Designation: E1706 – 20

Standard Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates[1]

This standard is issued under the fixed designation E1706; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope*

1.1 *Relevance of Sediment Contamination*—Sediment provides habitat for many aquatic organisms and is a major repository for many of the more persistent chemicals that are introduced into surface waters. In the aquatic environment, both organic and inorganic chemicals may accumulate in sediment, which can in turn serve as a source of exposure for organisms living on or in sediment. Contaminated sediments may be directly toxic to aquatic life or can be a source of contaminants for bioaccumulation in the food chain.

1.2 *Sediment Assessment Tools*—Several types of information may be useful in assessing the risk, or potential risk, posed by sediment contaminants, including: (*1*) chemical analysis of sediment contaminants; (*2*) sediment toxicity tests, (*3*) bioaccumulation tests; and (*4*) surveys of benthic community structure. Each of these provides a different type of information to the assessment, and integrating information from all four lines of evidence may often provide the most robust assessments.

1.3 *Strengths of Toxicity Testing of Contaminated Sediments*—Directly assessing the toxicity of contaminated sediments provides some of the same advantages to sediment assessment that whole effluent toxicity testing provides to management of industrial and municipal effluents. As for effluent tests, direct testing of sediment toxicity allows the assessment of biological effects even if: (*1*) the identities of toxic chemicals present are not (or not completely) known; (*2*) the influence of site-specific characteristics of sediments on toxicity (bioavailability) is not understood; and (*3*) the interactive or aggregate effects of mixtures of chemicals present are not known or cannot be adequately predicted. In addition, testing the response of benthic or epibenthic organisms exposed via sediment provides an assessment that is based on the same routes of exposure that would exist in nature, rather than only through water column exposure.

1.4 *Relating Sediment Exposure to Toxicity*—One of the challenges with sediment assessment is that the toxicity of sediment contaminants can vary greatly with differences in sediment characteristics; a bulk sediment concentration (normalized to dry weight) may be sufficient to cause toxicity in one sediment, while the same concentration in another sediment does not cause toxicity (for example, Adams et al. 1985) (**1**).[2] Factors such as the amount and characteristics of the organic carbon present in sediment can alter the bioavailability of many chemicals (Di Toro et al. 1991 (**2**); Ghosh 2007 (**3**)), as can other characteristics such as acid volatile sulfide or iron and manganese oxides (Di Toro et al. 1990 (**4**), Tessier et al. 1996 (**5**)). Direct measurement of toxicity in contaminated sediments can provide a means to measure the aggregate effects of such factors on the bioavailability of sediment toxicants.

1.5 *Understanding the Causes of Sediment Toxicity*—While direct testing of sediment toxicity has the advantage of being able to detect the effects of any toxic chemical present, it has the disadvantage of not providing any specific indication of what chemical or chemicals are causing the observed responses. Other techniques, such as spiked-sediment toxicity tests or Toxicity Identification Evaluation (TIE) methods for sediments have been developed and are available to help evaluate cause/effect relationships (USEPA 2007) (**6**).

1.6 *Uses of Sediment Toxicity Tests*—Toxicity tests conducted on sediments collected from field locations can be used to: (*1*) conduct surveys of sediment quality as measured by sediment toxicity; (*2*) prioritize areas of sediment for more detailed investigation of sediment contamination; (*3*) determine the spatial extent of sediment toxicity; (*4*) compare the sensitivity of different organisms to sediment contamination; (*5*) evaluate the relationship between the degree of sediment contamination and biological effects along a contamination gradient; (*6*) evaluate the suitability of sediments for removal and placement at other location (for example, dredged material disposal); (*7*) help establish goals for remedial actions; and (*8*) assess the effectiveness of remedial actions at reducing sediment toxicity. These applications are generally targeted at

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

***A Summary of Changes section appears at the end of this standard**

assessing the likely biological effects of bedded sediments at field sites at the time of sampling. However, toxicity testing of natural or artificial sediments spiked with known quantities of chemicals can also be used to evaluate additional questions such as: (*1*) determining the potency of a chemical to organisms exposed via sediment; (*2*) evaluating the effect of sediment composition on chemical bioavailability or toxicity; (*3*) informing chemical-specific risk assessments for chemicals that may accumulate and persist in sediments upon release; (*4*) establishing regulatory guidance for chemicals in water or sediment. Spiked sediment studies have the advantage of allowing uni-variate experiments in which exposure gradients can be reliably constructed; as such they lend themselves to the derivation of standardized point estimates of effect, such as a median lethal concentration (LC50) or concentration reducing sublethal performance by a specified amount, such as an effect concentration (for example, EC20 estimated to reduce weight of test organisms by 20 %).

1.7 *Limitations*—While some safety considerations are included in this standard, it is beyond the scope of this standard to encompass all safety requirements necessary to conduct sediment toxicity tests.

1.8 *This standard is arranged as follows:*

1.9 *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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.* Specific hazard statements are given in Section 8.

1.10 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]

D1129 Terminology Relating to Water

D4387 Guide for Selecting Grab Sampling Devices for Collecting Benthic Macroinvertebrates (Withdrawn 2003)[4]

E11 Specification for Woven Wire Test Sieve Cloth and Test Sieves

E456 Terminology Relating to Quality and Statistics

E729 Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians

E943 Terminology Relating to Biological Effects and Environmental Fate

E1241 Guide for Conducting Early Life-Stage Toxicity Tests with Fishes

E1325 Terminology Relating to Design of Experiments

E1367 Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Estuarine and Marine Invertebrates

E1383 Guide for Conducting Sediment Toxicity Tests with Freshwater Invertebrates (Withdrawn 1995)[4]

E1391 Guide for Collection, Storage, Characterization, and Manipulation of Sediments for Toxicological Testing and for Selection of Samplers Used to Collect Benthic Invertebrates

E1525 Guide for Designing Biological Tests with Sediments

E1688 Guide for Determination of the Bioaccumulation of Sediment-Associated Contaminants by Benthic Invertebrates

E1733 Guide for Use of Lighting in Laboratory Testing

E1847 Practice for Statistical Analysis of Toxicity Tests Conducted Under ASTM Guidelines

E1850 Guide for Selection of Resident Species as Test Organisms for Aquatic and Sediment Toxicity Tests

E2455 Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels

E3163 Guide for Selection and Application of Analytical Methods and Procedures Used during Sediment Corrective Action

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

3. Terminology

3.1 The words “must”, “should”, “may”, “can”, and “might” have very specific meanings in this standard. “Must” is used to express an absolute requirement, that is, to state that a test

[3] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard’s Document Summary page on the ASTM website.

[4] The last approved version of this historical standard is referenced on www.astm.org.

ought to be designed to satisfy the specified conditions, 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 a test. "Should" is used to state that the specified condition is recommended and ought to be met if possible. Although the 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 for either "may" or "can."

3.2 *Definitions*—For definitions of other terms used in this test method, refer to Guides E729 and E1241 and Terminology E943, E456, E1325, and D1129. For an explanation of units and symbols, refer to IEEE/ASTM-SI-10 .

3.3 *Definitions of Terms Specific to This Standard:*

3.3.1 *clean, n*—denotes a sediment or water that does not contain concentrations of test materials which cause apparent stress to the test organisms or reduce their survival.

3.3.2 *clean sediment and clean water, n*—denotes a sediment or water that does not contain concentrations of test materials which cause apparent stress to the test organisms or reduce their survival.

3.3.3 *concentration, n*—the ratio of weight or volume of test material(s) to the weight or volume of sediment.

3.3.4 *contaminated sediment, n*—sediment containing chemical substances at concentrations that pose a known or suspected threat to environmental or human health.

3.3.5 *control sediment, n*—a sediment that is essentially free of contaminants and is used routinely to assess the acceptability of a test. Any contaminants in control sediment may originate from the global spread of pollutants and does not reflect any substantial input 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. Control sediment is also called a negative control because no toxic effects are anticipated in this treatment.

3.3.6 *effect concentration (EC), n*—the toxicant concentration that would cause an effect in a given percent-age of the test population. Identical to lethal concentration (LC) when the observable adverse effect is death. For example, the EC50 is the concentration of toxicant that would cause a specified effect in 50 % of the test population.

3.3.7 *equilibrium partitioning sediment guidelines (ESGs), n*—numerical concentrations of chemical contaminants in sediment at or below which direct lethal or sublethal toxic effects on benthic organisms are not expected. ESGs are based on the theory that an equilibria exists among contaminant concentration in sediment pore water, contaminant associated with a binding phase in sediment, and biota. ESGs are derived by assigning a protective water-only effects concentration to the pore water (such as a Final Chronic Value), and expressing the associated equilibrium sediment concentration in terms of the principal binding phase that limits contaminant bioavailability (for example, total organic carbon for nonionic organics or acid volatile sulfides for metals).

3.3.8 *formulated sediment, n*—mixtures of materials used to mimic the physical components of a natural sediment.

3.3.9 *inhibition concentration (IC), n*—the toxicant concentration that would cause a given percent reduction in a non-quantal measurement for the test population. For example, the IC25 is the concentration of toxicant that would cause a 25 % reduction in growth for the test population, and the IC50 is the concentration of toxicant that would cause a 50 % reduction.

3.3.10 *interstitial water or pore water, n*—water occupying space between sediment or soil particles.

3.3.11 *lethal concentration (LC), n*—the toxicant concentration that would cause death in a given percentage of the test population. Identical to EC when the observable adverse effect is death. For example, the LC50 is the concentration of toxicant that would cause death in 50 % of the test population.

3.3.12 *lowest-observable-effect concentration (LOEC), n—in a toxicity test*, the lowest tested concentration of a material at which organisms were adversely affected compared to control organisms as determined by statistical hypothesis tests—should be accompanied by a description of the statistical tests and alternative hypotheses, levels of significance, and measures of performance, for example, survival, growth, reproduction, or development—and must be above any other concentration not producing statistically significant adverse effects.

3.3.13 *no-observable-effect concentration (NOEC), n—in a toxicity test*, the highest tested concentration of a material at which organisms did as well as control organisms as determined by statistical hypothesis tests—should be accompanied by a description of the statistical tests and alternative hypotheses, levels of significance, and measures of performance, for example, survival, growth, reproduction, or development—and must be below any other concentration producing statistically significant adverse effects.

3.3.14 *overlying water, n*—the water placed over sediment in a test chamber during a test.

3.3.15 *reference sediment, n*—a whole sediment near an area of concern used to assess sediment conditions exclusive of material(s) of interest. The reference sediment may be used as an indicator of localized sediment conditions exclusive of the specific pollutant input of concern. Such sediment would be collected near the site of concern and would represent the background conditions resulting from any localized pollutant inputs as well as global pollutant input. This is the manner in which reference sediment is used in dredge material evaluations.

3.3.16 *reference-toxicity test, n*—a test conducted with reagent-grade reference chemical to assess the sensitivity of the test organisms. Deviations outside an established normal range may indicate a change in the sensitivity of the test organism

population. Reference-toxicity tests are most often performed in the absence of sediment.

3.3.17 *sediment, n*—particulate material that usually lies below water. Formulated particulate material that is intended to lie below water in a test.

3.3.18 *spiked sediment, n*—a sediment to which a material has been added for experimental purposes.

3.3.19 *whole sediment, n*—sediment and associated pore water which have had minimal manipulation. The term bulk sediment has been used synonymously with whole sediment.

4. Summary of Test Method

4.1 *Method Description*—Procedures are described for testing freshwater organisms in the laboratory to evaluate the potential toxicity of chemicals associated with whole sediments or with water-borne exposures to contamiants. Sediments may be collected from the field or spiked with compounds in the laboratory. This standard is a companion to the USEPA (2019) **(7)** methods manual and both this standard and USEPA (2019) **(7)** were developed as revisions to the second edition of the USEPA (2000) **(8)** methods manual and a previous version of this standard (Test Method E1706-19). This standard and USEPA (2019) **(7)** have lead to the development of other methods for assessing sediment toxicity with invertebrates by other organizations (that is, Enviroment Canada 1997ab **(9, 10)**, 2007 **(11)**, 2013 **(12)**, 2017 **(13)**; OECD 2004a and b **(14, 15)**, 2006 **(16)**, 2010 **(17)**; ISO 2013 **(18)**; Test Method E1367).

4.2 *Short-term Toxicity Testing with the Amphipod Hyalella azteca*—Short-term 10-d sediment toxicity testing methods are outlined for the amphipod *Hyalella azteca* in Annex A1. The short-term sediment exposures with *H. azteca* are started with known-age organisms. Toxicity tests are conducted for 10 d in 300-mL chambers containing 100 mL of sediment and 175 mL of overlying water. Overlying water is renewed daily and chemistry of the overlying water is monitored. Food is provided daily. The endpoints in the 10-d toxicity test with *H. azteca* are survival, dry weight, and biomass. Procedures are primarily described for testing freshwater sediments; however, estuarine sediments (up to 15 ‰ salinity) can also be tested in 10-d toxicity tests with *H. azteca*. Also included is guidance on adapting this method for use in testing the toxicity of chemicals introduced via the water column rather than sediment.

4.3 *Long-term Toxicity Testing with the Amphipod Hyalella azteca*—Methods are described for conducting long-term sediment toxicity tests with *H. azteca* in Annex A2. Toxicity tests are conducted in 300-mL chambers containing 100 mL of sediment and 175 mL of overlying water. Overlying water is renewed daily and chemistry of the overlying water is monitored. Food is provided daily. The long-term sediment exposures with *H. azteca* are started with known-age 7- to 8-d-old amphipods. On Day 28, amphipods are isolated from the sediment and placed in water-only chambers where reproduction is measured on Day 35 and 42. Endpoints measured in the long-term amphipod test include survival (Day 28, 35, and 42), dry weight and biomass (Day 28 and 42), reproduction (number of young per female produced from Day 28 to 42, number of young/surviving female, and survival-normalized reproduction). Procedures are primarily described for testing freshwater sediments; however, estuarine sediments (up to 15 ‰ salinity) can also be tested in long-term toxicity tests with *H. azteca*. The longer-term method with *H. azteca* also include options for abbreviated versions of this test (for example, 28-d exposures measuring survival, dry weight, and biomass). Also included is guidance on adapting this method for use in testing the toxicity of chemicals introduced via the water column rather than sediment.

4.4 *Short-term Toxicity Testing with the Midge Chironomus dilutus*—Short-term 10-d sediment toxicity testing methods are outlined the midge *Chironomus dilutus* in Annex A2. The short-term sediment exposures with *C. dilutus* are started with known-age organisms. Toxicity tests are conducted for 10 d in 300-mL chambers containing 100 mL of sediment and 175 mL of overlying water. Overlying water is renewed daily and chemistry of the overlying water is monitored. Food is provided daily. The endpoints in the 10-d toxicity test with *C. dilutus* are survival, ash-free dry weight, and biomass. Also included is guidance on adapting this method for use in testing the toxicity of chemicals introduced via the water column rather than sediment.

4.5 *Long-term Toxicity Testing with the Midge Chironomus dilutus*—Methods are described for conducting long-term sediment toxicity tests with *C. dilutus* in Annex A4. Midge larvae are exposed to sediments beginning at 3 d old. After 14 d of exposure, a subset of replicates are destructively sampled to determine larval survival, ash-free-dry weight, and biomass. The remaining reproduction replicates are continued through emergence and reproduction of adult midges (for up to about 50 days in exposure started with about 3-d-old larvae), ending when no additional adult emergence has been recorded for 7 consecutive days. Overlying water is renewed daily and chemistry of the overlying water is monitored. Food is provided daily. Endpoints are larval survival, larval weight, larval biomass, percent adult emergence, time to adult emergence, number of egg masses per mated female, average eggs per egg mass, percent of eggs hatching, total young produced, and survival-normalized reproduction. The longer-term method with *C. dilutus* also include options for abbreviated versions of this test (for example, measuring survival, weight, biomass of larvae and emergence of adults but not measuring reproduction). Also included is guidance on adapting this method for use in testing the toxicity of chemicals introduced via the water column rather than sediment.

4.6 *Additional Species for Sediment Toxicity Testing*—Guidance is also provided for conducting sediment toxicity tests with juvenile freshwater mussels (Annex A5), with a second species of midge (*Chironomus riparius*, Annex A6), with a mayfly (*Hexagenia* spp., Annex A7), and with an oligochaete (*Tubifex tubifex*, Annex A8).

4.7 *Bioaccumulation Testing with Sediment*—Guidance for conducting 28-d sediment bioaccumulation tests with the oligochaete *Lumbriculus variegatus* is provided in Guide E1688 and in USEPA (2019) **(7)**.

4.8 The previous version of this standard (Test Method E1706-19) described methods for conducting whole-sediment toxicity tests with *Daphnia magna* and *Ceriodaphnia dubia* (cladocerans) and with *Diporeia* spp. (amphipod). Methods for conducting sediment toxicity tests with cladocerans and with *Diporeia* spp. are not included in the current version of the standard due to limited use of these methods over the past 25 years. A description of the methods for conducting sediment toxicity tests with *D. magna*, *C. dubia* and *Diporeia* spp. can be found in a historic version of the standard (E1706-19) at http://www.astm.org.

4.9 Results of toxicity tests, even those with the same species, using procedures different from those described in this standard may not be comparable and using these different procedures may alter bioavailability (Guide E1525). Comparison of results obtained using modified versions of these procedures might provide useful information concerning new concepts and procedures for conducting sediment tests with aquatic organisms. If toxicity tests are conducted with procedures different from those described in this test method, additional tests are required to determine comparability of results. General procedures described in this standard might be useful for conducting tests with other aquatic organisms; however, modifications may be necessary (Guide E1850).

4.10 *Selection of Test Organisms*—A previous version of this standard (Test Method E1706-19), Guide E1525, and USEPA (2000) **(8)** provide information that was used to select the test organisms in Annex A1 to Annex A8 for conducting sediment toxicity testing.

4.10.1 The choice of a sediment toxicity test organism has a major influence on the relevance, success, and interpretation of a test. Test organism selection should be based on both environmental relevance and practical concerns (Guide E1525, E1850). Ideally, a test organism should: (*1*) have a toxicological database demonstrating relative sensitivity and discrimination to a range of chemicals of concern in sediment; (*2*) have a database for inter-laboratory comparisons of procedures (for example, round-robin studies); (*3*) be in contact with sediment (for example, water column vs. benthic organism); (*4*) be readily available through culture or from field collection; (*5*) be easily maintained in the laboratory; (*6*) be easily identified; (*7*) be ecologically or economically important; (*8*) have a broad geographical distribution, be indigenous (either present or historical) to the site being evaluated, or have a niche similar to organisms of concern (for example, similar feeding guild or behavior to the indigenous organisms); (*9*) be tolerant of a broad range of sediment physico-chemical characteristics (for example, grain size); and (*10*) be compatible with selected exposure methods and endpoints (Table 1.3 in USEPA 2000) **(8)**. The method should also be (*11*) peer reviewed (for example, journal articles, USEPA or ASTM methods) and (*12*) confirmed with responses with natural populations of benthic organisms.

4.10.2 Of these criteria, a database demonstrating relative sensitivity to chemicals, contact with sediment, ease of culture in the laboratory, inter-laboratory comparisons, tolerance to varying sediment physico-chemical characteristics, and confirmation with responses of natural benthic populations were the primary criteria used for selecting *H. azteca*, *C. dilutus*, *C. riparius*, *Hexagenia* ssp., *T. tubifex*, and freshwater mussels for sediment toxicity testing (USEPA 2000 **(8)**, Test Method E1706-19, Guide E1525, Guide E2455).

5. Significance and Use

5.1 Sediment provides habitat for many aquatic organisms and is a major repository for many of the more persistent chemicals that are introduced into surface waters. In the aquatic environment, most anthropogenic chemicals and waste materials including toxic organic and inorganic chemicals can accumulate in sediment, which can in turn serve as a source of exposure for organisms living on or in sediment. Contaminated sediments may be directly toxic to aquatic life or can be a source of contaminants for bioaccumulation in the food chain.

5.2 The objective of a sediment test is to determine whether chemicals in sediment are harmful to or are bioaccumulated by benthic organisms. The tests can be used to measure interactive toxic effects of complex chemical mixtures in sediment. Furthermore, knowledge of specific pathways of interactions among sediments and test organisms is not necessary to conduct the tests. Sediment tests can be used to: (*1*) determine the relationship between toxic effects and bioavailability, (*2*) investigate interactions among chemicals, (*3*) compare the sensitivities of different organisms, (*4*) determine spatial and temporal distribution of contamination, (*5*) evaluate hazards of dredged material, (*6*) measure toxicity as part of product licensing or safety testing, (*7*) rank areas for clean up, and (*8*) estimate the effectiveness of remediation or management practices.

5.3 Results of toxicity tests on sediments spiked at different concentrations of chemicals can be used to establish cause and effect relationships between chemicals and biological responses. Results of toxicity tests with test materials spiked into sediments at different concentrations may be reported in terms of a LC50 (median lethal concentration), an EC50 (median effect concentration), an IC50 (inhibition concentration), or as a NOEC (no observed effect concentration) or LOEC (lowest observed effect concentration). However, spiked sediment may not be representative of chemicals associated with sediment in the field. Mixing time, aging and the chemical form of the material can affect responses of test organisms in spiked sediment tests (10.6).

5.4 Evaluating effect concentrations for chemicals in sediment requires knowledge of factors controlling their bioavailability. Similar concentrations of a chemical in units of mass of chemical per mass of sediment dry weight often exhibit a range in toxicity in different sediments (Di Toro et al. 1990 **(4)**, 1991 **(2)**). Effect concentrations of chemicals in sediment have been correlated to interstitial water concentrations, and effect concentrations in interstitial water are often similar to effect concentrations in water-only exposures. The bioavailability of nonionic organic compounds and metals in sediment is often inversely correlated with the organic carbon concentration; moreover, the bioavailability of metals in sediment are often inversely correlated with acid volatile sulfide. Whatever the route of exposure, these correlations of effect concentrations to

interstitial water concentrations indicate that predicted or measured concentrations in interstitial water can be used to quantify the exposure concentration to an organism. Therefore, information on partitioning of chemicals between solid and liquid phases of sediment is useful for establishing effect concentrations (DiToro et al. 1990 (**4**), 1991 (**2**); Wenning et al. 2005 (**19**)).

5.5 Field surveys can be designed to provide either a qualitative reconnaissance of the distribution of sediment contamination or a quantitative statistical comparison of contamination among sites. Surveys of sediment toxicity are usually part of more comprehensive analyses of biological, chemical, geological, and hydrographic data (USEPA 2002a, b, and c) (**20-22**). Statistical correlations may be improved and sampling costs may be reduced if subsamples are taken simultaneously for sediment tests, chemical analyses, and benthic community structure.

5.6 Table 1 lists several approaches used to assess of sediment quality. These approaches include: (*1*) equilibrium partitioning sediment guidelines (ESGs; USEPA 2003 (**23**), 2005 (**24**); Nowell et al. 2016 (**25**)), (*2*) empirical sediment quality guidelines (for example, probable effect concentrations, PECs; MacDonald et al. 2000 (**26**), Ingersoll et al. 2001 (**27**)), (*3*) tissue residues, (*4*) interstitial water toxicity, (*5*) whole-sediment toxicity with field-collected sediment tests and with sediment-spiking tests, (*6*) benthic community structure, and (*7*) sediment quality triad integrating data from sediment chemistry, sediment toxicity and benthic community structure (Burton 1991 (**28**), Chapman et al. 1997 (**29**), USEPA 2002a, b, and c (**20-22**)). The sediment assessment approaches listed in Table 1 can be classified as numeric (for example, ESGs), descriptive (for example, whole-sediment toxicity tests), or a combination of numeric and descriptive approaches (for example, PECs). Numeric methods can be used to derive chemical-specific effects-based sediment quality guidelines (SQGs). Although each approach can be used to make site-specific decisions, no one single approach can adequately address sediment quality. Overall, an integration of several methods using the weight of evidence is the most desirable approach for assessing the effects of contaminants associated with sediment (USEPA 2002a, b, and c (**20-22**), Wenning et al. 2005 (**19**), Guide E1525, Guide E3163). Hazard evaluations integrating data from laboratory exposures, chemical analyses, and benthic community assessments (the sediment quality triad) provide strong complementary evidence of the degree of pollution-induced degradation in aquatic communities (Burton 1991 (**28**), Chapman et al. 1997 (**29**)). Importantly, the weight

TABLE 1 Sediment Quality Assessment Procedures (Modified from USEPA 1992 (30))

Method	Type			Approach
	Numeric	Descriptive	Combination	
Equilibrium Partitioning Sediment Guidelines (ESGs)		*		An ESG for a given contaminant is determined by calculating the sediment concentration of the contaminant that corresponds to an interstitial water concentration equivalent to the USEPA water-quality criterion for the contaminant.
Emperical Sediment Quality Guidelines	*	*	*	The sediment concentration of contaminants associated with toxic responses measured in laboratory exposures or field assessments (that is, Apparent Effects Threshold (AET), Effect Range Median (ERM), Probable Effect Level (PEL), Probable Effect Concentration (PEC)).
Tissue Residues	*			Safe sediment concentrations of specific chemicals are established by determining the sediment chemical concentration that results in acceptable tissue residues.
Interstitial-water Toxicity	*	*	*	Toxicity of interstitial water isolated from sediment is quantified and identification evaluation procedures are applied to identify and quantify chemical components responsible for sediment toxicity.
Whole-sediment Toxicity with Field-collected Sediments and with Sediment Spiking	*	*	*	Test organisms are exposed to whole sediments that may contain known or unknown quantities of potentially toxic chemicals. Dose-response relationships can be established by exposing test organisms to whole sediments that have been spiked with known amounts of chemicals or mixtures of chemicals.
Benthic Community Structure		*		Environmental degradation is measured by evaluating alterations in resident benthic community structure.
Sediment Quality Triad	*	*	*	Sediment chemical contamination, sediment toxicity, and benthic community structure are measured on the same sediment sample from the site of interest. Correspondence between sediment chemistry, toxicity, and field effects is used to determine sediment concentrations that discriminate conditions of minimal, uncertain, and major biological effects.

of the evidence needed to make a decision (number of methods used) should be determined based on the weight (cost) of the decision.

6. Interferences

6.1 *General:*

6.1.1 *Interferences in the Context of Sediment Toxicity Testing*—In narrow terms, the purpose of a sediment toxicity test is to determine whether the constituents of a sediment sample reduce performance of the test organism relative to a control sediment, reference sediment, or some other sediment whose characteristics serve as a meaningful point of comparison. Sediments that reduce relative organism performance are generally considered to be "toxic", or at least to have greater toxicity that the sediment serving as a point of reference. Because the methods in this standard are intended to assess sediment-associated contaminants, reduced organism performance is generally attributed to the presence of those contaminants. So an interference in a sediment toxicity test can be thought of as a factor that causes a sediment to be judged non-toxic (for example, not different from control) when in fact the level of sediment contamination is sufficient that it should decrease performance (a false negative) or a factor that causes a sediment to indicate toxicity (for example, lower performance relative to control) when in fact the reduced performance is not caused by sediment contaminants. In cases where a gradient in response is being assessed rather than simply "toxic" or "not toxic", these effects could be viewed as causing greater or lesser response than would be expected absent the influence of the "interfering" factor.

6.1.2 *Differences in Responses to Field-collected Sediments when Tested in the Laboratory*—Many applications of sediment toxicity tests involve collection of bedded sediments from the field, that are subsequently tested in the laboratory. It is possible that differences between the physical or biological setting of the original field sediment and the conditions of a laboratory sediment toxicity test could create differences in the apparent adverse effects that might result from exposure; this is possible not only because of differences in the conditions themselves, but also because of changes to the sediment that result from its removal from the field, storage, and manipulation as part of the preparation for and conducting laboratory of sediment toxicity testing. Whether this potential is viewed as an "interference" or simply a consequence of the measurement depends on the presumption of the investigator. Laboratory sediment toxicity tests are often used as tools to assess the likely effects of sediment contaminants under field conditions, but this connection is not intrinsic to the test itself. The extrapolation of responses measured in the laboratory to those that might exist in the field is an important, but separate evaluation that is the responsibility of those designing and implementing the overall sediment assessment program. It is worth noting that the issue of laboratory to field extrapolation is by no means unique to contaminated sediment assessment, and while the issue is important to consider, the precedent of using laboratory tests to develop assessment guidance for pollution in natural systems is extensive, such as in the development of water quality guidelines from single species aquatic toxicity tests (for example, USEPA water quality criteria; Stephan et al. 1985 (**31**)).

6.1.3 *Scope of Interferences Discussion*—Because the definition of an interference in a sediment toxicity test is somewhat context sensitive, the remainder of this section does not attempt to define issues specifically as to whether these factors should be considered interferences. Instead, several factors are discussed that are known or suspected to be potential influences on the responses (including chemical accumulation) of organisms exposed as described in this standard. The importance and implications of these factors for specific studies is left to the investigator or the authority under which the study is conducted.

6.2 *Issues to Consider in Planning or Evaluating Sediment Tests:*

6.2.1 *Studies with Spiked Sediments versus Field-collected Sediments*—Typically, spiked sediment tests are structured so that the same sediment is tested with differing levels of chemical added. This provides much greater consistency in test conditions across treatments than may exist in studies of field samples collected at multiple sites, which may differ in many different characteristics (for example, grain size, organic carbon) beyond just contaminant concentration(s). As such, most of the issues discussed in 6.2.2 through 6.2.6 are likely to be of greater concern in studies involving multiple samples collected from field sites.

6.2.2 *Sediment Collection and Handling Procedures*—The processes involved in removing a bedded field sediment from its field location site, and transporting, storing, and preparing the sediment sample for testing have the potential to alter the characteristics of the sediment sample relative to conditions occurring in the field. Section 10 describes a number of steps that can be taken to minimize undesirable changes associated with these processes. Assessment of sediment chemistry at appropriate points in time from collection to completion of testing can provide important information on the degree to which handling, processing, and testing have affected test sediments.

6.2.3 *Grain Size*—The organisms used in sediment tests described in this standard were selected in part to be tolerant of a fairly wide range of grain size (see Section 1 in USEPA 2000 (**8**)). Because the interactions of sediment contaminants with sediment particles are generally surface-mediated processes, large particles (for example, <5 mm) with small surface area to volume ratios may have limited influence on the contaminant-related response of organisms, and coarse sieving to remove these large particles is discussed in Section 10 and in Annex A1 to Annex A8. If there is a reason for concern that grain size may be an important influence on study results, testing of field reference samples, or control sediments amended to adjust particle size, may be a useful addition to a study.

6.2.4 *Organic Carbon*—Organic carbon content of sediments is known to affect the bioavailability of many sediment-associated contaminants, both by the relative amount of organic carbon present (Di Toro et al 1991 (**2**), 2005 (**32**)), or by the nature and source of the particles comprising the organic carbon fraction (Ghosh 2007) (**3**). While the amount and nature

of organic carbon can affect the relationship between contaminant concentration and effect, this would not generally be considered an interference. Several studies have evaluated the effect of different types, concentrations, or sources of organic carbon on the survival and growth of *H. azteca* and *C. dilutus* (also *C. riparius*), and come to varying conclusions depending in part on how the effect is assessed (for example, Lacey et al. 1999 (**33**), Ristola et al. 1999 (**34**), Suedel and Rogers 1994 (**35**), Ankley et al. 1994a (**36**)). Some of these studies did not include supplemental feeding, and all were conducted before the development of the feeding regimes included in the current sediment toxicity test methods described in this standard.This is important because in studies with no feeding, or insufficient feeding, responses to varying organic carbon can reflect the potential for organic carbon to be a food source, in addition to any other influence on organism performance. In an interlaboratory study with *H. azteca* (Ivey et al. 2016) (**37**) which used the currently recommended feeding regimes described in Table A1.1 and Table A2.1, organisms provided with only silica sand as a substrate generally showed performance at or near that achieved using a field control sediment, demonstrating that there is no minimum organic carbon content for the substrate required for *H. azteca* to meet minimum control performance requirements (Tables A1.1 and A1.2). Likewise, *C. dilutus* fed as specified in Annex A3 and Annex A4 readily meet the control performance criteria associated with those tests (USEPA-Duluth, USGS-Columbia, unpublished data). Ankley et al. (1994a) (**36**) did not find a relationship between sediment organic content (up to 8 %) and 10-d growth of *H. azteca* or *C. dilutus* in a survey of 50 sediments. However, Sampson et al. (2009) (**38**) reported that very high organic carbon (for example, >20 %) was associated with decreased growth of *C. dilutus*. As for any sediment characteristic that lies outside a typical range, inclusion of control or reference sediments that represent extremes in sediment characteristics may be a useful addition to a study.

6.2.5 *Nutritional Content of Sediment*—For studies of field-collected sediments, the innate nutritional content of sediments may vary across sediments, which might influence organism performance, especially growth. There has been research evaluating the relationship between different measures of sediment chemistry and growth of benthic organisms (for example, Vos et al. 2000 (**39**), 2002 (**40**)). In studies supporting the development of the revised foods and rations described in this standard, a major emphasis was placed on finding feeding regimes that were sufficient to make organism performance largely independent of the presence of additional food sources within a sediment, and thereby reduce any confounding of the response to sediment contaminants. Particularly for *C. dilutus*, increasing rations beyond what is recommended in this standard resulted in even higher larval weights, but larger rations also caused large reductions in dissolved oxygen, and the recommended ration was developed as a compromise between increasing growth and limiting effects on dissolved oxygen. Accordingly, while improvements were made, there remains some potential for organism performance to be enhanced in sediments exceptionally rich in nutritional content. The significance of this as a confounding factor in assessing toxicity from sediment-associated contaminants is not clear; while clean sediments that have high nutritional content could result in growth above that found in control or reference treatments, it is not clear how much toxicant stress can be offset by the greater nutritional resources, thus potentially interfering with the ability of the test to detect toxicant stress. In the reverse case, where test sediments might have lower nutritional content than the control or reference, a non-nutritive substrate, such as a sand control, can be used as a point of comparison for organism performance when organisms have only the nutrition provided to all sediments. A sand control provides a reference for the low end of performance that might be expected based on limited nutritional content, but it is not necessary that a field sediment perform more poorly than a sand control to be considered potentially toxic.

6.2.6 *Density Dependent Growth*—As discussed in 6.2.5, the ration provided to *C. dilutus* does not support maximum growth rates. In cases where one or more midge larvae do not survive, there will be proportionately more food available to the survivors, raising the potential for higher growth. This potential was supported by a meta-analysis of growth and survival in control replicates from a large number of 10-d sediment tests with *C. dilutus*, which showed a general tendency toward higher than average weights in replicates with lower than average survival, and vice versa (Fig. A3.2; USEPA-Duluth unpublished data). The effect of density-dependent growth can be compensated for, to some degree, by analyzing total biomass in addition to average dry weight. In the same meta-analysis, control biomass was found to have a lower coefficient of variation than did average dry weight. A similar analysis of data from 10-d tests with *H. azteca* did not show a relationship between survival (density) and weight, indicating that this issue is of less concern for *H. azteca* (Fig. A1.3).

6.2.7 *Ammonia*—Ammonia is produced naturally as a by-product of microbial activity, and is commonly present in measurable amounts in the pore water of natural sediments. In some cases, ammonia concentrations can be high enough to be a potential influence on sediment test results, either through direct toxicity, or by inducing changes in organism behavior (for example, sediment avoidance; Whiteman et al. 1996 (**41**)). Depending on the nature of the assessment, toxicity caused by ammonia may be considered to be part of the aggregate effect of interest, while other assessments may be focused on more persistent chemicals (for example, metals, PAHs) and effects from ammonia may be viewed as an interference. The assessment and management of ammonia is discussed in greater detail within each individual test method (Annex A1 to Annex A5), including information on the sensitivity of each test organism to ammonia. Extending pre-test equilibration of test sediments may be useful in reducing ammonia concentrations in sediment through diffusion to and renewal of overlying water, provided it does not otherwise compromise study objectives. High concentrations of ammonia in field sediments may be indicative of nutrient enrichment; as the ecological effects of nutrient enrichment are far ranging, sediment toxicity testing may not be an appropriate tool for assessing nutrient enrichment as a stressor.

6.2.8 *Low Dissolved Oxygen*—Each of the test methods (Annex A1 to Annex A5) specifies a minimum dissolved oxygen concentration of 2.5 mg/L, a concentration that has been shown to be tolerated by all three of the test organisms described in this standard (Irving et al. 2004 (**42**), Mattson et al. 2009 (**43**)). Dissolved oxygen below 2.5 mg/L may reduce growth or survival. Because the food provided can add to oxygen demand, previous editions of this standard included direction that if dissolved oxygen approached 2.5 mg/L in any treatment, that feeding be reduced in all treatments, in an effort to maintain comparability among all treatments. In the current edition, a more nuanced approach to low dissolved oxygen is discussed, recognizing that having a small number of treatments compromised because of low dissolved oxygen may be preferable to altering the conditions (reduced food, aeration) in all treatments. Detailed discussion of this issue is contained within each individual test method in Annex A1 to Annex A5.

6.2.9 *Influence of Indigenous Organisms*—The potential effects of indigenous organisms, that is organisms present in the sediment at the time of collection, is difficult to study and not fully understood. Generally speaking, having sediments without other organisms present would allow a more standardized means of assessing the toxicity of sediment contaminants, but there is no widely accepted means of eliminating indigenous organisms from samples. Course sieving may be used to remove larger organisms, and extended storage under refrigeration may be effective in reducing the number of surviving indigenous organisms (10.3.2.3). Additional discussion is provided within each test method.

6.2.10 *Influence of Light Quality*—Some toxicants interact with certain wavelengths of light in a way that increases toxicity (for example, photo-induced toxicity of polycyclic aromatic hydrocarbons (PAHs); Davenport and Spacie 1991 (**44**), Ankley et al. 1994b (**45**)). Other toxicants may undergo photolysis if exposed to the appropriate wavelengths of light. Often such light-mediated effects are created by the higher-energy wavelengths present in sunlight, such as the ultraviolet range, which are generally not present to a similar extent in common fluorescent lighting as is typically used for laboratory sediment toxicity testing. Addressing this issue in the context of bedded sediments in the field is challenging, as the penetration of ultraviolet light can be greatly attenuated by the water column, and physical shading by sediment particles may further reduce exposure of benthic organisms and sediment associated. Where these processes may be important, study specific considerations will be required.

7. Water, Formulated Sediment, and Reagents

7.1 *Water:*

7.1.1 *Performance-based Requirements:*

7.1.1.1 *Supporting Organism Performance*—The primary requirement for water used to test and culture organisms is that it support satisfactory survival, growth, or reproduction of the test organisms. Test organisms should survive, grow, and reproduce at rates at or above those described in control performance criteria for long-term testing (for example, Table A2.1 and Table A4.1), and should not show signs of disease or apparent stress (for example, discoloration, unusual behavior). Control tests used to demonstrate the adequacy of the water used to conduct a test should not be conducted using a natural sediment or other substrate that would contribute additional ions to the water; instead, a more inert substrate, such as rinsed sand, should be used to ensure that the test water alone is adequate to support organism health. Note that a demonstration of adequacy in long-term testing is required even if only 10-d tests are being conducted; this is important because some shortcomings of overlying waters only become apparent during longer exposures, even though they undoubtedly cause some degree of shorter-term impact on organism health. If problems are observed in the culturing or testing of organisms, it is desirable to evaluate the characteristics of the water. See Guide E729 for recommendations on chemical analysis of the water supply.

7.1.1.2 *Bromide and Chloride Requirements for Hyalella azteca*—Research has shown that the strain of *Hyalella azteca* most commonly cultured for laboratory testing has more specific requirements for bromide and chloride than does *Chironomus dilutus* (Borgmann 1996 (**46**); Soucek et al. 2015 (**47**), Ivey et al. 2016 (**37**), Ivey and Ingersoll 2016 (**48**)). Special attention to these constituents is required when selecting culture and test water for *H. azteca*.

(1) Bromide—Borgmann (1996) (**46**) reported the essentiality of bromide to *H. azteca*, and proposed a water containing 0.8 mg/L Br, a concentration well above that in most natural waters. Ivey and Ingersoll (2016) (**48**) conducting additional studies to refine the range of Br required for *H. azteca*, and concluded that a minimum of about 0.02 mg Br/L was required for culturing and testing of *H. azteca*, with poor survival, growth and reproduction of *H. azteca* observed in reconstituted waters having less than 0.02 mg/L Br. The adequacy of 0.02 mg Br/L was further supported in inter-laboratory studies (Ivey et al. 2016) (**37**), although a concentration of 0.04 mg Br/L is recommended to assure adequate bromide is present (Table A1.1 and Table A2.1). The requirement of *H. azteca* for Br appears to exist for multiple strains of this amphipod, as different strains were used by Borgmann (1996) (**46**) and Ivey et al. (2016) (**37**).

(2) Chloride—In addition to Br, the "US Lab" strain of *H. azteca* (Major et al. 2013) (**49**) has an additional requirement for Cl concentrations higher than is present in many natural or reconstituted waters. Soucek et al. (2015) (**47**) showed that performance of *H. azteca* declines when chloride concentrations are below about 15 mg/L (Soucek et al. 2015) (**47**). This is consistent with previous reports of difficulty culturing or testing *H. azteca* in reconstituted waters with lower Cl, such as that derived from the original formulation proposed by Marking and Dawson (1973) (**50**) and recommended in some toxicity testing methods for other species (USEPA 2002d) (**51**). In addition to reduced growth and reproduction, the "US Lab" strain of *H. azteca* also showed greater sensitivity to the acute toxic effects of sodium sulfate and sodium nitrate at lower Cl, in a concentration-depend manner similar to that observed for control growth and reproduction. In contrast, the growth of a genetically distinct strain of *H. azteca* obtained from an Environment Canada laboratory in Burlington, Ontario, Canada, was not influenced by chloride concentration, nor was

the acute toxicity of sodium sulfate or sodium nitrate. These findings suggest that the chloride-dependence of toxicity shown for the US Lab strain of *H. azteca* may be an unusual feature of that strain and perhaps not broadly representative of aquatic organisms as a whole.

(3) Supplementing with NaBr and NaCl—If a laboratory's control water does not meet or exceed both 0.04 mg Br/L and 15 mg Cl/L, the waters can be supplemented with NaBr and NaCl to reach these minima for any toxicity tests conducted with *H. azteca* (Annex A1 and Annex A3). A minimum of 0.04 mg Br/L is recommended because the studies of Ivey and Ingersoll (2016) did not determine where between 0.01 and 0.02 mg Br/L performance decreased, and spiking to a target Br concentration of 0.04 mg/L avoids being too close to the minimum requirement. Although not required, waters so adjusted appear completely compatible for testing with *C. dilutus* (Soucek et al. 2015 (**47**), USEPA Duluth laboratory and USGS Columbia laboratory, unpublished data).

7.1.2 *Sources and Treatment:*

7.1.2.1 *Natural Waters*—A natural water is considered to be of uniform quality if monthly ranges of the hardness, alkalinity, and specific conductance are less than about 10 % of their respective averages and if the monthly range of pH is less than about 0.5 units. Uncontaminated wells or springs are likely sources of suitable waters that are stable in composition. Surface waters may be used, but the intake should be positioned to: (*1*) minimize fluctuations in quality and contamination, (*2*) avoid depths with low oxygen content, and (*3*) ensure low concentrations of sulfide and iron. Surface waters should have low concentrations of dissolved organic carbon (for example, less than 5 mg/L), should be filtered to remove particulates.

7.1.2.2 *Municipal Tap Water*—Municipal water supplies may be temporally variable and may contain unacceptably high concentrations of materials such as copper, lead, zinc, fluoride, chlorine, or chloramines. Chlorinated water should not be used for culturing or testing because residual chlorine and chlorine-produced oxidants are toxic to many aquatic organisms. Use of tap water is discouraged unless it is rigorously dechlorinated (USEPA 2002d) (**51**).

7.1.2.3 *Reconstituted Water*—Reconstituted waters may be prepared by adding reagent grade salts to de-ionized water. Details and recipes are provided in 7.1.3.

7.1.2.4 *Adjusting or Matching Water Chemistry*—If desired, natural (or treated tap) waters may have their chemistry adjusted by diluting natural water with deionized water (for example, Kemble et al. 1994 (**52**), Besser et al. 2011 (**53**)) or by adding salts to relatively dilute natural waters (for example, Kunz et al. 2013 (**54**)), or a combination of both. For site-specific investigations, it may be desirable to have the water quality characteristics of the overlying water similar to the site water, provided those characteristics do not adversely affect the test organisms. Water chemistry is known to affect the water-column toxicity of certain contaminants common to sediments, such as many cationic metals, though the influence of overlying water chemistry on the toxicity of sediment-associated metals is less understood.

7.1.2.5 *Dissolved Gases*—Water might need aeration using air stones, surface aerators, or column aerators. Adequate aeration will stabilize pH, bring concentrations of dissolved oxygen and other gases into equilibrium with air, and minimize oxygen demand and concentrations of volatiles. Waters from sources that are substantially colder than test temperature, or that are collected at depth from surface waters (for example, >5 m), may contain dissolved gases (for example, oxygen, nitrogen) at concentrations substantially above those in equilibrium with ambient air and pressure. Because some gases can be slow to come to equilibrium, mechanical degassing equipment may be necessary to bring dissolved gases into an appropriate range.

7.1.3 *Reconstituted Waters:*

7.1.3.1 *Background*—Reconstituted waters offer the ability for laboratories with different water sources to conduct testing using the same water chemistry, and also provides a means to conduct tests in laboratories that do not have access to an appropriate source of natural water. Early in the development of sediment test methods, particularly for *H. azteca*, control performance problems were noted when tests were conducted with some common reconstituted waters, notably those derived from the formulation of Marking and Dawson (1973) (**50**), which has been incorporated into several standard toxicity test methods (for example, USEPA 2002d (**51**), Guide E729). As discussed in 7.1.1.2, insufficient concentrations of Br (<0.02 mg/L) or Cl (<15 mg/L) were likely a cause of many such problems with *H. azteca*. For tests conducted with the US Laboratory strain of *H. azteca* (Major et al. 2013) (**49**), reconstituted waters should contain at least 0.04 mg Br/L and 15 mg Cl/L. As emphasized in 7.1.1.1, reconstituted waters should only be used for sediment toxicity testing after they have been shown to support adequate survival, growth, and reproduction, such as in control tests meeting the control performance criteria for long-term tests (Table A2.1 and Table A4.1).

7.1.3.2 *Base Water for Preparing Reconstituted Waters*—Reconstituted water is generally prepared by adding specified amounts of reagent-grade chemicals to deionized water. Deionized water should be obtained from a system capable of producing at least 1 mega-ohm water. If large quantities of high quality deionized water are needed, it may be advisable to supply the laboratory grade water deionizer with water that has been pre-treated to reduce ion content, such as by a lower-specification mixed-bed ion exchange treatment or a reverse osmosis system, to extend the life of the laboratory grade system. Water prepared by reverse osmosis, or by distillation, may also be used provided it is shown to produce water of similar quality, and to be comparably free of contaminants. Note that many older water distillation systems contained components that would allow elevated concentrations of metals or other contaminants to be present in the product water.

7.1.3.3 *General Procedures for Preparing Reconstituted Waters*—Published recipes for reconstituted waters may have accompanying procedural instructions, though there is no evidence that the way in which the component salts are dissolved into de-ionized water is important. Some recipes dissolve salts individually into separate aliquots of water, while others combine them in different subsets, or all together.

Calcium salts are sometimes dissolved in water separately from other salts (particularly bicarbonate (HCO_3)). All formulas recommended aeration for at least 24 h following salt addition to ensure complete dissolution and stabilization of pH. Salts should be sprinkled in gradually with mixing to avoid formation of a fused mass at the bottom of the container. In addition, dissolution of the salts should be visually verified, and further confirmed through characterization of the resulting water (for example, conductivity, hardness, alkalinity; 7.1.3.4).

7.1.3.4 *Characterization of Reconstituted Waters*—Conductivity, pH, hardness, dissolved oxygen, and alkalinity should be measured on each batch of reconstituted water. The reconstituted water should be aerated before use to stabilize pH and ensure dissolved oxygen is within acceptable ranges. USEPA (1991a) **(55)** recommends using a batch of reconstituted water for up to 2 weeks.

7.1.3.5 *Examples of Reconstituted Fresh Waters*—Information on several reconstituted waters that have been used successfully for sediment toxicity testing in at least some laboratories are provided below. No single water is uniquely recommended, and their order of appearance does not imply priority or preference. Aspects of different formulations that may affect water selection are noted. As per 7.1.1.1, any water selected needs to be shown to support organism health via control testing. It should be noted that most developmental work supporting the use of reconstituted waters in freshwater sediment toxicity test methods has been done in waters with a hardness at or near 100 mg/L as $CaCO_3$, such as in the waters described in the subsections that follow; laboratories using waters with substantially different hardness (or otherwise different composition) should take extra care to ensure that any conclusions or recommendations developed based on waters in the 100 mg/L hardness range are applicable to other water chemistries.

(1) Borgmann (1996) **(46)**—Several laboratories have successfully used the formulation from Borgmann (1996) **(46)** to conduct water or sediment toxicity tests with *H. azteca* or with other test organisms. The Borgmann (1996) **(46)** water ("SAM-5S" shown in Table 5 of the original publication) includes the addition of 0.8 mg/L bromide; that concentration of bromide is much higher than that found in most natural waters and may be undesirable for that reason. However, laboratory testing has indicated that bromide is not harmful to *H. azteca* at concentrations as high as 20 mg/L (Ivey and Ingersoll 2016) **(48)**. Later experiments by Ivey and Ingersoll (2016) **(48)** indicated that the Borgmann (1996) **(46)** water supported good survival, growth, and reproduction when the Br concentration was reduced to as low as 0.02 mg/L. For this reason, an alternative Br concentration of 0.04 mg/L is included here, which is closer to Br concentrations typical of North American surface waters, while still above the apparent minimum requirement (Table 2).

(2) Smith et al., (1997) **(56)** *with NaBr Amendment*—Smith et al. (1997) **(56)** reported on difficulties experienced with reconstituted water formulated after Marking and Dawson (1973) **(50)** and proposed a reformulation that both increased the Cl concentration of the water, and increased the Ca:Mg ratio, which is unusually low in the original Marking and Dawson (1973) **(50)** formula. The resulting formula is not markedly different in composition from that proposed by Borgmann (1996) **(46)**, with the exception that the original Smith et al. (1997) **(56)** formulation did not include any added Br, and some laboratories reported limited success with *H. azteca* in long-term testing using that original formulation. Ivey et al. (2016) **(37)** suggested that the absence of Br from the original formulation was the likely cause of those problems, and demonstrated good performance of *H. azteca* if Smith et al. (1997) **(56)** was supplemented to contain at least 0.02 mg Br/L. The formulation below contains 0.04 mg Br/L (Table 3). Note that Smith et al. (1997) **(56)** recommended dissolving the Ca salts in a separate aliquot of de-ionized water from the other salts, then combining the 2 solutions.

(3) USEPA Duluth Soluble Salt Formula—Because some existing reconstituted water formulas have ionic composition dissimilar from many natural waters, staff at the USEPA-Duluth laboratory developed a reconstituted water formulas intended to better match the characteristics of natural waters, based on surface water survey data collected by the U.S. Geological Survey (Alexander et al. 1996) **(57)**. Two versions were developed, one that uses only easily dissolved salts (this section and Table 4) and another that uses $CaCO_3$, which is more difficult to dissolve (7.1.3.5(*4*) and Table 5). While this

TABLE 2 Formula for Reconstituted Water after Borgmann (1996) (46) and Ivey and Ingersoll (2016) (48)

Salt	Concentration		Notes
	mM (millimolar)	mg/L	
$CaCl_2 \cdot 2H_2O$	1	141	Other hydration states may be used with appropriate adjustment of concentration.
$NaHCO_3$	1	84.0	
$MgSO_4 \cdot 7H_2O$	0.25	61.6	Other hydration states may be used with appropriate adjustment of concentration.
KCl	0.05	3.73	
NaBr	0.0005 (or 0.01)	0.0519 (or 1.04)	Top concentration is for 0.04 mg Br/L (Ivey and Ingersoll 2016) **(48)**; bottom concentration is original Borgmann (1996) **(46)** formulation with 0.8 mg Br/L.

TABLE 3 Formula for Reconstituted Water after Smith et al. (1997) (56); with NaBr per Ivey and Ingersoll (2016) (48)

Salt	Concentration		Notes
	mM (millimolar)	mg/L	
$CaCl_2 \cdot 2H_2O$	0.45	66.1	Other hydration states may be used with appropriate adjustment of concentration.
$CaSO_4$	0.37	50.0	
$NaHCO_3$	1.14	96.0	
$MgSO_4 \cdot 7H_2O$	0.25	61.6	Other hydration states may be used with appropriate adjustment of concentration.
KCl	0.054	4.00	
NaBr	0.0005	0.0519	For 0.04 mg Br/L (Ivey and Ingersoll 2016) **(48)**

TABLE 4 Formula for Soluble Salt Reconstituted Water Developed by USEPA-Duluth (100 mg/L hardness)

Salt	Concentration		Notes
	mM (millimolar)	mg/L	
$CaCl_2 \cdot 2H_2O$	0.363	53.4	Other hydration states may be used with appropriate adjustment of concentration.
$CaSO_4 \cdot 2H_2O$	0.338	58.2	Other hydration states may be used with appropriate adjustment of concentration.
$NaHCO_3$	1.14	129	
$MgSO_4 \cdot 7H_2O$	0.298	73.5	Other hydration states may be used with appropriate adjustment of concentration.
$KHCO_3$	0.0509	5.10	
NaBr	0.0005	0.0519	For 0.04 mg Br/L when testing *H. azteca* (Ivey and Ingersoll 2016) (**48**)

TABLE 5 Formula for Reconstituted Water with CO_2-aided Dissolution Developed by USEPA-Duluth (100 mg/L hardness)

Salt	Concentration		Notes
	mM (millimolar)	mg/L	
$CaCO_3$	0.701	70.2	
$MgCl_2 \cdot 6H_2O$	0.0655	13.2	Other hydration states may be used with appropriate adjustment of concentration.
$MgSO_4 \cdot 7H_2O$	0.233	57.4	Other hydration states may be used with appropriate adjustment of concentration.
$NaHCO_3$	0.342	28.7	
NaCl	.0755	4.41	
KCl	.0509	3.80	
If using water for *H. azteca* testing, also add the following:			
NaCl	0.165	9.67	
NaBr	0.0005	0.0519	For 0.04 mg Br/L (Ivey and Ingersoll 2016) (**48**))

soluble salt formula matches well with U.S. natural waters in several respects (for example, ratios of Ca:Mg, Cl:SO_4, hardness:alkalinity), the overall concentrations of Na+K are higher (relative to hardness) than in most surface waters. As for most reconstituted waters, this stems from adding alkalinity primarily as $NaHCO_3$, rather than from carbonates of Ca and Mg, as in most natural waters. $CaCO_3$ and $MgCO_3$, are not typically used in reconstituted waters because they dissolve extremely slowly if additional steps are not taken to dissolve them (for example, CO_2 enrichment; see 7.1.3.5(*4*)). Another consequence of adding alkalinity as $NaHCO_3$ (or $KHCO_3$) instead of $CaCO_3$ and $MgCO_3$ is that Ca and Mg must then be added as Cl or SO_4 salts, resulting in higher than typical concentrations of those anions. The formula developed by USEPA-Duluth reduces these problems by adding K as $KHCO_3$ instead of KCl (thus reducing $NaHCO_3$ addition), and by maintaining a typical Cl:SO_4 ratio even though Cl + SO_4 is high. When made to 100 mg/L hardness as shown below, this formula contains 25.7 mg Cl/L, thus exceeding the 15 mg/L minimum, and is supplemented with NaBr to achieve 0.04 mg Br/L. This water has been used successfully in long term testing with the US Laboratory strain of *H. azteca* (Soucek et al. 2013 (**58**), 2016 (**59**), Ivey et al. 2016 (**37**)).

(4) USEPA Duluth CO_2-aided Reconstituted Water—As discussed in 7.1.3.5(*3*), closely matching the chemistry of typical natural waters generally requires that most alkalinity be added as $CaCO_3$ or $MgCO_3$. While adding alkalinity in this way allows creation of waters that have chemistry that better aligns with most natural waters, it is more difficult to prepare because these salts, particularly $CaCO_3$, dissolve very slowly at neutral pH. The dissolution can be speeded by temporarily reducing the pH by bubbling CO_2 through the solution. After the $CaCO_3$ has dissolved, the water can be reaerated with ambient air to bring the pCO_2 and pH back into normal ranges. Also, the original formulation of this water has a Cl concentration of about 9.1 mg/L less than the 15 mg Cl/L recommended for *H. azteca*. To use this formulation with *H. azteca*, additional NaCl can be added to raise the Cl concentration to 15 mg/L, as noted in the detailed directions (Table 5).

Procedure for preparing USEPA Duluth CO_2-aided reconstituted water:

(a) Add salts at the concentrations indicated in Table 5 to the appropriate volume of deionized water. If water is intended for use with *H. azteca*, add the additional amounts of NaCl and NaBr noted at the bottom of Table 5.

(b) In a hood, or other ventilated workspace that will ensure workers are not exposed to elevated CO_2 concentrations, bubble 100 % CO_2 gas through the solution using an airstone or other diffuser to disperse the gas. Vessels that have a taller geometry (higher than wide) will speed dissolution of the CO_2 and, as a result, the salts. Stir the solution as needed to aid dissolution of the salts. Time for dissolution will dependent on the chamber geometry, gas flow, and bubble size, but dissolution should not usually take more than about 30 minutes.

(c) Bubbling with 100 % CO_2 will suppress pH considerably (for example, pH 5.0). After salts are dissolved, the solution is aerated with ambient air until appropriate pH (circa 6.8 to 8.3 is restored). Aeration may continue overnight if desired.

7.1.3.6 *Synthetic Seawater—Hyalella azteca* have been used to evaluate the toxicity of estuarine sediments using overlying waters up to 15 ‰ salinity in 10-d exposures (Nebeker and Miller 1988 (**60**), Roach et al. 1992 (**61**), Winger et al. 1993 (**62**), Ingersoll et al. 1996 (**63**)) and in 42-d exposures (Chris Ingersoll, USGS, Columbia, MO, unpublished data). It is important that high salinity testing be conducted using an ion mixture resembling seawater, rather than just NaCl, as *H. azteca* is considerably less tolerant of NaCl alone as compared to the broader mix of ions present in seawater; elevated K and Ca in seawater have been identified as important components of the increased tolerance of seawater (USEPA-Duluth, unpublished data). Introduction of seawater salts to *H. azteca* cultures should also be gradual, as sudden transfer to elevated salinity can induce toxicity (Soucek et al. 2013 (**58**), USEPA-Duluth, unpublished data). Ideally, the cultures of *H. azteca* should be slowly acclimated to elevated salinity over several weeks

before obtaining test organisms for conducting exposures in elevated salinity waters (7.1.3.6(*2*)).

(1) Seawater Salts—Reconstituted salt water can be prepared by adding commercial salt mixtures, such as FORTY FATHOMS®, HW MARINEMIX®, INSTANT OCEAN®, or equivalent to deionized water. A synthetic seawater formulation called GP2 is can also be prepared using reagent grade chemicals that can be diluted with deionized water to the desired salinity (USEPA 1994d) **(64)**.

(2) Culture at Elevated Salinity—Ingersoll et al. (1992) **(65)** describe procedures for culturing *H. azteca* at salinities up to 15 ‰. Reconstituted salt water was prepared by adding INSTANT OCEAN® salts to a 25:75 (v/v) mixture of freshwater (hardness 283 mg/L as $CaCO_3$) and deionized water that was held at least 2 weeks before use. Synthetic seawater was conditioned by adding 6.2 mL of Fritzyme® #9 nitrifying bacteria (*Nitromonas* sp. and *Nitrobacter* sp.; Fritz Chemical Company, Dallas, TX) to each liter of water. The cultures were maintained by using renewal procedures; 25 % of the culture water was replaced weekly.

7.2 *Formulated Sediment:*

7.2.1 *Background and Purpose*—Formulated sediments are mixtures of materials that are intended to mimic the physical components of natural sediments (for example, a mixture of mineral particle sizes and containing a source of organic carbon). A primary use of formulated sediment could be as a control sediment. Formulated sediments allow for standardization of sediment testing or provide a basis for conducting sediment research. Formulated sediment provides a basis by which any testing program can assess the acceptability of their procedures and facilities. In addition, formulated sediment provides a consistent measure evaluating criteria required to determine test acceptability. The use of formulated sediment eliminates interferences caused by the presence of indigenous organisms. For toxicity tests with sediments spiked with specific chemicals, the use of a formulated sediment eliminates or controls the variation in sediment physico-chemical characteristics and provides a consistent method for evaluating the fate of chemicals in sediment.

7.2.2 *Considerations in Formulation*—A variety of recipes for creating formulated sediments were described in USEPA (2000 **(8)**, 2019 **(7)**). A constraint on designing formulated sediments for broad use includes identifying materials that are broadly available, are representative of natural materials in field-collected sediments. For example, most all of the sources of silt, clay, and sand used to prepare formulated sediments described by Kemble et al. (1999) **(66)** are no longer commercially available. Moreover, the alpha cellulose used as a source of total organic carbon in the formulated sediment developed by Kemble et al. (1999) **(66)** has since been found to lack the binding characteristics and nutritional quality of naturally occurring sources of total organic carbon in natural sediments. Consistency of characteristics of the component materials is also important, meaning: (*1*) consistency of materials from batch to batch, (*2*) contaminant concentrations below concentrations of concern, and (*3*) availability to all individuals and facilities.

7.2.3 *Performance-based Acceptability*—Regardless of formulation, an essential characteristic of formulated sediments is that they be shown to support normal survival, growth, or reproduction for benthic invertebrates of interest. In the context of the toxicity methods described here, this generally means demonstrating that a control comprised of the formulated sediment can meet the control performance criteria for long-term sediment testing outlined in Annex A3 and Annex A4. Note that even if formulated sediments will only be used for 10-d sediment toxicity tests with *H. azteca* or *C. dilutus* (Annex A1 and Annex A2), the formulated sediment should still be shown to support adequate performance in longer term tests (Annex A3 and Annex A4) to ensure that the formulated sediment is fully capable of supporting organism health.

7.3 *Reagents:*

7.3.1 *Specifications*—Chemicals used as a part of sediment toxicity testing should be at least reagent grade, unless a test using a formulated commercial product, technical-grade, or use-grade material is specifically needed. Reagent containers should be dated when received from the supplier, and the shelf life of the reagent should not be exceeded. Working solutions should be dated when prepared and the recommended shelf life should not be exceeded.

8. Health, Safety, Waste Management, and Biosecurity

8.1 *General Precautions:*

8.1.1 *Scope*—Procedures described in this standard may involve hazardous materials, operations, and equipment, but it does not purport to address all of the safety problems associated with these activities. It is the responsibility of the user to establish appropriate safety and health practices, and determine the applicability of regulatory limitations before use. While some safety considerations are included, it is beyond the scope of this standard to encompass all safety requirements necessary to conduct sediment tests. Appropriate safety measures must be developed by each laboratory, and modified as necessary for studies posing unique hazards; the guidance in this section is only general.

8.1.2 *Components*—Development and maintenance of an effective health, safety, waste management, and biosecurity 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 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.

8.1.3 *Diversity of Potential Risks*—Collection and use of sediment may involve substantial risks to personal safety and health. Contaminants in field-collected sediment may include carcinogens, mutagens, and other potentially toxic compounds. Inasmuch as sediment toxicity testing is often begun before chemical analyses can be completed, worker contact with sediment may need to be minimized by: (*1*) using gloves, laboratory coats, safety glasses, face shields, and respirators as appropriate, (*2*) manipulating sediment under a ventilated hood or in an enclosed glove box, and (*3*) enclosing and ventilating

the exposure system. Personnel collecting sediment samples and conducting tests should take all safety precautions necessary for the prevention of bodily injury and illness that might result from contact with, or ingestion or inhalation of infectious agents, or corrosive or toxic substances, or asphyxiation because of lack of oxygen or presence of noxious gasses.

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

8.2 *Safety Equipment:*

8.2.1 *Personal Safety Gear*—Personnel should use appropriate safety equipment, such as laboratory coats, gloves, safety glasses, face shields, rubber aprons, respirators, and safety shoes.

8.2.2 *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. All laboratories should be equipped with a telephone to enable personnel to summon help in case of emergency.

8.3 *General Laboratory and Field Operations:*

8.3.1 *Training and Education*—Laboratory personnel should be trained in proper practices for handling and using chemicals that are encountered during procedures described in this standard. Routinely encountered chemicals include acids, organic solvents, and standard materials for reference-toxicity tests. Special handling and precautionary guidance in Safety Data Sheets (formerly known as Material Safety Data Sheets) should be reviewed and followed for reagents and other chemicals purchased from supply houses.

8.3.2 *Work Practices*—Work with some sediment may require compliance with regulations pertaining to the handling of hazardous materials. Personnel collecting samples and performing tests should not work alone. It is advisable to wash exposed parts of the body with antibacterial soap and water immediately after collecting or manipulating sediment samples.

8.3.3 *Chemical Safety*—Strong acids and volatile organic solvents should be used in a fume hood or under an exhaust canopy over the work area. To prepare dilute acid solutions, the concentrated acid should be added to water, not vice versa. Opening a bottle of concentrated acid and adding concentrated acid to water should be performed only under a fume hood. An acidic solution should not be mixed with a hypochlorite solution because hazardous vapors might be produced.

8.3.4 *Electrical Safety*—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.

8.3.5 *Labeling*—All containers should be adequately labeled to identify their contents.

8.4 *Disease Prevention:*

8.4.1 *Vaccination and Hygiene*—Personnel handling samples that are known or suspected to contain human wastes should be given the opportunity to be immunized against diseases such as hepatitis B, tetanus, typhoid fever, polio, and others as appropriate. Thorough washing of exposed skin with antibacterial soap should always follow handling these samples.

8.5 *Pollution Prevention, Waste Management, and Sample Disposal:*

8.5.1 *Compliance*—It is the laboratory's responsibility to comply with the federal, state, and local regulations governing the waste management, particularly hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. In addition, compliance is required with any sewage discharge permits and regulations.

8.5.2 *Handling of Hazardous Materials*—Guidelines for the handling and disposal of hazardous materials should be followed. As a handler of hazardous materials, it is a laboratory's responsibility to know and comply with the applicable federal and state regulations. Where possible, substitution of non-hazardous chemicals and reagents should be encouraged.

8.5.3 *Hazardous Waste*—Use of the methods described in this standard might result in wastes that are legally classified as "hazardous waste", and thereby be subject to additional federal and state regulations.

8.6 *Biosecurity and Non-native Species:*

8.6.1 *Concerns for Non-native Species*—The introduction of non-native aquatic organisms from sources such as culturing facilities or toxicity testing facilities into a watershed is an environmental concern as invasive species can alter existing habitats (Zhu et al. 2006) (**67**), out-compete or replace native species (Meffe 1985 (**68**), Fausch 1988 (**69**), Walser et al. 2000 (**70**)), introduce diseases (Hoffman and Schubert 1984) (**71**), or change the community composition (Lemly 1985) (**72**).

8.6.2 *Sediment Toxicity Testing and Biosecurity*—Biosecurity represents precautions that can be taken to reduce the likelihood of releasing non-native organisms or introduction of diseases associated with toxicity testing. Test organisms used to conduct a laboratory toxicity test may not be native to the watershed were testing is conducted. Field-collected sediments or effluent generated from laboratory testing have the potential to contain organisms that are not native to the watershed where laboratory testing is conducted.

8.6.3 *Minimizing Potential for Release from the Laboratory*—The potential for release of non-native organisms from a culturing or testing laboratory can be reduced by using physical or chemical treatment of water and sediments from sediment toxicity testing. For example, effluent water generated from laboratory testing can be passed through 100-µm mesh opening polypropylene screen to capture macroinvertebrates inadvertently released from the sediment (for example, during the exposures and during the sieving of sediments at the end of the exposures; see Ingersoll et al. 2013 (**73**)). Maximizing surface area can reduce clogging of the mesh screens (for example, Fig. 1). Effluents generated from laboratory testing can also be treated with chlorine to kill non-native organisms or to help destroy disease organisms (for example, Fig. 2). Effluent generated from sieving sediment at the end of an