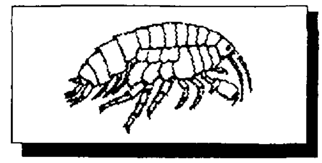

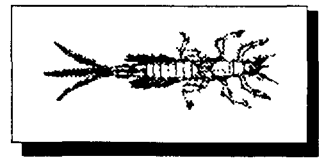

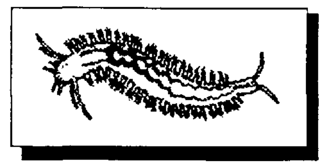

User's Guide: Evaluation of Sediment Toxicity Tests for Biomonitoring Programs

HEALTH AND ENVIRONMENTAL SCIENCES DEPARTMENT

API PUBLICATION NUMBER 4608

NOVEMBER 1994

STEP
Strategies for Today's Environmental Partnership

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

STEP
Strategies for Today's Environmental Partnership

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.

User's Guide:
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SCIENCES DEPARTMENT

API PUBLICATION NUMBER 4608

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OCTOBER 1994

American
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ACKNOWLEDGMENTS

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

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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.

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.

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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 documents 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 ($\geq$28 ppt)
- Estuarine ($>$0.5 ppt and $<$28 ppt)
- Freshwater ($\leq$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, cleanup 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

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

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

TABLE 3. EVALUATION OF SEDIMENT TOXICITY TESTS – ESTUARINE LETHAL

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	*Ampelisca abdita*	●	◕	◕	●	◕	◕	●	High	B,C,E
129	S	Amphipod	Large juveniles and adults	*Eohaustorius estuarius*	●	●	◑	●	◕	◕	●	High	C
003	S	Amphipod	Juveniles or young adults	*Eohaustorius washingtonianus*	●	●	◑	●	◕	◕	●	Med.	E
133	S	Amphipod	Juveniles	*Leptocheirus plumulosus*	●	●	◑	●	◕	◑	●	Low	B,E
135	S	Amphipod	Mixed sexes	*Leptocheirus plumulosus*	●	◕	◑	●	◕	◕	●	High	B
126	S	Amphipod	Juveniles or young adults	*Corophium volutator*	●	●	◑	◕	◕	◕	●	Med.	E
002	S	Amphipod	Juveniles or young adults	*Amphiporeia virginiana*	●	●	◑	●	◕	◕	◑	Med.	E
128	S	Amphipod	Adults	*Corophium volutator*	●	●	◑	◕	◕	◕	●	Low	E
134	S	Amphipod	Juveniles	*Leptocheirus plumulosus*	●	◕	◑	●	◕	◕	●	Low	B
131	S	Amphipod	7-14 days old	*Hyalella azteca*	●	◕	◑	◕	◑	●	●	High	B
020	S	Littleneck clam	Juveniles	*Protothaca staminea*	◕	◕	◕	●	◑	◕	○	Low	
017	EL	Blue mussel	Embryos	*Mytilus edulis*	◕	◑	●	○	◕	●	●	Med.	B
027	S	Blue crab	Juveniles	*Callinectes sapidus*	◕	◕	◕	◕	◔	●	◑	Low	
151	S	Grass shrimp	Post-hatch (1–4 days)	*Palaemonetes* sp.	◕	◕	◑	◕	◔	●	●	Low	
016	S	Blue mussel	Embryos	*Mytilus edulis*	◕	◑	●	◔	◕	○	●	Med.	B,C
009	EL	Eastern oyster	Embryos	*Crassostrea virginica*	◕	◑	●	○	◑	●	●	Med.	B
011	EL	Pacific oyster	Embryos	*Crassostrea gigas*	◕	◑	●	○	◑	●	●	Med.	B,D
030	S	Sand shrimp	Juveniles	*Crangon* sp.	◕	◕	◑	◕	◔	●	◑	Low	
153	EL	Sheepshead minnow	1–14 days old	*Cyprinodon variegatus*	◕	◕	◑	○	●	●	◔	Low	B
010	S	Pacific oyster	Embryos	*Crassostrea gigas*	◕	◑	●	◔	◑	○	●	Med.	B,C,D

Technical Rating: Overall Technical Rating through Chemical Responsiveness.

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

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TABLE 5. EVALUATION OF SEDIMENT TOXICITY TESTS – FRESHWATER LETHAL

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
241	S	Mayfly	Nymphs	*Hexagenia limbata*	●	◕	◕	●	◕	◕	●	Low	B,D
239	S	Mayfly	Young nymphs	*Hexagenia limbata*	●	◕	◕	●	◕	◕	●	Low	B,D
238	S	Midge	2nd instar larvae	*Chironomus tentans*	●	◕	◑	●	●	◑	●	High	B,D
237	S	Midge	Neonates or larvae	*Chironomus riparius*	●	◕	◑	●	●	◑	●	Med.	B
200	S	Amphipod	Juveniles	*Hyalella azteca*	●	◕	◑	◕	●	◕	●	High	B
242	S	Mayfly	Nymphs	*Hexagenia limbata*	●	◕	◑	●	◕	◕	●	Low	B,D
209	INT	Water flea	Neonates	*Daphnia magna*	●	●	◕	○	●	●	●	Low to Med.	B,D,E
256	S	Marsh grass	Seedlings	*Echinochloa crusgalli*	●	◕	●	●	◔	●	◑	Low	
250	S	Oligochaete	Large worms	*Lumbriculus variegatus*	●	◕	◑	●	◕	◕	◑	Low	B
249	S	Oligochaete	Mixed-age	*Lumbriculus variegatus*	●	◕	◑	●	◕	◕	◑	Low	B
212	EL	Water flea	Neonates (<24 hrs)	*Daphnia magna*	●	●	◑	○	●	●	●	Med.	B,D,E
240	INT	Mayfly	Nymphs	*Hexagenia limbata*	●	◕	◕	◑	◕	◑	●	Low	B,D
205	EL	Water flea	Neonates	*Ceriodaphnia dubia*	◕	●	◑	○	●	◕	●	Med.	B,E
213	EL	Water flea	Neonates (<24 hrs)	*Daphnia pulex*	◕	●	◑	○	●	◕	●	Med.	B,E
208	EL	Water flea	Neonates	*Daphnia magna*	◕	●	◑	○	●	◕	●	Med.	B,D,E
214	EL	Water flea	Neonates (<24 hrs)	*Daphnia pulex*	◕	◕	◑	○	●	●	●	Med.	B
202	S	Paper pondshell clam	Juveniles	*Anodonta imbecillis*	◕	◕	◑	●	◑	◕	◔	Low	
252	S	Oligochaete	Mixed age; similar size	*Pristina leidyi*	◕	◕	◑	◕	◑	●	◑	Low	B
255	S	Oligochaete	Mixed age	*Tubifex tubifex*	◕	◕	◑	◕	◑	●	◑	Low	B,D
222	EL	Bluegill	Approx. 4 days	*Lepomis macrochirus*	◕	●	◕	○	●	●	○	Low	B,E

Technical Rating

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

Test Number	Exposure Media	Common Name	Life Stage	Test Species	Technical Rating: Overall Technical Rating	Reliability	Ecological Relevance	Exposure Relevance	Availability	Interferences	Chemical Responsiveness	Regulatory Status	Comments
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
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◑		
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○	low	none

EL Elutriate
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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*, *Lytechinis 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 (*Hyalella 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.

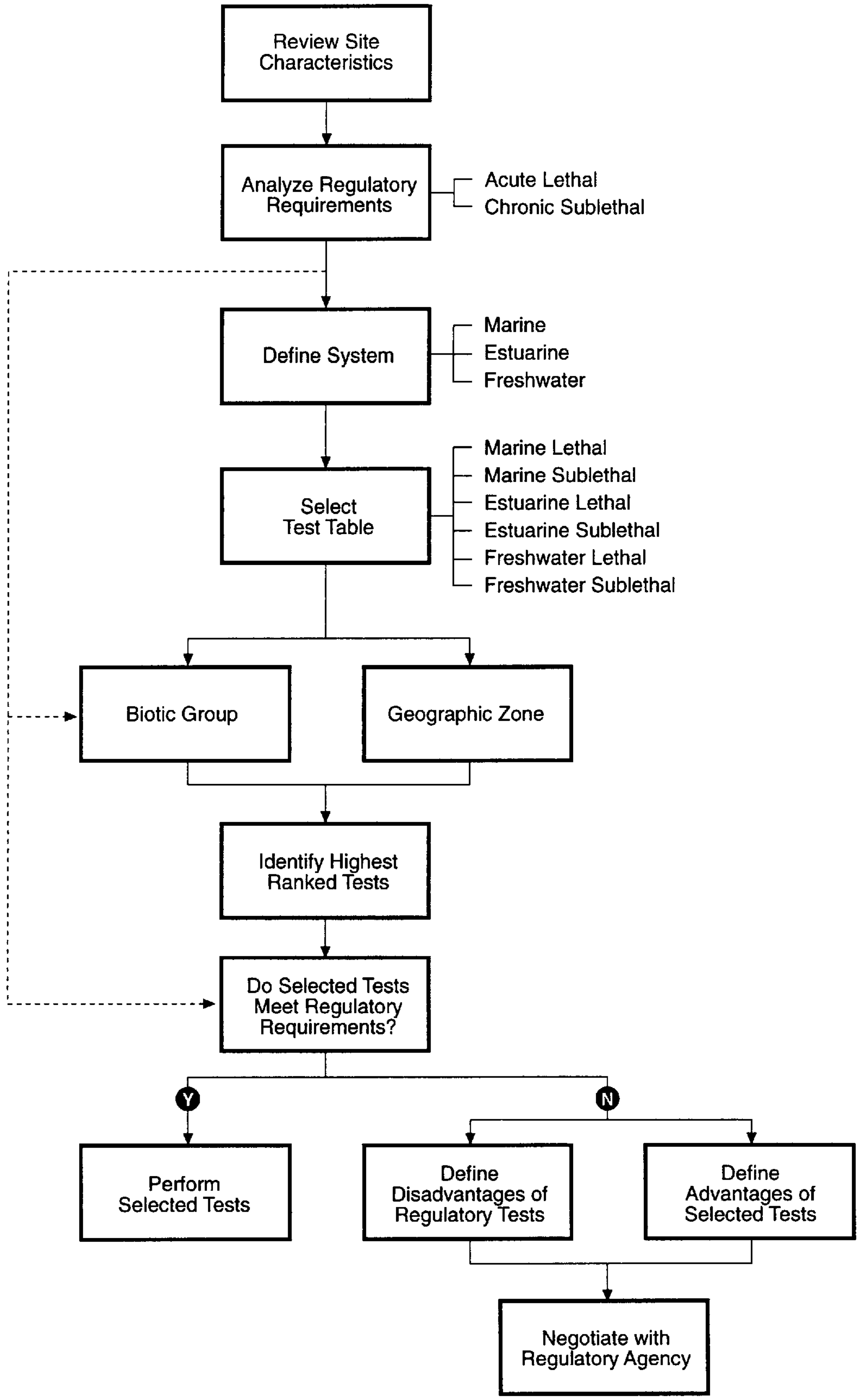

Figure 1. Approach to selection of sediment toxicity tests for a specific site.

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 AND REFERENCES

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., μg/L [micrograms of chemical per liter of water] or μg/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.
In situ	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.

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