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

This standard is issued under the fixed designation E1367; 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 estuarine or marine 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. General guidance is presented in Sections 1 – 15 for conducting sediment toxicity tests with estuarine or marine amphipods. Specific guidance for conducting 10-d sediment toxicity tests with estuarine or marine amphipods is outlined in Annex A1 and specific guidance for conducting 28-d sediment toxicity tests with *Leptocheirus plumulosus* is outlined in Annex A2.

1.2 Procedures are described for testing estuarine or marine amphipod crustaceans in 10-d laboratory exposures to evaluate the toxicity of contaminants associated with whole sediments (Annex A1; USEPA 1994a (**1**)). Sediments may be collected from the field or spiked with compounds in the laboratory. A toxicity method is outlined for four species of estuarine or marine sediment-burrowing amphipods found within United States coastal waters. The species are *Ampelisca abdita*, a marine species that inhabits marine and mesohaline portions of the Atlantic coast, the Gulf of Mexico, and San Francisco Bay; *Eohaustorius estuarius*, a Pacific coast estuarine species; *Leptocheirus plumulosus*, an Atlantic coast estuarine species; and *Rhepoxynius abronius*, a Pacific coast marine species. Generally, the method described may be applied to all four species, although acclimation procedures and some test conditions (that is, temperature and salinity) will be species-specific (Sections 12 and Annex A1). The toxicity test is conducted in 1-L glass chambers containing 175 mL of sediment and 775 mL of overlying seawater. Exposure is static (that is, water is not renewed), and the animals are not fed over the 10-d exposure period. The endpoint in the toxicity test is survival with reburial of surviving amphipods as an additional measurement that can be used as an endpoint for some of the test species (for *R. abronius* and *E. estuarius*). Performance criteria established for this test include the average survival of amphipods in negative control treatment must be greater than or equal to 90 %. Procedures are described for use with sediments with pore-water salinity ranging from >0 ‰ to fully marine.

1.3 A procedure is also described for determining the chronic toxicity of contaminants associated with whole sediments with the amphipod *Leptocheirus plumulosus* in laboratory exposures (Annex A2; USEPA-USACE 2001(**2**)). The toxicity test is conducted for 28 d in 1-L glass chambers containing 175 mL of sediment and about 775 mL of overlying water. Test temperature is 25° ± 2°C, and the recommended overlying water salinity is 5 ‰ ± 2 ‰ (for test sediment with pore water at 1 ‰ to 10 ‰) or 20 ‰ ± 2 ‰ (for test sediment with pore water >10 ‰). Four hundred millilitres of overlying water is renewed three times per week, at which times test organisms are fed. The endpoints in the toxicity test are survival, growth, and reproduction of amphipods. Performance criteria established for this test include the average survival of amphipods in negative control treatment must be greater than or equal to 80 % and there must be measurable growth and reproduction in all replicates of the negative control treatment. This test is applicable for use with sediments from oligohaline to fully marine environments, with a silt content greater than 5 % and a clay content less than 85 %.

1.4 A salinity of 5 or 20 ‰ is recommended for routine application of 28-d test with *L. plumulosus* (Annex A2; USEPA-USACE 2001 (**2**)) and a salinity of 20 ‰ is recommended for routine application of the 10-d test with *E. estuarius* or *L. plumulosus* (Annex A1). However, the salinity of the overlying water for tests with these two species can be adjusted to a specific salinity of interest (for example, salinity representative of site of interest or the objective of the study may be to evaluate the influence of salinity on the bioavailability of chemicals in sediment). More importantly, the salinity tested must be within the tolerance range of the test organisms (as outlined in Annex A1 and Annex A2). If tests are conducted with procedures different from those described in 1.3 or in Table A1.1 (for example, different salinity, lighting, temperature, feeding conditions), additional tests are required to determine comparability of results (1.10). If there is not a

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

TABLE 1 Rating of Selection Criteria for Estuarine or Marine Amphipod Sediment Toxicity Testing A "+" or "–" Rating Indicates a Positive or Negative Attribute

Criterion	*Ampelisca abdita*	*Eohaustorius estuarius*	*Leptocheirus plumulosus*	*Rhepoxynius abronius*
Relative sensitivity toxicity data base	+	+	+	+
Round-robin studies conducted	+	+	+	+
Contact with sediment	+	+	+	+
Laboratory culture	+/-	-	+	-
Taxonomic identification	+	+	+	+
Ecological importance	+	+	+	+
Geographical distribution	ATL, PAC, GOM	PAC	ATL	PAC
Sediment physicochemical tolerance	+	+	+	+
Response confirmed with benthos populations	+	+[A]	+	+
Peer reviewed	+	+	+	+
Endpoints monitored	Survival	Survival, reburial	Survival	Survival, reburial

[A] Anderson et al. (2001 (14)).

ATL = Atlantic Coast, PAC = Pacific Coast, GOM= Gulf of Mexico

need to make comparisons among studies, then the test could be conducted just at a selected salinity for the sediment of interest.

1.5 Future revisions of this standard may include additional annexes describing whole-sediment toxicity tests with other groups of estuarine or marine invertebrates (for example, information presented in Guide E1611 on sediment testing with polychaetes could be added as an annex to future revisions to this standard). Future editions to this standard may also include methods for conducting the toxicity tests in smaller chambers with less sediment (Ho et al. 2000 (3), Ferretti et al. 2002 (4)).

1.6 Procedures outlined in this standard are based primarily on procedures described in the USEPA (1994a (1)), USEPA-USACE (2001(2)), Test Method E1706, and Guides E1391, E1525, E1688, Environment Canada (1992 (5)), DeWitt et al. (1992a (6); 1997a (7)), Emery et al. (1997 (8)), and Emery and Moore (1996 (9)), Swartz et al. (1985 (10)), DeWitt et al. (1989 (11)), Scott and Redmond (1989 (12)), and Schlekat et al. (1992 (13)).

1.7 Additional sediment toxicity research and methods development are now in progress to *(1)* refine sediment spiking procedures, *(2)* refine sediment dilution procedures, *(3)* refine sediment Toxicity Identification Evaluation (TIE) procedures, *(4)* produce additional data on confirmation of responses in laboratory tests with natural populations of benthic organisms (that is, field validation studies), and *(5)* evaluate relative sensitivity of endpoints measured in 10- and 28-d toxicity tests using estuarine or marine amphipods. This information will be described in future editions of this standard.

1.8 Although standard procedures are described in Annex A2 of this standard for conducting chronic sediment tests with *L. plumulosus*, further investigation of certain issues could aid in the interpretation of test results. Some of these issues include further investigation to evaluate the relative toxicological sensitivity of the lethal and sublethal endpoints to a wide variety of chemicals spiked in sediment and to mixtures of chemicals in sediments from contamination gradients in the field (USEPA-USACE 2001 (2)). Additional research is needed to evaluate the ability of the lethal and sublethal endpoints to estimate the responses of populations and communities of benthic invertebrates to contaminated sediments. Research is also needed to link the toxicity test endpoints to a field-validated population model of *L. plumulosus* that would then generate estimates of population-level responses of the amphipod to test sediments and thereby provide additional ecologically relevant interpretive guidance for the laboratory toxicity test.

1.9 This standard outlines specific test methods for evaluating the toxicity of sediments with *A. abdita*, *E. estuarius*, *L. plumulosus*, and *R. abronius*. While standard procedures are described in this standard, further investigation of certain issues could aid in the interpretation of test results. Some of these issues include the effect of shipping on organism sensitivity, additional performance criteria for organism health, sensitivity of various populations of the same test species, and confirmation of responses in laboratory tests with natural benthos populations.

1.10 General procedures described in this standard might be useful for conducting tests with other estuarine or marine organisms (for example, *Corophium spp.*, *Grandidierella japonica*, *Lepidactylus dytiscus*, *Streblospio benedicti*), although modifications may be necessary. 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 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.

1.11 *Selection of Toxicity Testing Organisms:*

1.11.1 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). Ideally, a test organism should: *(1)* have a toxicological database demonstrating relative sensitivity to a range of

contaminants of interest in sediment, *(2)* have a database for interlaboratory comparisons of procedures (for example, round-robin studies), *(3)* be in direct contact with sediment, *(4)* be readily available from culture or through 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 (Guide E1525). Methods utilizing selected organisms should also be *(11)* peer reviewed (for example, journal articles) and *(12)* confirmed with responses with natural populations of benthic organisms.

1.11.2 Of these criteria (Table 1), a database demonstrating relative sensitivity to contaminants, contact with sediment, ease of culture in the laboratory or availability for field-collection, ease of handling in the laboratory, tolerance to varying sediment physico-chemical characteristics, and confirmation with responses with natural benthic populations were the primary criteria used for selecting *A. abdita*, *E. estuarius*, *L. plumulosus*, and *R. abronius* for the current edition of this standard for 10-d sediment tests (Annex A1). The species chosen for this method are intimately associated with sediment, due to their tube- dwelling or free-burrowing, and sediment ingesting nature. Amphipods have been used extensively to test the toxicity of marine, estuarine, and freshwater sediments (Swartz et al., 1985 (**10**); DeWitt et al., 1989 (**11**); Scott and Redmond, 1989 (**12**); DeWitt et al., 1992a (**6**); Schlekat et al., 1992 (**13**)). The selection of test species for this standard followed the consensus of experts in the field of sediment toxicology who participated in a workshop entitled "Testing Issues for Freshwater and Marine Sediments". The workshop was sponsored by USEPA Office of Water, Office of Science and Technology, and Office of Research and Development, and was held in Washington, D.C. from 16-18 September 1992 (USEPA, 1992 (**15**)). Of the candidate species discussed at the workshop, *A. abdita*, *E. estuarius*, *L. plumulosus*, and *R. abronius* best fulfilled the selection criteria, and presented the availability of a combination of one estuarine and one marine species each for both the Atlantic (the estuarine *L. plumulosus* and the marine *A. abdita*) and Pacific (the estuarine *E. estuarius* and the marine *R. abronius*) coasts. *Ampelisca abdita* is also native to portions of the Gulf of Mexico and San Francisco Bay. Many other organisms that might be appropriate for sediment testing do not now meet these selection criteria because little emphasis has been placed on developing standardized testing procedures for benthic organisms. For example, a fifth species, *Grandidierella japonica* was not selected because workshop participants felt that the use of this species was not sufficiently broad to warrant standardization of the method. Environment Canada (1992 (**5**)) has recommended the use of the following amphipod species for sediment toxicity testing: *Amphiporeia virginiana*, *Corophium volutator*, *Eohaustorius washingtonianus*, *Foxiphalus xiximeus*, and *Leptocheirus pinguis*. A database similar to those available for *A. abdita*, *E. estuarius*, *L. plumulosus*, and *R. abronius* must be developed in order for these and other organisms to be included in future editions of this standard.

1.11.3 The primary criterion used for selecting *L. plumulosus* for chronic testing of sediments was that this species is found in both oligohaline and mesohaline regions of estuaries on the East Coast of the United States and is tolerant to a wide range of sediment grain size distribution (USEPA-USACE 2001 (**2**), Annex Annex A2). This species is easily cultured in the laboratory and has a relatively short generation time (that is, about 24 d at 23°C, DeWitt et al. 1992a(**6**)) that makes this species adaptable to chronic testing (Section 12).

1.11.4 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. Several studies have evaluated the sensitivities of *A. abdita*, *E. estuarius*, *L. plumulosus*, or *R. abronius*, either relative to one another, or to other commonly tested estuarine or marine species. For example, the sensitivity of marine amphipods was compared to other species that were used in generating saltwater Water Quality Criteria. Seven amphipod genera, including *Ampelisca abdita* and *Rhepoxynius abronius*, were among the test species used to generate saltwater Water Quality Criteria for 12 chemicals. Acute amphipod toxicity data from 4-d water-only tests for each of the 12 chemicals was compared to data for *(1)* all other species, *(2)* other benthic species, and *(3)* other infaunal species. Amphipods were generally of median sensitivity for each comparison. The average percentile rank of amphipods among all species tested was 57 %; among all benthic species, 56 %; and, among all infaunal species, 54 %. Thus, amphipods are not uniquely sensitive relative to all species, benthic species, or even infaunal species (USEPA 1994a (**1**)). Additional research may be warranted to develop tests using species that are consistently more sensitive than amphipods, thereby offering protection to less sensitive groups.

1.11.5 Williams et al. (1986 (**16**)) compared the sensitivity of the *R. abronius* 10-d whole sediment test, the oyster embryo (*Crassostrea gigas*) 48-h abnormality test, and the bacterium (*Vibrio fisheri*) 1-h luminescence inhibition test (that is, the Microtox[2] test) to sediments collected from 46 contaminated sites in Commencement Bay, WA. *Rhepoxynius abronius* were exposed to whole sediment, while the oyster and bacterium tests were conducted with sediment elutriates and extracts, respectfully. Microtox[2] was the most sensitive test, with 63 % of the sites eliciting significant inhibition of luminescence. Significant mortality of *R. abronius* was observed in 40 % of test sediments, and oyster abnormality occurred in 35 % of sediment elutriates. Complete concordance (that is, sediments that were either toxic or not-toxic in all three tests) was observed in 41 % of the sediments. Possible sources for the lack of concordance at other sites include interspecific differences in sensitivity among test organisms, heterogeneity in contaminant types associated with test sediments, and differences in routes of exposure inherent in each toxicity test. These

[2] Microtox is a trademark of Strategic Diagnostics Inc. 111 Pencader Drive Newark, Delaware 19702-3322.