may be appropriate.

Exposure Medium

The kind of exposure medium was used to classify the various toxicity tests because each kind of exposure medium has favorable and unfavorable characteristics that can profoundly influence the toxicity test results. The four kinds of exposure media considered were as follows:

- Whole sediments
- Interstitial water
- Sediment elutriates
- Sediment extracts.

Whole Sediments—The use of whole sediments is probably the most realistic exposure scenario because it mimics the manner in which most organisms are exposed to chemicals in the

environment. Whole-sediment toxicity tests integrate multiple exposure routes, including chemical intake from dermal contact with sediment particles and interstitial water as well as ingestion of sediment particles, interstitial water, and food organisms (the food uptake route applies to at least some methods in which the test species is not fed). For most whole sediment tests, the sediments are carefully placed in the exposure chamber and the chamber is then filled with clean water. Resuspended particles are allowed to settle before initiation of exposure. In whole-sediment tests, infaunal test organisms are expected to have the highest potential for exposure to chemicals because they live within the sediments.

Interstitial Water—Interstitial water as an exposure medium is prepared by removing water from the test sediments by methods such as filtration and centrifugation. The test organisms are then introduced to the interstitial water in the absence of sediments. For infaunal organisms, interstitial water is a representative exposure medium for primarily one exposure route (i.e., dermal contact with the dissolved forms of chemicals). Interstitial water is not a representative exposure medium for epifaunal, planktonic, and nektonic organisms. The degree to which the sampling of interstitial waters or the elutriation process modifies the toxicity of the sample is usually unknown.

Sediment Elutriates—Sediment elutriates are prepared by mixing sediments and test water for a fixed period of time and then removing the sediments by methods such as filtration, centrifugation, and decanting after a settling period. The test organisms are then introduced to the test water in the absence of sediments. Elutriates are useful for representing the exposure to chemicals that can occur after sediments have been resuspended into the water column or after they have passed through the water column as part of dredged material disposal operations. Although the use of a sediment elutriate as an exposure medium is realistic for planktonic and nektonic test organisms, it is unrealistic for infaunal and epibenthic organisms. The degree to which the sampling of interstitial waters or

the elutriation process modifies the toxicity of the sample is usually unknown.

Sediment Extracts—Sediment extracts are prepared by mixing sediments with an organic solvent that is capable of removing specific kinds of chemicals from the sediments. After the extraction process is completed, the sediments are removed by methods such as filtration, centrifugation, and decanting after a settling period. The extractant and the extracted chemicals are diluted with water for testing. In some cases, the extracted chemicals are first exchanged with a less toxic carrier medium before the test concentrations are prepared. In either case, the test organisms are introduced to a solvent-water mixture containing the extracted chemicals. Because the test organisms are exposed to an unnatural exposure medium (organic solvent) in the absence of sediments, an extractant-prepared exposure medium is generally considered an unrealistic exposure scenario.

Endpoint Type

The major types of endpoints for most toxicity tests include the following:

- Lethal (i.e., mortality)
- Sublethal
 - Reduced growth
 - Reproductive effects
 - Developmental abnormality
 - Histopathological abnormalities.

The determination of the lethal endpoint is unambiguous and is clearly an adverse effect. The reliability of any sublethal endpoint test depends on use of experienced laboratory personnel (for details see *Endpoint Type* in the section *Classification of Available Test Methods, Classification Criteria* in the Technical Resource Document).

EVALUATION CRITERIA

A technical rating was assigned to each sediment toxicity test based on each of the following evaluation criteria:

- Reliability
 - The endpoint can be measured accurately
 - The results are repeatable
 - The negative control results generally meet quality assurance criteria
 - Intra- and interlaboratory variability studies indicate high precision
- Ecological relevance
 - The results of a test method are directly applicable to indigenous species under field conditions
 - Test organisms are species that are of commercial or ecological importance
- Exposure relevance
 - The pathway of exposure used in a test is analogous to exposure under field conditions
- Availability
 - Test organisms can be easily obtained or cultured
 - The method is standardized and well documented
 - Commercial laboratories routinely perform the test
- Interferences
 - Test methods have a low susceptibility to confounding physical or chemical factors
- Chemical discrimination
 - Test results are useful in defining gradients of sediment toxicity in the environment
 - Test methods and organisms are not overly sensitive or insensitive.

An overall technical rating was determined by summing the scores for each of the individual criteria. Because little information was available on interferences and chemical discrimination for most tests, their influence on the overall technical rating scores was moderated by use of a weighting factor (see the *Test Screening Approach*, PTI 1994, section of the Technical Resource Document).

The rating for regulatory status was based on information from regional and national EPA offices and whether a test was recommended in guidance documents for potential use in NPDES programs, clean-up assessments, baseline monitoring, and dredged material testing. The guidance documents considered as the basis for rating regulatory status included the method documents issued by the Canadian government (Environment Canada 1990a-e, 1992a-f), the dredged material testing documents issued by United States government agencies (U.S. EPA and U.S. COE 1991, 1993), and a major research and development planning document issued by EPA (U.S. EPA 1992). If a test was included in 3-4 of these document categories, it was assigned a rating of "high" for regulatory status. If a test was included in 1-2 of these document categories, it was assigned a rating of "medium." Toxicity tests that were not included in these documents and were not known to be required for use in current regulatory programs were assigned a rating of "low."

EVALUATION RESULTS

Results of the evaluation of sediment toxicity tests are presented in Tables 1 through 6. Most of the highly ranked marine and estuarine infaunal tests were based on the use of amphipods as test organisms, whereas most of the highly ranked freshwater infaunal tests were based on the use of insects (mayfly nymphs and midge larvae) as test organisms. These species groups are ecologically important, especially as key prey items for various fishes. In most cases, the highest ranking tests were the ones based on the exposure of infaunal organisms to whole sediments because: 1) exposure conditions closely mimic field conditions, 2) most of the test species are available by field collection during most of the year, and 3) many of the tests have well-developed methods.

TABLE 1. EVALUATION OF SEDIMENT TOXICITY TESTS – MARINE LETHAL

					Technical Rating								
Test Number	Exposure Media	Common Name	Life Stage	Test Species									
					Overall Technical Rating	Reliability	Ecological Relevance	Exposure Relevance	Availability	Interferences	Chemical Responsiveness	Regulatory Status	Comments
001	S	Amphipod	Juveniles or adult females	<i>Ampelisca abdita</i>	●	●	●	●	●	●	●	High	B,C,E
008	S	Amphipod	Adults	<i>Rhepoxynius abronius</i>	●	●	●	●	●	●	●	High	C,E
003	S	Amphipod	Juveniles or young adults	<i>Eohaustorius washingtonianus</i>	●	●	●	●	●	●	●	Med.	E
002	S	Amphipod	Juveniles or young adults	<i>Amphiporeia virginiana</i>	●	●	●	●	●	●	●	Med.	E
006	S	Amphipod	Juveniles or young adults	<i>Leptocheirus pinguis</i>	●	●	●	●	●	●	●	Med.	
005	S	Amphipod	Immature	<i>Grandidierella japonica</i>	●	●	●	●	●	●	●	Low	A,B
004	S	Amphipod	Juveniles or young adults	<i>Foxiphalus xiximeus</i>	●	●	●	●	●	●	●	Med.	E
020	S	Littleneck clam	Juveniles	<i>Protothaca staminea</i>	●	●	●	●	●	●	○	Low	
017	EL	Blue mussel	Embryos	<i>Mytilus edulis</i>	●	●	●	○	●	●	●	Med.	B
033	S	Shrimp	Juveniles	<i>Pandalus</i> sp.	●	●	●	●	●	●	●	Low	
029	S	Cancer crab	Juveniles	<i>Cancer</i> sp.	●	●	●	●	●	●	●	Low	
027	S	Blue crab	Juveniles	<i>Callinectes sapidus</i>	●	●	●	●	●	●	●	Low	
034	S	Shrimp	Post-larvae	<i>Penaeus</i> sp.	●	●	●	●	●	●	●	Low	
071	S	Polychaete	Juveniles	<i>Neanthes</i> sp.	●	●	●	●	●	●	●	Med.	B
021	S	Japanese clam	Juveniles	<i>Tapes japonica</i>	●	●	●	●	●	●	○	Low	
016	S	Blue mussel	Embryos	<i>Mytilus edulis</i>	●	●	●	●	●	○	●	Med.	B,C
011	EL	Pacific oyster	Embryos	<i>Crassostrea gigas</i>	●	●	●	○	●	●	●	Med.	B,D
009	EL	Eastern oyster	Embryos	<i>Crassostrea virginica</i>	●	●	●	○	●	●	●	Med.	B
055	EL	Sheepshead minnow	1-14 days old	<i>Cyprinodon variegatus</i>	●	●	●	○	●	●	●	Med.	B,E
037	S	Ridge-back prawn	Juveniles	<i>Sicyonia ingentis</i>	●	●	●	●	●	●	●	Low	A

Key

●	high	widely adopted
◐		
◑		
◒		
○	low	none

EL Elutriate
S Sediment

A Geographically restricted or alien to United States
B Widely distributed and/or cultured
C Potential sediment interferences
D Field validated to benthos
E Interlaboratory comparisons available

TABLE 2. EVALUATION OF SEDIMENT TOXICITY TESTS – MARINE SUBLETHAL

					Technical Rating								
Test Number	Exposure Media	Common Name	Life Stage	Test Species	Overall Technical Rating							Regulatory Status	Comments
					Reliability	Ecological Relevance	Exposure Relevance	Availability	Interferences	Chemical Responsiveness			
001	S	Amphipod	Juveniles or adult females	<i>Ampelisca abdita</i>	●	○	●	●	●	●	●	Low	B,C
121	S	Polychaete	Juveniles	<i>Neanthes sp.</i>	●	●	○	●	●	●	○	Low	B,E
109	EL	Purple sea urchin	Gametes	<i>Strongylocentrotus purpuratus</i>	●	●	●	○	●	●	●	Med.	E
008	S	Amphipod	Adults	<i>Rhepoxynius abronius</i>	●	○	○	●	●	●	●	Low	C
005	S	Amphipod	Immature	<i>Grandidierella japonica</i>	●	○	○	●	●	●	●	Low	B
017	EL	Blue mussel	Embryos	<i>Mytilus edulis</i>	●	○	●	○	●	●	●	Low	B,E
111	EL	Purple sea urchin	Gametes	<i>Strongylocentrotus purpuratus</i>	●	●	●	○	○	●	●	Low	E
047	EL	Purple sea urchin	Gametes	<i>Strongylocentrotus purpuratus</i>	●	●	●	○	○	●	●	Med.	
102	EL	Sand dollar	Gametes	<i>Dendraster excentricus</i>	●	●	○	○	●	●	●	Med.	E
100	EL	Atlantic urchin	Gametes	<i>Arbacia punctulata</i>	●	●	○	○	●	●	●	Med.	E
115	EL	Green sea urchin	Gametes	<i>Strongylocentrotus droebachiensis</i>	●	●	○	○	●	●	●	Med.	E
015	S	Clam	Juveniles	<i>Mulinia lateralis</i>	●	○	○	●	○	●	●	Low	
107	EL	White sea urchin	Gametes	<i>Lytechinus pictus</i>	●	●	○	○	●	●	●	Med.	A,E
112	EL	Purple sea urchin	Gametes	<i>Strongylocentrotus purpuratus</i>	●	○	●	○	○	●	●	Low	
011	EL	Pacific oyster	Embryos	<i>Crassostrea gigas</i>	●	○	●	○	○	●	●	Low	B,D,E
081	EL	Microtox	Cells	<i>Photobacterium phosphoreum</i>	●	○	○	○	●	●	●	Low	B,D
016	S	Blue mussel	Embryos	<i>Mytilus edulis</i>	●	○	●	○	●	○	●	Med.	B,C
009	EL	Eastern oyster	Embryos	<i>Crassostrea virginica</i>	●	○	●	○	○	●	●	Low	B
080	INT	Microtox	Cells	<i>Photobacterium phosphoreum</i>	●	○	○	○	●	●	●	Low	B,D
010	S	Pacific oyster	Embryos	<i>Crassostrea gigas</i>	●	○	●	○	○	○	●	Med.	B,C,D

Key

●	high	widely adopted
◐		
◑		
◒		
○	low	none

EL Elutriate

INT Interstitial water

S Sediment

A Geographically restricted or alien to United States

B Widely distributed and/or cultured

C Potential sediment interferences

D Field validated to benthos

E Interlaboratory comparisons available



Documents

Document Sharing Hub

TABLE 3. EVALUATION OF SEDIMENT TOXICITY TESTS – ESTUARINE LETHAL

					Technical Rating								
Test Number	Exposure Media	Common Name	Life Stage	Test Species	Overall Technical Rating							Regulatory Status	
					Reliability	Ecological Relevance	Exposure Relevance	Availability	Interferences	Chemical Responsiveness		Comments	
001	S	Amphipod	Juveniles or adult females	<i>Ampelisca abdita</i>	●	●	●	●	●	●	High	B,C,E	
129	S	Amphipod	Large juveniles and adults	<i>Eohaustorius estuarius</i>	●	●	○	●	●	●	High	C	
003	S	Amphipod	Juveniles or young adults	<i>Eohaustorius washingtonianus</i>	●	●	○	●	●	●	Med.	E	
133	S	Amphipod	Juveniles	<i>Leptocheirus plumulosus</i>	●	●	○	●	●	●	Low	B,E	
135	S	Amphipod	Mixed sexes	<i>Leptocheirus plumulosus</i>	●	●	○	●	●	●	High	B	
126	S	Amphipod	Juveniles or young adults	<i>Corophium volutator</i>	●	●	○	●	●	●	Med.	E	
002	S	Amphipod	Juveniles or young adults	<i>Amphiporeia virginiana</i>	●	●	○	●	●	○	Med.	E	
128	S	Amphipod	Adults	<i>Corophium volutator</i>	●	●	○	●	●	●	Low	E	
134	S	Amphipod	Juveniles	<i>Leptocheirus plumulosus</i>	●	●	○	●	●	●	Low	B	
131	S	Amphipod	7-14 days old	<i>Hyalella azteca</i>	●	●	○	●	○	●	High	B	
020	S	Littleneck clam	Juveniles	<i>Protothaca staminea</i>	●	●	●	●	○	○	Low		
017	EL	Blue mussel	Embryos	<i>Mytilus edulis</i>	●	○	●	○	●	●	Med.	B	
027	S	Blue crab	Juveniles	<i>Callinectes sapidus</i>	●	●	●	●	○	○	Low		
151	S	Grass shrimp	Post-hatch (1–4 days)	<i>Palaemonetes</i> sp.	●	●	○	●	○	●	Low		
016	S	Blue mussel	Embryos	<i>Mytilus edulis</i>	●	○	●	○	●	○	Med.	B,C	
009	EL	Eastern oyster	Embryos	<i>Crassostrea virginica</i>	●	○	●	○	○	●	Med.	B	
011	EL	Pacific oyster	Embryos	<i>Crassostrea gigas</i>	●	○	●	○	○	●	Med.	B,D	
030	S	Sand shrimp	Juveniles	<i>Crangon</i> sp.	●	●	○	●	○	○	Low		
153	EL	Sheepshead minnow	1–14 days old	<i>Cyprinodon variegatus</i>	●	●	○	○	●	○	Low	B	
010	S	Pacific oyster	Embryos	<i>Crassostrea gigas</i>	●	○	●	○	○	●	Med.	B,C,D	

Key		
●	high	widely adopted
◐		
◑		
◒		
○	low	none

- EL Elutriate
S Sediment
- B Widely distributed and/or cultured
C Potential sediment interferences
D Field validated to benthos
E Interlaboratory comparisons available

TABLE 4. EVALUATION OF SEDIMENT TOXICITY TESTS – ESTUARINE SUBLETHAL

Test Number	Exposure Media	Common Name	Life Stage	Test Species	Technical Rating							Regulatory Status	Comments
					Overall Technical Rating	Reliability	Ecological Relevance	Exposure Relevance	Availability	Interferences	Chemical Responsiveness		
001	S	Amphipod	Juveniles or adult females	<i>Ampelisca abdita</i>	●	●	●	●	●	●	●	Low	B,C
134	S	Amphipod	Juveniles	<i>Leptocheirus plumulosus</i>	●	●	●	●	●	●	●	Low	B
135	S	Amphipod	Mixed sexes	<i>Leptocheirus plumulosus</i>	●	●	●	●	●	●	●	Low	B
129	S	Amphipod	Large juveniles and adults	<i>Eohaustorius estuarius</i>	●	●	●	●	●	●	●	Low	C
128	S	Amphipod	Adults	<i>Corophium volutator</i>	●	●	●	●	●	●	●	Low	E
133	S	Amphipod	Juveniles	<i>Leptocheirus plumulosus</i>	●	●	●	●	●	●	●	Low	B
017	EL	Blue mussel	Embryos	<i>Mytilus edulis</i>	●	●	●	○	●	●	●	Low	B,E
011	EL	Pacific oyster	Embryos	<i>Crassostrea gigas</i>	●	●	●	○	●	●	●	Low	B,D,E
010	S	Pacific oyster	Embryos	<i>Crassostrea gigas</i>	●	●	●	○	●	○	●	Med.	B,C,D
016	S	Blue mussel	Embryos	<i>Mytilus edulis</i>	●	●	●	○	●	○	●	Med.	B,C
014	EL	Quahog clam	Embryos	<i>Mercenaria mercenaria</i>	●	●	●	○	●	●	●	Low	B
009	EL	Eastern oyster	Embryos	<i>Crassostrea virginica</i>	●	●	●	○	●	●	●	Low	B
187	EL	Sheepshead minnow	Adults	<i>Cyprinodon variegatus</i>	●	●	●	○	●	●	●	Low	B
176	EX	Microtox	Cells	<i>Photobacterium phosphoreum</i>	●	●	●	○	●	●	●	Low	B,D
191	INT	Polychaete	Females	<i>Dinophilus gyrociliatus</i>	●	●	●	○	●	●	●	Low	
175	S	Microtox	Cells	<i>Photobacterium phosphoreum</i>	●	●	●	○	●	○	●	Low	B,C

Key		
●	high	widely adopted
◐		
◑		
◒		
○	low	none

EL Elutriate
EX Extract
INT Interstitial water
S Sediment

B Widely distributed and/or cultured
C Potential sediment interferences
D Field validated to benthos
E Interlaboratory comparisons available

TABLE 5. EVALUATION OF SEDIMENT TOXICITY TESTS – FRESHWATER LETHAL

Test Number	Exposure Media	Common Name	Life Stage	Test Species	Technical Rating							Regulatory Status	Comments
					Overall Technical Rating	Reliability	Ecological Relevance	Exposure Relevance	Availability	Interferences	Chemical Responsiveness		
241	S	Mayfly	Nymphs	<i>Hexagenia limbata</i>	●	●	●	●	●	●	●	Low	B,D
239	S	Mayfly	Young nymphs	<i>Hexagenia limbata</i>	●	●	●	●	●	●	●	Low	B,D
238	S	Midge	2nd instar larvae	<i>Chironomus tentans</i>	●	●	○	●	●	○	●	High	B,D
237	S	Midge	Neonates or larvae	<i>Chironomus riparius</i>	●	●	○	●	●	○	●	Med.	B
200	S	Amphipod	Juveniles	<i>Hyalella azteca</i>	●	●	○	●	●	●	●	High	B
242	S	Mayfly	Nymphs	<i>Hexagenia limbata</i>	●	●	○	●	●	●	●	Low	B,D
209	INT	Water flea	Neonates	<i>Daphnia magna</i>	●	●	○	○	●	●	●	Low to Med.	B,D,E
256	S	Marsh grass	Seedlings	<i>Echinochloa crusgalli</i>	●	●	●	●	○	●	○	Low	
250	S	Oligochaete	Large worms	<i>Lumbriculus variegatus</i>	●	●	○	●	●	●	○	Low	B
249	S	Oligochaete	Mixed-age	<i>Lumbriculus variegatus</i>	●	●	○	●	●	●	○	Low	B
212	EL	Water flea	Neonates (<24 hrs)	<i>Daphnia magna</i>	●	●	○	○	●	●	●	Med.	B,D,E
240	INT	Mayfly	Nymphs	<i>Hexagenia limbata</i>	●	●	●	○	●	○	●	Low	B,D
205	EL	Water flea	Neonates	<i>Ceriodaphnia dubia</i>	○	●	○	○	●	●	●	Med.	B,E
213	EL	Water flea	Neonates (<24 hrs)	<i>Daphnia pulex</i>	○	●	○	○	●	●	●	Med.	B,E
208	EL	Water flea	Neonates	<i>Daphnia magna</i>	○	●	○	○	●	●	●	Med.	B,D,E
214	EL	Water flea	Neonates (<24 hrs)	<i>Daphnia pulex</i>	○	●	○	○	●	●	●	Med.	B
202	S	Paper pondshell clam	Juveniles	<i>Anodonta imbecillis</i>	○	●	○	●	○	●	○	Low	
252	S	Oligochaete	Mixed age; similar size	<i>Pristina leidyi</i>	○	●	○	○	○	●	○	Low	B
255	S	Oligochaete	Mixed age	<i>Tubifex tubifex</i>	○	●	○	○	○	●	○	Low	B,D
222	EL	Bluegill	Approx. 4 days	<i>Lepomis macrochirus</i>	○	●	○	○	○	○	○	Low	B,E

Key		
●	high	widely adopted
◐		
◑		
◒		
○	low	none

EL Elutriate
INT Interstitial water
S Sediment

B Widely distributed and/or cultured
C Potential sediment interferences
D Field validated to benthos
E Interlaboratory comparisons available

TABLE 6. EVALUATION OF SEDIMENT TOXICITY TESTS – FRESHWATER SUBLETHAL

					Technical Rating								
Test Number	Exposure Media	Common Name	Life Stage	Test Species	Overall Technical Rating							Regulatory Status	Comments
					Reliability	Ecological Relevance	Exposure Relevance	Availability	Interferences	Chemical Responsiveness			
238	S	Midge	2nd instar larvae	Chironomus tentans	●	●	●	●	●	●	●	Low	B,D
237	S	Midge	Neonates or larvae	Chironomus riparius	●	●	●	●	●	●	●	Low	B,D
200	S	Amphipod	Juveniles	Hyalella azteca	●	●	●	●	●	●	●	Low to Med.	B,D
242	S	Mayfly	Nymphs	Hexagenia limbata	●	●	●	●	●	●	●	Low	B
333	S	Aquatic vascular plant	Apical shoots	Hydrilla verticillata	●	●	●	●	●	●	●	Low	B
256	S	Marsh grass	Seedlings	Echinochloa crusgalli	●	●	●	●	●	●	●	Low	B,D
269	S	Amphipod	Juveniles (<1 week)	Hyalella azteca	●	●	●	●	●	●	●	Low	B,D,E
285	EL	Water flea	Neonates	Ceriodaphnia dubia	●	●	●	○	●	●	●	Med.	
330	S	Oligochaete	Adults	Tubifex tubifex	●	●	●	●	●	●	●	Low	B
304	INT	Midge	2nd instar larvae	Chironomus tentans	●	●	●	●	●	●	●	Low to Med.	B
250	S	Oligochaete	Large worms	Lumbriculus variegatus	●	●	○	●	●	●	●	Low	B,D,E
323	S	Oligochaete	Juveniles and adults	Lumbriculus variegatus	●	●	●	●	●	●	●	Low	B,D
324	S	Oligochaete	15 mm in length	Lumbriculus variegatus	●	●	●	●	●	●	●	Low	B,E
226	EL	Fathead minnow	Larvae (<24 hrs old)	Pimephales promelas	●	●	●	○	●	●	●	Med.	B,E
260	EL	Green algae	Cells	Selenastrum capricornutum	●	●	●	○	●	●	●	Med.	B,D,E
272	INT	Microtox	Cells	Photobacterium phosphoreum	●	●	●	○	●	●	●	Low to Med.	B
235	S	Rainbow trout	Egg-sac stage	Oncorhynchus mykiss	●	●	●	●	●	●	●	Low	
261	INT	Green algae	Cells	Selenastrum capricornutum	●	●	●	○	●	●	●	Low	B
252	S	Oligochaete	Mixed age; similar size	Pristina leidyi	●	●	●	●	●	●	●	Low	B,D
176	EX	Microtox®	Cells	Photobacterium phosphoreum	●	●	●	○	●	●	●	Low	B,E

Key

●	high	widely adopted
◐		
◑		
◒		
○	low	none

EL Elutriate

EX Extract

INT Interstitial water

S Sediment

B Widely distributed and/or cultured

D Field validated to benthos

E Interlaboratory comparisons available



Documents

Document Sharing Hub

Many of the lowest ranking toxicity tests involved exposure of planktonic organisms to whole sediments. The exposure relevance of these tests is relatively low because the test species are rarely exposed to sediments in the field and they may be sensitive to interference of suspended sediments with feeding mechanisms.

The species included in the highest ranking marine and estuarine tests for lethality include the following amphipods: *Ampelisca abdita*, *Rhepoxynius abronius*, *Grandidierella japonica*, *Eohaustorius washingtonianus*, *Eohaustorius estuarius*, *Amphiporeia virginiana*, *Foxiphalus xiximeus*, *Corophium volutator*, *Leptocheirus pinguis*, and *Leptocheirus plumulosus*. Reproductive endpoints are also well developed for the *L. plumulosus* test. Although behavioral endpoints (e.g., reburial at exposure termination) are used in many of these amphipod tests, the behavioral endpoints have generally not been field validated. The tests based on *A. abdita* and *R. abronius* are the only ones with a high regulatory status.

Taxonomic groups other than amphipods also ranked high among the marine and estuarine sublethal tests, including the polychaete (*Neanthes* sp.) growth test based on a 20–28 day exposure to whole sediments, the echinoderm (*Strongylocentrotus purpuratus*, *S. droebachiensis*, *Dendraster excentricus*, *Arbacia punctulata*, *Lytechinus pictus*) fertilization test of sediment elutriates, and the bivalve (*Mytilus edulis*, *Crassostrea gigas*, *C. virginica*) larval abnormality test of sediment elutriates. Although these elutriate tests have a lower exposure relevance than the whole sediment tests, they use sensitive life stages of ecologically important species, are widely available, and have well developed methods. Although these elutriate tests are generally reliable, their variability can be high and the negative controls fail quality assurance limits more frequently than those in the tests involving juveniles and adults of these or other species. Other high-ranking tests in the marine and estuarine sublethal category included the juvenile clam (*Mulinia lateralis*) test with whole sediments and the Microtox® (*Photobacterium phosphoreum*) test with sediment elutriates or interstitial water.

The highest ranking freshwater tests for lethal and sublethal endpoints were based on the exposure of infaunal insects (i.e., nymphs of the mayfly *Hexagenia limbata* and larvae of the midges *Chironomus riparius* and *Chironomus tentans*) and an epifaunal amphipod (*Hyaella azteca*) to whole sediments. Only the *H. azteca* and *C. tentans* lethal tests have high regulatory status. Whole sediment tests with vascular plants (*Hydrilla verticillata* and *Echinochloa crusgalli*) were among the top six ranked tests in the freshwater lethal category. These tests ranked high primarily because of their high degrees of exposure and ecological relevance and their relatively low susceptibility to interferences. The high ecological relevance of the two plant tests is based on the importance of the plants in providing habitat for other organisms. The major drawback of these two tests is their infrequent use in regulatory programs.

There is a relative lack of information on interferences and chemical discrimination for sediment toxicity tests. Further research in these areas and more comparative studies of toxicity tests with corresponding data on the bioavailability of sediment chemicals are needed.

SITE-SPECIFIC SELECTION OF SEDIMENT TOXICITY TESTS

The selection of toxicity test methods for application at a particular site involves consideration of many factors, including physical, chemical, and biological conditions at the site; regulatory requirements at federal, state, and local levels; and specific objectives for a monitoring program. Procedures for selecting sediment toxicity tests for use in biomonitoring programs are outlined in this section. First, the factors to be considered in test selection are defined. Second, the steps for selecting a test or battery of tests for application at a given site are described.

DEFINITION OF SELECTION CRITERIA

The selection of sediment toxicity tests for use in a biomonitoring program depends on site-specific characteristics, regulatory requirements, and other factors that are important in test evaluation (Table 7). Many of the decisions based on these factors may be constrained by technical specifications of a permit or monitoring program requirements.

OVERVIEW OF TEST SELECTION PROCESS

The process for selecting the most appropriate sediment test for a given study is illustrated in the decision tree shown in Figure 1. As users progress through each decision point within the tree, the number of candidate tests is reduced until the final sediment test(s) have been selected. Habitats and endpoints desired for the biomonitoring program should be matched to one of the six tables for test selection (Tables 1 through 6). Information on biotic group and geographic range for each of the tests is found in Appendix D of the Technical Resource Document. Also included in Appendix D are important comments regarding sensitivity to

chemicals and interferences that, when combined with the known chemical and physical characteristics of the study site, provide critical information in the selection process. An overview of how to use the decision-making framework in selecting toxicity tests is provided in the following sections.

Site Characteristics

A review of available information on the characteristics of the discharge site to be monitored and the organisms living at the study site is the fundamental first step in the selection process. Available data on site-specific chemicals and physical properties of the sediments can be useful in selecting test species that are sensitive to the presence of the site-specific chemicals, yet have minimal interferences to other properties of the sediment (e.g., grain size, organic carbon, ammonia). Knowing what organisms live at the study site can help guide the selection of appropriate species. If, for example, polychaete worms and bivalves dominate the benthic community in a marine study area and echinoderms (sea urchins and sand dollars) are absent, it is likely that the most appropriate test would include either polychaetes or bivalves as receptors, not echinoderms. Other important information that should be assembled includes regional water quality data, sediment characteristics, habitat types, and seasonal patterns in biological or physical/chemical characteristics.

Regulatory Requirements

An equally important step in the selection of sediment toxicity tests is a thorough understanding of the applicable regulatory requirements that are driving the testing program. Regulatory programs frequently include explicit requirements that immediately limit the field of potential toxicity tests. These confining factors can include specifications for lethal or sublethal tests, exposure duration, seasons for testing, single species vs. a battery of species for testing, and data quality objectives. Guidelines for selecting toxicity tests can also be included as part of regulatory programs. Knowledge of the regulatory requirements or guidelines

TABLE 7. SELECTION OF SEDIMENT TOXICITY TESTS

Decision Factor	Alternatives	
Objectives	Single species vs. test battery Season(s) for testing Site-specific chemicals, receptors, and sediment types Data quality objectives	
Regulatory Requirements	Various state and EPA regulations	
Geographic Zone	West Coast (north or south) East Coast (north or south) Gulf Coast (east or west)	
Habitat Type	Marine Estuarine Freshwater	
Biotic Group	Bacteria	Polychaete
	Eukaryotic cells	Oligochaete
	Algae	Mollusc
	Vascular plant	Echinoderm
	Crustacean	Amphibian
	Insect	Fish
	Nematode	
Species/Life Stage	Various species Gametes Embryos/Larvae Juveniles Adults	
Exposure Duration	Acute Chronic	
Endpoint	Lethal Sublethal	
Habitat Group ^a	Infauna Epifauna Plankton/nekton	
Exposure Medium ^a	Whole sediment Sediment elutriate Interstitial water Sediment extract	
Potential Interferences ^a	Grain size Organic carbon Acid-volatile sulfides Ammonia Mold, pathogens	

Note: EPA - U.S. Environmental Protection Agency

^a These decision factors were considered in ranking sediment toxicity tests. All other factors should be explicitly considered when selecting the sediment toxicity tests on a site-specific basis.

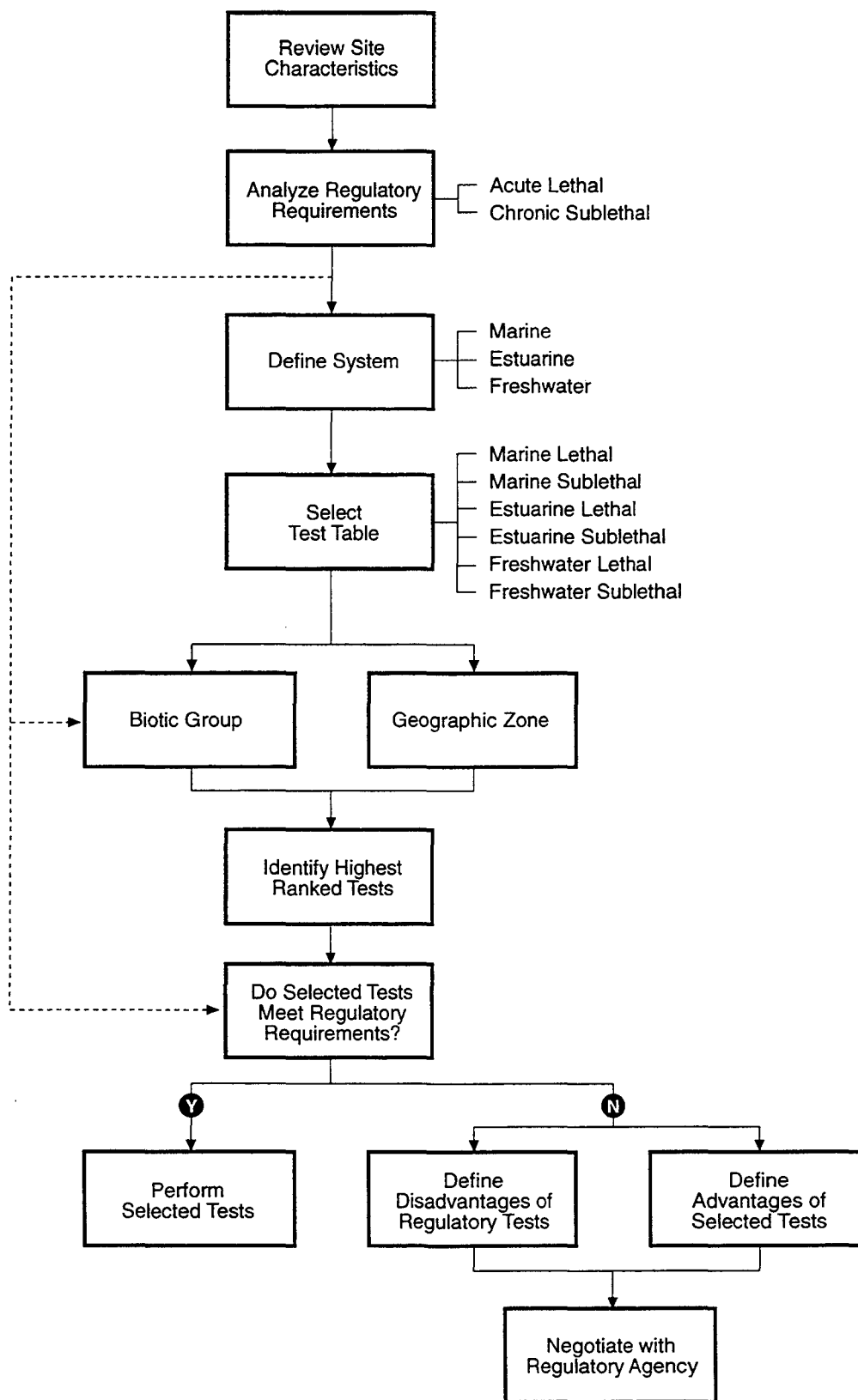


Figure 1. Approach to selection of sediment toxicity tests for a specific site.

for these or other toxicity test parameters is fundamental to the ultimate selection of the appropriate test. It is also important to have a full understanding of these regulatory requirements so that they can be evaluated in the context of the overall decision framework.

Selection of Evaluation Tables

Based on the habitat (marine, estuarine, or freshwater) and endpoint type (lethal and sublethal), one or more of the evaluation tables (Tables 1 through 6) is used to select appropriate tests. Important ancillary information relevant to each test is included in the Technical Resource Document (see especially Appendix D).

Biotic Group and Geographic Zone

A wide variety of biotic groups is represented in the listing of tests for each habitat and endpoint type. The list of candidate tests can be further reduced by deciding which organisms and which geographic zones are most relevant. The location of the study site will provide the information required to select a geographic zone. In addition, knowledge of the regulatory requirements may direct the selection of the species. If, for example, emphasis is on organisms that may be consumed by humans, then crabs, large bivalves, or fish are likely candidates for testing. If emphasis is on ecological risks, then other biotic groups such as algae, amphipods, insects, or polychaete worms become good candidate organisms.

Identify Highest Rank Tests

In the evaluation tables (Tables 1 through 6), tests are ranked from best overall candidate tests to least appropriate overall tests for each habitat/endpoint type. In most cases, the higher ranked tests may have very similar total scores. The user should select the most appropriate high-ranked test based on a consideration of site-specific factors or regulatory considerations.

Compare Selected Test(s) with Regulatory Requirements

The candidate toxicity test(s) tentatively selected should be matched with the regulatory requirements. If the test(s) meet these requirements, then the selection process is complete and the actual test(s) can be performed. If the selected toxicity test(s) do not meet the requirements of the applicable regulatory program, then low-ranked tests may need to be considered.

APPLICATION OF SEDIMENT TOXICITY TESTS

After the selected sediment toxicity tests are approved for a biomonitoring program, a sampling and analysis plan should be developed. The sampling and analysis plan specifies the study design for the field sampling program (see the *Application of Sediment Toxicity Tests* section in the Technical Resource Document), methods for implementing the toxicity tests, quality assurance procedures, and data analysis approaches. Issues related to quality assurance, sampling, and data analysis are discussed below.

METHODS AND QUALITY ASSURANCE ISSUES

The use of acceptable and well-documented laboratory methods is essential for ensuring that the results of toxicity testing are meaningful estimates of toxicity and that the tests are repeatable. Except for experimental studies, the tests that should be used for toxicity evaluations are those that have detailed, peer-reviewed methods to ensure that the testing is conducted properly and that the data will be comparable with data from other studies that use the same methods. Many of the well-standardized tests are documented in methods or guidance manuals developed by the American Society of Testing and Materials (ASTM), the U.S. Environmental Protection Agency (EPA), and Environment Canada.

It is essential that the performance of laboratory testing be monitored using quality assurance and quality control procedures to document the quality of results and determine whether the results are acceptable for their intended use (e.g., U.S. EPA 1991b; Moore et al. 1994). The major quality assurance and quality control procedures for toxicity testing are as follows:

- The use of negative controls to ensure that the test organisms are suitably healthy for testing

- The use of positive controls (i.e., reference toxicants) to ensure that the test organisms are suitably sensitive to toxic chemicals
- The monitoring of key test conditions (e.g., water temperature, dissolved oxygen) to ensure that the test results are not influenced by factors other than chemical toxicity
- The evaluation of variability among replicates and possibly tests for outliers.

Certain factors intrinsic to natural sediment samples may confound the relationship between the concentrations of sediment contaminants and toxicity. The objective of sediment toxicity testing is to evaluate the response of the test species to target chemicals contained in the sediment sample. It is preferable that the species not be responsive to other sediment characteristics such as grain size or organic carbon content. If such responses occur, toxicity may be incorrectly attributed to target chemicals. Changes in the following factors can restrict the application of a particular test or have a confounding effect on test results:

- Sediment grain size
- Organic carbon content
- Oxidation-reduction conditions
- pH
- Alkalinity
- Temperature
- Turbidity
- Water hardness
- Ultraviolet light intensity
- Mold or pathogens.

Information on potential interferences in sediment toxicity tests is provided in Appendix D of the Technical Resource Document.

SAMPLING ISSUES

The collection of representative sediment samples is essential for ensuring that the results of the subsequent toxicity tests are indicative of the true conditions in the field. A representative sample is one that is collected in a relatively undisturbed state from the intended field location; one that is collected using an appropriate collection device; and one for which proper handling, preservation, and documentation procedures have been observed after collection. A deficiency in any one of the above elements can affect the integrity of the sample and thereby influence the results of the toxicity testing so that they are not indicative of the true field conditions. Each of these elements is described below.

Sample Location

Sediment samples should be collected as close to their intended locations as required to satisfy the study objectives. This usually means that accurate positioning methods should be used both to locate the station initially and to allow the station to be revisited, if necessary, for subsequent sample collection.

Sample Collection

Sediment samples should be collected using appropriate collection devices that ensure that the sediment is collected with minimal disturbance, that an adequate penetration depth is achieved, and that the sample is retrieved in a relatively undisturbed state. When the results for different samples will be compared with each other (e.g., along spatial gradients, during different time periods), it is advisable to use the same sampling device to collect all of the samples so that biases that may occur from the use of different sample collection devices can be avoided.

Sediment samples should be collected in a relatively undisturbed state. The most common means of disturbing sediments are by excessive bow wake in front of the sample collection device immediately before the device contacts the sediment and by leakage of overlying water from the sample collection

device as it is retrieved. In both cases, fine-grained surface organic material can be lost from the sample, thus biasing the grain-size characteristics of the sample toward the coarse mineral fraction.

Sample Handling

Sediment samples should be subsampled and homogenized in a controlled and noncontaminating manner. To avoid contaminating sediments, all utensils should be constructed of stainless steel and should be chemically cleaned between different samples. Sediments should be removed from the sampling device in an unbiased manner, especially if the characteristics of the sediments are heterogeneous. In general, all of the sediment collected from a station that will be evaluated for toxicity, chemical concentrations, and sediment conventional variables should be pooled and homogenized prior to being distributed to sample jars. This process ensures that the various kinds of analytical results will be related as closely as possible. Homogenization is considered complete when the sediments are visually uniform with respect to texture and color.

Sediments that will be analyzed for unstable chemicals such as volatile organic compounds and acid volatile sulfides should not be homogenized prior to distribution because the resulting sample disturbance could alter those chemicals. Therefore, sediments that are suspected to contain unstable chemicals should be transferred directly from the sampling device to the sample jar, leaving minimal or no headspace. To provide representative sediments for unstable chemicals, it is best to take several random subsamples from various parts of each sediment sample.

Chemicals in interstitial water samples are likely to be modified during the collection and preparation process. Guidance on sample collection procedures for interstitial water samples is contained in Burton (1992).

Sample Preservation

Sediment samples should be preserved in a manner that maintains their integrity during storage prior to

laboratory analysis and should be analyzed within the specified maximum holding times. Proper sample preservation is essential for minimizing potential changes in the toxicity of the sediments during storage. Typically, sediments should be held unfrozen at 4°C for toxicity tests that rely on exposure to whole sediments. The maximum allowable holding time prior to testing for those sediments is generally specified as 2 weeks. However, sediment characteristics change during storage, even under controlled conditions. Therefore, it is preferable to conduct toxicity testing as soon as possible after field collection. For toxicity tests that rely on exposure to sediment extracts, sediments can sometimes be stored frozen if the test method allows.

Sample Documentation

All field collection procedures should be properly documented to verify that appropriate methods were used and that the security of samples was maintained at all times. Proper documentation generally involves the use of a field logbook to record pertinent information for each station and sediment sample and the use of chain-of-custody forms to document the transfer of samples among different parties.

DATA ANALYSIS ISSUES

Toxicity data should be analyzed using methods that are appropriate for the kinds of data available. To ensure that the data are appropriate for the planned analytical methods, it is essential that those methods be identified when the toxicity study is being designed. The study design specifications can then be tailored to provide data that are appropriate for the planned data analysis methods.

In monitoring programs and cleanup assessments, hypotheses regarding the toxicity of sediments at a specific site are usually tested using statistical methods to provide an objective analysis of the data. Statistical analysis allows quantification of the uncertainty associated with test results and typically ensures that several investigators would reach the same conclusions if each one analyzed the data

separately. Statistical analyses are especially important for determining whether the results of a site-specific toxicity test differ significantly from the reference sediment results. For example, a statistical approach might be used to evaluate the following null hypothesis: *There is no significant ($P > 0.05$) difference between the site and a reference area in sediment toxicity as measured by the amphipod mortality test.* Rejection of the null hypothesis based on statistical comparison of the sediment toxicity test data from the site with data from the reference area generally leads to acceptance of the alternative hypothesis that the site sediments are toxic (at least as measured by a laboratory toxicity test). However, a regulatory program may require further analyses to assess the implications of the laboratory test results. A specific probability level ($P \leq 0.05$ in the example above) is associated with the statistical test to quantify the level of confidence in the result if the null hypothesis is rejected. If the null hypothesis is not rejected, acceptance of the alternative hypothesis of “significant toxicity” may be supported by a further evaluation technique known as statistical power analysis that determines the probability of detecting a specified level of toxicity.

When designing a study for which the data will be analyzed statistically, there are two major considerations that should be addressed. One consideration is whether to use parametric or nonparametric statistical methods. The parametric tests assume a normal frequency distribution for the data, whereas the nonparametric tests make no assumptions about the form of the data distribution. Typically, it is desirable to use parametric methods because they generally are more powerful than nonparametric methods. However, it is important to evaluate the assumptions of the selected statistical test for each data set. If one or more parametric assumptions are not met, the data can be transformed and the assumptions can then be evaluated for the transformed data. If the transformed data satisfy the assumptions, they can be evaluated using parametric methods. Otherwise, nonparametric methods should be used to evaluate untransformed data.

A second consideration is the specific kind of statistical test that will be used to analyze the data. The

kind of test is usually determined by the study objectives. If the objective is to compare the toxicity results between a potential problem area and a reference area, analysis of variance can be used to conduct the evaluation. If the objective is to evaluate whether a gradient of toxicity exists with distance from a potential problem area, an analysis of variance or a correlation analysis can be used. In many cases, the kinds of statistical procedures that are used to analyze toxicity test results will be specified in a permit. Other details such as sample comparisons, statistical confidence levels, and other interpretive guidelines may also be specified. For an evaluation of permit specifications or design of testing programs refer to Gad and Weil (1986).

GLOSSARY

Acute toxicity	The ability of a chemical to cause a toxic response in organisms immediately or shortly after exposure to the chemical.
Adverse effect	An impairment of biological functions or description of ecological processes that results in unfavorable changes in an ecological system.
Amphipod	A small shrimp-like member of one subgroup of the large group of animals called Crustacea, which includes crayfish, lobsters, shrimps, and crabs.
Aquatic	Living or growing in water.
Benthic	Pertaining to, or associated with, the bottom of a body of water.
Biomass	The total weight of live organisms in a sampled population.
Biotic group	A group of related organisms with generally similar body structure and function.
Chronic toxicity	The ability of a chemical to produce a toxic response when an organism is exposed over a long period of time, generally corresponding to a substantial part of the organism's life cycle.
Concentration	The amount of a chemical expressed relative to amount of environmental medium (e.g., $\mu\text{g/L}$ [micrograms of chemical per liter of water] or $\mu\text{g/g}$ [micrograms of chemical per gram of sediment]).
Control sediment	A sediment essentially free of chemicals and compatible with the biological needs of the test organisms such that it has no discernable influence on the response being measured in the test. Control sediment may be the sediment from which the test organisms are collected or a laboratory sediment, provided the organisms meet control standards. Test procedures are conducted with the control sediment in the same way as the reference sediment and test material. The purpose of the control sediment is to confirm the biological acceptability of the test conditions and to help verify the health of the organisms during the test. Excessive mortality in the control sediment indicates a problem with the test conditions or organisms and can invalidate the results of the corresponding test.
Ecosystem	An ecological community, together with its physical habitat, considered as a unit.

Embryo	A plant or animal in the very early stages of development following fertilization of the egg.
Elutriate	A liquid solution used for toxicity testing, which is prepared by adding water to the sediment, shaking, and centrifuging to separate the solids.
Endpoint	The biological or ecological unit or variable being measured or assessed. The number of organisms dead at the end of an exposure is a lethal endpoint.
Epibenthic	Inhabiting the sediment surface, or closely associated with the sediment surface, rather than dwelling buried within the sediments.
Estuarine	Surface water containing greater than 0.5 parts per thousand (ppt) salinity and less than 28 ppt salinity.
Exposure	Contact between an organism and a chemical in the environment.
Fresh water	Surface water containing less than or equal to 0.5 ppt salinity.
Foundation species	A species that provides important physical habitat for other species in a biological community (e.g., marsh grass).
Hardness	A measure of the calcium and magnesium concentrations in water.
<i>In situ</i>	In the natural or original position (occurring in nature, and not in the laboratory).
Infaunal	Refers to animals living in the sediments, including such forms as worms and clams.
Interference	Physical elements or chemical compounds that cause bias in the results of a toxicity test.
Keystone species	A species that controls the species composition and relative abundances of species in a community by its predatory (or grazing) effects (e.g., by grazing on kelp, purple urchins prevent the establishment of kelp beds and maintain open rocky subtidal communities).
Interstitial water	Water that fills the spaces between sediment particles. Often referred to as "pore water."
Larval	Relating to the juvenile form of certain invertebrate animals that must undergo metamorphosis before assuming adult characteristics.

Lethal	Causing death; mortality (or survival) is the endpoint for lethal toxicity tests.
Life stage	A developmental stage of an organism (e.g., egg, larva, embryo, juvenile, adult).
Macroinvertebrate	An invertebrate (without a backbone) organism visible to the naked eye (e.g., > 1.0 mm). Often refers to animals such as insects, worms, clams, and snails.
Marine	Surface water containing 28 ppt salinity or greater.
Medium (plural: media)	The substance in which a chemical may exist. Air, sediment, and water are all media.
Midge	A group of true flies (similar to mosquitos) that have aquatic larvae and non-biting adults. They are one of the most abundant groups of aquatic insects.
Monitoring	Periodic testing of water and sediment quality or of biota to verify continued compliance with the requirements of a discharge permit or other authorization.
Nektonic	Refers to the nekton, the group of active swimmers that are capable of strong, independent movement in the water. Examples include many juvenile and adult fishes and large invertebrates (e.g., squid).
Organism	An individual plant or animal.
Ovigerous	Refers to females bearing eggs.
Planktonic	Refers to the plankton, the group of small plants and animals that are weak swimmers and tend to drift with the current.
Population	A group of individuals of the same species interacting within a given habitat.
Precision	The ability to replicate a value; the degree to which observations or measurements of the same property, usually obtained under similar conditions, conform to themselves. Usually expressed as standard deviation, variance, or range.
Quality assurance and quality control	A system of procedures, checks, audits, and corrective actions to ensure that all research design and performance, environmental monitoring and sampling, and other technical and reporting activities are of the highest achievable quality.

Reference sediment	A sediment, substantially free of chemicals, that is as similar as practicable to the grain size of the test material and the sediment at the disposal site and that reflects the conditions that would exist in the vicinity of the site had no anthropogenic activity ever taken place but had all other influences on sediment condition taken place.
Reference area	An area that has similar characteristics to a site being evaluated but that is unaffected by chemicals of potential concern. The reference area is compared to the site to assess the effects of chemicals of potential concern.
Route	The mechanism of contact between an organism and a toxic chemical (e.g., ingestion or dermal contact).
Site-specific	Of or relating to a particular area or location.
Sediments	Material, such as sand, silt, or clay, suspended in or settled on the bottom of a water body.
Sublethal	Causing an endpoint other than death; growth is a sublethal endpoint in toxicity tests.
Terrestrial	Living or growing on land.
Toxicity test	A test in which organisms are exposed to chemicals in a test medium (e.g., waste, sediment, soil) to determine the effects of exposure.
Trophic	Relating to food or feeding relationships. Trophic levels consist of producers (plants), herbivores or primary consumers, carnivores or secondary consumers, and top carnivores or tertiary consumers.

REFERENCES AND OTHER KEY LITERATURE

ASTM. 1980. *Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians*. E729-80. American Society for Testing and Materials, Philadelphia, PA.

ASTM. 1984. Standard Practice for Conducting Acute Toxicity Tests on Wastewaters with *Daphnia*. *Annual Book of ASTM Standards*. D4229-84. American Society for Testing and Materials, Philadelphia, PA. pp. 64-76.

ASTM. 1989. *Standard Guide for Conducting Static Acute Toxicity Tests Starting with Embryos of Four Species of Saltwater Bivalve Molluscs*. E724-89. American Society for Testing and Materials, Philadelphia, PA.

ASTM. 1990. *Standard Guide for Conducting 10-day Static Sediment Toxicity Tests with Marine and Estuarine Amphipods*. E1367-90. American Society for Testing and Materials, Philadelphia, PA.

ASTM. 1991a. *Standard Guide for Collection, Storage, Characterization, and Manipulation of Sediments for Toxicological Testing*. E1391-90. American Society for Testing and Materials, Philadelphia, PA.

ASTM. 1991b. *Standard Guide for Conducting Sediment Toxicity Tests with Freshwater Invertebrates*. E1383-90. American Society for Testing and Materials, Philadelphia, PA.

Burton, G.A., Jr. 1991. Assessing the Toxicity of Freshwater Sediments. *Environ. Toxicol. Chem.* 10:1585-1627.

Burton, G.A., Jr. 1992. Sediment Collection and Processing: Factors Affecting Realism. In: *Sediment Toxicity Assessment*. G.A. Burton (ed). Lewis Publishers, Inc., Ann Arbor, MI. pp. 167-182.

Chapman, P.M., R.N. Dexter, and E.R. Long. 1987. Synoptic Measures of Sediment Contamination, Toxicity, and Infaunal Community Structure (the Sediment Quality Triad) in San Francisco Bay. *Mar. Ecol. Prog. Ser.* 37:75-96.

Chapman, P.M., E.A. Powers, and G.A. Burton, Jr. 1992. Integrative Assessments in Aquatic Ecosystems. In: *Sediment Toxicity Assessment*. G.A. Burton (ed). Lewis Publishers, Boca Raton, FL. pp. 313-340.

Dillon, T.M. and A.B. Gibson. 1990. *Review and Synthesis of Bioassessment Methodologies for Freshwater Contaminated Sediments*. Final Report. Misc. Paper EL-90-2. Prepared for U.S. Environmental Protection Agency, Great Lakes National Program Office, Chicago, IL. U.S. Army Corps of Engineers, Waterways Experiment Stations, Vicksburg, MS. 26 pp. + appendices.

Environment Canada. 1990a. *Biological Test Method: Acute Lethality Test Using *Daphnia* spp.* Report EPS 1/RM/11. Environment Canada, Conservation and Protection, Environmental Protection, Ottawa, Ontario.

Environment Canada. 1990b. *Biological Test Method: Acute Lethality Test Using Rainbow Trout*. Report EPS 1/RM/9. Environment Canada, Conservation and Protection, Environmental Protection, Ottawa, Ontario.

Environment Canada. 1990c. *Biological Test Method: Acute Lethality Test Using Threespine Stickleback*. Report EPS 1/RM/10. Environment Canada, Conservation and Protection, Environmental Protection, Ottawa, Ontario.

Environment Canada. 1990d. *Biological Test Method: Reference Method for Determining Acute Lethality of Effluents to *Daphnia magna**. Report EPS 1/RM/14. Environment Canada, Conservation and Protection, Environmental Protection, Ottawa, Ontario.

Environment Canada. 1990e. *Biological Test Method: Reference Method for Determining Acute Lethality of Effluents to Rainbow Trout*. Report EPS 1/RM/13. Environment Canada, Conservation and Protection, Environmental Protection, Ottawa, Ontario.

Environment Canada. 1992a. *Biological Test Method: Acute Test for Sediment Toxicity Using Marine or Estuarine Amphipods*. Report EPS 1/RM/26. Environment Canada, Conservation and Protection, Environmental Protection, Ottawa, Ontario. 83 pp.

Environment Canada. 1992b. *Biological Test Method: Fertilization Assay Using Echinoids (Sea Urchins and Sand Dollars)*. Report EPS 1/RM/27. Environment Canada, Conservation and Protection, Environmental Protection, Ottawa, Ontario.

Environment Canada. 1992c. *Biological Test Method: Growth Inhibition Test Using Freshwater Alga *Selenastrum capricornutum**. Report EPS 1/RM/25. Environment Canada, Conservation and Protection, Environmental Protection, Ottawa, Ontario.

Environment Canada. 1992d. *Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows*. Report EPS 1/RM/22. Environment Canada, Conservation and Protection, Environmental Protection, Ottawa, Ontario. 70 pp.

Environment Canada. 1992e. *Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia**. Report EPS 1/RM/21. Environment Canada, Conservation and Protection, Environmental Protection, Ottawa, Ontario. 72 pp.

Environment Canada. 1992f. *Biological Test Method: Toxicity Test Using Luminescent Bacteria (*Photobacterium phosphoreum*)*. Report EPS 1/RM/24. Environment Canada, Conservation and Protection, Environmental Protection, Ottawa, Ontario.

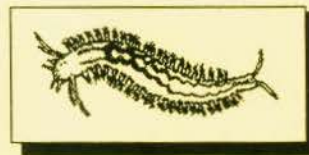
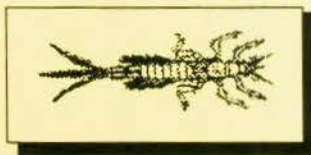
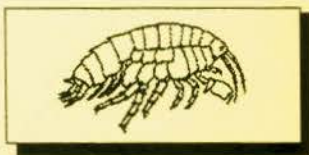
Gad, S.C., and C.S. Weil. 1986. *Statistics and Experimental Design for Toxicologists*. Telford Press, Caldwell, NV.

Giesy, J.P., and R.A. Hoke. 1990. Freshwater Sediment Quality Criteria: Toxicity Assessment. In: *Sediments: Chemistry and Toxicity of In-Place Pollutants*. R. Baudo, J. Giesy, and H. Muntau (eds). Lewis Publishers, Inc., Ann Arbor, MI. pp. 265-348.

Hill, I.R., P. Matthiessen, and F. Heimbach. 1993. *Guidance Document on Sediment Toxicity Tests and Bioassays for Freshwater and Marine Environments*. Presented at the Workshop on Sediment Toxicity Assessment, November 8-10, Slot Moermond Congresscentrum, Renesse, The Netherlands. Society of Environmental Toxicology and Chemistry, European Chapter.

- Johns, D.M., T.C. Ginn, and D.J. Reish. 1992. *Protocol for Juvenile Neanthes Sediment Bioassay*. Prepared by PTI Environmental Services, Bellevue, WA. EPA 910/9-90-011. U.S. Environmental Protection Agency, Office of Puget Sound, Puget Sound Estuary Program, Seattle, WA. 17 pp.
- Lamberson, J.O., T.H. DeWitt, and R.C. Swartz. 1992. Assessment of Sediment Toxicity to Marine Benthos. In: *Sediment Toxicity Assessment*. G.A. Burton (ed). Lewis Publishers, Ann Arbor, MI. pp. 183-211.
- McGee, B.L., C.E. Schlekot, and E. Reinharz. 1993. Assessing the Sublethal Levels of Sediment Contamination Using the Estuarine Amphipod *Leptocheirus plumulosus*. *Environ. Toxicol. Chem.* 12:577-587.
- Moore, D.W., T.M. Dillon, J.Q. Word, and J.A. Ward. 1994. *Quality Assurance/quality Control (QA/QC) Guidance for Laboratory Dredged Material Bioassays*. Miscellaneous Paper D-94-3. U.S. Army Corps of Engineers, Waterways Experiment Station, Vicksburg, MS.
- PTI Environmental Services. 1994. *User's Guide and Technical Resource Document: Evaluation of Sediment Toxicity Tests for Biomonitoring Programs*. (published separately) API Publication No. 4607. American Petroleum Institute. Washington, D.C.
- Suedel, B.C., and J.H. Rodgers, Jr. 1994. Development of Formulated Reference Sediments for Freshwater and Estuarine Sediment Testing. *Environ. Toxicol. Chem.* 13:1163-1175.
- U.S. EPA. 1991a. *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*. EPA 600/4-90-027. U.S. Environmental Protection Agency, Office of Research and Development, Washington, D.C. 293 pp.
- U.S. EPA. 1991b. *Recommended Guidelines for Conducting Laboratory Bioassays on Puget Sound Sediments*. Recommended Protocols for Measuring Environmental Variables in Puget Sound. U.S. Environmental Protection Agency Region 10, Office of Puget Sound, Puget Sound Estuary Program. Seattle, WA.
- U.S. EPA. 1991c. *Technical Support Document for Water Quality-based Toxics Control*. EPA 505/2-90-001. U.S. Environmental Protection Agency, Office of Research and Development, Washington, D.C. 145 pp. + appendices.
- U.S. EPA. 1992. *Proceedings of the EPA Workshop on Tiered Testing Issues for Freshwater and Marine Sediments*. September 16-18, 1992. U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology and Office of Research and Development, Washington, D.C.
- U.S. EPA and U.S. COE. 1991. *Evaluation of Dredged Material Proposed for Ocean Disposal. Testing Manual*. EPA 503/8-91-001. U.S. Environmental Protection Agency, Office of Water and U.S. Army Corps of Engineers, Washington, D.C.
- U.S. EPA and U.S. COE. 1993. *Evaluation of Dredged Material Proposed for Discharge in Inland and Near Coastal Waters—Testing Manual*. Draft Report. U.S. Environmental Protection Agency, Washington, D.C.

**Technical Resource Document:
Evaluation of Sediment
Toxicity Tests for
Biomonitoring Programs**



ABSTRACT

Sediment toxicity test methods are available for marine, estuarine, and freshwater sediments and organisms. The methods can be used for a variety of purposes: for example, assessment of existing environmental conditions, monitoring changes with time, or for NPDES permit compliance. Use of inappropriate test methods or species for a given purpose can impact the toxicity results and their interpretation. This Technical Resource document has been prepared as a detailed resource for environmental support staff for petroleum industry facilities (refineries, marketing terminals, and production locations) in their selection of sediment toxicity test methods and species which are reliable, scientifically valid, and appropriate for the habitat. Test methods are evaluated for their reliability, ecological relevance, exposure relevance, availability, interferences, and ability to discriminate toxicants. The tests are categorized by habitat type (marine, estuarine, or freshwater) and test endpoint (lethal or sublethal). Species' descriptions, test protocols, and documentation of method evaluations are contained in appendices. A companion report (User's Guide) has been prepared to provide an introduction to sediment toxicity test methods and to summarize their use.

CONTENTS

	<u>Page</u>
EXECUTIVE SUMMARY	ES-1
INTRODUCTION	1
OBJECTIVES	1
USE OF DOCUMENT	1
USER'S GUIDE AND RELATED DOCUMENTS	2
REPORT ORGANIZATION	2
TEST SCREENING APPROACH	3
INFORMATION REVIEW	3
CLASSIFICATION OF TESTS	3
SELECTION OF TESTS FOR EVALUATION	3
EVALUATION CRITERIA AND APPROACH	4
Reliability	4
Ecological Relevance	5
Exposure Relevance	5
Availability	5
Interferences	5
Chemical Discrimination	6
Regulatory Status	6
CLASSIFICATION OF AVAILABLE TEST METHODS	7
CLASSIFICATION CRITERIA	7
Habitat Type	7
Endpoint Type	7
KEY CHARACTERISTICS	8
Exposure Medium	8
Characteristics of Test Organisms	10
Test Duration	11
EVALUATION FOR TEST SELECTION	12
MARINE LETHAL TESTS	12
MARINE SUBLETHAL TESTS	24
ESTUARINE LETHAL TESTS	25
ESTUARINE SUBLETHAL TESTS	25
FRESHWATER LETHAL TESTS	25



	<u>Page</u>
FRESHWATER SUBLETHAL TESTS	26
APPLICATION OF SEDIMENT TOXICITY TESTS	27
STUDY DESIGN SPECIFICATIONS	27
GENERAL ASSESSMENT APPROACH	27
SELECTION OF TOXICITY TESTS	28
EFFECTS ASSESSMENT	28
ANCILLARY INFORMATION	29
Supplemental Sediment Variables	29
Sediment Chemical Concentrations	30
<i>In Situ</i> Biological Effects	30
CONCLUSIONS	32
GLOSSARY	34
REFERENCES	38

APPENDIX A - Classification of Sediment Toxicity Tests
APPENDIX B - Species Summaries
APPENDIX C - Overview of Test Methods
APPENDIX D - Detailed Evaluation Tables
APPENDIX E - User's Guide

LIST OF TABLES

	<u>Page</u>
Table 1. Sediment phases used in toxicity tests	9
Table 2. Evaluation of sediment toxicity tests - marine lethal	13
Table 3. Evaluation of sediment toxicity tests - marine sublethal	15
Table 4. Evaluation of sediment toxicity tests - estuarine lethal	17
Table 5. Evaluation of sediment toxicity tests - estuarine sublethal	19
Table 6. Evaluation of sediment toxicity tests - freshwater lethal	20
Table 7. Evaluation of sediment toxicity tests - freshwater sublethal	22



EXECUTIVE SUMMARY

Federal and state agencies are developing and implementing water quality-based approaches to regulate permitted wastewater discharges. As part of this overall approach, sediment toxicity testing may be required as part of discharge monitoring programs. Toxicity tests of sediments provide an integrated measure of sediment contamination that eliminates much of the uncertainty associated with theoretical predictions of sediment toxicity at a specific site. The value of the biological effects-based approach can be diminished, however, by using inappropriate test methods or species or by not considering the many physical and chemical factors that can affect biological testing. To date, the selection of a sediment toxicity test(s) for a specific site has not been a straightforward process. The selection process is complicated by the constant introduction of new test species and methods. There have been few objective comparisons among alternative tests and there is a general lack of recommendations for the most appropriate sediment test procedures for a given situation.

OBJECTIVES AND APPROACH

The purpose of this document is to provide a resource tool that will enable environmental staff at petroleum facilities to readily select sediment toxicity tests and test methods that are reliable, scientifically valid, and appropriate for a specific site.

The test methods and species identified during the information review were classified into six groups based on the following physical and biological test characteristics:

- Habitat type (marine, estuarine, or fresh water)
- Test endpoint (lethal or sublethal).

Each toxicity test within each of the six groups is summarized in a classification table that also contains information on the organisms, including the broad taxonomic group, common name, scientific name, and life stage of the species used in the test; exposure type (whole sediment, interstitial water,

water elutriate, or solvent extract); the exposure duration; and the primary literature references. In some cases, tests differed only by the species, life stage, or exposure period used. Even so, the tests were maintained as separate entities for the evaluation because minor differences in organisms or exposure duration may significantly influence test sensitivity.

The tests in the initial screening were evaluated according to various technical criteria. An overall technical rating was based on the following evaluation criteria:

- Reliability
- Ecological relevance
- Exposure relevance
- Availability
- Interferences
- Chemical discrimination.

RECOMMENDED TESTS

This report contains a series of evaluation tables that describe the group of relevant toxicity tests for each habitat (marine, estuarine, or freshwater) and for each endpoint type (lethal and sublethal). In most cases, the highest ranking tests were the ones based on the exposure of infaunal organisms to whole sediments. These kinds of tests were ranked highly because they rely on exposure conditions that closely mimic field conditions. Many of the test species are available by field collection during most of the year. The highly ranked marine infaunal tests predominantly used amphipods as test organisms, whereas most of the highly ranked freshwater infaunal tests predominantly used insects (mayfly nymphs and midge larvae) as test organisms.

In many cases, the lowest ranking toxicity tests were those that rely on the exposure of planktonic organisms to whole sediments. Those tests have a relatively low exposure relevance because the test organisms are rarely exposed to sediments in the field. In addition, the tests are sensitive to potential interferences from sediments in the test chambers.

In some cases, a highly ranked lethal test was also a highly ranked sublethal test. The inclusion of a test in both categories is a particularly desirable characteristic when both lethal and sublethal endpoints will be evaluated in a biomonitoring program. By using the same species to evaluate both endpoints, potential interpretive problems related to interspecific differences in factors such as sensitivity to toxicity can be avoided.

USER'S GUIDE

A companion document, *User's Guide: Evaluation of Sediment Toxicity Tests for Biomonitoring Programs* (PTI, 1994) has been prepared to provide an introduction to sediment toxicity testing and to describe how to use this resource manual for those readers who are unfamiliar with sediment toxicity testing.

INTRODUCTION

The U.S. Environmental Protection Agency (EPA) has established a water-quality based approach to control potentially toxic substances in wastewater effluents that are discharged to inland and coastal waters (U.S. EPA 1991c). Toxicity testing of effluents and ambient water or sediments in the vicinity of wastewater discharges is a key element in predicting the potential biological effects of discharges and in monitoring existing impacts. For example, requirements for toxicity testing of whole effluents are being incorporated into many wastewater discharge permits under the National Pollutant Discharge Elimination System (NPDES). At the same time, development of sediment toxicity tests that could be used to monitor discharge sites is proceeding at a relatively rapid pace at the federal and state levels (U.S. EPA 1992).

Toxicity tests of sediments provide an integrated measure of sediment contamination that eliminates much of the uncertainty associated with theoretical predictions of sediment toxicity at a specific site. The value of the biological effects-based approach can be diminished, however, by using inappropriate test methods or species, or by not considering the many physical and chemical factors that can affect biological testing. To date, the selection of a sediment toxicity test for a specific site has not been a straightforward process.

OBJECTIVES

The purpose of this resource document is to provide information that will enable environmental staff at petroleum facilities to select sediment toxicity tests and test methods that are reliable, scientifically valid, and appropriate for a specific site. This document also summarizes the technical requirements and appropriate conditions for use of sediment toxicity test methods anticipated for future NPDES permit compliance. This document was developed specifically for use by petroleum industry operations (refineries, marketing terminals, and

production facilities) that have effluent discharges to surface waters. However, this Technical Resource Document and the User's Guide contain information that is applicable to other industries and could be used by any wastewater discharger.

USE OF DOCUMENT

The first step when using the decision framework and information in this document is a **review of available information** on the ecological characteristics of the discharge site. Available data on site-specific chemicals and physical properties of the sediments can be useful in selecting test species that are sensitive to the chemicals of concern at the site, but that are minimally affected by the natural properties of the sediment (e.g., grain size, organic carbon, ammonia). An equally important step in the selection of sediment toxicity tests is a **thorough understanding of the applicable regulatory requirements** that are driving the testing program. Regulatory programs frequently include explicit requirements that immediately limit the field of potential toxicity tests. These confining factors can include specifications for lethal or sublethal tests, exposure duration, seasons for testing, single species vs. a battery of species for testing, and data quality objectives.

This report contains a series of evaluation tables that describe the group of relevant toxicity tests for each habitat system (marine, estuarine, or fresh water) and for each test type (lethal and sublethal). These tables summarize the key information used in selecting toxicity tests. **Users enter the evaluation tables for a given system and test type** (e.g., estuarine/lethal). In each evaluation table, candidate tests are ranked from best overall tests to least appropriate tests. In most cases, the higher ranked tests may have very similar overall scores. Therefore, **the user should select the most appropriate high-ranked test** for a particular program based on a consideration of site-specific factors and regulatory considerations. The selected test should match the site-specific requirements for the biotic group and geographic zone.

The candidate toxicity test(s) that were tentatively selected in the previous step should be compared with the regulatory requirements identified. If the test (or tests) meets these requirements, then the selection process is complete and the actual test (or tests) can be performed. If the selected toxicity test (or tests) does not meet the requirements of the applicable regulatory program, then users have the option of entering into negotiations with the regulatory agencies. The information provided in the evaluation tables and the appendices will be useful in developing the arguments for and against particular tests. Detailed guidance on test selection is provided in a companion document, *User's Guide: Evaluation of Sediment Toxicity Tests for Biomonitoring Programs* (PTI, 1994).

USER'S GUIDE AND RELATED DOCUMENTS

A companion document, *User's Guide: Evaluation of Sediment Toxicity Tests for Biomonitoring Programs* (PTI, 1994) has been prepared to provide an introduction to sediment toxicity testing and to present how to use this resource manual for those readers who are unfamiliar with sediment toxicity testing. The User's Guide contains descriptions of habitat type, sediment test systems, and biological endpoints. Site-specific concerns are identified to aid in test selection. Lastly, brief summaries of sampling and data analysis issues are presented.

The Society of Environmental Toxicology and Chemistry has recently released a guidance document on use of sediment toxicity tests (Hill *et al.* 1993). Other useful review documents include Dillon and Gibson (1990), Burton (1991), and U.S. EPA (1992).

REPORT ORGANIZATION

The next section presents the approach used to compile and evaluate information on sediment toxicity test methods. The following sections classify available test methods using technical criteria. Technical evaluation results and guidance on application of toxicity tests in the context of

NPDES programs are then provided in a sequence of tables for each system and toxicity test type (e.g., freshwater/sublethal). The highest ranked tests are shown first. Supporting summary materials are in appendices. Appendix A classifies the available sediment toxicity tests that were considered in this evaluation. Appendix B presents profiles of the species commonly used in the tests that ranked highest in the evaluation. Appendix C provides an overview of the key test methods. Appendix D is a summary table of the rationale for the evaluation results, organized by evaluation criteria. Key technical terms used herein are defined in the *Glossary*.

TEST SCREENING APPROACH

Toxicity test information, the classification of tests, the selection of tests for evaluation, and the development and application of evaluation criteria are described below.

INFORMATION REVIEW

The sources consulted to identify available test methods and species included Current Contents (a compilation of information contained in agricultural, biological, and environmental science journals); a bibliography of the Ecological Risk Assessment Library at PTI Environmental Services; and recent reviews of test methods, including Giesy and Hoke (1990), Burton (1991, 1992a), Burton *et al.* (1992), Lamberson *et al.* (1992), Bennett and Cabbage (1992), and the proceedings of the EPA workshop on *Tiered Testing Issues for Freshwater and Marine Sediments* (U.S. EPA 1992). Major documents discussing test methods and test strategies (e.g., U.S. EPA 1991b; U.S. EPA and U.S. COE 1991, 1993; Environment Canada 1990a–e, 1992a–f) were also reviewed to identify candidate tests. Finally, information from colleagues and agencies that are conducting ongoing investigations of test methods was collected and used to identify relatively new tests that show promise for testing of contaminated sediments.

CLASSIFICATION OF TESTS

The toxicity test methods and species identified during the information review were classified into six groups based on a series of physical and biological test characteristics. The objective of this step was to identify each group of test methods that have common characteristics and that, therefore, should be considered as possible alternatives for use at all facilities with similar receiving waters. The characteristics used to classify available test methods and species into the six groups are:

- Habitat type (marine, estuarine, or freshwater)
- Toxicity test endpoint (lethal or sublethal).

Habitat type is a key physical factor in narrowing the list of appropriate test species for use at a facility. For example, few marine sediment toxicity tests will be applicable at a facility that discharges to fresh water (one exception is the Microtox® test, which can be adjusted for different salinity regimes). For purposes of this study, habitat categories were defined as follows:

- Marine (≥ 28 ppt)
- Estuarine (> 0.5 and < 28 ppt)
- Freshwater (≤ 0.5 ppt)

Because the division between habitat categories is an artificial distinction, use of a particular habitat designation for a test in this report should not necessarily preclude the application of a test to sediments in other habitats. For example, some tests that are classified as marine tests may be applied to high salinity estuarine sediments, and in some cases, adjusting the salinity of a sediment sample to allow the use of a particular test may be appropriate. The test endpoint is a key biological factor in comparing and evaluating performance data among toxicity tests and satisfying regulatory requirements. Each toxicity test is summarized in a classification table that also contains information on the organisms (see *Classification of Available Test Methods* section and Appendix A).

SELECTION OF TESTS FOR EVALUATION

All of the individual toxicity tests identified in this review are listed in the classification tables (Appendix A). To facilitate detailed consideration of the most promising tests, tests were selected for further evaluation based on the following criteria:

- Tests recommended in guidance documents issued recently by national agencies in the United States and Canada (e.g., Dillon and Gibson 1990; U.S. EPA and U.S. COE 1991,

1993; U.S. EPA 1991b, 1992; Environment Canada 1990a-e, 1992a-f)

- Tests recommended in recent evaluations of sediment toxicity tests (e.g., Dillon and Gibson 1990; Giesy and Hoke 1990; Bennett and Cubbage 1992)
- Tests that are under development in several laboratories and show promise for application to NPDES sediment testing for petroleum refineries (e.g., relatively new tests included in EPA research and development; U.S. EPA 1992).

Tests that were not evaluated (Appendix A, Attachment 1) included relatively old ones (>10 years old) that are generally not used now, very new ones that need substantial method development, and tests with inadequate specification of the method to classify them.

EVALUATION CRITERIA AND APPROACH

Criteria to evaluate sediment toxicity tests include repeatability, precision, dose-responsiveness, discrimination among chemicals or sites, sensitivity, ecological relevance, appropriateness of the sample phase (e.g., particulate, extract), exposure period, relationship to field effects, availability, ease of use, and stage of method development (Pastorok and Becker 1989; Giesy and Hoke 1990; Burton 1991). An overall technical rating was based on the following technical evaluation criteria:

- Reliability
- Ecological relevance
- Exposure relevance
- Availability
- Interferences
- Chemical discrimination.

Each test was rated according to each criterion using a scoring system of 0 to 4, with 4 being the most favorable score. The overall technical rating was

derived by summing the scores for the individual criteria. The scores for "interferences" and "chemical discrimination" were weighted by a factor of 0.5 to reduce their influence on the overall rating. Less information was available for these two criteria and, therefore, confidence in their scores was lower than those for other criteria. Because the cost of a given sediment toxicity test varies greatly with its status for regulatory use and with the laboratory conducting the test, cost was not considered in the overall rating. Details of the scoring system used to rank the toxicity tests are presented in Appendix D.

Factors considered in evaluating the tests relative to the technical criteria listed above and relative to the regulatory status of tests are described in more detail in the following sections.

Reliability

The reliability of a test method is high when the endpoint can be measured accurately, the results are repeatable, and the negative control results generally meet quality assurance criteria. High reliability ratings were assigned to tests that have been subjected to an interlaboratory comparison and found to have an acceptable level of variability within and among laboratories (e.g., ≤ 50 percent coefficient of variation). However, most sediment toxicity tests have not undergone interlaboratory studies. A test method was assigned low reliability if determination of the endpoint involves a high degree of subjectivity, if the response is easily biased by laboratory artifacts (e.g., organism stress due to confinement), or if repeated tests yield highly variable results. For example, many lethal tests involve a relatively straightforward determination of whether a fish or larger invertebrate is dead or alive. Such situations have minimal potential for error or bias on the part of the observer. Alternatively, some sublethal tests have a relatively subjective endpoint such as "abnormality" of a microscopic larvae. In such cases, the potential for individual bias is much greater. Tests were also assigned a low rating for reliability if the negative controls are prone to failures (e.g., high susceptibility of test organisms to laboratory stress).

Ecological Relevance

The ecological relevance of a test method is high if the results of a test method are directly applicable to indigenous species under field conditions. A test method has low ecological relevance if there is little or no basis for associating the method to the field conditions or if the method results display no concordance with the responses of indigenous organisms. Field validation studies provide the best evidence for judging ecological relevance, but most sediment toxicity tests have not undergone field validation tests. In lieu of information on field validation, any test that uses a species that is commercially harvested (e.g., oysters) or a species that provides habitat structure for other species (e.g., marsh grass) was assigned a high rating for ecological relevance.

Exposure Relevance

The exposure relevance of a test method is high when the pathway of exposure used in a test is analogous to exposure under field conditions (e.g., whole sediment exposure for chemicals that may be taken up by several routes such as pore water and ingestion of sediment particles). Other test procedures involve exposure of water column organisms to extracts of sediments or sediment interstitial water, which is not necessarily a good analog of field conditions. Exposure relevance is, therefore, low when test conditions mimic only some of the exposure pathways applicable to field conditions. The general ecological niche of the test organism was also considered in assessing exposure relevance. Thus, exposure of infaunal test organisms to whole sediment would have a higher exposure relevance than exposure of planktonic organisms to any of the test media.

Availability

The availability of a test method is high when test organisms can be easily obtained, the method is standardized and well documented, and commercial laboratories routinely perform the test. Test organisms that are easily cultured or are available from

the field throughout all seasons in a broad geographic area were assigned high ratings for this criterion. Test methods that are constrained by a limited supply of organisms (by season or geography) or a lack of experienced laboratories were assigned low scores for availability. A low rating was assigned to test methods that are still in the early stages of documentation and standardization; these methods will be difficult or costly to develop and use as quickly as routine methods.

Interferences

The interference criterion is a measure of the extent of confounding physical or chemical factors. The objective of sediment toxicity testing is to evaluate the response of the test species to target chemicals contained in the sediment sample. It is preferable that the species not be responsive to other sediment characteristics such as particle size or organic content. If such responses occur, toxicity may be incorrectly attributed to target chemicals. Changes in the following factors can restrict the application of a particular test or have a confounding effect on test results:

- Sediment grain size
- Organic carbon content
- Oxidation-reduction conditions
- pH
- Alkalinity
- Temperature
- Turbidity
- Water hardness
- Ultraviolet light intensity
- Mold or pathogens.

A high rating for interference was assigned to test methods that have a low susceptibility to interferences. A low rating for interference was assigned to test methods that exhibit a large number of interferences or a narrow range of applicable environmental conditions.

Chemical Discrimination

The chemical discrimination of a test method is high if the test results are dose-responsive over at least a moderate range of chemical concentrations. Test methods that exhibit high chemical discrimination are expected to be useful in defining gradients of sediment toxicity in the environment. Test methods that are either insensitive or always highly-sensitive to chemical contamination were assigned a low rating for chemical discrimination.

Regulatory Status

The rating for regulatory status was based on information from national and regional EPA offices and whether a test was recommended in guidance documents for potential use in NPDES programs, clean-up assessments, baseline monitoring, or dredged material testing. The guidance documents considered as the basis for rating regulatory status included the method documents issued by the Canadian government (Environment Canada 1990a–e, 1992a–f), the dredged material testing documents issued by United States government agencies (U.S. EPA and U.S. COE 1991, 1993), and a major research and development planning document issued by EPA (U.S. EPA 1992). If a test was included in 3–4 of these document categories, it was assigned a rating of “high” for regulatory status. If a test was included in 1–2 of these document categories, it was assigned a rating of “medium.” Toxicity tests that were not included in these documents and were not known to be recommended for use in current regulatory programs were assigned a rating of “low.”

CLASSIFICATION OF AVAILABLE TEST METHODS

The available toxicity test methods were classified by type of habitat (marine, estuarine, and fresh water) to which each method applies and the general endpoint type (lethal or sublethal) specified for each test. This classification scheme resulted in the following six major categories of tests:

- Marine lethal
- Marine sublethal
- Estuarine lethal
- Estuarine sublethal
- Freshwater lethal
- Freshwater sublethal.

Within each category, tests are distinguished by three key characteristics: 1) the exposure medium, 2) characteristics of the test organisms, and 3) the test duration.

Using the classification criteria and key characteristics described above, 336 unique tests were identified (Appendix A). Each test was assigned a number to allow users of this document to track a given test through the various evaluation tables. In many cases, several of the specified tests were actually variations of a single test method and were assigned the same test number. For example, if the test species is tolerant of both marine and estuarine conditions and if both lethal and sublethal endpoints are possible (e.g., the amphipod test using *Ampelisca abdita*), then the test can be classified four different ways.

CLASSIFICATION CRITERIA

Use of general habitat type and endpoint type to classify sediment toxicity tests provides a broad

classification scheme. This approach should facilitate review of evaluation results and selection of tests at specific sites.

Habitat Type

The primary characteristic that distinguishes marine, estuarine, and freshwater habitat types is water salinity. Salinity strongly influences the distributions of most of the test organisms. In some cases, test organisms are tolerant of both marine and estuarine conditions or both estuarine and freshwater conditions. However, few test organisms tolerate both marine and freshwater conditions.

Endpoint Type

The two major types of endpoints for most toxicity tests are lethal and sublethal. The lethal endpoint is based on the percentage of test organisms that die during the exposure period. The use of mortality as a test endpoint has several advantages. Mortality represents an unambiguous adverse effect (i.e., death). In addition, the ecological significance of the endpoint is relatively certain. If the test organisms cannot survive in association with the test sediments, it is likely that significant alterations of aquatic assemblages would be found in the environment. Although mortality may appear to not be an environmentally protective endpoint, tests that use a sensitive species may be protective of less-sensitive species.

Sublethal endpoints represent a wide variety of organism responses other than mortality. Some of the most commonly measured sublethal endpoints include:

- Reduced growth
- Reproductive effects
- Developmental abnormality
- Histopathological abnormalities.

The reliability of any sublethal endpoint test depends on the use of experienced laboratory personnel.

Reduced growth is generally measured in juvenile organisms (i.e., when growth is generally expected to be rapid) and can be estimated directly by measuring the size of organisms (e.g., length, biomass) prior to and following exposure to test media. Growth can also be represented as an instantaneous measurement called "scope for growth," which is based on physiological variables such as feeding rate, absorption efficiency, respiration rate, and excretion. Growth itself does not require extensive expertise to measure, but scope for growth does. Both measures of growth are quantitative and objective. However, the ecological relevance of reductions in organism growth is uncertain or varies considerably among test species. If organisms can maintain their normal level of fecundity, then no reductions in population levels may be experienced. A reduction in size of adult organisms may cause organisms to be less desirable to some predators and more desirable to others, thereby influencing trophic relationships. Growth reductions may also enhance the risk of being preyed on because organisms cannot reach a size large enough to escape predation.

Reproductive effects are frequently measured in adult female organisms as number of eggs per individual, percentage of ovigerous individuals, and time to sexual maturity. These measurements can be made in a relatively quantitative and objective manner. Reproductive effects may be more ecologically relevant than growth because they imply that the local supply of recruits to adult populations may be reduced. However, adult populations could be sustained by recruitment of pelagic larvae from surrounding areas or immigration by adult organisms.

Developmental abnormalities are generally evaluated in the early life stages (e.g., embryos, larvae) of the test organisms. Bivalve molluscs and echinoderms are two groups of organisms that are frequently used to evaluate developmental abnormalities. Because the determination of abnormal development can be somewhat subjective, the abnormality endpoint can be somewhat ambiguous. Much of the potential subjectivity of the abnormality endpoint can be avoided by standardizing and clearly defining abnormalities. The ecological relevance of the abnormality endpoint is uncertain. Although the presence of

abnormalities in early life stages suggests that recruitment to adult assemblages may be curtailed, it is possible that those assemblages could be maintained by a limited level of recruitment or by immigration of adult organisms (for mobile species). Abnormality endpoints are typically selected to represent conditions that limit the successful growth or reproduction of individuals. Because larvae represent a sensitive life stage and because abnormalities may be expected to occur prior to the onset of mortality, the abnormality endpoint can generally be considered environmentally protective.

Histopathological abnormalities include measures of degeneration, necrosis, and other abnormalities in cells and tissues. The determination of these disorders requires a highly trained pathologist and frequently is subjective. The ecological relevance of histopathological disorders are uncertain because they may or may not influence an organism's life functions. For example, a malignant tumor will lead to negative consequences. With other kinds of abnormalities, the affected individual may experience no negative consequences and may eventually recover.

KEY CHARACTERISTICS

Exposure Medium

The kind of exposure medium was used to classify the various toxicity tests because each kind of exposure medium has favorable and unfavorable characteristics that can profoundly influence the toxicity test results. The four kinds of exposure media considered are whole sediments, sediment elutriates, interstitial water, and sediment extracts (Table 1). The first two media are commonly used in tests, whereas use of the latter two media is infrequent.

The use of whole sediments is probably the most realistic exposure scenario because it mimics the manner in which most organisms are exposed to chemicals in the environment. Ankley *et al.* (1991) and Green *et al.* (1993) showed that toxicity may be detected in pore water or interstitial water tests