



Standard Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Estuarine and Marine Invertebrates¹

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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 $25^{\circ} \pm 2^{\circ}\text{C}$, and the recommended overlying water salinity is $5\text{‰} \pm 2\text{‰}$ (for test sediment with pore water at 1 ‰ to 10 ‰) or $20\text{‰} \pm 2\text{‰}$ (for test sediment with pore water $>10\text{‰}$). 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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