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Standard Guide for Collection, Storage, Characterization, and Manipulation of Sediments for Toxicological Testing¹

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1. Scope

1.1 This guide covers procedures for obtaining, storing, characterizing, and manipulating saltwater and freshwater sediments, for use in laboratory sediment toxicity evaluations. It is not meant to provide guidance for all aspects of sediment assessments, such as chemical analyses or monitoring, geo-physical characterization, or extractable phase and fractionation analyses. However, some of this information might have applications for some of these activities. A variety of test methods are reviewed in this guide. A statement on the consensus approach then follows this review of the test methods. This consensus approach has been included in order to foster consistency among studies. The state-of-the-art is currently in its infancy, and the development of standard test methods is not feasible; however, it is crucial that there be an understanding of the significant effects that these test methods have on sediment quality evaluations. It is anticipated that recommended test methods and this guide will be updated routinely to reflect progress in our understanding of sediments and how to best study them.

1.2 There are several regulatory guidance documents concerned with sediment collection and characterization procedures that might be important for individuals performing federal or state agency-related work. Discussion of some of the principles and current thoughts on these approaches can be found in Dickson, et al (1).²

1.3 Three documents, (Environment Canada (2), USEPA (3) and Test Method E 1706) provide supplemental guidance on procedures dealing with the collection, storage, characterization, and manipulation of sediments used in toxicological assessments.

1.4 This guide is arranged as follows:

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1.5 Field-collected sediments might contain potentially toxic materials and should thus be treated with caution to minimize occupational exposure to workers. Worker safety must also be considered when working with spiked sediments containing various organic, inorganic, or radiolabeled contaminants, or some combination thereof. Careful consideration should be given to those chemicals that might biodegrade, volatilize, oxidize, or photolyze during the exposure.

1.6 The values stated in either SI or inch-pound units are to be regarded as the standard. The values given in parentheses are for information only.

1.7 *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.* Specific hazards statements are given in Section 8.

2. Referenced Documents

2.1 ASTM Standards:

- D 1129 Terminology Relating to Water³
- D 4387 Classification of Grab Sampling Devices for Collecting Benthic Macroinvertebrates⁴
- D 4822 Guide for Selection of Methods of Particle Size Analysis of Fluvial Sediments (Manual Methods)⁵
- D 4823 Guide for Core-Sampling Submerged, Unconsolidated Sediments⁵
- E 729 Guide for Conducting Acute Tests with Fishes, Macroinvertebrates, and Amphibians⁴

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² *Annual Book of ASTM Standards*, Vol 11.01.

³ *Annual Book of ASTM Standards*, Vol 11.05.

⁴ *Annual Book of ASTM Standards*, Vol 11.02.

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



E 943 Terminology Relating to Biological Effects and Environmental Fate⁴

E 1367 Guide for Conducting 10-Day Static Sediment Toxicity Tests with Marine and Estuarine Amphipods⁴

E 1383 Guide for Conducting Sediment Toxicity Tests with Freshwater Invertebrates⁴

E 1706 Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates³

IEEE/ASTM SI 10 Standard for Use of the International System of Units (SI): The Modern Metric System⁵

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 the specified condition, unless the purpose of the test requires a different design. “Must” is used only in connection with the factors that relate directly to the acceptability of the test. “Should” is used to state that the specified condition is recommended and ought to be met in most tests. Although the violation of one “should” is rarely a serious matter, the 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 for 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 Classification D 4387; for an explanation of units and symbols, refer to IEEE/ASTM SI 10.

4. Summary to Guide

4.1 This guide provides a review of widely used test methods for collecting, storing, characterizing, and manipulating sediments for toxicity testing. Where the science permits, recommendations are provided on which procedures are appropriate, while identifying their limitations.

5. Significance and Use

5.1 Sediment toxicity evaluations are a critical component of environmental quality and ecosystem impact assessments, used to meet a variety of research and regulatory objectives. The manner in which the sediments are collected, stored, characterized, and manipulated can influence the results of any sediment quality or process evaluation greatly. Addressing these variables in a systematic and uniform manner will aid the interpretations of sediment toxicity or bioaccumulation results and may allow comparisons between studies.

6. Interferences

6.1 Maintaining the integrity of a sediment sample relative to ambient environmental conditions during its removal, transport, and testing in the laboratory is extremely difficult. The sediment environment is composed of a myriad of microenvi-

ronments, redox gradients, and other interacting physicochemical and biological processes. Many of these characteristics influence sediment toxicity and bioavailability to benthic and planktonic organisms, microbial degradation, and chemical sorption. Any disruption of this environment complicates interpretations of treatment effects, causative factors, and in situ comparisons. See Section 9 for additional information.

7. Apparatus

7.1 A variety of sampling, characterization, and manipulation methods exist using different equipment. These are reviewed in Sections 9 and 14.

7.2 *Cleaning*—Test chambers and equipment used to collect and store sediment samples, prepare and store dilution water and stock solutions, and expose test organisms should be cleaned before use. New glassware and plasticware should be soaked in 1:1 concentrated acid prior to use. Soaking overnight is adequate for glassware. Soaking for seven days in HCl, followed by seven days in HNO₃, followed by seven days in deionized water is recommended for plasticware. Used sample containers should be washed following these steps: (1) non-phosphate detergent wash, (2) triple water rinse, (3) water-miscible organic solvent wash (acetone followed by pesticide-grade hexane (4, 5)), (4) water rinse, (5) acid wash (such as 5 % concentrated hydrochloric acid), and (6) triple rinse with deionized-distilled water. Altering this cleaning procedure might result in problems. Many organic solvents might leave a film that is insoluble in water (Step 3). A dichromate-sulfuric acid cleaning solution can generally be used in place of both the organic solvent and the acid (Steps 3 through 5), but it might attack silicone adhesive. (See 9.10 for cleaning during sample collection.)

8. Safety Hazards

8.1 Many substances can affect humans adversely if adequate precautions are not taken. Information on the toxicity to humans (6) and recommended handling procedures of toxicants (7) should be studied before tests are begun with any contaminant or sediment. Health and safety precautions should be incorporated into any study plan prior to initiating any work with contaminants or sediments.

8.2 Field-collected sediments might contain a mixture of hazardous contaminants or disease-causing agents such that proper handling to avoid human exposure is critical. Skin contact with all test materials and solutions should therefore be minimized by such means as wearing appropriate protective gloves, especially when putting hands into sediments, overlying water, or washing equipment. Proper handling procedures might include the following: (1) sieving and distributing sediments under a ventilated hood or enclosed glove box; (2) enclosing and ventilating the toxicity test water bath; and (3) using respirators, aprons, safety glasses, and gloves when handling potentially hazardous sediments. Special procedures might be necessary with radiolabeled test materials (8) and materials that are, or are suspected of being, carcinogenic (7).

8.3 The disposal of sediments, dilution water over sediments, and test organisms containing hazardous compounds might pose special problems. Removal or degradation of the toxicant(s) before disposal is sometimes desirable for test

involving spiking sediments with known toxicants. Disposal of all hazardous wastes should adhere to the requirements and regulations of the Resource Conservation and Recovery Act and any relevant state or local regulations.

9. Sampling and Transport

9.1 Sediments have been collected for a variety of chemical, physical, toxicological, and biological investigations. The sediments should be collected from depositional zones in which fine-grained sediments accumulate. Site selection should also consider the location of pollutant loadings and hydrological flow patterns. The site selection may also need to be of a random or stratified random nature, depending on the study objectives. Sediment variability must be considered since most sediments are very heterogeneous (both vertically and horizontally) in nature. A preliminary survey or review of background data may therefore be required to determine accurately the appropriate number of sediment replicates to collect.

9.2 Sediment collections have been made with grab and dredge sampling devices and core samplers (see Table 1 and Guide D 4823). The advantages and disadvantages of the various collection methods have been reported previously (9, 10) and are summarized in Table 2. All sampling methods disturb the sediment integrity to a degree. It is important to obtain sediments with as little disruption as possible when

using sediment toxicity evaluations for realistic laboratory evaluations of in situ conditions. Core sampling is preferred above other methods for this reason. Choosing the most appropriate sediment sampler for a study will depend on the sediment's characteristics, efficiency required, and study objectives. Several references are available that discuss the various collection devices (9-13). Grab samplers can penetrate sediments to depths of 10 to 50 cm. Dredge samplers collect to a depth of 10 cm and disrupt sediment integrity. Core samplers collect up to 1 or 2 m when collected by hand or gravity. However, vibratory or piston corers can reach depths of 10 m. The depth of penetration is limited to 10 core diameters in sandy substrates and 20 diameters in predominately clay sediments. The efficiency of these samplers for benthic collections has been compared, and the grab samplers are less efficient collectors than the corers in general, but they are easier to handle in rough water, often require fewer personnel, and are obtained more easily (11, 13, 14). Most of the reported studies used grab samplers, although box corers (15-17), gravity corers (18), and hand collection (19-21) test methods are reported with increasing frequency.

9.3 The disadvantages of grab and dredge samplers (Table 2) include a shallow depth of penetration and the presence of a shock wave that results in loss of the fine surface sediments. Murray and Murray (22), however, described a grab sampler

**TABLE 1 Sampling Containers, Preservation Requirements, and Holding Times for Sediment Samples^A (EPA, 196, 197).
See also Rochon and Chevalier (160)**

Contaminant	Container ^B	Preservation	Holding Time
Acidity	P, G	Cool, 4°C	14 days
Alkalinity	P, G	Cool, 4°C	14 days
Ammonia	P, G	Cool, 4°C	28 days
Sulfate	P, G	Cool, 4°C	28 days
Sulfide	P, G	Cool, 4°C	28 days
Sulfite	P, G	Cool, 4°C	48 h
Nitrate	P, G	Cool, 4°C	48 h
Nitrate-nitrite	P, G	Cool, 4°C	28 days
Nitrite	P, G	Cool, 4°C	48 h
Oil and grease	G	Cool, 4°C	28 days
Organic	P, G	Cool, 4°C	28 days
<i>Metals^C</i>			
Chromium VI	P, G	Cool, 4°C	48 h
Mercury	P, G		8 days
Metals (except Cr or Hg)	P, G		6 months
<i>Organic Compounds^C</i>			
Extractables (including phthalates, atrosamines organochlorine pesticides, PCB's artoaromatics, isophorone, Polynuclear aromatic hydrocarbons, haloethers, chlorinated hydrocarbons, and TCDD)	G, PTFE-lined cap	Cool, 4°C	7 days (until extraction) 30 days (after extraction)
Extractables (phenols)	G, PTFE-lined cap	Cool, 4°C	7 days (until extraction) 30 days (after extraction)
Purgables (halocarbons and aromatics)	G, PTFE-lined septum	Cool, 4°C	14 days
Purgables (acrolein and acrylonitrile)	G, PTFE-lined septum	Cool, 4°C	3 days
Orthophosphate	P, G	Cool, 4°C	48 h
Pesticides	G, PTFE-lined cap	Cool, 4°C	7 days (until extraction) 30 days (after extraction)
Phenols	P, G	Cool, 4°C	28 days
Phosphorus (elemental)	G	Cool, 4°C	48 h
Phosphorus, total	P, G	Cool, 4°C	28 days
Chlorinated organic compounds	G, PTFE-lined cap	Cool, 4°C	7 days (until extraction) 30 days (after extraction)

^A Taken from EPA 600-4-84-075 and EPA 600-4-85-048. See also Ref (85) and USEPA/COE 1991.

^B Polyethylene (P) or Glass (G).

^C Freezing is recommended by some for metals and organics with holding times of 30 and 10 days, respectively (USEPA/COE 1991).

TABLE 2 Summary of Bottom Sampling Equipment^A

Device	Use	Advantages	Disadvantages
PTFE or glass tube	Shallow wadeable waters or deep waters if SCUBA available. Soft or semi-consolidated deposits.	Preserves layering and permits historical study of sediment deposition. Rapid—Samples immediately ready for laboratory shipment. Minimal risk of contamination.	Small sample size requires repetitive sampling.
Hand corer with removable PTFE or glass liners	Same as above except more consolidated sediments can be obtained.	Handles provide for greater ease of substrate penetration. Above advantages.	Careful handling necessary to prevent spillage. Requires removal of liners before repetitive sampling. Slight risk of metal contamination from barrel and core cutter.
Box corer	Same as above.	Collection of large sample undisturbed, allowing for subsampling.	Hard to handle.
Gravity corers, that is, Phleger Corer	Deep lakes and rivers. Semi-consolidated sediments.	Low risk of sample contamination. Maintains sediment integrity relatively well.	Careful handling necessary to avoid sediment spillage. Small sample, requires repetitive operation and removal of liners. Time consuming.
Young Grab (PTFE- or kynar-lined modified 0.1-m ² van Veen) Ekman or box dredge	Lakes and marine areas. Soft to semisoft sediments. Can be used from boat, bridge, or pier in waters of various depths.	Eliminates metal contamination. Reduced bow wake. Obtains a larger sample than coring tubes. Can be subsampled through box lid.	Expensive. Requires winch. Possible incomplete jaw closure and sample loss. Possible shock wave, which may disturb the fines. Metal construction may introduce contaminants. Possible loss of "fines" on retrieval.
PONAR Grab Sampler	Deep lakes, rivers, and estuaries. Useful on sand, silt, or clay.	Most universal grab sampler. Adequate on most substrates. Large sample obtained intact, permitting subsampling.	Shock wave from descent may disturb "fines." Possible incomplete closure of jaws results in sample loss. Possible contamination from metal frame construction. Sample must be further prepared for analysis.
BMH-53 Piston Corer	Waters of 4 to 6 ft deep when used with extension rod. Soft to semi-consolidated deposits.	Piston provides for greater sample retention.	Cores must be extruded on site to other containers. Metal barrels introduce risk of metal contamination.
Van Veen	Deep lakes, rivers, and estuaries. Useful on sand, silt, or clay.	Adequate on most substrates. Large sample obtained intact, permitting subsampling.	Shock wave from descent may disturb "fines." Possible incomplete closure of jaws results in sample loss. Possible contamination from metal frame construction. Sample must be further prepared for analysis.
BMH-60	Sampling moving waters from a fixed platform.	Streamlined configuration allows sampling where other devices could not achieve proper orientation.	Possible contamination from metal construction. Subsampling difficult. Not effective for sampling fine sediments.
Petersen Grab Sampler	Deep lakes, rivers, and estuaries. Useful on most substrates.	Large sample; can penetrate most substrates.	Heavy. May require winch. No cover lid to permit subsampling. All other disadvantages of Ekman and Ponar.
Shipek Grab Sampler	Used primarily in marine waters and large inland lakes and reservoirs.	Sample bucket may be opened to permit subsampling. Retains fine-grained sediments effectively.	Possible contamination from metal construction. Heavy. May require winch.
Orange-Peel Grab Smith-McIntyre Grab	Deep lakes, rivers, and estuaries. Useful on most substrates.	Designed for sampling hard substrates.	Loss of fines. Heavy. May require winch. Possible metal contamination.
Scoops, Drag Buckets	Various environments, depending on depth and substrate.	Inexpensive, easy to handle.	Loss of fines on retrieval through water column.

^A Comments represent subjective evaluations.

usable in rough water that samples the top 1 cm of sediment quantitatively and retains fine materials. Other grab samplers that sample surface sediments quantitatively have been described by Grizzle (23). The depth profile of the sample may be lost in removal of the sample from the sampler. Grab sampling promotes the loss of not only fine sediments (Table 2), but also water-soluble compounds and volatile organic compounds present in the sediment. Dredge samplers are appropriate only for collecting sediments that are to be dredged because they disrupt sediment integrity severely and lose surficial fines.

9.4 Studies of macroinvertebrate sampling efficiency with various grab samplers have provided useful information for sampling in sediment toxicity and sediment quality evaluations. These data provide information that would indicate

sampler efficiency at retaining surficial sediment layers. The modified van Veen is used commonly in coastal sampling (24). The Ekman grab is a commonly used sampler for benthic investigations (23). The Ekman's efficiency is limited to less compacted, fine-grained sediments, as are the corer samplers. Blomqvist (25) reviewed the various Ekman modifications and their associated problems and concluded that the Ekman grab could be used reliably if caution was used during operation. The most commonly used corer is the Kajak-Brinkhurst corer. The Petersen, PONAR, and Smith-McIntyre grabs are used most often (11) in more resistant sediments. Based on studies of benthic macroinvertebrate populations the sediment corers are the most accurate samplers, in most cases followed by the Ekman grab (11). The PONAR grab was the most accurate and

the Petersen the least for compacted sediments (11). A comparison of sampler precision indicated the van Veen sampler to be the least precise; the most precise were the corers and Ekman grab (11).

9.5 Many of the problems associated with grab and dredge samplers are largely overcome with the corers. The best corers for most sediment studies are hand-held poly tetrafluoroethylene (PTFE) plastic, high-density polyethylene, glass corers (liners), or large box-corers. The corers can maintain the integrity of the sediment surface while collecting a sufficient depth. Furthermore, the box core can be sub-cored or sectioned at specific depth intervals, as required by the study. Unfortunately, the box corer is large and cumbersome; it is thus difficult to use. Freefall or gravity cores tend to cause compaction, disrupting the vertical gradients in the sediment. Compaction is reduced using the piston corer. Other coring devices that have been used successfully include the percussion corer (26) and vibratory corers (27-29).

9.6 Corer samplers have several limitations. Most corers do not work well in sandy sediments; grab samplers or diver-collected material remain the only current alternatives. In general, corers collect less sediment than grab samplers, which may provide inadequate quantities for some studies. Small cores tend to increase bow waves (that is, disturbance of surface sediments) and compaction, thus altering the vertical profile. However, these corers provide better information on spatial variation when multiple cores are obtained (11, 30-34). As shown by Rutledge and Fleeger (35) and others, care must be taken in subsampling from core samples since surface sediments might be disrupted in even hand-held core collection. They recommend subsampling in situ or homogenizing core sections before subsampling. See Ref (10) for additional information of various core types.

9.7 Core sampling should be used to best maintain the complex integrity of the sediment for studies of sediment toxicity, interstitial waters, microbiological processes, or chemical fate. When obtaining cores from shallow waters, one must ensure that the vessel does not disturb the sediments before sampling (36). If core sampling is not possible due to an inability of the core to penetrate the sediment (for example, highly compacted sediment) or retain the sample (for example, primarily sand composition), grab samplers should be used that reduce the loss of fine-grained surficial sediments.

9.8 Subsampling, compositing, or homogenization of sediment samples is often necessary, and the optimal methods will depend on the study objectives. Important considerations include the following: loss of sediment integrity and depth profile; changes in chemical speciation by means of oxidation and reduction or other chemical interactions; chemical equilibrium disruption resulting in volatilization, sorption, or desorption; changes in biological activity; completeness of mixing; and sampling container contamination. It is advantageous in most studies of sediment toxicity to subsample the inner core area (not contacting the sampler) since this area is most likely to have maintained its integrity and depth profile and not be contaminated by the sampler. Subsamples from the depositional layer of concern, for example, the top 1 or 2 cm, should be collected with a nonreactive sampling tool such as a

PTFE-lined calibration scoop (37). Samples are frequently of a mixed depth; however, a 2-cm sample (38) is the most common depth obtained, although depths up to 12 m have been used in some dredging studies. It is advantageous or necessary for some studies to composite or mix single sediment samples (37, 39, 40). Composites usually consist of three to five grab samples. An advantage of composited samples is that they reduce the likelihood of missing a “hot spot” due to site heterogeneity. However, a disadvantage is the loss of information on the spatial variability at the site and reduction of the toxicity of hot spot samples when they are diluted with cleaner samples. This is a more critical issue in the boundary areas of the site contamination, also known as the grey zone. Subsamples are collected with a nonreactive sampling scoop and placed in a nonreactive bowl or pan. The composite sample should be mixed until the texture and color appear uniform.

9.9 The assessment of in situ sediment toxicity or bioaccumulation is aided by the collection and testing of reference and control samples. For the purposes of this guide, a reference sediment is defined as a whole sediment near an area of contamination used to assess sediment condition exclusive of the material(s) of interest. It should contain characteristics similar to those of the test sediment. Sediment characteristics, such as particle size distribution and percent organic carbon, should bracket that of the test sediment. If a wide range of test sediment types exists, the reference sediment characteristics should be in an intermediate range unless the test species is affected by particle size. The appropriate ASTM guides for marine (Guide E 1367) and freshwater (Guide E 1383) invertebrates should then be consulted to determine the particle size requirements of the test species. It is preferable that reference sediments be collected from the same aquatic system and be located close to and have physical, chemical, and biological characteristics similar to those of the test sediment. The reference sediment test results might be analyzed as either a treatment or a control variable, depending on the study objectives. The reference sediment might be toxic in some situations due to naturally occurring chemical, physical, or biological properties. It is important for this reason to also use control sediments in the evaluation of test sediments. A control sediment is defined as a sediment that is essentially free of contamination and is used routinely to assess the acceptability of a test. Control sediments have been used successfully in toxicity evaluations (41).

9.10 When collecting sediment grab samples, it is important to clean the sampling device, scoop, spatula, and mixing bowls between sample sites. The cleaning procedure can follow that outlined in Section 7 or the following (42): (1) soap and water wash, (2) distilled water rinse, (3) methanol rinse, (4) methylene chloride rinse, and (5) site water rinse. Waste solvents should be collected in labeled hazardous waste containers.

9.11 The transport conditions for the samples were not specified in the references reviewed in most cases. Where conditions were specified, the sediments were usually transported whole, in both plastic, polyethylene (43-45), and glass (20, 21, 46) containers, and transported under refrigeration or on ice (20, 21, 38, 46-51).

9.12 The collection, transport, storage, and test chamber material composition should be chosen based on a consideration of sorption effects, sample composition, and contact time (Table 1). For example, in sediments in which organics are of concern, brown borosilicate glass containers with PTFE lid liners are optimal, while plastic containers are recommended for metal samples. PTFE or high-density polyethylene containers are relatively inert and optimal for samples contaminated with multiple chemical types. Additionally, polycarbonate containers have been shown not to sorb metal species (52). However, Moody and Lindstrom (53) found that all plastics (including PTFE) leached elements and should be preconditioned with a seven-day soaking in 1:1 HCl, HNO₃, and deionized water. Shipping containers with insulation 1 in. (25.4 mm) in thickness kept samples at 4°C for 21 h, while insulation of 2-in. (51-mm) thickness maintained 4°C for 60 to 82 h (54). Additional information regarding chemical analyses on sample containers, preservation, storage times, and volume requirements is available in other guidance documents (9, 10, 39, 55-57). These criteria are applicable to toxicity test requirements in many cases.

10. Storage

10.1 Containers for storage were generally not specified, although it was assumed that the containers were the same as the transport containers, where specified, and were generally high-density polyethylene (see 9.12). Where sediments contain volatile compounds, transport and storage should be in airtight PTFE or glass containers with PTFE-lined screw caps. Volatile and semi-volatile compounds must be stored at 4°C and are lost in seven or eight days, respectively (54). See Table 1 for further information on the storage requirements for chemical analyses.

10.2 Drying, freezing, and cold storage conditions all affect toxicity and bioavailability (58-63). The storage time of sediments used in toxicity tests was often not specified and, where specified, ranged from a few days (64) to one year (44). The storage of sediments after arrival at the laboratory was generally by refrigeration at 4°C (43-45, 47-51, 61, 64-67). Significant changes in metal toxicity to cladocerans and microbial activity have been observed in stored sediments (62, 68). Recommended limits for the storage of metal-spiked sediments have ranged from within two days (58) to five days (64) and seven days (69, 70). Cadmium toxicity in sediments has been shown to be related to acid volatile sulfide (AVS) complexation (71). When anoxic sediments were exposed to air, AVS was volatilized rapidly. AVS is apparently the reactive solid phase sulfide pool that binds metal, thus reducing toxicity. If a study objective is to investigate metal toxicity and the sediment environment is anoxic, exposure to air might reduce or increase toxicity due to the oxidation and precipitation of the metal species or loss of acid volatile sulfide complexation. A study of sediments contaminated with nonpolar organics found that the interstitial water storage time did not affect toxicity to polychaetes when samples were frozen (72); however, it is generally agreed that sediments to be used for toxicity testing must not be frozen (61, 63, 64, 69, 73).

10.3 Although risking changes in sediment composition, several studies elected to freeze samples (38, 61, 74-78).

Fast-freezing of sediment cores has been recommended for some metal and organic chemical analyses (24, 65, 79); however, this alters the sediment structure and profile distortion occurs (35). Freezing has been reported to inhibit the oxidation of reduced iron and manganese compounds (75). It has also been recommended for stored sediments that are to be analyzed for organics and nutrients (80). Thomson, et al (79) found that no storage method for sediments preserved the initial chemical and physical characteristics of the sediment. Freezing was adequate for the chemical analyses of several metals and organic material (65, 79). Changes were observed at 15 days in sediments stored at 4°C. Oxidation was greater than reduction during storage (79). Carr and Wilkniss (81) showed no mercury loss in sediments acidified to pH 1 for up to eight days, but the sorbed fraction decreased from 80 to 15 % of the total concentration. If sediments are to be frozen for chemical analyses, they should be a split sample from those used for toxicity testing that are kept at 4°C.

10.4 Interstitial water chemistry can change significantly after 24-h storage (82, 83), even when stored at in situ temperatures (83). The coagulation and precipitation of humic material was noted when interstitial water was stored at 4°C for more than one week (84). Oxidation of reduced arsenic species in the pore water of stored sediments was unaffected for up to six weeks when samples were acidified and kept near 0°C, without deoxygenation. Deoxygenation was necessary when samples were not acidified (85). See also Section 11.

10.5 In summary, it is recommended that sediments for toxicity tests and chemical analyses be refrigerated or placed on ice in polyethylene containers during transport. In addition, if samples are to be used for chemical analyses, the appropriate container and holding time should be used as previously described and in Table 1. The storage conditions should be refrigeration at 4°C and under anoxic conditions, if appropriate (39, 55, 86). It has been shown that some contaminated sediments can be stored at 4°C for up to 12 months without significant alterations in toxicity (87). Limits to storage time before testing therefore appear to be a function of both the sediment and contaminant characteristics. Storage should be limited to a two-week period at 4°C unless previous data exist that indicate that the study site sediments can be stored without affecting toxicity.

11. Collection of Interstitial Water

11.1 Interstitial water (pore water), defined as the water occupying the space between sediment or soil particles, is often isolated to provide either a matrix for toxicity testing or an indication of the concentration and partitioning of contaminants within the sediment matrix. There is some indication that the interstitial water may be as useful as whole sediment for evaluating the toxicity of some sediment-associated compounds, for example, those that are not sorbed strongly to particles and where the ingestion of contaminated particles is not a major route of accumulation. The isolation of sediment interstitial water can be accomplished by several methods: centrifugation, squeezing, gas pressurization, suction, and equilibrium dialysis. These techniques have been reviewed recently by Adams (88) and Burton (89). In general, when

relatively large volumes of water are required only centrifugation (for example, **46, 84, 90-98**) and sediment squeezing (**72, 99**) can provide large quantities. Other methods, such as suction (**100-103**) and in situ samplers (**88**), do not produce sufficient volumes from most sediments easily.

11.2 Most collection methods have been shown to alter interstitial water chemistry and therefore may alter toxicity. There are a number of precautions that one should take to reduce the likelihood of causing significant sample change from in situ conditions. Some interstitial water constituents, for example, dissolved organic carbon, dimethylsulfide, ammonia, and major cations, can be altered significantly by the collection method (**104-107**). Increased sample handling by means of methods such as centrifugation or squeezing, compared to in situ “peepers” or core-port suction, may cause increased ammonia and decreased sulfide concentrations (**105**). Other constituents, such as salinity, dissolved inorganic carbon, ammonia, sulfide, and sulfate, might not be affected by collection, providing that oxidation is prevented (**105**). If the sediments are anoxic, all of the steps involved in sample processing should be conducted in inert atmospheres or by limited contact with the atmosphere in order to prevent oxidation (and the subsequent sorption and precipitation) of reduced species (**104, 105, 108**). Immediate collection of the interstitial water is recommended since chemical changes might occur even when the sediments are stored for short periods of time (for example, 24 h) at in situ temperatures (**82, 83**). Toxicity changes have been observed in interstitial water stored for less than 24 h (**109**). The coagulation and precipitation of humic material was noted when interstitial water was stored at 4°C for more than one week (**84**). The oxidation of reduced arsenic species in the interstitial water of stored sediments was unaffected for up to six weeks when the samples were acidified and kept near 0°C, without deoxygenation. Deoxygenation was necessary when the samples were not acidified, (**85**). Others have recommended that interstitial waters be frozen after extraction, prior to toxicity testing, to prevent changes (**72**). The optimal collection method will depend on the intended use of the sample (for example, acidification for metal analysis and not toxicity testing), characteristics of the sediment, and contaminants of concern.

11.3 The conditions for isolation of interstitial waters by centrifugation have varied considerably. Interstitial waters have been isolated for toxicity testing over a range of centrifugal forces and temperatures (**46, 84, 90-95**) with centrifuge bottles of various compositions. When centrifugation followed by filtration has been compared with in situ dialysis, higher speed centrifugation followed by filtration with 0.2 membrane filters has produced results that were more comparable for metals and organic carbon (**75, 109, 110**). Centrifuging at low speeds or the use of a 0.45- μm pore size membrane will result in the collection of both dissolved contaminants, colloidal materials, and aquatic bacteria (**75**). High-speed centrifugation (for example, $10\,000 \times g$) is necessary to remove colloids and dispersible clays (**88, 111, 112**). The duration of the centrifugation has varied in the literature, but 30 min is relatively common and is the recommended time. The temperature for the centrifugation should reflect the ambient temperature of

collection to ensure that the equilibrium between the particles and interstitial water is not shifted. Since trace metals and organics concentrate on solids, their removal is important in sorption and partitioning studies (**75, 111**). However, filtration through a wide range of filter types such as glass fiber or polycarbonate membranes may be inappropriate since they sorb some dissolved metals and organics (**113**). If filtration is used, a nonfiltered sample should also be tested for toxicity and contaminant concentrations. The effects of centrifugation speed, filtration, and oxic conditions on some chemical concentrations in interstitial waters have been well documented (for example, **88, 114, 115**). It is recommended that sediments should be centrifuged at $10\,000 \times g$ for a 30-min period for routine toxicity testing of interstitial waters.

11.4 It is difficult to collect interstitial water from sediments that are predominately coarse sand. A modified centrifuge bottle has been developed, with an internal filter that can recover 75 % of the interstitial water, compared to 25 to 30 % from squeezing (**116**).

11.5 If sorptive organic compounds or mixtures of inorganic and organic compounds are to be isolated, PTFE centrifuge bottles should be used. Polytetrafluorethylene bottles will collapse at $3000 \times g$ but have been used successfully in the range of 2500 g when filled to 80 % of capacity (**90**). So, in this case, the isolation of interstitial water should be at the temperature of collection, at a slower speed of 2500 g for 30-min duration. This material will contain colloidal material as well as dissolved compounds. Removal of the colloids may not be possible at low centrifugation speeds, without filtration. The influence of dissolved and colloidal organic carbon may be estimated by measuring the organic carbon content. Centrifugation can be performed with glass tubes (up to $10\,000 \times g$) (**113**) if small volumes of water are required, for example, 50 mL, for testing higher speed. High-speed centrifugation in stainless steel centrifuge tubes can be performed if metals are not an issue.

11.6 The isolation of interstitial water by squeezing has been performed by means of a variety of practices (**72, 88, 96-99, 117**). In all cases, the interstitial water is passed through a filter that is a part of the apparatus. Filters have different sorptive capacities for different compounds. The characteristics of filters and the filtering apparatus should be considered carefully based on the types of contaminants expected. Squeezing has been demonstrated to yield results equivalent to those for other methods for silica (**118**) but not for sulfide (**119**). However, squeezing has been shown to produce a number of artifacts due to shifts in equilibrium from pressure, temperature, and gradient changes (for example, **97, 106, 107, 120-122**). Squeezing can affect the electrolyte concentration in the interstitial water with a drop near the end of the squeezing process. It is therefore recommended that moderate pressures be used with electrolyte (conductivity) monitoring during extraction (**121**). Several studies revealed significant alterations to the interstitial water composition when squeezing was at temperatures that differed from ambient temperatures (for example, **106, 107**). The major sources of alteration of the interstitial water, when using the squeezing method, are as follows: contamination from overlying water, interstitial water