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.

Designation: E1611 – 21

Standard Guide for Conducting Sediment Toxicity Tests with Polychaetous Annelids[1]

This standard is issued under the fixed designation E1611; 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 guide covers procedures for obtaining laboratory data concerning the adverse effects of potentially contaminated sediment, or of a test material added experimentally to contaminated or uncontaminated sediment, on marine or estuarine infaunal polychaetes during 10-day or 20 to 28-day exposures. These procedures are useful for testing the effects of various geochemical characteristics of sediments on marine and estuarine polychaetes and could be used to assess sediment toxicity to other infaunal taxa, although modifications of the procedures appropriate to the test species might be necessary. Procedures for the 10-day static test are described for *Neanthes arenaceodentata* and *Alitta virens* [2] (formerly *Nereis virens* and *Neanthes virens*) and for the 20 to 28-day static-renewal sediment toxicity for *N. arenaceodentata*.

1.2 Modifications of these procedures might be appropriate for other sediment toxicity test procedures, such as flow-through or partial life-cycle tests. The methods outlined in this guide should also be useful for conducting sediment toxicity tests with other aquatic taxa, although modifications might be necessary. Other test organisms might include other species of polychaetes, crustaceans, and bivalves.

1.3 Other modifications of these procedures might be appropriate for special needs or circumstances. Although using appropriate procedures is more important than following prescribed procedures, the results of tests conducted using unusual procedures are not likely to be comparable to those of many other tests. Comparisons of the results obtained using modified and unmodified versions of these procedures might provide useful information concerning new concepts and procedures for conducting sediment tests with infaunal organisms.

1.4 These procedures are applicable to sediments contaminated with most chemicals, either individually or in formulations, commercial products, and known or unknown mixtures. These procedures can be used with appropriate modifications to conduct sediment toxicity tests on factors such as temperature, salinity, dissolved oxygen (DO), and natural sediment characteristics (for example, particle size distribution, organic carbon content, and total solids). These procedures can also be used to conduct bioconcentration tests and in situ tests, and to assess the toxicity of potentially contaminated field sediments, or of materials such as sewage sludge, oils, particulate matter, and solutions of toxicants added to sediments. A median lethal concentration (LC50) or median sublethal effect concentration (EC50) of toxicants or of highly contaminated sediment mixed into uncontaminated sediment can be determined. Materials adhering to sediment particles or dissolved in interstitial water can be tested.

1.5 The results of 10-day toxicity tests with contaminated sediments can be reported as a LC50 if a series of concentrations is tested or as a percent mortality relative to a control or reference sediment. The results of 20 to 28-day toxicity tests with contaminated sediments can be reported as a LC50 if a series of concentrations is tested or as a percent mortality or growth relative to a control or reference sediment.

1.6 This guide is arranged as follows:

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

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[2] World Register of Marine Species (WoRMS) at https://www.marinespecies.org/aphia.php?p=taxdetails&id=234851

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

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1.7 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.8 *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 hazards statements are given in Section 8.

1.9 *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

D3976 Practice for Preparation of Sediment Samples for Chemical Analysis

D4447 Guide for Disposal of Laboratory Chemicals and Samples

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

E943 Terminology Relating to Biological Effects and Environmental Fate

E1023 Guide for Assessing the Hazard of a Material to Aquatic Organisms and Their Uses

E1192 Guide for Conducting Acute Toxicity Tests on Aqueous Ambient Samples and Effluents with Fishes, Macroinvertebrates, and Amphibians

E1241 Guide for Conducting Early Life-Stage Toxicity Tests with Fishes (Withdrawn 2022)[4]

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

E1525 Guide for Designing Biological Tests with Sediments

E1706 Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates

E1733 Guide for Use of Lighting in Laboratory Testing

E1847 Practice for Statistical Analysis of Toxicity Tests Conducted Under ASTM Guidelines (Withdrawn 2022)[4]

SI10-02 IEEE/ASTM SI 10 American National Standard for Use of the International System of Units (SI): The Modern Metric System

3. Terminology

3.1 *Definitions:*

3.1.1 The words “must,” “should,” “may,” “can,” and “might” have very specific meanings in this guide. “Must” is used to express the strongest possible recommendation, just short of an absolute requirement, that is, to state that this test ought to be designed to satisfy the specific condition, unless the purpose of the test requires a different design. “Must” is used only in connection with factors that relate directly to the acceptability of the test (see Section 14). “Should” is used to state that the specific condition is recommended and ought to be met if possible. Although the violation of one “should” is rarely a serious matter, the violation of several will often render the results questionable. Terms such as “is desirable,” “is often desirable,” and “might be desirable” are used in connection with less important factors. “May” is used to mean “is (are) allowed to,” “can” is used to mean “is (are) able to,” and “might” is used to mean “could possibly.” Thus the classic distinction between “may” and “can” is preserved, and “might” is never used as a synonym for either “may” or “can.”

3.1.2 For definitions of other terms used in this guide, refer to Terminologies D1129 and E943, Guides E729, E1023, E1192, E1367, and E1525. For an explanation of units and symbols, refer to SI10-02 IEEE/ASTM SI 10.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *ash-free dry weight*—Organism weight determined by subtracting the standard dry weight from the ashed (550 °C dried) weight to remove the inorganic contribution.

3.2.1.1 *Discussion*—AFDW is therefore the weight of the organic content of the organism.

3.2.2 *clean sediment, n*—sediment that does not contain concentrations of toxicants that cause apparent stress to the test organism or reduce their survival.

[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.

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

3.2.4 *estimated individual dry weight, n*—a value that is calculated by dividing the total dry weight by the number of surviving worms within a replicate.

3.2.5 *exposure, n*—contact with a chemical or physical agent (see Terminology E943).

3.2.6 *interstitial water, n*—water occupying the space between sediment or soil particles; a synonym for *pore water*.

3.2.7 *overlying water, n*—the water added to the test chamber over the solid phase of the sediment in a toxicity test.

3.2.8 *pore water, n*—water occupying the space between sediment particles; a synonym for *interstitial water*.

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

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

3.2.11 *short-term toxicity tests, n*—generally used to determine the concentration of test material that produces a specific adverse effect on a specific percentage of test organisms during a short exposure (for example, 10 days).

3.2.11.1 *Discussion*—Because death is obviously an important adverse effect and is detected easily for many species, the most common end point is survival. Both survival and growth are used as end points in the 20 to 28-day test. Effect on 50 % of a group of test organisms is the most experimentally reproducible and easily determined measure of toxicity, and 96 h is often a convenient, useful exposure duration. The measure used most often in acute tests is therefore the 96-h LC50 value. In tests with contaminated sediment, however, the exposure period is generally 10 days or 20 to 28 days. Death is used as the measure of toxicity in the 10-day test; the results are reported as a 10-day LC50 or response relative to a control or reference sediment. Dry body weight is used as the measure of effect in the 20 to 28-day test or the 20 to 28-day LC50 if dilutions are tested. Ash-free dry weight may also be measured to differentiate the influence of the gut contents from final tissue mass.

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

3.2.13 *toxicity, n*—the property of a material or combination of materials that affects organisms adversely (see Terminology E943).

3.2.14 *whole sediment, n*—sediment that has not had material extracted or removed.

4. Summary of Guide

4.1 Two procedures are used to measure the relative toxicity of marine or estuarine sediments to polychaetes: (*1*) the 10-day test, which measures the effect of contaminated sediment on survival; and (*2*) the 20-day to 28-day test, which determines the effect of contaminated sediment on survival and growth. If smaller worms are used, such as *N. arenaceodentata*, a minimum of five worms are placed in a 1-L glass test chamber with a minimum sediment depth of 2 cm to 3 cm and the overlying water aerated. The survival of the worm exposed to the test sediment is compared with the survival in a negative control or reference sediment in the 10-day test. The same procedure is used in the 20-day to 28-day test, except for the test duration (see Annex A1). If larger worms are used, such as *A. virens*, a minimum of ten worms are placed in beakers or glass aquaria (4 L to 37 L) with a minimum sediment depth of 5 cm and the overlying water aerated. A negative control or reference sediment is used to give a measure of the acceptability of the test by (*1*) providing evidence of the health and relative quality of the test organisms, suitability of the overlying water, test conditions, and handling procedures, etc.; and (*2*) providing a basis for interpreting data obtained from the test sediments.

4.1.1 The percent survival of polychaetes exposed to field-collected sediment is compared to those exposed to a negative control or reference sediment in 10-day tests. The survival and body weight of the animals surviving in field-collected sediment is compared to those exposed to negative control or reference sediment in 20 to 28-day tests. The toxicity of field sediments may also be assessed by testing dilutions of highly toxic test sediments with clean sediments to obtain information on the toxicity of proportions of that sediment.

4.1.2 The toxicity of a material added experimentally to sediments can be expressed by analyzing the survival and growth data to determine a LC50 for the material for the duration of exposure.

4.2 The annexes at the end of this guide outline the techniques for collecting, identifying, holding, and testing *N. arenaceodentata* and *A. virens* and culturing *N. arenaceodentata*.

5. Significance and Use

5.1 The test procedure covered in this guide is not intended to simulate exactly the exposure of benthic polychaetes to chemicals under natural conditions, but rather to provide a conveniently rapid, standard toxicity test procedure yielding a reasonably sensitive indication of the toxicity of materials in marine and estuarine sediments.

5.2 The protection of a community of organisms requires averting detrimental contaminant-related effects on the number and health of individuals and species within that population. Sediment toxicity tests provide information on the toxicity of test materials in sediments. Theoretically, projection of the most sensitive species within a community will protect the community as a whole.

5.3 Polychaetes are an important component of the benthic community. They are preyed upon by many species of fish, birds, and larger invertebrate species, and they are predators of smaller invertebrates, larval stages of invertebrates, and, in