Designation: E1706 – 19

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 This test method covers procedures for testing freshwater organisms in the laboratory to evaluate the toxicity of contaminants associated with whole sediments. Sediments may be collected from the field or spiked with compounds in the laboratory.

1.1.1 Test methods are described for two toxicity test organisms, the amphipod *Hyalella azteca* (*H. azteca*) (see 13.1.2) and the midge *Chironomus dilutus* (formerly known as *C. tentans*; Shobanov et al. 1999.(**1**) (see 14.1.2). The toxicity tests are conducted for 10 days in 300-mL chambers containing 100 mL of sediment and 175 mL of overlying water. Overlying water is renewed daily and test organisms are fed during the toxicity tests. Endpoints for the 10-day toxicity tests are survival and growth. These test methods describe procedures for testing freshwater sediments; however, estuarine sediments (up to 15 ppt salinity) can also be tested with *H. azteca.* In addition to the 10-day toxicity test method outlined in 13.1.2 and 14.1.2, general procedures are also described for conducting 10-day sediment toxicity tests with *H. azteca* (see 13.1.2) and *C. dilutus* (see 14.1.2).

NOTE 1—Morphological comparison of populations of *Chironomus (Camptochironomus) tentans*(Fabricius) from Europe, Asia, and North America have confirmed cytogenetic evidence that two distinct species inhabit the Palearctic and Nearctic under this name. The Palearctic species is the true *C. tentans* and the Nearctic populations constitute a new species described under the name *Chironomus (Camptochironomus) dilutus* (Shobanov et al. 1999 (**1**).”

1.1.2 Guidance for conducting sediment toxicity tests is outlined in Annex A1 for *Chironomus riparius,* in Annex A2 for *Daphnia magna* and *Ceriodaphnia dubia,* in Annex A3 for *Hexagenia* spp., in Annex A4 for *Tubifex tubifex,* and in Annex A5 for the *Diporeia* spp. Guidance is also provided in Annex A6 for conducting long-term sediment toxicity tests with *H. azteca* by measuring effects on survival, growth, and reproduction. Guidance is also provided in Annex A7 for conducting long-term sediment toxicity tests with *C. dilutus* by measuring effects on survival, growth, emergence, and reproduction. 1.6 outlines the data that will be needed before test methods are developed from the guidance outlined in Annex A1 to Annex A7 for these test organisms. General procedures described in Sections 1 – 14 for sediment testing with *H. azteca* and *C. dilutus* are also applicable for sediment testing with the test organisms described in Annex A1 to Annex A7.

1.2 Procedures outlined in this test method are based primarily on procedures described in the United States Environmental Protection Agency (USEPA) (**2-9**)[2], Test Method E1367, and Guides E1391, E1525 and E1688.

1.3 Additional research and methods development are now in progress to: *(1)* evaluate additional test organisms, *(2)* further evaluate the use of formulated sediment, *(3)* refine sediment dilution procedures, *(4)* refine sediment toxicity identification evaluation (TIE) procedures (**10**), *(5)* refine sediment spiking procedures, *(6)* develop *in situ* toxicity tests to assess sediment toxicity and bioaccumulation under field conditions, *(7)* evaluate relative sensitivities of endpoints measured in tests, *(8)* develop methods for new species, *(9)* evaluate relationships between toxicity and bioaccumulation, and *(10)* produce additional data on confirmation of responses in laboratory tests with natural populations of benthic organisms. Some issues that may be considered in interpretation of test results are the subject of continuing research including the influence of feeding on bioavailability, nutritional requirements of the test organisms, and additional performance criteria for organism health. See Section 6 for additional detail. This information will be described in future editions of this standard.

1.4 The USEPA (**2**) and Guide E1688 also describes 28-day bioaccumulation methods for the oligochaete *Lumbriculus variegatus*.

1.5 Results of tests, even those with the same species, using procedures different from those described in the test method may not be comparable and using these different procedures

[1] This test method is under the jurisdiction of ASTM Committee E50 on Environmental Assessment, Risk Management and Corrective Action and are the direct responsibility of Subcommittee E50.47 on Biological Effects and Environmental Fate.

Current edition approved April 1, 2019. Published April 2019. Originally approved in 1995. Last previous edition approved in 2010 as E1706 – 05(2010) which was withdrawn January 2019 and reinstated in April 2019. DOI: 10.1520/E1706-19.

[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**

may alter bioavailability. 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 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 test method might be useful for conducting tests with other aquatic organisms; however, modifications may be necessary.

1.6 *Selection of Toxicity Testing Organisms:*

1.6.1 The choice of a test organism has a major influence on the relevance, success, and interpretation of a test. Furthermore, no one organism is best suited for all sediments. The following criteria were considered when selecting test organisms to be described in this standard (Table 1 and Guide E1525). A test organism should: *(1)* have a toxicological data base demonstrating relative sensitivity and discrimination to a range of chemicals of concern in sediment, *(2)* have a database for interlaboratory comparisons of procedures (for example, round-robin studies), *(3)* be in contact with sediment [e.g., water column vs benthic organisms], *(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. The method should also be *(11)* peer reviewed and *(12)* confirmed with responses with natural populations of benthic organisms (see 1.6.8).

1.6.2 Of the criteria outlined in Table 1, a data base demonstrating relative sensitivity to contaminants, contact with sediment, ease of culture in the laboratory, interlaboratory comparisons, tolerance of varying sediment physico-chemical characteristics, and confirmation with responses of natural benthos populations were the primary criteria used for selecting *H. azteca* and *C. dilutus* to be described as test methods in the current version of this standard (see Sections 13 and 14). Procedures for conducting sediment tests with organisms in accordance with Annex A1 to Annex A7 do not currently meet all the required selection criteria listed in Table 1. A similar data base must be developed before these or other test organisms can be included as standard test methods instead of as guidance in future versions of these this method.

1.6.3 An important consideration in the selection of specific species for test method development is the existence of information concerning relative sensitivity of the organisms both to single chemicals and complex mixtures. A number of studies have evaluated the sensitivity of *H. azteca, C. dilutus*, and *L. variegatus,* relative to one another, as well as other commonly tested freshwater species. For example, Ankley et al **(11)** found *H. azteca* to be as, or slightly more, sensitive than *Ceriodaphnia dubia* to a variety of sediment elutriate and pore-water samples. In that study, *L. variegatus* were less sensitive to the samples than either the amphipod or the cladoceran. West et al **(12)** found the rank sensitivity of the three species to the lethal effects of copper in sediments from the Keweenaw Waterway, MI was (from greatest to least): *H. azteca* > *C. dilutus* > *L. variegatus*. In short-term (48 to 96 h) exposures, *L. variegatus* generally was less sensitive than *H. azteca, C. dubia,* or *Pimephales promelas* to cadmium, nickel, zinc, copper, and lead **(13)**. Of the latter three species, no one species was consistently the most sensitive to the five metals.

1.6.3.1 In a study of contaminated Great Lakes sediment, *H. azteca, C. dilutus,* and *C. riparius* were among the most sensitive and discriminatory of 24 organisms tested **(14-17)**. Kemble et al **(18)** found the rank sensitivity of four species to metal-contaminated sediments from the Clark Fork River, MT to be (from greatest to least): *H. azteca* > *C. riparius* > *Oncorhynchus mykiss* (rainbow trout) > *Daphnia magna*. Relative sensitivity of the three endpoints evaluated in the *H. azteca* test with Clark Fork River sediments was (from greatest to least): length > sexual maturation > survival.

1.6.3.2 In 10-day water-only and whole-sediment tests, *Hyalella azteca* and *C. dilutus* were more sensitive than *D. magna* to fluoranthene-spiked sediment **(19)**.

TABLE 1 Rating of Selection Criteria for Freshwater Sediment Toxicity Testing Organisms. A "+" or "–" Rating Indicates a Positive or Negative Attribute ("NA" = Not Applicable)

Criterion	*Hyalella azteca*	*Diporeia spp.*	*Chironomus dilutus*	*Chironomus riparius*	*Lumbriculus variegatus*	*Tubifex tubifex*	*Hexagenia spp.*	Molluscs	*Daphnia spp.* and *Ceriodaphnia spp.*
Relative sensitivity toxicity data base	+	–	+	–	+	–	–	–	–
Round-robin studies conducted	+	–	+	–	–	–	–	–	–
Contact with sediment	+	+	+	+	+	+	+	+	–
Laboratory culture	+	–	+	+	+	+	–	–	+
Taxonomic identification	+	+/–	+/–	+/–	+	+	+	+	+
Ecological importance	+	+	+	+	+	+	+	+	+
Geographical distribution	+	+/–	+	+	+	+	+	+	+/–
Sediment physicochemical tolerance	+	+	+/–	+	+	+	–	+	NA
Response confirmed with benthos populations	+	+	+	+	+	+	+	–	+
Peer reviewed	+	+	+	+	+	+	+	–	+/–
Endpoints monitored	S,G,M	S,B,A	S,G,E	S,G,E	B,S	S,R	S,G	B	S,G,R

S = survival, G = Growth, B = Bioaccumulation, A = avoidance
R = Reproduction, M = Maturation, E = Emergence

1.6.3.3 Ten-day, water-only tests also have been conducted with a number of chemicals using *H. azteca, C. dilutus,* and *L. variegatus* ((**19**) and Table 2). These tests all were flow-through exposures using a soft natural water (Lake Superior) with measured chemical concentrations that, other than the absence of sediment, were conducted under conditions (for example, temperature, photoperiod, feeding) similar to those being described for the standard 10-day sediment test in 13.1.2. In general, *H. azteca* was more sensitive to copper, zinc, cadmium, nickel, and lead than either *C. dilutus* or *L. variegatus. Chironomus dilutus* and *H. azteca* exhibited a similar sensitivity to several of the pesticides tested. *Lumbriculus variegatus* was not tested with several of the pesticides; however, in other studies with whole sediments contaminated by dichlorodiphenyltrichloroethane (DDT) and associated metabolites, and in short-term (96-h) experiments with organophosphate insecticides (diazinon, chlorpyrifos), *L. variegatus* has proved to be far less sensitive than either *H. azteca* or *C. dilutus*. These results highlight two important points germane to these test methods. First, neither of the two test species selected for estimating sediment toxicity (*H. azteca, C. dilutus*) was consistently most sensitive to all chemicals, indicating the importance of using multiple test organisms when performing sediment assessments. Second, *L. variegatus* appears to be relatively insensitive to most of the test chemicals, which perhaps is a positive attribute for an organism used for bioaccumulation testing (**9**).

1.6.3.4 Using the data from Table 2, sensitivity of *H. azteca, C. dilutus*, and *L. variegatus* can be evaluated relative to other freshwater species. For this analysis, acute and chronic toxicity data from water quality criteria (WQC) documents for copper, zinc, cadmium, nickel, lead, DDT, dieldrin, and chlorpyrifos, and toxicity information from the AQUIRE data base (**20**) for 1,1,dichloro-2,2-bis(p-chlorophenyl)ethane (DDD) and dichlorodiphenyldichloroethylene (DDE), were compared to assay results for the three species (**19**). The sensitivity of *H. azteca* to metals and pesticides, and *C. dilutus* to pesticides was comparable to chronic toxicity data generated for other test species. This was not completely unexpected given that the 10-day exposures used for these two species are likely more similar to chronic partial life-cycle tests than the 48 to 96-h exposures traditionally defined as acute in the WQC documents. Interestingly, in some instances (for example, dieldrin and chlorpyrifos), LC50 data generated for *H. azteca* or *C. dilutus* were comparable to or lower than any reported for other freshwater species in the WQC documents. This observation likely is a function not only of the test species, but of the test conditions; many of the tests on which early WQC were based were static, rather than flow-through, and report unmeasured contaminant concentrations.

TABLE 2 Water-Only, 10-Day LC50 (μg/L) Values for *Hyalella azteca, Chironomus dilutus*, and *Lumbriculus variegatus* for Chemicals Tested at ERL-Duluth in Soft Water (Hardness 40 mg/L as $CaCO_3$; (19))

Chemical	*H. azteca*	*C. dilutus*	*L. variegatus*
Copper	35	54	35
Zinc	73	1125[1]	2984
Cadmium	2.8[2]	NT[3]	158
Nickel	780	NT	12 160
Lead	<16	NT	794
p,p'-DDT	0.07	1.23	NT
p,p'-DDD	0.17	0.18	NT
p,p'-DDE	1.39	3.0	>3.3
Dieldrin	7.6	1.1	NT
Chlorpyrifos	0.086	0.07	NT

[1] 50 % mortality at highest concentration tested.
[2] 70 % mortality at lowest concentration tested.
[3] NT, not tested.

1.6.3.5 Measurable concentrations of ammonia are common in the pore water of many sediments and have been found to be a common cause of toxicity in pore water (**21 , 22, 23**). Acute toxicity of ammonia to *H. azteca, C. dilutus*, and *L. variegatus* has been evaluated in several studies. As has been found for many other aquatic organisms, the toxicity of ammonia to *C. dilutus* and *L. variegatus* has been shown to be dependent on pH. Four-day LC50 values for *L. variegatus* in water-column (no sediment) exposures ranged from 390 to 6.6 mg/L total ammonia as pH was increased from 6.3 to 8.6 Schubauer-Berigan et al.(**24**). For *C. dilutus*, 4-day LC50 values ranged from 370 to 82 mg/L total ammonia over a similar pH range (Schubauer-Berigan et al.) (**24**). Ankley et al. (**25**) reported that the toxicity of ammonia to *H. azteca* (also in water-only exposures) showed differing degrees of pH-dependence in different test waters. In soft reconstituted water, toxicity was not pH dependent, with 4-day LC50 values of about 20 mg/L at pH ranging from 6.5 to 8.5. In contrast, ammonia toxicity in hard reconstituted water exhibited substantial pH dependence with LC50 values decreasing from >200 to 35 mg/L total ammonia over the same pH range. Borgmann and Borgmann (**26**) later showed that the variation in ammonia toxicity across these waters could be attributed to differences in sodium and potassium content, which appear to influence the toxicity of ammonia to *H. azteca.*

(1) Although these studies provide benchmark concentrations that may be of concern in sediment pore waters, additional studies by Whiteman et al. (**27**) indicated that the relationship between water-only LC50 values and those measured in sediment exposures differs among organisms. In sediment exposures, the 10-day LC50 for *L. variegatus* and *C. dilutus* occurred when sediment pore water reached about 150 % of the LC50 determined from water-only exposures. However, experiments with *H. azteca* showed that the 10-day LC50 was not reached until pore water concentrations were nearly 10× the water-only LC50, at which time the ammonia concentration in the overlying water was equal to the water-only LC50. The authors attribute this discrepancy to avoidance of sediment by *H. azteca*. Thus, it appears that water-only LC50 values may provide suitable screening values for potential ammonia toxicity, higher concentrations may be necessary to actually induce ammonia toxicity in sediment exposures, particularly for *H. azteca*. Further, these data underscore the importance of measuring the pH of pore water when ammonia toxicity may be of concern. Ankley Schubauer-Bergian (**28**) and Besser et al. (**29**) describe procedures for conducting toxicity identification evaluations (TIEs) for pore-water or whole-sediment samples to determine if ammonia is contributing to the toxicity of sediment samples.

1.6.4 Relative species sensitivity frequently varies among chemicals; consequently, a battery of tests including organisms representing different trophic levels may be needed to assess sediment quality (**14, 17, 30-33**). For example, Reish (**34**) reported the relative toxicity of six metals (arsenic, cadmium, chromium, copper, mercury, and zinc) to crustaceans, polychaetes, pelecypods, and fishes and concluded that no one species or group of test organisms was the most sensitive to all of the metals.

1.6.4.1 Sensitivity of a species to chemicals is also dependent on the duration of the exposure and the endpoints evaluated. Annex A6 and Annex A7 describe results of studies which demonstrate the utility of measuring sublethal endpoints in sediment toxicity tests with the amphipod *H. azteca* and the midge *C. dilutus*.

1.6.5 The sensitivity of an organism to chemicals should be balanced with the concept of discrimination (**14**). The response of a test organism should provide discrimination between different levels of contamination. However, insensitive organisms may be preferred for determining bioaccumulation. The use of indigenous organisms that are ecologically important and easily collected is often very straightforward; however, indigenous organisms at a site may be insensitive to the chemicals of concern. Indigenous organisms might be more important for evaluation of bioaccumulation (**9**). See Guides E1525, E1688, and E1850 for additional detail on selection of test organisms.

1.6.6 Sensitivity of an organism is related to route of exposure and biochemical sensitivity to chemicals. Sediment-dwelling organisms can receive a dose from three primary sources: interstitial water, sediment particles, and overlying water. Food type, feeding rate, assimilation efficiency, and clearance rate will control the dose of chemicals from sediment (Guide E1688). Benthic invertebrates often selectively consume different particle sizes (**35**) or particles with higher organic carbon concentrations which may have higher chemical concentrations. Detrital feeders may receive most of their body burden directly from sediment ingestion. In amphipods (**36**) and clams (**37**) uptake through the gut can exceed uptake across the gills for certain hydrophobic compounds. Organisms in direct contact with sediment may also accumulate chemicals by direct adsorption to the body wall or by absorption through the integument (**38**).

1.6.7 Despite the potential complexities in estimating the dose that an animal receives from sediment, the toxicity and bioaccumulation of many chemicals in sediment such as chlordecone, fluoranthene, organochlorines, and metals have been correlated with either the concentration of these chemicals in interstitial water or in the case of nonionic organic chemicals, concentrations of an organic-carbon basis (**39, 40**). The relative importance of whole sediment and interstitial water routes of exposure depends on the test organism and the specific contaminant (**35, 38**). Because benthic communities contain a diversity of organisms, many combinations of exposure routes may be important. Therefore, behavior and feeding habits of a test organism can influence its ability to accumulate contaminants from sediment and should be considered when selecting test organisms for sediment testing.

1.6.8 The response of *H. azteca* and *C. dilutus* in laboratory toxicity studies has been compared to the response of natural populations of benthic organisms to potentially contaminated sediments.

1.6.8.1 Chironomids were not found in sediment samples that decreased the growth of *C. dilutus* by 30 % or more in 10-day laboratory toxicity tests (**41**). Wentsel et al (**42-44**) reported a correlation between effects on *C. dilutus* in laboratory tests and the abundance of *C. dilutus* in metal-contaminated sediments.

1.6.8.2 Canfield et al. (**45,46,47**) evaluated the composition of benthic invertebrate communities in sediments for the following areas: (1) three Great Lakes Areas of Concern (AOC; Buffalo River, NY: Indiana Harbor, IN: Saginaw River, MI), (2) the upper Mississippi River, and (3) the Clark Fork River located in Montana. Results of these benthic community assessments were compared to sediment chemistry and toxicity (28-day sediment exposures with *H. azteca* which monitored effects on survival, growth, and sexual maturation). Good concordance was evident between measures of laboratory toxicity, sediment contamination, and benthic invertebrate community composition in extremely contaminated samples. However, in moderately contaminated samples, less concordance was observed between the composition of the benthic community and either laboratory toxicity test results or sediment contaminant concentration. Laboratory sediment toxicity tests better identified chemical contamination in sediments compared to many of the commonly used measures of benthic invertebrate community composition. Benthic measures may reflect other factors such as habitat alteration in addition to responding to contaminants. Canfield et al. (**45, 46, 47**) identified the need to better evaluate non-contaminant factors (i.e., TOC, grain size, water depth, habitat alteration) in order to better interpret the response of benthic invertebrates to sediment contamination.

1.6.8.3 Results from laboratory sediment toxicity tests were compared to colonization of artificial substrates exposed *in situ* to Great Lakes sediment (**14**) Burton et al. (**17**) Survival or growth of *H. azteca* and *C. dilutus* in 10–28-day laboratory exposures were negatively correlated to percent chironomids and percent tolerant taxa colonizing artificial substrates in the field. Schlekat et al (**48**) reported general good agreement between sediment toxicity tests with *H. azteca* and benthic community responses in the Anacostia River in Washington, DC.

1.6.8.4 Sediment toxicity with amphipods in 10-day toxicity tests, field contamination, and field abundance of benthic amphipods were examined along a sediment contamination gradient of DDT (**48**). Survival of *Eohaustorius estuarius, Rhepoxynius abronius*, and *H. azteca* in laboratory toxicity tests was positively correlated to abundance of amphipods in the field and negatively correlated to DDT concentrations. The threshold for 10-day sediment toxicity in laboratory studies was about 300 µg DDT (+metabolites)/g organic carbon. The threshold for abundance of amphipods in the field was about 100 µg DDT (+metabolites)/g organic carbon. Therefore, correlations between toxicity, contamination, and field populations indicate that short-term sediment toxicity tests can

provide reliable evidence of biologically adverse sediment contamination in the field, but may be underprotective of sublethal effects.

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 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]

D4447 Guide for Disposal of Laboratory Chemicals and Samples

E29 Practice for Using Significant Digits in Test Data to Determine Conformance with Specifications

E105 Practice for Probability Sampling of Materials

E122 Practice for Calculating Sample Size to Estimate, With Specified Precision, the Average for a Characteristic of a Lot or Process

E141 Practice for Acceptance of Evidence Based on the Results of Probability Sampling

E177 Practice for Use of the Terms Precision and Bias in ASTM Test Methods

E178 Practice for Dealing With Outlying Observations

E456 Terminology Relating to Quality and Statistics

E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

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

E943 Terminology Relating to Biological Effects and Environmental Fate

E1193 Guide for Conducting *Daphnia magna* Life-Cycle Toxicity Tests

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

E1295 Guide for Conducting Three-Brood, Renewal Toxicity Tests with *Ceriodaphnia dubia*

E1325 Terminology Relating to Design of Experiments

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

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

E1402 Guide for Sampling Design

E1525 Guide for Designing Biological Tests with Sediments

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

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

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 ought to be designed to satisfy the specified conditions, unless

[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.

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

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

3.3.4 *control sediment*—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.

3.3.5 *EC50*—a statistically or graphically estimated concentration that is expected to cause one or more specified effects in 50 % of a group of organisms under specified conditions.

3.3.6 *Formulated sediment*—Mixtures of materials used to mimic the physical components of a natural sediment.

3.3.7 *IC50*—a point estimate of the toxicant concentration that would cause a 50 % reduction in a non-quantal measurement such as fecundity or growth.

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

3.3.9 *LC50*—a statistically or graphically estimated concentration that is expected to be lethal to 50 % of a group of organisms under specified conditions.

3.3.10 *lowest-observable-effect concentration (LOEC)*—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.

3.3.11 *no-observable-effect concentration (NOEC)*— 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.

3.3.12 *overlying water*—the water placed over sediment in a test chamber during a test.

3.3.13 *reference sediment*—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.

3.3.14 *reference-toxicity test*—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.

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

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

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

4. Summary of Standard

4.1 *Method Description*—Procedures are described for testing freshwater organisms in the laboratory to evaluate the toxicity of contaminants associated with whole sediments. Sediments may be collected from the field or spiked with compounds in the laboratory.

4.1.1 Test methods are described for conducting toxicity tests with two organisms: the amphipod *Hyalella azteca* (see 13.1.2) and the midge *Chironomus dilutus* (formerly known as *C. tentans*; Shobanov et al. 1999.(**1**), (see 14.1.2). The toxicity tests are conducted for 10 days in 300-mL chambers containing 100 mL of sediment and 175 mL of overlying water. Overlying water is renewed daily and test organisms are fed during the toxicity tests. Endpoints for the 10-day toxicity tests are survival and growth. Length or weight is reported as the average of the surviving organisms at the end of the test (Sections 13 and 14). Another approach for reporting growth might be as biomass (dry weight of surviving organisms

divided by the initial number of organisms). The rationale for evaluating biomass in toxicity testing is that small differences in either growth or survival may not be statistically significantly different from the control; however, a combined estimate of biomass may increase the statistical power of the test. While USEPA **(3)** recommend reporting biomass as a measure of growth in effluent toxicity tests, the approach has not yet been routinely applied in sediment testing. Therefore, biomass is not listed as a primary endpoint in the methods described in Sections 13 and 14 or in Annex A1 to Annex A7. The standard describes procedures for testing freshwater sediments; however, estuarine sediments (up to 15 ppt salinity) can also be tested with *H. azteca*. In addition to the 10-day toxicity test methods outlined in 13.1.2 and 14.1.2, general procedures are also described for conducting sediment toxicity tests with *H. azteca* (see 13.1.2) and *C. dilutus* (see 14.1.2).

4.1.2 Guidance for conducting sediment toxicity tests is provided in Annex A1 for *Chironomus riparius,* in Annex A2 for *Daphnia magna* and *Ceriodaphnia dubia,* in Annex A3 for *Hexagenia spp.*, in Annex A4 for *Tubifex tubifex,* and in Annex A5 for the *Diporeia spp.*

4.1.3 Guidance for conducting long-term sediment toxicity tests with *H. azteca* by measuring effects on survival, growth, and reproduction is provided in Annex A6. The long-term sediment exposures with *H. azteca* are started with 7- to 8-day-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), growth (Day 28 and 42), and reproduction (number of young/female produced from Day 28 to 42). Guidance for conducting long-term sediment toxicity tests with *C. dilutus* by measuring effects on survival, growth, emergence, and reproduction is provided in Annex A7. The long-term sediment exposures with *C. dilutus* start with newly hatched larvae (<24-h old) and continue through emergence, reproduction, and hatching of the F_1 generation (about 60-day exposures). Survival and growth are determined at 20 day. Starting on Day 23 to the end of the test, emergence and reproduction of *C. dilutus* are monitored daily. The number of eggs/female is determined for each egg case, which is incubated for 6 day to determine hatching success.

4.1.3.1 The long-term toxicity testing methods for *Hyalella azteca* (Annex A6) and *Chironomus dilutus* (Annex A7) can be used to measure effects on reproduction as well as long-term survival and growth. Reproduction is a key variable influencing the long-term sustainability of populations (Rees and Crawley, **(49)**) and has been shown to provide valuable and sensitive information in the assessment of sediment toxicity Derr and Zabik, **(50)**; Wentsel et al., **(51)** ; Williams et al., **(52)**; Postma et al., **(53)**; Sibley et al., **(54)**, **(55)**; Ingersoll et al., **(56)**. Further, as concerns have emerged regarding the environmental significance of chemicals that can act directly or indirectly on reproductive endpoints (e.g., endocrine disrupting compounds), the need for comprehensive reproductive toxicity tests has become increasingly important. Reproductive endpoints measured in sediment toxicity tests with *H. azteca* and *C. dilutus* tend to be more variable compared to survival or growth (Section A6.4.6 and A7.5.4.6). Hence, additional replicates would be required to achieve the same statistical power as for survival and growth endpoints (Section 16). The procedures described in Annex A6 and Annex A7 include measurement of a variety of lethal and sublethal endpoints; minor modifications of the basic methods can be used in cases where only a subset of these endpoints is of interest (A6.1.3 and A7.1.2).

4.1.4 Paragraph 1.6 outlines the data that will be needed before test methods are developed from the guidance outlined for these test organisms in Annex A1 to Annex A7. General procedures described in Sections 1 to 14 for sediment testing with *H. azteca* and *C. dilutus* are also applicable for sediment testing with the test organisms described in Annex A1 to Annex A7.

4.2 *Experimental Design*—The following section is a general summary of experimental design. See Section 15 for additional detail.

4.2.1 *Control and Reference Sediment:*

4.2.1.1 Sediment tests include a control sediment (sometimes called a negative control). A control sediment is a sediment that is essentially free of contaminants and is used routinely to assess the acceptability of a test and is not necessarily collected near the site of concern. Any contaminants in control sediment are thought to originate from the global spread of pollutants and do not reflect any substantial inputs from local or non-point sources **(9)**. Comparing test sediments to control sediments is a measure of the toxicity of a test sediment beyond inevitable background contamination and organism health **(9)**. A control sediment provides a measure of test acceptability, evidence of test organism health, and a basis for interpreting data obtained from the test sediments. A reference sediment is collected near an area of concern and is used to assess sediment conditions exclusive of material(s) of interest. Testing a reference sediment provides a site-specific basis for evaluating toxicity.

(1) In general, the performance of test organisms in the negative control is used to judge the acceptability of a test, and either the negative control or reference sediment may be used to evaluate performance in the experimental treatments, depending on the purpose of the study. Any study in which organisms in the negative control do not meet performance criteria must be considered questionable because it suggests that adverse factors affected the response of test organisms. Key to avoiding this situation is using only control sediments that have a demonstrated record of performance using the same test procedure. This includes testing of new collections from sediment sources that have previously provided suitable control sediment.

(2) Because of the uncertainties introduced by poor performance in the negative control, such studies should be repeated to insure accurate results. However, the scope or sampling associated with some studies may make it difficult or impossible to repeat a study. Some researchers have reported cases where performance in the negative control is poor, but performance criteria are met in reference sediment included in the study design. In these cases, it might be resonable to infer that other samples that show good performance are probably not

toxic; however, any samples showing poor performance should not be judged to have shown toxicity, since it is unknown whether the adverse factors that caused poor control performance might have also caused poor performance in the test treatments.

4.2.1.2 Natural physico-chemical characteristics such as sediment texture may influence the response of test organisms (**57**). The physico-chemical characteristics of test sediment need to be within the tolerance limits of the test organism. Ideally, the limits of a test organism should be determined in advance; however, controls for factors including grain size and organic carbon can be evaluated if the limits are exceeded in a test sediment. See 12.1 for information on physico-chemical requirements of test organisms. If the physico-chemical characteristics of a test sediment exceed the tolerance range of the test organism, a control sediment encompassing these characteristics can be evaluated. The effects of sediment characteristics on the results of sediment tests can be addressed with regression equations (**57, 58**). The use of formulated sediment can also be used to evaluate physico-chemical characteristics of sediment on test organisms (**59, 60, 61,62**).

4.2.2 The experimental design depends on the purpose of the study. Variables that need to be considered include the number and type of control sediments, the number of treatments and replicates, and water quality characteristics. For instance, the purpose of the study might be to determine a specific endpoint such as an LC50 and may include a control sediment, a positive control, a solvent control, and several concentrations of sediment spiked with a chemical (see Section 10.3.2). A useful summary of field sampling design is presented by (**63**). See Section 15 for additional guidance on experimental design and statistics.

4.2.2.1 The purpose of the study might be to determine if field-collected sediments are toxic and may include controls, reference sediments, and test sediments. Controls are used to evaluate the acceptability of the test (see 13.3, 14.3, Annex A1 to Annex A7) and might include a control sediment, a formulated sediment (Section 7.2), a sand substrate (for *C. dilutus*; see 13.2, A7.2), or water-only exposures (for *H. azteca*; Section A6.3.7.8). Testing a reference sediment provides a site-specific basis for evaluating toxicity of the test sediments. Comparisons of test sediments to multiple reference or control sediments representative of the physical characteristics of the test sediment (i.e., grain size, organic carbon) may be useful in these evaluations. A summary of field sampling design is presented by Green (**63**). See Section 15 for additional guidance on experimental design and statistics.

4.2.2.2 If the purpose of the study is to conduct a reconnaissance field survey to identify sites for further investigation, the experimental design might include only one sample from each site to allow for sampling a larger area. The lack of replication at a site usually precludes statistical comparisons (for example, analysis of variance (ANOVA)), but these surveys can be used to identify sites for further study or may be evaluated using regression techniques.

4.2.2.3 In other instances, the purpose of the study might be to conduct a quantitative sediment survey of chemistry and toxicity to determine statistically significant differences between effects among control and test sediments from several sites. The number of replicates/site should be based on the need for sensitivity or power (see Section 15). In a quantitative survey, field replicates (separate samples from different grabs collected at the same site) would need to be taken at each site. Chemical and physical characterizations of each of these grabs would be required for each of these field replicates used in sediment testing. Separate subsamples might be used to determine within-sample variability or for comparisons of test procedures (for example, comparative sensitivity among test organisms), but these subsamples cannot be considered to be true field replicates for statistical comparisons among sites.

4.2.2.4 Sediments often exhibit high spatial and temporal variability (**64**). Therefore, replicate samples may need to be collected to determine variance in sediment characteristics. Sediment should be collected with as little disruption as possible; however, subsampling, compositing, or homogenization of sediment samples may be required for some experimental designs.

4.2.2.5 Site locations might be distributed along a known pollution gradient, in relation to the boundary of a disposal site, or at sites identified as being contaminated in a reconnaissance survey. Comparisons can be made in both space and time. In pre-dredging studies, a sampling design can be prepared to assess the contamination of samples representative of the project area to be dredged. Such a design should include subsampling cores taken to the project depth.

4.2.2.6 The primary focus of the physical and experimental test design and statistical analysis of the data, is the experimental unit, which is defined as the smallest physical entity to which treatments can be independently assigned (Guide E1241). Because overlying water or air cannot flow from one test chamber to another the test chamber is the experimental unit. The experimental unit is defined as the smallest physical entity to which treatments can be independently assigned and to which air and water exchange between test chambers are kept to a minimum. Because of factors that might affect results within test chambers and results of a test, all test chambers should be treated as similarly as possible. Treatments should be randomly assigned to individual test chamber locations. Assignment of test organisms to test chambers should be impartial (see Guide E729). As the number of test chambers/treatment increases, the number of degrees of freedom increases, and, therefore, the width of the confidence interval on a point estimate, such as an LC50, decreases, and the power of a significance test increases (see Section 15).

5. Significance and Use

5.1 *General:*

5.1.1 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 eventually accumulate in sediment. Mounting evidences exists of environmental degradation in areas where USEPA Water Quality Criteria (WQC; (**65**)) are not exceeded, yet organisms in or near sediments are adversely affected (**66**). The WQC

were developed to protect organisms in the water column and were not directed toward protecting organisms in sediment. Concentrations of contaminants in sediment may be several orders of magnitude higher than in the overlying water; however, bulk sediment concentrations have not been strongly correlated to bioavailability (**67**). Partitioning or sorption of a compound between water and sediment may depend on many factors including: aqueous solubility, pH, redox, affinity for sediment organic carbon and dissolved organic carbon, grain size of the sediment, sediment mineral constituents (oxides of iron, manganese, and aluminum), and the quantity of acid volatile sulfides in sediment (**40, 41**). Although certain chemicals are highly sorbed to sediment, these compounds may still be available to the biota. Chemicals in sediments may be directly toxic to aquatic life or can be a source of chemicals for bioaccumulation in the food chain.

5.1.2 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 (**68**). 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.

5.1.3 A variety of methods have been developed for assessing the toxicity of chemicals in sediments using amphipods, midges, polychaetes, oligochaetes, mayflies, or cladocerans (Section 13 and 14; Annex A1 to Annex A5; (**2**), (**4**), (**69**), (**70**). Several endpoints are suggested in these methods to measure potential effects of contaminants in sediment including survival, growth, behavior, or reproduction; however, survival of test organisms in 10-day exposures is the endpoint most commonly reported. These short-term exposures which only measure effects on survival can be used to identify high levels of contamination in sediments, but may not be able to identify moderate levels of contamination in sediments (USEPA (**2**); Sibley et al., (**54**); Sibley et al., (**55**); Sibley et al., (**71**); Benoit et al., (**72**); Ingersoll et al., (**56**)). Sublethal endpoints in sediment tests might also prove to be better estimates of responses of benthic communities to contaminants in the field (**18**). The previous version of this standard (Test Method E1706-95b) described 10-day toxicity tests with the amphipod *Hyalella azteca* and midge *Chironomus dilutus* (formerly known as *C. tentans*; Shobanov et al. 1999.(**1**), (see Section 13 and 14). This version of the standard now outlines approaches for evaluating sublethal endpoints in longer-term sediment exposures with these two species (Annex A6 and Annex A7).

5.1.3.1 The decision to conduct short-term or long-term toxicity tests depends on the goal of the assessment. In some instances, sufficient information may be gained by measuring sublethal endpoints in 10-day tests. In other instances, the 10-day tests could be used to screen samples for toxicity before long-term tests are conducted. While the long-term tests are needed to determine direct effects on reproduction, measurement of growth in these toxicity tests may serve as an indirect estimate of reproductive effects of contaminants associated with sediments (A6.4.5 and A7.4.6.2). Additional studies are ongoing to more thoroughly evaluate the relative sensitivity between lethal and sublethal endpoints measured in 10-day tests (Sections 13 and 14) and between sublethal endpoints measured in the long-term tests. Results of these studies and additional applications of the methods described in Annex A6 and Annex A7 will provide data that can be used to assist in determining where application of long-term tests will be most appropriate.

5.1.3.2 Use of sublethal endpoints for assessment of contaminant risk is not unique to toxicity testing with sediments. Numerous regulatory programs require the use of sublethal endpoints in the decision-making process (Pittinger and Adams (**73**)) including: (1) Water Quality Criteria (and State Standards); (2) National Pollution Discharge Elimination System (NPDES) effluent monitoring (including chemical-specific limits and sublethal endpoints in toxicity tests); (3) Federal Insecticide, Rodenticide and Fungicide Act (FIFRA) and the Toxic Substances Control Act (TSCA, tiered assessment includes several sublethal endpoints with fish and aquatic invertebrates); (4) Superfund (Comprehensive Environmental Responses, Compensation and Liability Act; CERCLA); (5) Organization of Economic Cooperation and Development (OECD, sublethal toxicity testing with fish and invertebrates); (6) European Economic Community (EC, sublethal toxicity testing with fish and invertebrates); and (7) the Paris Commission (behavioral endpoints).

5.1.4 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 an 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 (**74**), aging (**36, 75, 76**), and the chemical form of the material can affect responses of test organisms in spiked sediment tests.

5.1.5 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 (**39, 40**). 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 in sediment is often inversely correlated with the organic carbon concentration. 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 (**40**).

5.1.6 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.

5.1.7 Surveys of sediment toxicity are usually part of more comprehensive analyses of biological, chemical, geological, and hydrographic data. 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.

5.1.8 Table 3 lists several approaches the USEPA has considered for the assessment of sediment quality (**77**). These approaches include: *(1)* equilibrium partitioning, *(2)* tissue residues, *(3)* interstitial water toxicity, *(4)* whole-sediment toxicity and sediment-spiking tests, *(5)* benthic community structure, *(6)* effect ranges (for example, effect range median, ERM), and *(7)* sediment quality triad (see **78-81**) for a critique of these methods). The sediment assessment approaches listed in Table 3 can be classified as numeric (for example, equilibrium partitioning), descriptive (for example, whole-sediment toxicity tests), or a combination of numeric and descriptive approaches (for example, ERM, (**82**). Numeric methods can be used to derive chemical-specific sediment quality guidelines (SQGs). Descriptive methods such as toxicity tests with field-collected sediment cannot be used alone to develop numerical SQGs for individual chemicals. 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 (**83**, **84**, **85**, **86**). 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 (**67**, **87**, **88**).

5.2 *Regulatory Applications of Sediment Tests:*

5.2.1 The USEPA has authority under a variety of statutes to manage contaminated sediments (Table 4). USEPA's Contaminated Sediment Management Strategy (**89**, **90**) establishes the following four goals for contaminated sediments and describes actions that the Agency intends to take to accomplish these goals: (1) to prevent further contamination of sediments that may cause unacceptable ecological or human health risks; (2) when practical, to clean up existing sediment contamination that adversely affects the Nation's waterbodies or their uses, or that causes other significant effects on human health or the environment; (3) to ensure that sediment dredging and the disposal of dredged material continue to be managed in an environmentally sound manner; and (4) to develop and consistently apply methodologies for analyzing contaminated sediments. The Agency plans to employ its pollution prevention and source control programs to address the first goal. To accomplish the second goal, USEPA will consider a range of risk management alternatives to reduce the volume and effects of existing contaminated sediments, including *in-situ* containment and contaminated sediment removal. Finally, the Agency is developing tools for use in pollution prevention, source control, remediation, and dredged material management to meet the collective goals. These tools include national inventories of sediment quality and environmental releases of contaminants, numerical assessment guidelines to evaluate contaminant concentrations, and standardized bioassays to evaluate the bioaccumulation and toxicity potential of sediment samples.

TABLE 3 Sediment Quality Assessment Procedures (Modified from USEPA (82))

Method	Type			Approach
	Numeric	Descriptive	Combination	
Equilibrium Partitioning		*		A sediment quality value 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.
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 is quantified and identification evaluation procedures are applied to identify and quantify chemical components responsible for sediment toxicity.
Benthic Community Structure		*		Environmental degradation is measured by evaluating alterations in benthic community structure.
Whole-sediment Toxicity and Sediment Spiking	*	*	*	Test organisms are exposed to sediments that may contain known or unknown quantities of potentially toxic chemicals. At the end of a specified time period, the response of the test organisms is examined in relation to a specified endpoint. Dose-response relationships can be established by exposing test organisms to sediments that have been spiked with known amounts of chemicals or mixtures of chemicals.
Sediment Quality Triad	*	*	*	Sediment chemical contamination, sediment toxicity, and benthic community structure are measured on the same sediment sample. Correspondence between sediment chemistry, toxicity, and field effects is used to determine sediment concentrations that discriminate conditions of minimal, uncertain, and major biological effects.
Sediment Quality Guidelines	*	*	*	The sediment concentration of contaminants associated with toxic responses measured in laboratory exposures or field assessments (i.e., Apparent Effects Threshold (AET), Effect Range Median (ERM), Probable Effect Level (PEL).

TABLE 4 Statutory Needs for Sediment Quality Assessment (Modified from Dickson et al (91) and Southerland et al (89))

Law[A]	Area of need
CERCLA	—Assess need for remedial action with contaminated sediments; assess degree of cleanup required; disposition of sediment
CWA	—NPDES permitting, especially under Best Available Technology (BAT) in water-quality-limited water —Section 403(c) criteria for ocean discharges; mandatory additional requirements to protect marine environment —Section 301(g) waivers for publically owned treatment works (PTOWS) discharging to marine waters —Section 404 permits to dredge and fill activities (administered by the Corps of Engineers)
FIFRA	—Review uses of new and existing chemicals —Pesticide labeling and registration
MPRSA	—Permits for ocean dumping
NEPA	—Preparation of environmental impact statements for projects with surface water discharges
TSCA	—Section 5: Pre-manufacture notice reviews for new chemicals —Section 4,5,6: Reviews for existing industrial chemicals
RCRA	—Assess suitability (and permit) on-land disposal or beneficial use of contaminated sediments considered "hazardous"

[A] CERCLA Comprehensive Environmental Response, Compensation and Liability Act ("Superfund")
CWA Clean Water Act
FIFRA Federal Insecticide, Fungicide, and Rodenticide Act
MPRSA Marine Protection, Resources and Sanctuary Act
NEPA National Environmental Policy Act
TSCA Toxic Substances Control Act
RCRA Resource Conservation and Recovery Act

5.2.2 The Clean Water Act (CWA) is the single most important law dealing with environmental quality of surface waters in the United States. The objective of the CWA is to restore and maintain the chemical, physical, and biological integrity of the nation's waters (CWA, Section 101). Federal and state monitoring programs traditionally have focused on evaluating water column problems caused by point source dischargers. Findings in the National Sediment Quality Survey, volume I of the first biennial report to Congress on sediment quality in the U.S., indicate that this focus needs to be expanded to include sediment quality impacts (Section 1.1.2 and (92).

5.2.3 The Office of Water (OW), the Office of Prevention, Pesticides, and Toxic Substances (OPPTS), the Office of Solid Waste (OSW), and the Office of Emergency and Remedial Response (OERR) are all committed to the principle of consistent tiered testing described in the Contaminated Sediment Management Strategy (USEPA, (90)). Agency-wide consistent testing is desirable because all USEPA programs will use standard methods to evaluate health risk and produce comparable data. It will also provide the basis for uniform cross-program decision-making within the USEPA. Each program will, however, retain the flexibility of deciding whether identified risks would trigger regulatory actions.

5.2.4 Tiered testing refers to a structured, hierarchial procedure for determining data needs relative to decision-making that consists of a series of tiers, or levels, of investigative intensity. Typically, increasing tiers in a tiered testing framework involve increased information and decreased uncertainty (USEPA, (90)). Each EPA program office intends to develop guidance for interpreting the tests conducted within the tiered framework and to explain how information within each tier would trigger regulatory action. Depending on statutory and regulatory requirements, the program specific guidance will describe decisions based on a weight of evidence approach, a pass-fail approach, or comparison to a reference site. The following two approaches are currently being used by USEPA: (1) the Office of Water-U.S. Army Corps of Engineers dredged material testing framework and (2) the OPPTS ecological risk assessment tiered testing framework. USEPA-USACE (93) describes the dredged material testing framework and Smrchek and Zeeman (94) summarizes the OPPTS testing framework. A tiered testing framework has not yet been chosen for agency-wide use, but some of the components have been identified to be standardized. These components are toxicity tests, bioaccumulation tests, chemical criteria, and other measurements that may have ecological significance including benthic community structure, colonization rate, and *in situ* testing within a mesocosm (77).

5.3 *Performance-based criteria:*

5.3.1 The USEPA's Environmental Monitoring Management Council (EMMC) recommended the use of performance-based methods in developing standards (95). Performance-based methods were defined by EMMC as a monitoring approach which permits the use of appropriate methods that meet preestablished demonstrated performance standards (see 11.2).

5.3.2 The USEPA Office of Water, Office of Science and Technology, and Office of Research and Development held a workshop to provide an opportunity for experts in the field of sediment toxicology and staff from USEPA's Regional and Headquarters Program offices to discuss the development of standard freshwater and marine sediment testing procedures (77, 96). Workgroup participants arrived at a consensus on several culturing and testing methods. In developing guidance for culturing test organisms to be included in the USEPA's methods manual for sediment tests, it was agreed that no one method should be required to culture organisms. However, the consensus at the workshop was that success of a test depends on the health of the cultures. Therefore, having healthy test organisms of known quality and age for testing was determined to be the key consideration relative to culturing methods. A performance-based criteria approach was selected in USEPA (2) as the preferred method through which individual laboratories could use unique culturing methods rather than requiring use of one culturing method.

5.3.3 This standard recommends the use of performance-based criteria to allow each laboratory to optimize culture methods and minimize effects of test organism health on the reliability and comparability of test results. See 13.1.2 and 14.1.2 and Annex A1 to Annex A7 for a listing of performance criteria for culturing and testing.

6. Interferences

6.1 *General Interferences:*

6.1.1 An interference is a characteristic of a sediment or a test system that can potentially affect test organism response aside from those related to sediment-associated contaminants. These interferences can potentially confound interpretation of test results in two ways: *(1)* toxicity is observed in the test sediment when contamination is low or there is more toxicity

than expected, and *(2)* no toxicity is observed when contaminants are present at elevated concentrations or there is less toxicity than expected.

6.1.2 Because of the heterogeneity of natural sediments, extrapolation from laboratory studies to the field can sometimes be difficult (Table 5; (**67**)). Sediment collection, handling, and storage may alter bioavailability and concentration by changing the physical, chemical, or biological characteristics of the sediment. Maintaining the integrity of a field-collected sediment during removal, transport, mixing, storage, and testing is extremely difficult and may complicate the interpretation of effects. See (**62**) and E1391.

6.1.3 Depletion of aqueous and sediment-sorbed chemicals resulting from uptake by an organism or test chamber may also influence availability. In most cases, the organism is a minor sink for chemicals relative to the sediment. However, within the burrow of an organism, sediment desorption kinetics may limit uptake rates. Within minutes to hours, a major portion of the total chemical may be inaccessible to the organisms because of depletion of available residues. The desorption of a particular compound from sediment may range from easily reversible (labile; within minutes) to irreversible (non-labile; within days or months (**98**)). Interparticle diffusion or advection and the quality and quantity of sediment organic carbon can also affect sorption kinetics.

6.1.4 Testing sediments at temperatures different from the field might affect contaminant solubility, partitioning coefficients, or other physical and chemical characteristics. Interaction between sediment and overlying water and the ratio of sediment to overlying water may influence bioavailability (**74**).

TABLE 5 Advantages and Disadvantages for Use of Sediment Tests (Modified from Swartz (97))

Advantages
—Measure bioavailable fraction of contaminant(s).
—Provide a direct measure of benthic effects, assuming no field adaptation or amelioration of effects.
—Limited special equipment is required.
—Methods are rapid and inexpensive.
—Legal and scientific precedence exist for use; ASTM standard guides are available.
—Measure unique information relative to chemical analyses or benthic community analyses.
—Tests with spiked chemicals provide data on cause-effect relationships.
—Sediment-toxicity tests can be applied to all chemicals of concern.
—Tests applied to field samples reflect cumulative effects of contaminants and contaminant interactions.
—Toxicity tests are amenable to confirmation with natural benthos populations.

Disadvantages
—Sediment collection, handling, and storage may alter bioavailability.
—Spiked sediment may not be representative of field contaminated sediment.
—Natural geochemical characteristics of sediment may affect the response of test organisms.
—Indigenous animals may be present in field-collected sediments.
—Route of exposure may be uncertain and data generated in sediment toxicity tests may be difficult to interpret if factors controlling the bioavailability of contaminants in sediment are unknown.
—Tests applied to field samples may not discriminate effects of individual chemicals.
—Few comparisons have been made of methods or species.
—Only a few chronic methods for measuring sublethal effects have been developed or extensively evaluated.
—Laboratory tests have inherent limitations in predicting ecological effects.
—Tests do not directly address human health effects.

6.1.5 Results of sediment tests can be used to predict effects that may occur with aquatic organisms in the field as a result of exposure under comparable conditions. However, motile organisms might avoid exposure in the field. Photoinduced toxicity may be important for some compounds associated with sediment (for example, polycyclic aromatic hydrocarbons (PAHs) (**99**)). However, lighting typically used to conduct laboratory tests does not include the appropriate spectrum of ultraviolet radiation to photoactivate compounds (**100**, **101**) and thus laboratory tests may not account for toxicity expressed by this mode of action.

6.1.6 Natural physico-chemical characteristics such as sediment texture may influence the response of test organisms (**57**). The physico-chemical characteristics of test sediment need to be within the tolerance limits of the test organism. Ideally, the limits of the test organism should be determined in advance; however, control samples reflecting differences in factors such as grain size and organic carbon can be evaluated if the limits are exceeded in the test sediment (see 12.1). The effects of sediment characteristics can also be addressed with regression equations (**57**, **58**). The use of formulated sediment can also be used to evaluate physico-chemical characteristics of sediment on test organisms (**59**, **60**).

6.1.7 Indigenous organisms may be present in field-collected sediments. An abundance of the same organism or organisms taxonomically similar to the test organism in the sediment sample may make interpretation of treatment effects difficult. For example, growth of amphipods, midges, or mayflies may be reduced if high numbers of oligochaetes are in a sediment sample (**102**). Previous investigators have inhibited the biological activity of sediment with sieving, heat, mercuric chloride, antibiotics, or gamma irradiation (Guide E1391, (**103**)). However, further research is needed to determine effects on contaminant bioavailability or other modifications of sediments from treatments such as those used to remove or destroy indigenous organisms.

6.1.8 The route of exposure may be uncertain and data from sediment tests may be difficult to interpret if factors controlling the bioavailability of chemicals in sediment are unknown. Bulk-sediment chemical concentrations may be normalized to factors other than dry weight. For example, concentrations of nonionic organic compounds might be normalized to sediment organic-carbon content (**82**) and certain metals normalized to acid volatile sulfides (**39**). Even with the appropriate normalizing factors, determination of toxic effects from ingestion of sediment or from dissolved chemicals in the interstitial water can still be difficult (**104**).

6.1.9 The addition of food, water, or solvents to the test chambers might obscure the bioavailability of chemicals in sediment or might provide a substrate for bacterial or fungal growth. Without addition of food, the test organisms may starve during exposures (**58**, **105**). However, the addition of the food may alter the availability of the chemicals in the sediment (**35**, **106**) depending on the amount of food added, its composition (for example, total organic carbon (TOC)), and the chemical(s) of interest.

6.1.10 Laboratory sediment testing with field-collected sediments may be useful in estimating cumulative effects and

interactions of multiple contaminants in a sample. Tests with field samples usually cannot discriminate between effects of individual chemicals. Many sediment samples contain a complex matrix of inorganic and organic chemicals with many unidentified compounds. The use of Toxicity Identification Evaluations (TIE) procedures including sediment tests with spiked chemicals may provide evidence of causal relationships and can be applied to many chemicals of concern (**10**). Laboratory studies that test single compounds spiked into the sediment can be used to determine more directly the specific chemicals causing a toxic response (**107**).

6.1.11 Sediment spiking can also be used to investigate additive, antagonistic, or synergistic effects of specific chemical mixtures in a sediment sample (**107**). However, spiked sediment may not be representative of contaminated sediment in the field. Mixing time (**64**) and aging (**36, 75, 76**) of spiked sediment can affect responses of organisms.

6.1.12 Most assessments of contaminated sediment rely on acute-lethality testing methods (for example, $\leq$10 days; (**93, 108, 109**). Acute-lethality tests are useful in identifying "hot spots" of sediment contamination, but may not be sensitive enough to evaluate moderately contaminated areas. Sediment quality assessments using sublethal responses of benthic organisms such as effects on growth and reproduction have been used to successfully evaluate moderately contaminated areas (**110, 18, 56**), Annex A6 and Annex A7.

6.1.13 Despite the interferences previously listed, existing sediment testing methods that include measurement of sublethal endpoints may be used to provide a rapid and direct measure of effects of contaminants on benthic communities (e.g., Canfield et al. (**46**)). Laboratory tests with field-collected sediment can also be used to determine temporal, horizontal, or vertical distribution of contaminants in sediment. Most tests can be completed within two to four weeks. Legal and scientific precedence exist for use of sediment tests in regulatory decision making (for example, (**111, 97**)). Furthermore, sediment tests with complex contaminant mixtures are important tools for making decisions about the extent of remedial action for contaminated aquatic sites and for evaluating the success of remediation activities.

6.2 *Species-Specific Interferences*—Interferences of tests for each species are described in Sections 13 and 14 and in Annex A1 to Annex A7.

7. Reagents and Materials

7.1 *Water:*

7.1.1 *Requirements:*

7.1.1.1 Water used to test and culture organisms should be uniform in quality. Acceptable water should allow satisfactory survival, growth, or reproduction of the test organisms. Test organisms should not show signs of disease or apparent stress (for example, discoloration, unusual behavior). If problems are observed in the culturing or testing of organisms, it is desirable to evaluate the characteristics of the water. See USEPA (**3**) and Test Method E1367 for a recommended list of chemical analyses of the water supply.

7.1.1.2 When deionized water is required, the water-deionizing system should provide sufficient quantity of at least 1 MΩ of water. If large quantities of high-quality deionized water are needed, it may be advisable to supply the laboratory-grade water deionizer with preconditioned water from a mixed-bed water treatment system. Some investigators have observed that holding reconstituted water prepared from deionized water for several days before use in sediment tests may be improve performance of test organisms (C. Hickey, National Institute of Water and Atmospheric Research, Hamilton, New Zealand, personal communication).

7.1.2 *Source:*

7.1.2.1 A natural water is considered to be of uniform quality if monthly ranges of the hardness, alkalinity, and specific conductance are <10 % of their respective averages and if the monthly range of pH is <0.4. Natural waters should be obtained from an uncontaminated well or spring, if possible, or from a surface-water source. If surface water is used, the intake should be positioned to: *(1)* minimize fluctuations in quality and contamination, *(2)* maximize the concentration of dissolved oxygen, and *(3)* ensure low concentrations of sulfide and iron. Municipal-water supplies may be 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. Dechlorinated water should only be used as a last resort since dechlorination is often incomplete (Guide E1241, (**3**)).

7.1.2.2 For site-specific investigations, it is desirable to have the water-quality characteristics of the overlying water as similar as possible to the site water. For certain applications the experimental design might require use of water from the site from which sediment is collected. When distilled water was added to sediment, contaminant and organic carbon distributed on smaller sediment particles (perhaps resulting from disaggregation of particles). Therefore, it may be advisable to conduct sediment tests with water representative of the site of concern (**2**).

7.1.2.3 Water that might be contaminated with facultative pathogens may be passed through a properly maintained ultraviolet sterilizer equipped with an intensity meter and flow controls or passed through a filter with a pore size of $\leq$0.45 µm.

7.1.2.4 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. Excessive aeration may reduce hardness and alkalinity of hard water. The concentration of dissolved oxygen in source water should be between 90 to 100 % saturation to help ensure that dissolved oxygen concentrations are acceptable in test chambers. It may be desirable to aerate dechlorinated water before use (for example, 3 days).

7.1.3 *Reconstituted Water:*

7.1.3.1 Ideally, reconstituted water is prepared by adding specified amounts of reagent-grade[5] chemicals to high-purity distilled or deionized water (Guide E729, **(3)**). Problems have been observed with the use of reconstituted water in long-term exposures with *H. azteca* (Section 7.1.3.4.3). In some applications, acceptable high-purity water can be prepared using deionization, distillation, or reverse-osmosis units (see 9.3, **(3)**). Test water can also be prepared by diluting natural water with deionized water **(18)** or by adding salts to relatively dilute natural waters.

7.1.3.2 Deionized water should be obtained from a system capable of producing at least 1 MΩ water.

7.1.3.3 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 adjust pH and dissolved oxygen to the acceptable ranges (for example, see 7.1.3.4). USEPA **(3)** recommends using a batch of reconstituted water for less than two weeks.

7.1.3.4 *Reconstituted Fresh Water*—To prepare 100 L of reconstituted fresh water described in Smith et al. **(112)**, use the reagent grade chemicals as follows:

(1) Place about 75 L of deionized water in a properly cleaned container.

(2) Add 5 g of $CaSO_4$ and 5 g of $CaCl_2$ to a 2-L aliquot of deionized water and mix (for example, on a stir plate) for 30 min.

(3) Add 3 g of $MgSO_4$, 9.6 g $NaHCO_3$, and 0.4 g KCl to a second 2-L aliquot of deionized water and mix on a stir plate for 30 min or until the salts dissolve.

(4) Pour the two 2-L aliquots containing the dissolved salts into the 75 L of deionized water and fill the carboy to 100 L with deionized water.

(5) Aerate the mixture for at least 24 h before use.

(6) The water quality of the reconstituted water (reformulated moderately hard reconstituted water described by Smith et al. **(112)** and in USEPA **(2)**) should be about: hardness, 90 to 100 mg/L as $CaCO_3$, alkalinity 50 to 70 mg/L as $CaCO_3$, conductivity 330 to 360 µS/cm, and pH 7.8 to 8.2.

(7) McNulty et al. **(105)**and Kemble et al. **(113)**, **(61)**observed poor survival of *H. azteca* in tests conducted 14 to 28 days using a variety of reconstituted waters including the reconstituted water described by Smith et al. **(112)** in 7.1.3.4. Borgmann **(114)**described a reconstituted water that was used successfully to maintain *H. azteca* in culture; however, some laboratories have not had success with reproduction of the *H. azteca* when using this reconstituted water in the 42-day test (T.J. Norberg-King, USEPA, Duluth, MN, personal communication). Research is ongoing to develop additional types of reconstituted waters suitable for *H. azteca*. Until an acceptable reconstituted water has been developed for long-term exposures with *H. azteca*, a natural water demonstrated to support adequate survival, growth, and reproduction of amphipods is recommended for use in long-term *H. azteca* exposures (Annex A6.2; **(56, 113, 61)**).

7.1.3.5 *Synthetic Seawater*—Reconstituted salt water can be prepared by adding commercial sea salts to deionized water. A synthetic seawater formulation can be prepared with reagent grade chemicals which can be diluted with deionized water to the desired salinity **(115)**. Ingersoll et al **(116)**describes procedures for culturing *H. azteca* at salinities up to 15 ppt. Reconstituted salt water was prepared by adding commercial 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 two weeks before use. Synthetic seawater was conditioned by adding 6.2 mL of nitrifying bacteria No. 9[6] (*Nitromonas sp.* and *Nitrobacter sp.*) to each liter of water. The cultures were maintained by using renewal of water (25 % of the culture water was replaced weekly). *Hyalella azteca* have been used to evaluate the toxicity of estuarine sediments up to 15 ppt salinity in 10-day exposures **(48, 85, 117-119)**.

7.2 *Formulated Sediment:*

7.2.1 *General Requirements:*

7.2.1.1 Formulated sediments are mixtures of materials which mimic the physical components of natural sediments. Formulated sediments have not been routinely applied to evaluate sediment contamination. 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 performance-based criteria necessary for test acceptability. The use of formulated sediment eliminates interferences caused by the presence of indigenous organisms. Spiking formulated sediments with specific chemicals would reduce variation in sediment physico-chemical characteristics and would provide a consistent method for evaluating the fate of chemicals in sediment. See **(62)** and Guide E1391 for additional detail regarding uses of formulated sediment.

7.2.1.2 Ideally, a formulated sediment should: (*1*) support the survival, growth, or reproduction of a variety of benthic invertebrates, (*2*) provide consistent acceptable biological endpoints for a variety of species, and (*3*) be composed of materials that have consistent characteristics. Consistent material characteristics include: (*1*) consistency of materials from batch to batch, (*2*) contaminant concentrations below concentrations of concern, and (*3*) availability to all individuals and facilities **(61)**.

7.2.1.3 Physico-chemical characteristics which might be considered when evaluating the appropriateness of a formulated sediment include: percent sand, percent clay, percent silt, organic carbon content, cation exchange capacity (CEC), oxidation reduction potential (redox), pH, and carbon: nitrogen:phosphorus ratios.

7.2.2 *Sources of Materials:*

[5] *Reagent Chemicals, American Chemical Society Specifications,* American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals,* BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary,* U.S. Pharmaceutical Convention, Inc. (USPC), Rockville, MD.

[6] Nitrifying bacteria (*Nitromonas sp.* and *Nitrobacter sp.*) such as Frit-zyme® No. 9, available from Fritz Chemical Company, Dallas, TX.