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# Standard Guide for Designing Biological Tests with Sediments[1]

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## 1. Scope

1.1 As the contamination of freshwater and saltwater ecosystems continues to be reduced through the implementation of regulations governing both point and non-point source discharges, there is a growing emphasis and concern regarding historical inputs and their influence on water and sediment quality. Many locations in urban areas exhibit significant sediment contamination, which poses a continual and long-term threat to the functional condition of benthic communities and other species inhabiting these areas **(1)**.[2] Benthic communities are an important component of many ecosystems and alterations of these communities may affect water-column and nonaquatic species.

1.2 Biological tests with sediments are an efficient means for evaluating sediment contamination because they provide information complementary to chemical characterizations and ecological surveys **(2)**. Acute sediment toxicity tests can be used as screening tools in the early phase of an assessment hierarchy that ultimately could include chemical measurements or bioaccumulation and chronic toxicity tests. Sediment tests have been applied in both saltwater and freshwater environments **(2-6)**. Sediment tests have been used for dredge material permitting, site ranking for remediation, recovery studies following management actions, and trend monitoring. A particularly important application is for establishing contaminant-specific effects and the processes controlling contaminant bioavailability **(7)**.

1.3 This guide is arranged as follows:



1.4 The values stated in SI units are to be regarded as the standard. The values given in parentheses are for information only.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* For specific hazard statements, see Section 7.

## 2. Referenced Documents

2.1 *ASTM Standards:*

D 1129 Terminology Relating to Water[3]

D 4447 Guide for the Disposal of Laboratory Chemicals and Samples[4]

E 724 Guide for Conducting Static Acute Toxicity Tests Staring with Embryos of Four Species of Saltwater Bivalve Mollusc[4]

E 729 Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians[4]

E 943 Terminology Relating to Biological Effects and Environmental Fate[4]

E 1023 Guide for Assessing the Hazard of a Material to Aquatic Organisms and Their Uses[4]

E 1367 Guide for Conducting 10-Day Static Sediment Toxicity Tests with Marine and Estuarine Amphipods[4]

E 1383 Guide for Conducting Sediment Toxicity Tests with Freshwater Invertebrates[4]

E 1391 Guide for Collection, Storage, Characterization, and Manipulation of Sediments for Toxicological Testing[4]

E 1563 Guide for Conducting Static Acute Toxicity Tests with Echinoid Embryos[4]

E 1611 Guide for Conducting Sediment Toxicity Tests with Polychaetous Annelids[4]

E 1676 Guide for Conducting a Laboratory Soil Toxicity Test with the Lumbricid Earthworm *Eisenia foetida*[4]

E 1688 Guide for Determination of the Bioaccumulation of Sediment-associated Contaminants by Benthic Invertebrates[4]

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[2] The boldface numbers in parentheses refer to the list of references at the end of this standard.

[3] *Annual Book of ASTM Standards*, Vol 11.01.

[4] *Annual Book of ASTM Standards*, Vol 11.05.

E 1706 Test Methods for Measuring the Toxicity of Sediment-associated Contaminates with Freshwater Invertebrates[4]

IEEE/ASTM SI-10 Standard for Use of the International System of Units (SI): The Modern Metric System[5]

2.2 *Other Standards:*

Title 29 Code of Federal Regulations 1910.132 (f)[6]

## 3. Terminology

3.1 *Definitions:*

3.1.1 The words "must," "should," "may," "can," and "might" have very specific meanings in this guide. "Must" is used to express an absolute requirement, that is, to state that the test ought to be designed to satisfy a specific condition, unless the purpose of the test requires a different design. "Must" is used only in connection with the factors that apply directly to the acceptability of the test. "Should" is used to state that the specified conditions are recommended and ought to be met in most tests. Although a violation of one "should" is rarely a serious matter, violation of several will often render the results questionable. Terms such as "is desirable," "is often desirable," and "might be desirable" are used in connection with less important factors. "May" is used to mean "is (are) allowed to," "can" is used to mean" is (are) able to," and "might" is used to mean "could possibly." Thus, the classic distinction between "may" and" can" is preserved, and "might" is never used as a synonym of either "may" or "can."

3.1.2 For definitions of terms used in this guide, refer to Guide E 729, Terminologies D 1129 and E 943, and Guide E 1023. For an explanation of the units and symbols, refer to IEEE/ASTM SI-10.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *bioaccumulation*—the net uptake of a material by an organism from its environment through exposure by means of water and food.

3.2.2 *concentration*—the ratio of the weight or volume of test material(s) to the weight or volume of test sample.

3.2.3 *control sediment*—a sediment that is essentially free of contaminants and is used routinely to assess the acceptability of a test.

3.2.4 *elutriate*—the water and soluble portion extracted from the sediment.

3.2.5 *exposure*—contact with a chemical or physical agent.

3.2.6 *overlying water*—the water placed over the solid phase of a sediment in the test chamber for the conduct of the biological test; this may also include the water used to manipulate the sediments. In field situations, the water column above the sediment/water interface.

3.2.7 *pore water/interstitial water*—water occupying space between sediment or soil particles.

3.2.8 *reference sediment*—a whole sediment near the area of concern used to assess sediment conditions exclusive of material(s) of interest.

3.2.9 *sediment*—(1) particulate material that usually lies below water and (2) formulated paticulate matter that is intended to lie below water in a test.

3.2.10 *spiked sediment*—a sediment to which a material has been added for experimental purposes.

3.2.11 *suspension*—a slurry of sediment and water.

3.2.12 *toxicity*—the property of a material or combination of materials to affect organisms adversely.

3.2.13 *whole sediment*—sediment and associated pore water that has had minimal manipulation following collection or formulation.

## 4. Application

4.1 An ASTM guide outlines a series of options or instructions and does not recommend a specific course of action. The purpose of a guide is to offer guidance, based on a consensus of viewpoints, but not to establish a fixed procedure. A guide is intended to increase the awareness of the user to available techniques in a given subject area and to provide information from which subsequent evaluation and standardization can be derived.

4.2 This guide provides general interpretative guidance on the selection, application, and interpretation of biological tests with sediments. As such, this guide serves as a preface to other ASTM documents describing methods for sediment collection, storage, and manipulation (Guide E 1391); and toxicity or bioaccumulation tests with sediment ( Guides E 724, E 1367, E 1391, E 1611, E 1563, E 1688, and Test Method E 1706). Much of the guidance presented in this standard is also applicable to toxicity testing of soils (Guide E 1676). This guide serves as an introduction and summary of sediment testing and is not meant to provide specific guidance on test methods. Rather, its intent is to provide information necessary to accomplish the following:

4.2.1 Select a sediment exposure strategy appropriate to the assessment need. For example, a suspended phase exposure is relevant to the evaluation of dredged sediments for disposal at a dispersive aquatic site. (See Annex A1).

4.2.2 Select the test organism and biological endpoints appropriate to the desired exposure and aquatic resources at risk. For example, the potential for water quality problems and subsequent effects on oyster beds may dictate the use of sediment elutriate exposures with bivalve larvae (Guide E 724).

4.2.3 Establish an experimental design consistent with the objectives of the sediment evaluation. The use of appropriate controls is particularly important for evaluating sediment contamination (see Section 11).

4.2.4 Determine which statistical procedures should be applied to analysis of the data, and define the limits of applicability of the resultant analyses in data interpretation (Test Method E1706).

## 5. Summary of Guide

5.1 This guide provides general guidance and objectives for conducting biological tests with sediments. Detailed technical information on the conduct and evaluation of specific sediment tests is included in other documents referenced in this guide.

5.2 Neither this guide nor any specific test methodology can adequately address the multitude of technical factors that must be considered when designing and conducting a specific

[5] *Annual Book of ASTM Standards*, Vol 14.02.

[6] Available from Superintendent of Documents, U.S. Government Printing Office, Washington DC 20402.

investigation. The intended use of this document is therefore not to provide detailed guidance, but rather to assist the investigator in developing technically sound and environmentally relevant biological tests that adequately address the questions being posed by a specific investigation.

## 6. Significance and Use

6.1 Contaminated sediments may affect natural populations of aquatic organisms adversely. Sediment-dwelling organisms may be exposed directly to contaminants by the ingestion of sediments and by the uptake of sediment-associated contaminants from interstitial and overlying water. Contaminated sediments may affect water column species directly by serving as a source of contaminants to overlying waters or a sink for contaminants from overlying waters. Organisms may also be affected when contaminated sediments are suspended in the water column by natural or human activities. Water column species and nonaquatic species may also be affected indirectly by contaminated sediments by the transfer of contaminants through ecosystems **(7, 8)**.

6.2 The procedures described in this guide may be used and adapted for incorporation in basic and applied research to determine the ecological effects of contaminated sediments. These same methods may also be used in the development and implementation of monitoring and regulatory programs designed to prevent and manage sediment contamination.

6.3 Sediment tests with aquatic organisms can be used to quantify the acute and chronic toxicity and the bioavailability of new and presently used materials. Sediment toxicity may also result from environmental processes such as ammonia generation, pH shifts, or dissolved oxygen fluctuation. In many cases, consideration of the adverse effects of sediment-associated contaminants is only one part of a complete hazard assessment of manufactured compounds that are applied directly to the environment (for example, pesticides) and those released (for example, through wastewater effluents) as by-products from the manufacturing process or from municipalities **(7)**.

6.4 Sediment tests can be used to develop exposure-response relationships for individual toxicants by spiking clean sediments with varying concentrations of a test chemical and determining the concentration that elicits the target response in the test organism (Guide E 1391). Sediment tests can also be designed to determine the effects that the physical and chemical properties of sediments have on the bioavailability and toxicity of compounds.

6.5 Sediment tests can provide valuable information for making decisions regarding the management of contaminated sediments from hazardous waste sites and other contaminated areas. Biological tests with sediments can also be used to make defensible management decisions on the dredging and disposal of potentially contaminated sediments from rivers and harbors. (**(7, 8)**, Test Method E 1706.)

## 7. Hazards

7.1 General Precautions:

7.1.1 Development and maintenance of an effective health and safety program in the laboratory requires an ongoing commitment by laboratory management and includes: *(1)* the appointment of a laboratory health and safety officer with the responsibility and authority to develop and maintain a safety program, *(2)* the preparation of a formal, written health and safety plan, which is provided to each laboratory staff member, *(3)* an ongoing training program on laboratory safety, and *(4)* regular safety inspections.

7.1.2 Collection and use of sediments may involve substantial risk to personal safety and health. Chemicals in field-collected sediment may include carcinogenics, mutagens, and other potentially toxic compounds. Inasmuch as sediment testing is often started before chemical analysis can be completed, worker contact with sediment needs to be minimized by (1) using gloves, laboratory coats, safety glasses, face shields and respirators as appropriate, (2) manipulating sediments under a ventilated hood or in an enclosed glove box, and (3) enclosing and ventilating the exposure system. Personal collecting sediment samples and conducting tests should take all safety precautions necessary for the prevention of bodily injury and illness which might result from ingestion or invasion of infectious agents, inhaltion or absorption of corrosive or toxic substances through skin contact, and asphixiation because of lack of oxygen or precense of noxious gases.

7.1.3 Before beginning sample collection and laboratory work, personnel should determine that all the required safety equipment and materials have been obtained and are in good condition.

7.2 *Safety Equipment:*

7.2.1 *Personal Safety Gear*—Personnel should use safety equipment, such as, rubber aprons, laboratory coats, respirators, gloves, safety glasses, face shields, hard hats, and safety shoes. Before beginning sample collection and laboratory work, personnel should be properly trained in the following: *(1)* when and what personal protective equipment (PPE) is necessary, *(2)* How to properly wear PPE, *(3)* limitations to the PPE, and proper care maintenance, useful life, and *(4)* disposal of PPE (29 CFR 1910.132(f) ).

7.2.2 *Laboratory Safety Equipment*—Each laboratory should be provided with safety equipment such as first-aid kits, fire extinguishers, fire blankets, emergency showers, and eye wash stations. Mobile laboratories should be equipped with a telephone to enable personnel to summon help in case of emergency.

7.3 *General Laboratory and Field Operations:*

7.3.1 Special handling and precautionary guidance in Material Safety Data Sheets (MSDS) should be followed for reagents and other chemicals purchased from supply houses.

7.3.2 Work with some sediments may require compliance with rules pertaining to the handling of hazardous material. Personnel collecting samples and performing tests should not work alone.

7.3.3 It is adviseable to wash the exposed parts of the body with bacterial soap and water immediately after collecting or manipulating sediment samples.

7.3.4 Strong acids and volatile organic solvents should be used in a fume hood or under an exhaust canopy over the work area.

7.3.5 An acidic solution should not be mixed with a

hypochlorite solution because hazardous fumes might be produced.

7.3.6 To prepare and dilute acid solutions, concentrated acid should be added to water, not vise versa. Opening a bottle of concentrated acid and adding concentrated acid to water should be preformed only under a fume hood.

7.3.7 Use of ground-fault systems and leak detectors is strongly recommended to help prevent electrical shocks. Electrical equipment or extension cords not bearing the approval of Underwriter Laboratories should not be used. Ground-Fault interrupters should be installed in all "wet" laboratories where electrical equipment is used.

7.3.8 All containers should be adequately labeled to indicate their contents.

7.3.9 A clean well-organized work place contributes to safety and reliable results.

7.4 *Disease Prevention*—Personnel handling samples which are known or suspected to contain human wastes should be immunized against hepatitis B, tetanus, typhoid fever and polio. Thorough washing of exposed skin with bacterial soap should follow handling of samples collected in the field.

7.5 *Safety Manuals*— For further guidance on safe practices when handling sediment samples and conducting toxicity tests, check with the permittee and consult general industrial safety manuals including **(9, 10)**.

7.6 *Pollution Prevention, Waste Management and Sample Disposal*— Guidelines for the handling and disposal of hazardous material should be strictly followed (Guide D 4447). The Federal Government has published regulations for the management of hazardous waste and has given the States the option of either adopting those regulations or developing their own. If States develop their own regulations they are required to be as stringent as the Federal regulations. As a handler of hazardous materials, it is your responsibility to know and comply with the pertinent regulations applicable in the State in which you are operating. Refer to **(11)** for the citations of the Federal requirements.

## 8. Sediment Test Types

8.1 Many methods for assessing the toxicity of saltwater and freshwater sediments to benthic organisms have been reported. Those methods are provided in Table 1 for saltwater tests and in Table 2, for freshwater tests, respectively.

8.2 The selection of a specific toxicity test type is intimately related to the objectives of the sediment evaluation program. These assessments, whether they be for monitoring, regulatory, or research purposes, should be guided by a set of null hypotheses that define the appropriate exposure route and the endpoint of interest.

8.3 Organism exposure methods most commonly employ the whole sediment in the bedded phase (solid phase), but pore water, suspended and elutriate phase exposures have also been used **(7)**.

8.4 Programs seeking to characterize or rank sediments on a basin-wide or regional scale typically use whole sediment, solid-phase exposures. Regulatory or permitting programs for dredged material disposal at a containment site may also evaluate this exposure route **(8, 12)**. Disposal at a dispersive site, or concerns over the resuspension and transport of in-place sediments, would suggest the use of suspended phase

**TABLE 1 Organisms Used in Assessing the Toxicity of Saltwater Sediments**[A]

| Taxa | Exposure | Reference |
|---|---|---|
| *Mortality* | | |
| Amphipods | So[B] | **(12,28, 29 62-67),** Guide E 1367 |
| | Su[C] | **(54, 67-70)** |
| Bivalves | So | **(63, 67)** Guide E 724 |
| | Su | **(67,71,72)** |
| Copepods | So | **(62)** |
| | Su | **(62)** |
| Crab | Su | **(72)** |
| Cumaceans | So | **(29, 64-66** |
| Fish | El[D] | **(73,74)** |
| | So | **(67,75)** |
| | Su | **(67,71)** |
| Isopods | So | **(62)** |
| | Su | **(62)** |
| Lobster | Su | **(72)** |
| Mysids | So | **(67)** |
| | Su | **(67-70)** |
| Polychaetes | So | **(63,76,77)** Guide E 1611 |
| Phytoplankton | El | **(78)** |
| Shrimp | So | **(62, 76-80)** |
| | Su | **(62,74,79,80)** |
| Tunicate | Su | **(72)** |
| *Avoidance/behavior* | | |
| Amphipods | So | **(81,82)** |
| Bivalves | So | **(81,83,86-88)** |
| Crab | So | **(81,82)** |
| Echinoderm | So | **(81)** |
| Fish | So | **(83,84)** |
| Lobster | So | **(81)** |
| Polychaetes | So | **(83,85)** |
| Shrimp | So | **(81,83)** |
| *Growth/reproduction/life cycle* | | |
| Amphipods | Su | **(70)** |
| Bivalves | Su | **(89)** Guide E 724 |
| Copepods | So | **(90)** |
| Fish | Su | **(91)** |
| Mysids | Su | **(68,69,92)** |
| Nematodes | So | **(93)** |
| Polychaetes | So | **(91,94,95)** Guide E 1611 |
| | Su | **(91,94,95)** |
| Sea urchin | El | **(96)** Guide E 1563 |
| *Pathology* | | |
| Amphipods | So | **(97)** |
| | Su | **(97)** |
| Bivalves | So | **(97)** |
| | Su | **(97)** |
| Fish | So | **(73,98,99)** |
| | Su | **(98)** |
| Oyster | So | **(98)** |
| | Su | **(98)** |
| Polychaetes | So | **(97)** |
| | Su | **(97)** |
| *Physiology* | | |
| Fish | Su | **(100)** |
| Oligochaetes | El | **(101)** |
| Polychaetes | So | **(94)** |
| | Su | **(94,102)** |
| Shrimp | Su | **(102)** |
| *Chromosome damage* | | |
| Fish | El | **(103-105)** |
| Polychaetes | Su | **(106)** |
| *Bacterial activity* | | |
| Bacteria | El | **(49,107)** |
| *Community recolonization* | | |
| Macrobenthos | So | **(108-114)** |

[A] Many of these species have a wide salinity tolerance and therefore may be suitable for testing estuarine sediments.
[B] So—solid-phase sediment exposure.
[C] Su—suspended sediment exposure.
[D] El—elutriate, extract, pore water exposure.

**TABLE 2 Organisms Used in Assessing the Toxicity of Freshwater Sediments[A]**

| Taxa | Exposure | Reference |
|---|---|---|
| *Mortality* | | |
| Amphipods | El | **(115)** |
| | So | **(5,6,8,30,115-117)** Test Method E 1706 |
| Cladocerans | El | **(115)** |
| | So | **(5,115,116,118-128)** Test Method E 1706 |
| | Su | **(126)** |
| | El | **(115)** |
| | So | **(115,116, 118-121)** |
| Insect larvae | El | **(115)** |
| | So | **(5,8,18, 115-125, 129)** Test Method E 1706 |
| Isopods | So | **(118-121)** |
| Oligochaetes | So | **(130-132)** Guide E 1688 |
| *Growth/reproduction* | | |
| Amphipods | So | **(5,6,30)** Test Method E 1706 |
| Bacteria | El | **(133)** |
| | So | **(133)** |
| Cladocerans | El | **(133)** Test Method E 1706 |
| | So | **(5,133)** |
| Fish | El | **(133)** |
| | So | **(133)** |
| Insect larvae | So | **(18,129,134,135)** Test Method E 1706 |
| Nematodes | El | **(136)** |
| *Physiology* | | |
| Oligochaetes | El | **(137,138)** |
| *Genetic damage* | | |
| Fish | El | **(2,103,104,137,138)** |
| Nematodes | El | **(136)** |
| *Bacterial activity* | | |
| Bacteria | El | **(60,141)** |
| *Behavior* | | |
| Oligochaetes | So | **(36)** |

[A] Many of these species have a salinity tolerance and therefore may be suitable for testing estuarine sediments.

or elutriate exposures (Annex A1).

8.5 Methods have been developed to isolate and test the toxicity of elutriates **(13)** or sediment interstitial water **(14)** to aquatic organisms. The elutriate test was developed for assessing the potential acute effects of open-water disposal of dredged material. Tests with elutriate samples are used to estimate the water-soluble constituents that may be released from sediment to the water column during disposal operations **(15)**. Toxicity tests of the elutriate with water column organisms have generally indicated that little toxicity is associated with the discharge material **(4)**. However, elutriates have been reportedly more toxic than interstitial water samples **(16)**.

8.5.1 For many benthic invertebrates, the toxicity and bioaccumulation of sediment-associated contaminants, such as metals and non-ionic organic contaminants, may be correlated with the concentration of these chemicals in the interstitial water **(14, 17)**. The sediment interstitial water toxicity test was developed for assessing the potential *in situ* effects of contaminated sediment on aquatic organisms. Once the interstitial water (or elutriate) has been isolated from the whole sediment, the toxicity testing procedures are similar to effluent toxicity testing with non-benthic species. If benthic species are used as test animals, they may be stressed by the absence of sediment **(4)**.

8.5.2 The examination of organic extracts may have specific uses. However, caution should be exercised in the use of organic extracts since the availability of sediment contaminants to organisms may have been altered **(7)**.

## 9. Biological Responses

9.1 Toxicity endpoints in sediment tests range from lethality, growth, reproductive impairment, and physiological responses to alterations in community levels of organization (Table 1 and Table 2). Selection of the proper toxic endpoint is predicated largely on the objectives of the evaluation program and the available resources, time, and available methods. Several endpoints are suggested in published methods to measure the potential effects of contaminants in sediment including, survival, growth, behavior, or reproduction; however, survival of test organisms in 10–d exposures is the endpoint most commonly reported (Tables 1 and 2). These short-term exposures which only measure effects on survival can be used to identify high levels of contamination on sediments, but may not be able to identify moderate levels of contamination in sediments (Test Method E1706, **(8)**). Sublethal endpoints in sediment tests might also prove to be better estimates of reponses if benthic communities to contaminates in the field **(18-21)**.

9.2 The decision to conduct short-term or long-term toxicity tests depends on the goal of the assessment. In some instances, sufficient information may be gained by measuring sublethal endpoints in 10-d tests. In other instances, the 10-d test could be used to screen samples for toxicity before long-term tests are conducted. While the long-term tests are needed to determine direct effects on reproduction, measurement of growth in these toxicity tests may serve as an indirect estimate of reproductive effects of contaminates associated with sediments (Test Method E1706, **(8)**).

9.3 Use of sublethal endpoints for assessment of contaminate risk is not unique to toxicity testing with sediments. Numerous regulatory programs require the use of sublethal endpoints in the decision-making process **(7)** including: (1) Water Quality Criteria (and State Standards), (2) National Pollution Discharge Elimination System (NPDES) effluent monitoring (including chemical-specific limits and sublethal endpoints in toxicity tests); (3) Federal Insecticide, Rodenticide and Fungicide Act (FIFRA) and the Toxic Substances Control Act (TSCA, tiered assessment includes several sublethal endpoints with fish and aquatic invertebrates); (4) Superfund (Comprehensive Environmental Response, Compensation and Liability Act, CERCLA); (5) Organization of Economic Cooperation and Development (OECD, sublethal toxicity testing with fish an invertebrates); (6) European Economic Community (EC, sublethal toxicity testing with fish and invertebrates); and (7) the Paris Commission, (behavioral endpoints).

## 10. Test Organisms

10.1 Once the exposure routes and endpoints of interest have been established, several criteria should be considered when selecting appropriate species **(3, 8, 22 )** and Test Method E 1706 for which tests can be conducted that have ecologically relevant endpoints. Ideally, the test species should meet the following criteria:

10.1.1 Have a toxicological (sediment) database demonstrating sensitivity to a range of contaminants or the contaminant of interest, and be taxonomically identified;

10.1.2 Be readily available through field collection or culture;

10.1.3 Be easily maintained in the laboratory;

10.1.4 Be ecologically or economically important;

10.1.5 Have a broad geographical distribution, or be indigenous to the site being evaluated or have a similar niche, be in the same feeding guild, or be similar in behavior to an inhabitant (species);

10.1.6 Be tolerant to a broad range of sediment physicochemical characteristics (for example, organic carbon and grain size);

10.1.7 Be compatible with selected exposures and endpoints; and

10.1.8 Be tolerant of a range of different water quality characteristics.

10.2 Of these criteria, demonstrated sensitivity to contaminants, ecological relevance, and tolerance to varying sediment physico-chemical characteristics are the most important. The sensitivity of a species to contaminants should be balanced with the concept of discrimination. Species responses may need to provide discrimination between different levels of contamination. Additionally, insensitive species may be preferred for determining bioaccumulation potential. The use of indigenous species that are ecologically important and collected easily is often very straightforward; however, many indigenous species at a contaminated site may be insensitive to contaminants (Guide E 1688). Indigenous species might present a greater concern relative to bioaccumulation potential. With the exception of some saltwater amphipods, few test species have broad sediment toxicity databases. Additionally, many species can be maintained in the laboratory long enough for acclimation to test conditions, but very few are cultured easily. Widespread toxicity testing will require cultured organisms or the use of standard source populations that can be transported without experiencing excessive stress.

10.3 Toxicity is related to the species-specific physiological and biochemical response to a toxicant and the degree of contact between the sediment and the organism. Feeding habits, including the type of food and feeding rate, will influence the exposure of contaminants from sediment **(23)**. Infaunal deposit-feeding species can receive an exposure of sediment contaminants by means of three exposure routes: interstitial water, sediment particles, and overlying water. Benthic invertebrates may selectively consume particles with higher organic carbon and higher contaminant concentrations. Organisms in direct contact with sediment may also accumulate contaminants by direct adsorption to the body wall or exoskeleton, or by absorption through the integument **(24)**. Estimates of bioavailability will thus be more complex for epibenthic animals that inhabit both the sediment and the water column. Some benthic species are exposed primarily by detrital feeding **(25)**. Detrital feeders may not receive most of their body burden directly from interstitial water. For certain higher Kow compounds, uptake by the gut can exceed uptake across the gill **(26, 27)**. However, for many benthic invertebrates, the toxicity and bioaccumulation of sediment-associated contaminants such as metals, kepone, fluoranthene, and organochlorines are highly correlated with the concentration of these chemicals in the interstitial water **(14)**.

10.4 The saltwater test species include a broad spectrum of taxa and feeding types including crustaceans, bivalves, polychaetes, and fish (Table 1). Tests using amphipods have received a great deal of attention because of their overall sensitivity and because they are often absent from contaminated sites **(28)**. This sensitivity has led to the development of routine methods using the burrowing amphipod *Rheopoxynius abronius*. This 10-day acute toxicity test has recently been adapted for use with other amphipod species and has been established (Guide E 1367, **(29,12)**). Since 1977, the U.S. Army Corps of Engineers dredging permit program has routinely required tests with three species: a bivalve, a polychaete, and a fish or shrimp, incorporating both species that burrow into the sediment and those which inhabit the water column. Broad applications of these protocols reveal that these tests are not as sensitive as those with amphipods, and the latter have recently been recommended for permit programs.

10.5 Freshwater sediment tests use a number of different species, including amphipods, midges, mayflies, cladocerans, and oligochaetes (Table 2). Whole sediment tests with the amphipod *Hyalella azteca* generally start with juvenile animals and are Typically conducted for 10 to 14–d with measurement of survival or growth (Test Method E 1706 , **(8,30)**). Methods for conducting 42-d tests with *H. azteca* have been described in Test Method E 1706 and **(8)**. Endpoints measured in these long-term tests with *H. azteca* include survival, growth, and reproduction.

10.6 Tests with midge *Chironomus tentans* are generally started with second instar larvae (10 to 14 days old) and continued for 10 to 17 days until the fourth instar; larval survival or growth is the measure of toxicity (Test Method E 1706 **(8, 18)**). Methods for conducting 60–d tests with *C. tentans* have been described in Test Method E 1706 and **(8)**. Exposures start with first instar *C. tentans* and endpoints measured in these long-term tests include survival, growth, emergence, reporduction, and egg hatching. Whole sediment testing procedures with the midge *C. riparius* are started with 1 to 3-day-old larvae and may continue through pupation and adult emergence (**(6)** Test Method E 1706). Midge exposures started with older larvae may underestimate midge sensitivity to toxicants. For instance, first instar *C. tentans* larvae were 6 to 27 times more sensitive than fourth instar larvae to acute copper exposure **(5, 32)**, and first instar *C. riparius* larvae were 127 times more sensitive than second instar larvae to acute cadmium exposure **(33)**.

10.7 Sediment toxicity tests with mayflies and cladocerans are generally conducted for up to 10 days **(5, 34, 35)** and Test Method E 1706. Survival and molting frequency are the toxicity endpoints monitored in the mayfly tests, and survival, growth, and reproduction are monitored in the cladoceran tests. While cladocerans are not in direct contact with the sediment, they are frequently in contact with the sediment surface and are probably exposed to both water-soluble and particulate bound contaminants in the overlying water and surface sediment (Test Method E 1706). Cladocerans are also one of the more sensitive groups of species used in aquatic toxicity testing.

10.8 The most frequently described sediment testing procedures for oligochaetes are acute toxicity testing methods **(36 , 8)** also see, Guide E 1688. However, methods for conducting up to 500-day oligochaete exposures, with growth and reproduction as the toxicity endpoints, have been described **(37)**. A shorter 28-d test starting with sexually mature *Tubifex tubifex* has been described **(38)**. Effects on growth and reproduction are monitored in this shorter test, and the duration of the exposure makes the test more useful for routine sediment toxicity assessments with oligochaetes (Test Method E 1706). Many oligochaetes have complex life cycles and reproductive strategies, and therefore laboratory culturing requirements have prohibited their use in toxicity testing **(39)**. However, culturing procedures have been described for *Lumbriculus variegatus* and *Tubifex tubifex* **(8, 40,41)** (See also, Test Method E 1706 and Guide E 1688).

10.9 Because of the database that has been developed with existing tests, it is recommended that, for whole sediment exposures, either phoxocephalid, ampeliscid, or haustoriid amphipods be used in saltwater tests. For freshwater applications, hyalellid amphipods, midge larvae, or mayfly larvae would be appropriate. As new methods are developed, it will be important to establish the sensitivity of each method relative to a benchmark procedure for comparative purposes **(2)**. The whole sediment benchmark for saltwater tests should be the *Rheopoxynius abronius* survival 10-day acute test, and for freshwater tests it should be *Hyalella azteca* survival and growth in 28-d exposures **(31)**. While chronic tests with whole sediments have been described for a variety of freshwater tests, research is ongoing to describe chronic tests with marine amphipods.

10.10 Multispecies and microcosm tests can also be used to evaluate potential ecosystem responses to contaminated sediments. The use of multi-species tests may provide toxicity information not available from single-species tests since relative species sensitivity may vary among contaminants **(6)**. However, results from multi-species or microcosm tests are more difficult to interpret due to interactions and limited reference literature **(42, 43)**.

## 11. Experimental Design Considerations

11.1 *Sampling Methods*:

11.1.1 Sampling methods are dependent on the purpose and design of the study. The probable source and type of contamination and the objectives of the study should be evaluated before developing a sediment sampling regime. The number and type of samples taken depends on the objectives of the study **(44-47)**.

11.1.2 The number of replicate samples taken at a site should be determined based on the objectives of the study and a preliminary survey of sediment variability at the site. Information from the preliminary survey and the objectives of the study can be used to determine the minimum number of replicates that should be sampled at each site **(45, 46)**.

11.1.3 In general, both toxicity and bioaccumulation tests require at least two exposures: a control and one or more test treatments (see 11.3.12). The experimental unit for each test is the exposure chamber. A sediment sample is typically split into four or more test chambers. Individual observations obtained from within an individual chamber should not be used as replicate observations. Replicate chambers for a particular sediment provide an estimate of the variability within the test system and are not considered sediment sample or location replicates.

11.1.4 There are several acceptable methods of sampling sediments, for example, corers and grabs or dredges. Grabs or dredges (for example, Ponar or Ekman) are appropriate when sediments are known to be unstratified with respect to the contaminants of concern. If the contaminants are in strata, or if their accumulation rates are of interest, one of several core samplers should be used. $Pb^{210}$ or $Cs^{137}$ dating can be performed on cores to identify the thickness of the mixed layer **(44, 47)**. See Guide E 1391 for additional details.

11.2 *Sample Handling*:

11.2.1 Sample handling and preservation are discussed in Guide E 1391 and Test Method E 1706, and depend on the type of chemical characterization that will be performed. Any sediment disturbance may alter the chemical characterization of that sediment from *in situ* conditions. The use of clean sampling devices and sample containers is essential to ensure the accurate determination of sediment contamination **(45, 47)**.

11.2.2 Physical and chemical characterization of sediments is highly dependent on the needs of the investigator, but it may include loss on ignition, percent water, grain size, total organic carbon, total phosphorus, nitrogen forms, trace metals and organic compounds, pH, total volatile solids, biological oxygen demand, chemical oxygen demand, cation exchange capacity, Eh, pE, total inorganic carbon, acid volatile sulfides, and ammonia **(44, 46, 47)**. Many times, a sediment of concern has some historical data that are used as a basis for selection.

11.2.3 Indigenous organisms may be present in field-collected sediments. An abundance of the same organism or organisms taxonomically similar to the test organism in the sediment sample may make interpretation of treatment effects difficult. Previous investigators have inhibited the biological activity of sediment with sieving, heat, mercuric chloride, antibiotics, or gamma irradiation. (Guide E 1391.) However, further research is needed to determine effects on contaminate bioavailability or other modifications of sediments from treatments such as those used to remove or destroy indigenous organisms.

11.2.4 Field-collected sediment samples tend to settle during shipment. As a result, water above the sediment should not be discarded, but should be mixed back into the sediment during homogenization (Test Method E 1706). Sediment samples should not be routinely sieved to remove indigenous organisms unless there is a good reason to believe they will influence the response of the test organisms. Large indigenous organisms and large debris can be removed using forceps. Reynoldson et al. **(48)**, observed reduced growth of amphipods, midges, and mayflies in sediments with elevated numbers of oligochaetes and recommended sieving sediments suspected to have high numbers of indigenous oligochaetes. One approach might be to sieve an aliquot of each sediment before the start of a test. If potential predators are recovered from a sediment, it may be desirable to sieve all of that sample before the start of the test. Depending on the objective of the test, it

may be necessary to sieve all sediments or run a sieved and un-sieved treatment in parallel to account for potential affects of sieving on test results and subsequent comparisons. The size of the sieve used will depend on the size of the organisms in the sediment sample. If a sediment must be sieved, it is desirable to analyze a sample before and after sieving (for example, measure pore-water metals, dissolved organic carbon (DOC), acid volatile sulfide (AVS), total organic carbon (TOC)) to document the influence of sieving on sediment chemistry.

11.3 *Exposure Design*:

11.3.1 In addition to being available in adequate supply, overlying water used in toxicity tests, and water used to hold organisms before testing, should be acceptable to the test species and uniform in quality. To be acceptable the water must allow the test species to survive and grow without showing signs of disease or apparent stress, such as discoloration or unusual behavior.

11.3.2 Natural overlying water should be uncontaminated and of constant quality and should meet the specifications established in Guide E 729. Water should be characterized in accordance with Guide E 729 at least twice each year and more often if (*1*) such measurements have not been determined semiannually for at least two years or ( *2*) surface water is used.

11.3.3 A natural overlying water is considered to be of uniform quality if the monthly ranges of hardness and alkalinity are less than 5 mg/L or 10 % of their respective averages, whichever is higher, and if the monthly range of pH is less than 0.4 units. Natural overlying waters should be obtained from an uncontaminated well or spring, if possible, or from a surface water source. If surface water is used, the intake should be positioned to minimize fluctuations in quality and the possibility of contamination and maximize the concentration of dissolved oxygen and to help ensure low concentrations of sulfide and iron. For sediment studies with saltwater, the range of salinity should be less than 10 % of the average. In addition, the ion concentrations of the water should be within 10 % of the ion concentrations (adjusted for the salinity) listed in Guide E 729. Chlorinated water should not be used for, or in the preparation of, overlying water because residual chlorine and chlorine-produced oxidants are toxic to many aquatic animals and dechlorination is often incomplete.

11.3.4 For certain applications, the experimental design might require the use of water from the test sediment collection site.

11.3.5 Reconstituted fresh and salt water is prepared by adding specified amounts of reagent grade chemicals to high-quality distilled or deionized water (see Guide E 729 and Test Method E 1706). Acceptable water can be prepared using deionization, distillation, or reverse-osmosis units. Conductivity, pH, hardness, and alkalinity should be measured on each batch of reconstituted water. If the water is prepared from a surface water, the total organic carbon or chemical oxygen demand should be measured on each batch. Filtration through sand, rock, bag, or depth-type cartridge filters may be used to keep the concentration of particulate matter acceptably low. The reconstituted water should be intensively aerated before use, except that buffered soft fresh waters should be aerated before, but not after, the addition of buffers. Problems have been encountered with some species in some fresh reconstituted waters, but these problems can be overcome by aging the reconstituted water for one or more weeks (Guide E 729).

11.3.6 Materials used to construct test chambers may include glass, stainless steel, silicone, plastics, and fiberglass that have been prepared properly and tested for toxicity (Guides E 1367 and Test Method E 1706). The materials selected to construct test chambers may differ, depending on the types of contaminants in the sediments. Within a test, chambers need to be of the same material.

11.3.7 The use of site water or reconstituted water in toxicity tests may depend on the type of test to be performed and the time lapse between sample collection and start of the test.

11.3.8 Static sediment toxicity tests are the simplest to perform and have been used commonly. In such tests, water overlying the sediment is not changed during the test period, but it may be added to replace that which has evaporated. Since changes in water quality may affect the availability of contaminants to the test species, static exposures are more appropriate for acute tests (7 to 10 days).

11.3.9 Flow-through exposure chambers are suggested for use in chronic tests or with larger animals. Since water is renewed on a continual basis, fewer water quality changes are likely due to the buildup of waste products or interactions between the sediment and overlying water. Flow-through exposures may bias the results of the test by either encouraging the continual release of water-soluble contaminants throughout the test, or by depleting water-soluble contaminants from the sediment early in the test.

11.3.10 General water quality (variables such as pH, salinity, dissolved oxygen, ammonia, and temperature) in the test chambers should meet culture and maintenance requirements for the test species. These parameters should be monitored and recorded on a frequency appropriate to the test length. For example, if the test duration is only a few days, daily monitoring should be performed. However, if the test will continue for weeks or months, measurements may be reduced to every other day or every few days.

11.3.11 The depth of sediment in test chambers may vary depending on the species being tested, its size and degree of burrowing activity, and its sediment processing rate. The latter should be determined prior to the beginning of a sediment toxicity test **(45)**.

11.3.12 Sediment tests includes a control sediment, (sometimes called a negative control). A control sediment is a sediment that is essentially free of contaminates and is used routinely to assess the acceptability of a test and is not necessarily collected near the site of concern. Any contaminates in control sediment are thought to originate from the global spread of pollutants and do not reflect any substainal inputs from local or non-point sources. Comparing test sediments to control sediments is a measure of the toxicity of a test sediment beyond inevitable background contamination and organism health. A control sediment provides a measure of test acceptability, evidence of test organism health, and a basis for interpreting data obtained from the test sediments. A reference sediment is collected near the area of concern and is used to

assess sediment conditions exclusive of materials(s) of interest. Testing a reference sediment provides a site–specific basis for evaluating toxicity (Test Method E 1706, **(8)**). (1) In general, the performance of test organisms in the negative control is used to judge the acceptability of a test, and either the negative control or reference sediment may be used to evaluate performance in the experimental treatments, depending on the purpose of the study. Any study in which organisms in the negative control do not meet performance criteria must be considered questionable because it suggests that adverse factors affected the response of test organisms. Key to avoiding this situation is using only control sediments that have demonstrated record of performance using the same test procedure. This includes testing of new collections from sediment sources that have previously provided suitable control sediment. (2) Because of the uncertainties introduced by poor performance in the negative control, such studies should be repeated to insure accurate results. However, the scope or sampling associated with some studies may make it difficult or impossible to repeat a study. Some researchers have reported cases where performance in the negative control is poor, but performance criteria are met in a reference sediment included in the study design. In these cases, it might be reasonable to infer that other samples that show good performance are probably not toxic; however, any samples showing poor performance should not be judged to have shown toxicity, since it is unknown whether the adverse factors that caused poor control performance might have also caused poor performance in the test treatments. (3) Natural physico-chemical characteristics such as sediment texture may influence the response of test organisms (Guide E 1367). The physico-chemical characteristics of test sediment need to be within the tolerance limits of the test organism. Ideally, the limits of a test organism should be determined in advance; however, controls for factors including grain size and organic carbon can be evaluated if the limits are exceeded in a test sediment. If the physico-chemical characteristics of a test sediment exceed the tolerance range of the test organism, a control sediment encompassing these characteristics can be evaluated. The effects of sediment characteristics on the results of sediment tests can be addressed with regression equations. The use of formulated sediment can also be used to evaluate physico-chemical characteristics of sediment on test organisms (Guide E 1367, Test Method E 1706) (4) The experimental design depends on the purpose of the study. Variables that need to be considered include the number and type of control sediments, the number of treatments and replicates, and water quality characteristics. For instance, the purpose of the study might be to determine a specific endpoint such as an LC50 and may include a control sediment, a positive control, a solvent control, and several concentrations of sediment spiked with chemical (Test Method E 1706).

11.3.13 Test temperature should be chosen based on conditions of particular interest or to match the conditions at the sample site. In either case, the choice of temperature and test species should be compatible.

11.3.14 Dissolved oxygen in overlying water should be maintained between 40 and 100 % saturation.

11.3.15 Light quality (including wavelength composition) and daylength are important because of their impacts on both chemical degradation and organism health. Light should be provided from cool-white fluorescent lamps at an intensity appropriate for the test species.

11.3.16 The photoperiod can be selected to mimic that experienced at the sample site, or to simulate a particular season. Suggested periods of daylight and darkness include 16 h light/8 h dark, 14 h light/10 h dark, 12 h light/12 h dark, 24 h light/0 h dark, or 0 h light/24 h dark. Selection should be based on test needs and species.

11.3.17 Whether test organisms should be fed during the test depends on the test duration and type of test species in use. The addition of food can complicate the interpretation of test results because it adds new particulate material, and the food may interact in unknown ways with contaminants in the sediments **(45)**. Additionally, feeding uncontaminated food may reduce exposure. For acute tests ($\leq$1 week), most organisms can survive without being fed. If the species process sediments directly, and enough sediment has been provided to ensure adequate nutrition, feeding may not be necessary. If the species are fish or filter feeders, food may be required, especially during long tests. If organisms are fed during a sediment test, the excess food is typically not removed.

11.3.18 Test water and sediments should be analyzed for contaminants of concern if the objectives of the study are to determine the sources and concentrations of contaminants. If the test is designed to assess toxicity only, the identification of sources of toxicity is not necessary.

11.3.19 Analyses of specific contaminants in tissues of the test species are necessary if bioaccumulation is of interest. If the measurement of organic chemicals, metals, or other contaminants is desirable, appropriate preservation methods should be followed when the samples are collected.

## 12. Data Interpretation

12.1 Data interpretation must be considered in the initial stages of designing an experimental protocol for a specific investigation. Researchers must be aware that all aspects of an experimental protocol, including sampling techniques, number of test replicates, exposure routes, statistical methods, and selection of test species, will place constraints on data interpretation. Data interpretation must be consistent with the goal of the research program and experimental protocol to ensure the ecological significance and environmental relevance of the results of a specific investigation.

12.2 Bioaccumulation and toxicity of sediment-associated contaminants are important to the individuals of a particular species, however, interpreting the ecological significance of those data are difficult to evaluate (**(49)** see also, Guide E 1688 and Test Method E 1706). Toxic effects observed in laboratory exposures may not reflect effects on natural populations. However, bioaccumulation of a contaminant, or a toxic response when compared to that same response in a population exposed to a control sediment, is often undesirable.

12.2.1 Swartz et al. **(28)** evaluated sediment quality conditions along a sediment contaminated gradient of total DDT using information from 10-d toxicity tests with benthic amphipods, sediment chemistry, and the abundance of benthic amphipods in the field. Survival of amphipods, (*Eohaustorius*

*estaurius, Rhepoxynius abronius,* and *H.azteca*) in laboratory toxicity tests was positively correlated to the abundance of amphipods in the field and negatively correlated to total DDT concentrations. The toxicity threshold for amphipods in 10-d sediment toxicity test was about 300 ug total DDT/g organic carbon. The threshold for reduction in abundance of amphipod in the field was about 100 ug total DDT/g organic carbon. Therefore, correlations between toxicity contamination, and the status of benthic macroinvertebrates in the field indicate that 10-d sediment toxicity tests can provide a reliable indicator of the presence of adverse levels of sediment contamination in the field. However, these short-term toxicity tests may be under protective of sublethal effects of contaminants in benthic communities in the field.

12.2.2 Similarly, Canfield et al. **(19, 20, 21)** evaluated the composition of benthic invertebrate communities in sediments in a variety of locations including the Great Lakes, the upper Mississippi River, and the Clark Fork River in Montana. Results of these benthic invertebrate community assessments were compared to sediment quality guidelines (SQGs) and 28-d sediment toxicity tests with *H. azteca*. Good concordance was evident between measures of laboratory toxicity, SQGs, and benethic invertebrate composition in extremely contaminated samples. However, in moderately contaminated samples, less concordance was observed between the composition of the benthic community and either laboratory toxicity test or SQGs. The laboratory toxicity tests better identified chemical contamination in sediments compared to many of the commonly used measures of benthic invertebrate community structure. As the status of benthic invertebrates communities may reflect other factors such as habitat alteration in addition to effects of contaminants, the use of longer-term toxicity tests in combination with SQGs may provide a more sensitive and protective measure of potential toxic effects of sediment contamination on benthic communities compared to use of 10-d toxicity tests.

12.2.3 Numerical SQGs have been developed by a variety of federal, state, and provincial agencies across North America using matching sediment chemistry and biological effects data. These SQGs have been routinely used to interpret historical data, identify potential problem chemicals or areas at a site, design monitoring programs, classify hot spots and rank sites, and make decisions for more detailed studies **(50, 51, 52, 17)** Additional suggested uses for SQGs include identifying the need for source controls of problem chemicals before release, linking chemical sources to sediment contamination, triggering regulatory action, and establishing target remediation objectives **(8)**. Numerical SQGs, when used with other tools such as sediment toxicity tests, bioaccumulation, and benthic community surveys, can provide a powerful weight of evidence for assessing the hazards associated with contaminated sediments **(7)**.

12.3 The calculation procedure(s) and interpretation of the results should be appropriate to the experimental design. Statistical procedures used to calculate test results can be divided into two categories: those that test hypotheses and those that provide point estimates. No procedure should be used without careful consideration of (*1*) the advantages and disadvantages of various alternative procedures and (*2*) appropriate preliminary tests, such as those for outliers and heterogeneity (Test Method E 1706).

12.4 When samples from field sites are replicated (that is, separate samples from different grabs taken at the same site), site effects (bioaccumulation and toxicity endpoints) can be compared statistically by a one-tailed t-test, analysis of variance (ANOVA), or regression analysis. Analysis of variance is used to determine whether any of the sites are different from the control. This is a test of the null hypothesis, that no differences exist in effects observed among the sites and controls. If the F-test is not statistically significant ($P > 0.05$), it can be concluded that the effects observed in the sites were not large enough to be detected as statistically significant by the experimental design and hypothesis test used. Non-rejection does not mean that the null hypothesis is true. The amount of effect that occurred should be considered.

12.4.1 All exposure concentration effects (or field sites) can be compared with the control effects by using mean separation techniques such as those explained by Chew orthogonal contrasts, Fisher's methods, Dunnett's procedure, or Williams' method **(53, 54)**. The lowest concentration for which the difference in observed effect exceeds the statistical significant difference is defined as the LOEC (lowest observed effect concentration) for that endpoint. The highest concentration for which the difference in effect is not greater than the statistical significant difference is defined as the NOEC (no observed effect concentration) for that endpoint **(53)**.

12.5 In cases in which serial dilution sediment toxicity studies are conducted, the LC50 (median lethal concentration) or EC50 (median effect concentration) and its 95 % confidence limits should be calculated (when appropriate) on the basis of the following: (*1*) the measured initial sediment concentrations of test material, if available, or the nominal initial sediment concentrations for static tests; and (*2*) the average measured sediment concentrations of test material, if available, or the nominal average sediment concentrations for flow-through tests. If other LCs or ECs are calculated, their 95 % confidence limits should also be calculated (see Guide E 729).

12.6 Most toxicity tests produce quantal data, that is, counts of the number of responses in two mutually exclusive categories, such as alive or dead. A variety of methods **(55)** can be used to calculate an LC50 or EC50 and 95 % confidence limits from a set of quantal data that is binomially distributed and contains two or more concentrations at which the percent dead or affected is between 0 and 100. The most widely used are the probit, moving average, Spearman-Karber, and Litchfield-Wilcoxon methods. The method used should appropriately take into account the number of test organisms per chamber. The binomial test can also be used to obtain statistically sound information on the LC50 or EC50 even when there are less than two effective concentrations between 0 and 100 %, assuming mortalities of 0 and 100 % mortality are observed at two different concentrations. The binomial test provides a range within which the LC50 or EC50 should lie.

## 13. Keywords

13.1 bioaccumulation; contamination; experimental design; freshwater; saltwater; sediment; toxicity

# ANNEX

**(Mandatory Information)**

## A1. SEDIMENT RESUSPENSION TESTS

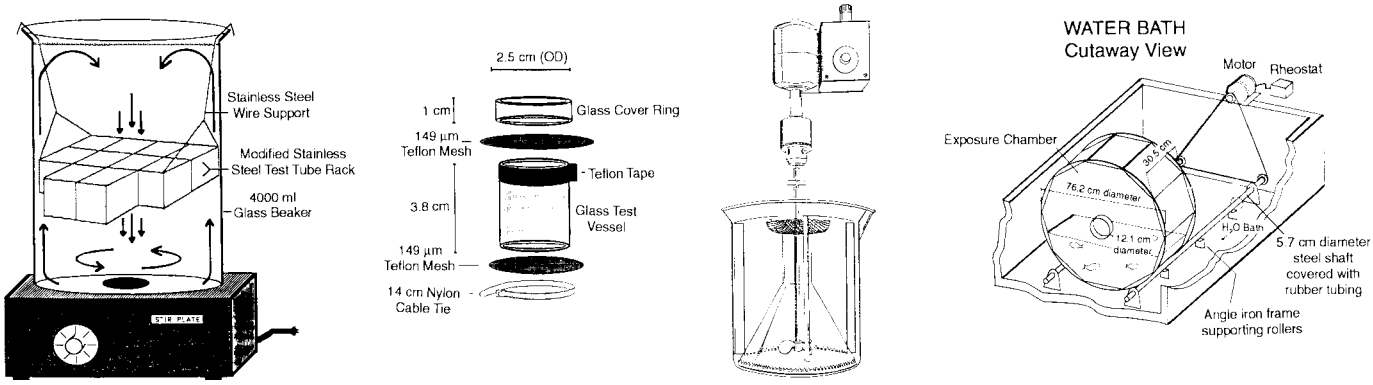
### A1.1 Scope

A1.1.1 This annex briefly describes twelve systems for evaluating the effects of suspended solids and their associated contaminants (soluble and insoluble) on aquatic organisms using static, recirculating, or flow-through exposure systems. The main objective, organisms, and apparatus used in these tests are detailed. A brief description of how the apparatus works and any discussion or conclusions reported (see Tables A1.1-A1.3) for these studies is also included. The following information will strictly provide a general guide to aid future research endeavors.

A1.1.2 Sediment suspension and resuspension tests provide information about the bioavailability of contaminants associated with sediments to aquatic organisms. Water column organisms can be exposed to contaminated bottom sediments that are resuspended into the water column by natural processes (bioturbation, wind-induced turbulence) or by human disturbances (dredging, vessel passage). Sediment resuspension tests can be used to evaluate the following: the desorptive nature of sediment associated contaminants and the effect of suspended solids that are not contaminated; the sub-lethal effects of intermittent suspended solids exposure on organisms; the importance of suspended solids levels in altering the bioavailability of contaminants to a water column organism; the responses of animals to actual mass concentration of particles; the relationship between contaminant, sediment, water column, and affected biota; horizontal and vertical gradients of contamination; the sensitivities of different species; the effects of various environmental factors; the biological availability of test materials; and structure-activity relationships.

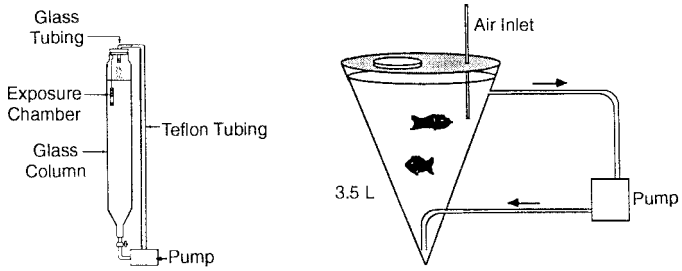
A1.1.3 Results from sediment suspension and resuspension tests may be important when assessing the hazards of materials to aquatic organisms or when deriving sediment quality criteria for aquatic organisms. Considerations for test designs may include the following: maintenance of a constant level of suspended solids without stressing test organisms; method of preparing/maintaining the suspension; consistency of environmental parameters with the dredge site; volatilization/degradation, oxidation/reduction of the sediment; length of test; and organisms used.

A1.1.4 Resuspension tests are usually a part of more comprehensive analyses of biological, chemical, geological, and hydrographic conditions. Statistical correlation can be increased and costs reduced if subsamples for sediment tests, geochemical analyses, and benthic community structure are taken simultaneously from the same grab of the same site. Sediment resuspension can be an important tool for making decisions regarding the extent of remedial action needed for contaminated aquatic sites



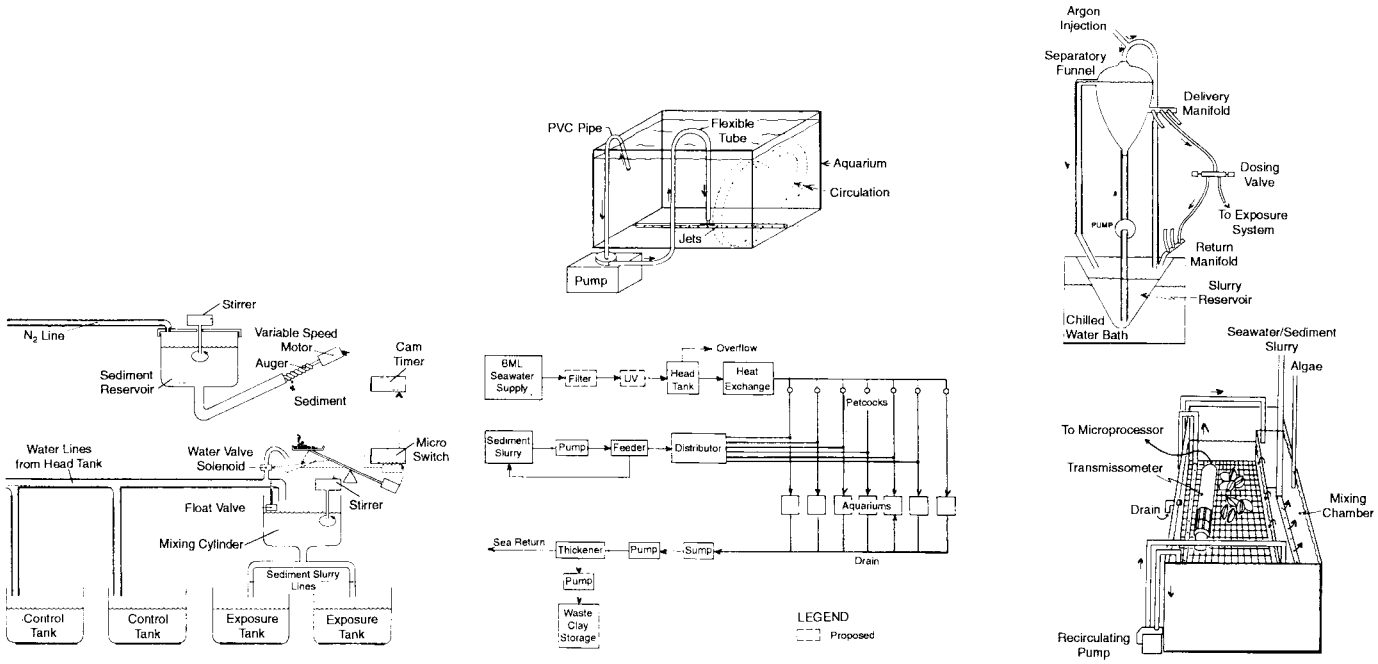

(see Ref. A/ ) (see Ref. B/Table A1.1) (see Ref. D/Table A1.1)[A]

**FIG. A1.1 Static/Renewal Tests**




(see Ref. A/Table A1.2)[A] (see Ref. B/Table A1.2)[A]

**FIG. A1.2 Recirculating Tests**




(see Ref. A/Table A1.3)[A] (see Ref. B/Table A1.3)[A] (see Ref. D/Table A1.3)

[A] Reprinted with permission from the publisher. Copyright 1993, National Research Council of Canada (Fig. A1.1, Ref. D); Copyright 1986, Springer-Verlag New York Inc. (Fig. A1.2, Ref. A); Copyright 1990, SETAC (Fig. A1.2, Ref. B); Copyright 1982, American Chemical Society (Fig. A1.3, Ref. A); Copyright 1971, Offshore Technology Conference (Fig. A1.3, Ref. B). See the specified table for full citation.

**FIG. A1.3 Flow-Through Tests**

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**TABLE A1.1 Static/Renewal Tests (see Fig. A1.1)**

| Ref. | Purpose | Organisms | Apparatus/Description | Comments |
|---|---|---|---|---|
| [A] | To maintain a constant level of suspended solids without stressing organisms. | Fresh water zooplankton (*D. pulex*) | • 4-L beaker with 3750 mL moderately hard water.<br>• Organism vessel is a glass tube (2.2-cm ID, 2.5-cm OD, 3.8-cm tall) with PTFE mesh (149 µm) connected by means of a glass cover ring (2.6-cm ID, 1-cm tall) on the top, and nylon cable tie (14-cm) on the bottom. PTFE tape is wrapped around the top of the vessel to improve the seal between the vessel and cover ring.<br>• Suspended sediment solution mixes at 250 r/min by means of a stir plate/PTFE-coated stir bar (replace stir bar as necessary, if PTFE erodes).<br>• A stainless steel wire support with two positions (for ease of observations) is connected to a “modified” stainless steel test tube rack that holds vessels. | • Inert, cleanable.<br>• Prevents organism interaction.<br>• Allows individual organism monitoring.<br>• Ease of observation.<br>• Large number of pieces (tedious).<br>• Water changes every 48 h (due to algae and solids buildup).<br>• Water flow through vessels constant but not equal (outer vessels slower rate). |
| [B] | To develop a suspension system for larval and juvenile fish. | Fresh water fish (*Pimephales promelas*, fathead minnows) | • 4-L beaker containing an inverted glass funnel (modified to allow passage of water and suspended sediment by cutting notches at the mouth).<br>• Glass baffles (1/12 of inner diameter of beaker) inhibited formation of a vortex.<br>• A stainless steel propeller-tipped stir rod driven by an electric motor with a rheostat provided the suspension.<br>• Stainless steel mesh screen at the top of the funnel inhibited impingement and entrainment of fish by the propeller system. | • Used 4-week-old fish.<br>• Some sediment was trapped on the screen; mesh size would need to be increased/determined based on particle size.<br>• Further modification of the system is necessary (high mortality in preliminary tests due to stress). |
| [C] | To evaluate effects of intermittent suspended solids exposure on feeding rate or efficiency of unionid clams. | Fresh water bivalves (clams: Unionidae: *Quadrula pustulosa, Fusconaia cerina, Pleurobema beadleanum*) | • Glass aquaria (25 by 51 by 20 cm) with 30 L of constantly aerated water.<br>• Two centrifuge water pumps per tank (alternate and overlap to prevent settling in one area of tank).<br>• Electric timer controls pumps.<br>• Food clearance rates provide estimates of feeding state of clams in different treatments.<br>• $O_2$ uptake and $N_2$ excretion give O:N ratios that provide assessment of relative contribution of protein to total catabolism. (Protein-based catabolism is indicated by an O:N ratio <30). If higher levels are obtained, catabolism can move to non-proteinaceous body stores.<br>• Maximum potential food ingestion rates were determined and converted to food clearance rates.<br>• Evaluated metabolic activity ($O_2$ uptake).<br>• Detect shift in catabolic substrates (using $N_2$ excretion rates).<br>• Determined dry weight of tissue. | • Unable to obtain zero solids levels between successive turbulent episodes.<br>• May require lengthy test to obtain significant endpoints (no reduction in growth was seen in a 10-day period).<br>• Following turbulence, solids levels peaked (600 to 700 mg/L), then fell to 10 % within 15 min. |

**TABLE A1.1** *Continued*

| Ref. | Purpose | Organisms | Apparatus/Description | Comments |
|---|---|---|---|---|
| [A,B,C,D] | To assess the bioaccumulation of cadmium in fish exposed to suspended river sediments, and to evaluate whole-body cadmium, hepatic metal-binding proteins (MBP), and hepatic nonthionein cytosolic cadmium as indicators of exposure to sediment-associated cadmium. | Fresh water fish (*Lepomis macrochirus*, bluegill sunfish) | • Exposure chamber was a decahedron (each glass plate 30.5-cm wide), the sides were circular pieces of glass (76.2-cm diameter). One circular piece had a centered 12.1-cm opening for introduction of water, sediment, and fish.<br>• Each chamber revolved (3.3 r/min) on motor-driven supports (5.7-cm diameter steel shafts covered with rubber tubing) in a water bath.<br>• Motors were controlled by means of a rheostat.<br>• Each chamber contained 45 L of water.<br>• Wet sediment (mass based on wet weight to dry weight ratio) was added to achieve 1000 mg/L total suspended solids (TSS).<br>• Chambers revolved 24 h, were emptied, rinsed, refilled, and organisms added.<br>• Two thirds of the suspension was renewed on days 7, 14, and 21 of the test.<br>• 25 juveniles per chamber were exposed for 28 days.<br>• Alkalinity, hardness, conductivity, TSS, turbidity, ammonia, cadmium dissolved in water and suspended with sediment were measured weekly; pH, temperature, and dissolved oxygen were measured daily.<br>• Total recoverable cadmium was measured in bulk sediment; sediment texture was determined for both bulk and suspended sediment.<br>• Fish were analyzed for nonthionein cytosolic cadmium, hepatic MBP, or whole-body cadmium. Saturated and unsaturated MBP assays were performed on livers.<br>• Whole-body cadmium concentration was the most sensitive indicator of cadmium exposure in this study.<br>• Growth was reduced significantly by suspended sediment exposure (due to physical effects and sediment-associated contaminants). | • The utility of MBP as a biomarker of exposure to sediment-associated cadmium may be hampered when certain other metals are present and when MBP is quantified by the cadmium-saturation method.<br>• Bioaccumulation of cadmium in comparable field situations may be increased due to dietary uptake of cadmium.<br>• Test system is appropriate for examination of a single concentration of suspended sediment of differing composition, or a series of concentrations of suspended sediment of a given composition.<br>• The size of the chamber and volume of water is sufficient for long-term tests with large or many small fish.<br>• Fish are not physically harmed; there is no impingement or entrainment of test organisms in the chamber. |

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**TABLE A1.2 Recirculating Tests (seeFig. A1.2)**

| Ref. | Purpose | Organisms | Apparatus/Description | Comments |
|---|---|---|---|---|
| [A] | To assess the importance of suspended solid levels and their organic carbon content in altering bioavailability of a neutral organic to a water column organism. | Fresh water zooplankton (*Daphnia magna*) | • 11.2-L recirculation system (9 L of test solution): a long glass column, solution is pumped from the bottom of the column through glass and PTFE tubing through the stopper at the top of the column.<br>• Variable speed PTFE/stainless steel gear pumps.<br>• Daphnid vessel (glass with 500-µm PTFE screen) suspended with stainless steel wire just below the water level.<br>• Recirculation rate 600 to 720 mL/min.<br>• Particle size< 180 µm (100 % clay).<br>• Chemical stock solutions prepared by stirring then filtering (0.45 µm) to remove undissolved chemical.<br>• Solids were stirred in diluent 24 h.<br>• Contact time (chemical/solids) was 1 h prior to introduction of organisms.<br>• Suspended solids levels were measured at the start of the test. | • A reduction in aqueous phase chlordane reduced toxicity when sediment threshold level was met.<br>• Stress from recirculation created a more toxic effect (than acute testing).<br>• Total and aqueous phase chemical concentrations were measured at 1-h contact time and at the end of the study (aqueous phase chlordane was defined as that fraction that will pass through a 0.45-µm filter). |
| [B] | To describe and compare bioconcentration of hydrophobic organic chemicals from water or a sediment suspension by fish. | Fresh water fish *Poecilia reticulata* (male guppies) | • 3.5-L vessel with pump and air inlet.<br>• Each vessel and pump constituted a closed system with essentially no head-space.<br>• Conical-shaped vessel; water was circulated from an outlet on the side, near the top of the vessel by means of a pump to the bottom (angled to prevent settling); an air inlet was added to provide oxygen.<br>• Performed a sediment control and sediment/chemical replicate (to control for volatilization/degradation).<br>• Water was spiked using a generator column and was divided into replicates.<br>• Oven-dried sediment (700 mg dry wt/L) was added to a replicate.<br>• After 48 h, fish were added. | • After 1 week, sediment adhered to vessel walls and showed an inhomogeneous suspension in replicates containing fish (however, chemical concentrations were not significantly different in upper versus lower portions of the vessel).<br>• Can obtain increased levels of chemicals in whole fish due to presence of sediment in the intestines (for lower hydrophobic compounds will not get the increased concentration in whole fish because of the low affinity of the compounds to the sediment). |
| [C] | To evaluate lethality of a suspended clay mineral texturally representative of the sediment-size fraction with which contaminants are most commonly associated. To relate responses of animals to actual mass concentration of particles in suspension (rather than turbidity). | Marine and estuarine organisms | • Water and suspended kaolin were metered into the aquaria using two sets of a modified version of the serial dilution apparatus of Mount and Brungs (1967).<br>• Suspension of kaolin in the test aquaria was maintained by individual circulation pumps that continuously withdrew water from the side of a tank and returned it through a disperser head in the tank bottom. Heat introduced by this method was removed by passing the circulating aquarium water through an electronically controlled heat-exchanger system.<br>• Median particle size 4.5 µm (high concentration 117 g/L).<br>• Concentrations of kaolin were maintained in aquaria at a replacement rate of 90 % in 12 h. | • The system produced stable temperatures in all aquaria at a chosen set-point, maintained homogeneous suspensions of kaolin near the desired concentrations, and allowed continual atmospheric exchange at the air-water interface. |

**TABLE A1.2** *Continued*

| Ref. | Purpose | Organisms | Apparatus/Description | Comments |
|---|---|---|---|---|
| [A,B,C,D] | To define relationships between contaminants, the sediment, the water column, and the affected biota. (Hypothesize that the free aqueous cadmium ion is the predominant toxic species, and that lower toxicity would be present in the sediment system due to a reduced free ion concentration from elevated organic ligand concentration.) | Fresh water zooplankton (*Daphnia magna*) | • A modified Prater-Anderson type apparatus.<br>• Rectangular glass chamber (23 by 6.4 by 16 cm).<br>• 7750 mL, circulated volume 60 mL/min.<br>• Daphnid vessel (~90-mL volume with No. 60 stainless steel mesh covering bottom) positioned under the water delivery tube.<br>• Air and water lift tubes (angled so water was pumped from the bottom of one end of the chamber up to the daphnid vessel at the opposite end).<br>• Additional glass tube (1-cm ID) conveyed water from the delivery tube to the vessel.<br>• Mean and median grain size tested: 6.1 and 2.0 µm.<br>To prepare slurry:<br>• Mix sediment and water to suspended solids levels with sonic dismembrator and mechanical stirrer;<br>• Dispense suspended solid aliquots into polypropylene centrifuge tubes;<br>• Dose with cadmium solution to attain total cadmium levels; adjust pH to 7.1;<br>• Mix tubes ≥12 h at 20°C;<br>• Centrifuge tubes at 10 000 r/min for 30 min;<br>• Measure supernatant soluble cadmium concentration;<br>• Dispense appropriate wet mass of sediment into test water, dose with cadmium and equilibrate based on the conditional adsorption constant to obtain the final targeted soluble cadmium concentration.<br>• The contents of the recirculating test chamber was also stirred hourly (manually) during the day and once at night. | • Some settling occurred at the bottom of the static test chamber (1-L Glass beakers aerated through a glass tube).<br>• Following turbulence, solids levels peaked (600 to 700 mg/L), then fell to 10 % within 15 min.<br>• The adsorbed isotherm (adsorbed versus soluble) used for conditional adsorption constant is calculated from the slope of the linear portion of the isotherm. |

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**TABLE A1.3 Flow-through Tests (see Fig. A1.3)**

| Ref. | Purpose | Organisms | Apparatus/Description | Comments |
|---|---|---|---|---|
| [A] | To simulate conditions fish would encounter during dredging and to ascertain whether chemical pollutants associated with bottom sediments are accumulated by fish in a slurry exposure. | Fresh water fish (*Perca flavescens*, yellow perch) | • A pulsed addition of sediment with continuous flow of water.<br>• $N_2$ is run over the sediment reservoir.<br>• Degassed distilled water is added to the sediment to obtain consistency needed to flow freely through the tubing.<br>• Sediment is drawn from the reservoir (12-L fiberglass cylinder with acrylic stirrer) through a PVC pipe (40-cm length, 3.75-cm ID) using an acrylic spiral auger (auger is controlled by means of a variable speed motor).<br>• Sediment falls into a cup attached to a counter-balanced lever.<br>• The lever activates a microswitch after about 25 g of sediment has fallen into the cup.<br>• The microswitch activates a CAM timer that shuts off the auger motor, opens a water valve solenoid (that rinses sediment from the cup) and starts the mixing cylinder stirrer.<br>• When the mixing cylinder (fiberglass) is full, a float valve stops the water flow.<br>• The sediment slurry then flows through PVC tubing into the exposure tanks (oval fiberglass tanks containing 120 L of water) at a rate of 2 L/min (regulated by pipette tips connected to the end of the tubing).<br>• A continuous flow turbidimeter monitored turbidity in the exposure tanks.<br>• Used both 74 to 124 mg suspended solids (SS)/L during 10-min sediment addition cycles, 165 mg SS/L and 266 mg SS/L during 5-min sediment addition cycles. | • Suggest using water the same hardness and salinity as dredge site (since metals may be more toxic in soft water).<br>• Suggest consistency in handling sediment (in terms of oxidation/reduction) and the amount of sediment used (aerated sediments were denser, resulting in a higher dose of sediments).<br>• Suggest consistency in size and origin of test organisms (different uptake by laboratory reared versus site collected).<br>• Bioaccumulation tests should be long (at least >10 days) or collect time-series data to enable estimation of steady-state concentrations (to define pattern of chemical accumulation in organisms). |
| [B] | To design an open-circuit seawater system for conducting bioassays on marine organisms while exposed to concentrations of suspended fine-grained mineral particles. | A wide variety of marine organisms (invertebrates and fish) | • Temperature control is maintained with an electronically proportioned hot/cold ambient water bath.<br>• Uniform seawater flow is maintained with a constant head tank with excess input and an overflow; aliquots for each aquarium are set with a separate valve.<br>• Sediment is continually added to the water flow (0.75 gal/min) into the aquaria (50 gal).<br>• 25 % sediment slurry is suspended with a heavy duty stirrer.<br>• A diaphragm pump delivers slurry into a reagent feeder; a cupwheel (driven by a gear motor) delivers slurry to a flow splitter that delivers 375 ml/min of slurry through a revolving turret over apertures in a circular receiving manifold.<br>• The metered seawater is run into each aperture, where it washes the sediment into the appropriate aquarium.<br>• A gentle upwelling is created in each aquarium by circulating the water through a manifold jet pipe at the bottom of a small submersible pump (pumps are placed in the water bath alongside aquariums with suction and discharge over the side to reduce the noise level in the aquariums).<br>• A 2-ton chiller and 8-KW heater are used for temperature control (temperature is adjusted prior to addition to aquaria). | • Proportional-turbidity-introduction system maintains constant turbidity levels flowing to each aquarium.<br>• Problems of an open seawater system (temperature fluctuations, disease or undesirable organisms, silt, and pollutants) can be controlled by use of a filter, ultraviolet light, and a temperature-controlled water bath.<br>• Need ocean site that does not greatly change environmental parameters diurnally/seasonally. |

**TABLE A1.3** *Continued*

| Ref. | Purpose | Organisms | Apparatus/Description | Comments |
|---|---|---|---|---|
| [A,B,C] | To investigate bioaccumulation potential with the ability to control suspended sediment loading in a flow-through system using a microcomputer. | Fresh or salt water organisms | • Water is gravity fed from large (2000 gal) water storage tanks (polyethylene) through charcoal and sand filters, an ultraviolet sterilizer to the system.<br>• A seawater stock (≥62 g/kg) is held in a storage tank, and then mixed with aged tap water to achieve the desired salinity.<br>• Water flow is controlled by electronic solenoid valves (600 mL every 2 min). Manual valves allow water flow for flushing between experiments. Volume is controlled through the computer program.<br>• Each aquaria is equipped with a circulating pump. Plumbing is routed through a heat exchanger for temperature control.<br>• A suspended sediment slurry is created and held in a cone-bottom hopper (630-L stainless steel) provided with air driven from a diaphragm pump to maintain circulation; argon gas (2 psi) prevents oxidation. The slurry is pumped from the bottom of the hopper, past the aquaria, and returned to the top of the hopper to prevent settling and provide slurry to the aquaria.<br>• The computer monitors suspended solids concentrations (every 8 min) through a transmissometer head in each aquarium. When low, a slurry valve opens, allowing addition of slurry to aquarium.<br>• A sump pit collects slurry that is then forced through a mud/water separator to remove sediment for disposal. | • 95 % of the water is replaced in each aquarium every 12 h.<br>• Thermocouples in heat exchangers send data to the computer, which manipulates pneumatic valves that provide either hot or cold water to flow to the heat exchangers.<br>• The computer system continuously monitors temperature, suspended solid levels, water supply levels, compressed air, and electricity.<br>• pH, dissolved oxygen, conductivity, and total organic carbon levels are monitored at 6-h intervals. |

**TABLE A1.3** *Continued*

| Ref. | Purpose | Organisms | Apparatus/Description | Comments |
|---|---|---|---|---|
| [D] | To adapt existing toxicological protocols for use with solid and suspended particulate phase flow-through tests for both indigenous and“ surrogate” test species. | Annelids, molluscs, arthropods, fishes | • Conical-shaped slurry reservoirs (40-cm diameter by 55 cm high) containing 40 L of slurry (37.7 L of seawater and 2.3 L of sediment) placed in a fiberglass chamber (94 by 61 by 79 cm), maintained at 4 to 10°C, were connected by polypropylene pipes (3.8-cm diameter) to PTFE diaphragm pumps (16 to 40 L/min capacity) for circulation. The pumps lead to 4-L separatory funnels (ensures constant head pressure by the overflow and serves as a connection for the manifold). The manifold distributes the slurry through PTFE dosing valves and back to the reservoir. At the dosing valves, sediment slurry is mixed with seawater.<br>• Argon gas (200 mL/min) minimized oxidation of the slurry in the reservoir and separatory funnel.<br>• Seawater was filtered through 15-µm sand filters.<br>• A microprocessor (connected to a transmissometer) controlled the dosing valves to deliver a pulse every 0.1 s to continuous delivery (once every second or hour).<br>• A fiberglass resin-coated plywood tank (123-L) was partitioned into two compartments for exposure apparatus.<br>• Filtered seawater (2 L/min) was combined with sediment slurry and food (as required) and delivered to the tank.<br>• A manifold collected the tank water and returned it to the chambers at 38 L/min. | • The system maintains reservoirs of reference sediment and dredged material under anoxic conditions and quantitatively delivers them through recirculating loops to test systems.<br>• As suspended particles were removed by the mussels, the microprocessor opened the dosing valve and turned on a peristaltic pump to deliver algae to the chamber.<br>• Twice per week, suspended particulate concentrations were analyzed by dry weight and electronic particle counting.<br>• The particulate concentration can generally be maintained within 10 % of the desired values. |

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## SUMMARY OF CHANGES

Committee E47 has identified the location of selected changes to this standard since the last issue (E1525–94a), that may impact the use of this standard. Additional guidance has been provided on:

*(1)* Hazards (Section 7)
*(2)* Chronic tests (Section 9)
*(3)* Control and reference sediments (Section 11.3.12), and
*(4)* Data interpretation (Section 12.2)