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~~Standard Guide for Conducting 10-day Static Sediment Toxicity Tests with Marine and Estuarine Amphipods~~Designation: E 1367 – 03 (Reapproved 2008)

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

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1. ~~Scope~~

~~1.1This guide~~ 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;

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

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 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 E 1611 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)**~~describes procedures for obtaining laboratory data concerning the short-term adverse effects of potentially contaminated sediment, or of a test material experimentally added to contaminated or uncontaminated sediment, on marine or estuarine infaunal amphipods during static 10-day exposures. These procedures are useful for testing the effects of various geochemical characteristics of sediments on marine and estuarine amphipods, 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 10-day static sediment toxicity tests are described for the following species: *Rhepoxynius abronius*~~) , 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 E 1706, and Guides E 1391, E 1525, E 1688, 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*, ~~*Eohaustorius estuarius*~~E. estuarius, ~~*Ampelisca abdita, Grandidierella japonica*~~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 E 1525). 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 E 1525). 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 **(14)**). 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 **(15)**) 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 results highlight the importance of using multiple assays when performing sediment assessments.

1.11.6 Several studies have compared the sensitivity of combinations of the four amphipods to sediment contaminants. For example, there are several comparisons between *A. abdita* and *R. abronius*, between *E. estuarius* and *R. abronius*, and between *A. abdita* and *L. plumulosus*. There are fewer examples of direct comparisons between *E. estuarius* and *L. plumulosus* , and no

[3] Boldface numbers in parentheses refer to the list of references at the end of this guide.

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

examples comparing *L. plumulosus* and *R. abronius*. There is some overlap in relative sensitivity from comparison to comparison within each species combination, which appears to indicate that all four species are within the same range of relative sensitivity to contaminated sediments.

1.11.6.1 Word et al. (1989 **(16)**) compared the sensitivity of *A. abdita* and *R. abronius* to contaminated sediments in a series of experiments. Both species were tested at 15°C. Experiments were designed to compare the response of the organism rather than to provide a comparison of the sensitivity of the methods (that is, *Ampelisca abdita* would normally be tested at 20°C). Sediments collected from Oakland Harbor, CA, were used for the comparisons. Twenty-six sediments were tested in one comparison, while 5 were tested in the other. Analysis of results using Kruskal Wallace rank sum test for both experiments demonstrated that *R. abronius* exhibited greater sensitivity to the sediments than *A. abdita* at 15°C. Long and Buchman (1989 **(17)**) also compared the sensitivity of *A. abdita* and *R. abronius* to sediments from Oakland Harbor, CA. They also determined that *A. abdita* showed less sensitivity than *R. abronius*, but they also showed that *A. abdita* was less sensitive to sediment grain size factors than *R. abronius*.

1.11.6.2 DeWitt et al. (1989 **(11)**) compared the sensitivity of *E. estuarius* and *R. abronius* to sediment spiked with fluoranthene and field-collected sediment from industrial waterways in Puget Sound, WA, in 10-d tests, and to aqueous cadmium ($CdCl_2$) in a 4-d water-only test. The sensitivity of *E. estuarius* was from two (to spiked-spiked sediment) to seven (to one Puget Sound, WA, sediment) times less sensitive than *R. abronius* in sediment tests, and ten times less sensitive to $CdCl_2$ in the water-only test. These results are supported by the findings of Pastorok and Becker (1990 **(18)**) who found the acute sensitivity of *E. estuarius* and *R. abronius* to be generally comparable to each other, and both were more sensitive than *Neanthes arenaceodentata* (survival and biomass endpoints), *Panope generosa* (survival), and *Dendraster excentricus* (survival).

1.11.6.3 *Leptocheirus plumulosus* was as sensitive as the freshwater amphipod *Hyalella azteca* to an artificially created gradient of sediment contamination when the latter was acclimated to oligohaline salinity (that is, 6 ‰; McGee et al., 1993 **(19)**). DeWitt et al. (1992b **(20)**) compared the sensitivity of *L. plumulosus* with three other amphipod species, two mollusks, and one polychaete to highly contaminated sediment collected from Baltimore Harbor, MD, that was serially diluted with clean sediment. *Leptocheirus plumulosus* was more sensitive than the amphipods *Hyalella azteca* and *Lepidactylus dytiscus* and exhibited equal sensitivity with *E. estuarius*. Schlekat et al. (1995 **(21)**) describe the results of an interlaboratory comparison of 10-d tests with *A. abdita*, *L. plumulosus* and *E. estuarius* using dilutions of sediments collected from Black Rock Harbor, CT. There was strong agreement among species and laboratories in the ranking of sediment toxicity and the ability to discriminate between toxic and non-toxic sediments.

1.11.6.4 Hartwell et al. (2000 **(22)**) evaluated the response of *Leptocheirus plumulosus* (10-d survival or growth) to the response of the amphipod *Lepidactylus dytiscus* (10-d survival or growth), the polychaete *Streblospio benedicti* (10-d survival or growth), and lettuce germination (Lactuca sativa in 3-d exposure) and observed that *L. plumulosus* was relatively insensitive compared to the response of either L. dytiscus or S. benedicti in exposures to 4 sediments with elevated metal concentrations.

1.11.6.5 Ammonia is a naturally occurring compound in marine sediment that results from the degradation of organic debris. Interstitial ammonia concentrations in test sediment can range from <1 mg/L to in excess of 400 mg/L (Word et al., 1997 **(23)**). Some benthic infauna show toxicity to ammonia at concentrations of about 20 mg/L (Kohn et al., 1994 **(24)**). Based on water-only and spiked-sediment experiments with ammonia, threshold limits for test initiation and termination have been established for the *L. plumulosus* chronic test. Smaller (younger) individuals are more sensitive to ammonia than larger (older) individuals (DeWitt et al., 1997a **(7)**, b **(25)**. Results of a 28-d test indicated that neonates can tolerate very high levels of pore-water ammonia (>300 mg/L total ammonia) for short periods of time with no apparent long-term effects (Moore et al., 1997 **(26)**). It is not surprising *L. plumulosus* has a high tolerance for ammonia given that these amphipods are often found in organic rich sediments in which diagenesis can result in elevated pore-water ammonia concentrations. Insensitivity to ammonia by *L. plumulosus* should not be construed as an indicator of the sensitivity of the *L. plumulosus* sediment toxicity test to other chemicals of concern.

1.11.7 Limited comparative data is available for concurrent water-only exposures of all four species in single-chemical tests. Studies that do exist generally show that no one species is consistently the most sensitive.

1.11.7.1 The relative sensitivity of the four amphipod species to ammonia was determined in ten-d water only toxicity tests in order to aid interpretation of results of tests on sediments where this toxicant is present (USEPA 1994a **(1)**). These tests were static exposures that were generally conducted under conditions (for example, salinity, photoperiod) similar to those used for standard 10-d sediment tests. Departures from standard conditions included the absence of sediment and a test temperature of 20°C for *L. plumulosus*, rather than 25°C as dictated in this standard. Sensitivity to total ammonia increased with increasing pH for all four species. The rank sensitivity was *R. abronius* = *A. abdita* > *E. estuarius* > *L. plumulosus*. A similar study by Kohn et al. (1994 **(24)**) showed a similar but slightly different relative sensitivity to ammonia with *A. abdita* > *R. abronius* = *L. plumulosus* > *E. estuarius*.

~~1.2Two documents (USEPA 1994 (~~

1.11.7.2 Cadmium chloride has been a common reference toxicant for all four species in 4-d exposures. DeWitt et al. (1992a **(6)**) reports the rank sensitivity as *R. abronius* > *A. abdita* > *L. plumulosus* > *E. estuarius* at a common temperature and salinity of 15°C and 28 ‰ . A series of 4-d exposures to cadmium that were conducted at species-specific temperatures and salinities showed the following rank sensitivity: *A. abdita* = *L. plumulosus* = *R. abronius* > *E. estuarius* (USEPA 1994a **(1)**~~, USEPA-USACE 1999 (~~**).**

1.11.7.3 Relative species sensitivity frequently varies among contaminants; consequently, a battery of tests including organisms

representing different trophic levels may be needed to assess sediment quality (Craig, 1984 **(27)**; Williams et al. 1986 **(15)**; Long et al., 1990 **(28)**; Ingersoll et al., 1990 **(29)**; Burton and Ingersoll, 1994 **(31)**). For example, Reish (1988 **(32)**) 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.

1.11.8 The sensitivity of an organism is related to route of exposure and biochemical response to contaminants. Sediment-dwelling organisms can receive exposure 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 contaminants from sediment. Benthic invertebrates often selectively consume different particle sizes (Harkey et al. 1994 **(33)**) or particles with higher organic carbon concentrations which may have higher contaminant concentrations. Grazers and other collector-gatherers that feed on aufwuchs and detritus may receive most of their body burden directly from materials attached to sediment or from actual sediment ingestion. In some amphipods (Landrum, 1989 **(34)**) and clams (Boese et al., 1990 **(35)**) uptake through the gut can exceed uptake across the gills for certain hydrophobic compounds. Organisms in direct contact with sediment may also accumulate contaminants by direct adsorption to the body wall or by absorption through the integument (Knezovich et al. 1987 **(36)**).

1.11.9 Despite the potential complexities in estimating the dose that an animal receives from sediment, the toxicity and bioaccumulation of many contaminants in sediment such as Kepone®, fluoranthene, organochlorines, and metals have been correlated with either the concentration of these chemicals in interstitial water or in the case of non-ionic organic chemicals, concentrations in sediment on an organic carbon normalized basis (Di Toro et al. 1990 **(37)**; Di Toro et al. 1991 **(38)**). The relative importance of whole sediment and interstitial water routes of exposure depends on the test organism and the specific contaminant (Knezovich et al. 1987 **(36)**). 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.

1.11.10 The use of *A. abdita*, *E. estuarius*, *R. abronius*, and *L. plumulosus* in laboratory toxicity studies has been field validated with natural populations of benthic organisms (Swartz et al. 1994 **(39)** and Anderson et al. 2001 **(40)** for *E. estuarius*, Swartz et al. 1982 **(43)** and Anderson et al. 2001 **(40)** for *R. abronius*, McGee et al. 1999 **(41)** and McGee and Fisher 1999 **(42)** for *L. plumulosus*).

1.11.10.1 Data from USEPA Office of Research and Development's Environmental Monitoring and Assessment program were examined to evaluate the relationship between survival of *Ampelisca abdita* in sediment toxicity tests and the presence of amphipods, particularly ampeliscids, in field samples. Over 200 sediment samples from two years of sampling in the Virginian Province (Cape Cod, MA, to Cape Henry, VA) were available for comparing synchronous measurements of *A. abdita* survival in toxicity tests to benthic community enumeration. Although species of this genus were among the more frequently occurring taxa in these samples, ampeliscids were totally absent from stations that exhibited *A. abdita* test survival <60 % of that in control samples. Additionally, ampeliscids were found in very low densities at stations with amphipod test survival between 60 and 80 % (USEPA 1994a **(1)**). These data indicate that tests with this species are predictive of contaminant effects on sensitive species under natural conditions.

1.11.10.2 Swartz et al. (1982 **(43)**) compared sensitivity of *R. abronius* to sediment collected from sites in Commencement Bay, WA, to benthic community structure at each site. Mortality of *R. abronius* was negatively correlated with amphipod density, and phoxocephalid amphipods were ubiquitously absent from the most contaminated areas.

1.11.10.3 Sediment toxicity to amphipods in 10-d toxicity tests, field contamination, and field abundance of benthic amphipods were examined along a sediment contamination gradient of DDT (Swartz et al. 1994 **(39)**). Survival of *E. estuarius* and *R. abronius* in laboratory toxicity tests was positively correlated to abundance of amphipods in the field and along with the survival of *H. azteca*, was negatively correlated to DDT concentrations. The threshold for 10-d sediment toxicity in laboratory studies was about 300 ug DDT (+metabolites)/g organic carbon. The threshold for abundance of amphipods in the field was about 100 ug DDT (+metabolites)/g organic carbon. Therefore, correlations between toxicity, contamination, and biology indicate that acute 10-d sediment toxicity tests can provide reliable evidence of biologically adverse sediment contamination in the field.

1.11.10.4 As part of a comprehensive sediment quality assessment in Baltimore Harbor, MD, McGee et al. (1999 **(41)**) conducted 10-d toxicity tests with *L. plumulosus* . Negative relationships were detected between amphipod survival and concentrations of select sediment-associated contaminants, whereas a very strong positive association existed between survival in laboratory exposures and field density of *L. plumulosus* at test sites. A field validation study of the 10- and 28-d *L. plumulosus* tests by McGee and Fisher (1999 **(42)**) in Baltimore Harbor, also indicated good agreement between acute toxicity, sediment associated contaminants and responses of the *in situ* benthic community. In this study, the chronic 28-d test was less sensitive to sediment contamination than the acute 10-d test; however, the feeding regime used in this evaluation is different than the one currently recommended in Annex A2 and may have influenced the test results. Field validation studies with the revised 28-d test outlined in Annex A2 have not been conducted.

1.12 *Chronic Sediment Methods with Leptocheirus plumulosus*:

1.12.1 Most standard whole sediment toxicity tests have been developed to produce a lethality endpoint (survival/mortality) with potential for a sublethal endpoint (reburial) in some species (USEPA 1994a (**1**), USEPA-USACE 2001 (**2**)) ~~provide additional guidance on methods for conducting sediment toxicity tests with estuarine and marine amphipods. This additional guidance includes supplemental information on: 1. sediment collection and storage (Section 10.4), 2. sediment spiking (Section 10.6), 3.~~

collection, handling, and culturing of amphipods (Section 11.4), and 4. statistical analyses (Section 16). USEPA-USACE (1999) also provides guidance on a method for conduction 28–d sediment toxicity tests with the amphipod)). Methods that measure sublethal effects have not been available or have not been routinely used to evaluate sediment toxicity in marine or estuarine sediments (Scott and Redmond, 1989 **(12)** ; Green and Chandler, 1996 **(44)** ; Levin et al., 1996 **(45)**; Ciarelli et al., 1998 **(46)**; Meador and Rice, 2001 **(47)**). Most assessments of contaminated sediment rely on short-term lethality tests (for example, ≤10 d; USEPA-USACE, 1991 **(48)**; 1998 **(49)**). Short-term lethality tests are useful in identifying "hot spots" of sediment contamination, but might not be sensitive enough to evaluate moderately contaminated areas. However, 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 (Ingersoll et al., 1998 **(50)**; Kemble et al., 1994 **(51)**; McGee et al., 1995 **(52)** ; Scott, 1989 **(53)**). The 28-d toxicity test with *Leptocheirus plumulosus* . Endpoints measured in this 28–d test include survival, growth, and reproduction.

1.3Modifications of these procedures might be appropriate for other sediment toxicity test procedures such as flow-through or partial life-cycle tests. 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 amphipods, other crustaceans, polychaetes, and bivalves.

1.4Other modifications of these procedures might be justified by special needs or circumstances. Although using appropriate procedures is more important than following prescribed procedures, results of tests conducted using unusual procedures are not likely to be comparable to results of many other tests. Comparisons of 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.

1.5These procedures are applicable to sediments containing most chemicals, either individually or in formulations, commercial products, and known or unknown mixtures. With appropriate modifications these procedures can be used to conduct sediment toxicity tests on factors such as temperature, salinity, dissolved oxygen, and natural sediment characteristics (for example, particle size distribution, organic carbon content, total solids). These methods can also be used to conduct bioconcentration tests and in situ tests, and to assess the toxicity of potentially contaminated field sediments, or of such materials 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 either adhering to sediment particles or dissolved in interstitial water can be tested.

1.6Results of short-term toxicity tests with test materials experimentally added to sediments may be reported in terms of an LC50, and sometimes an EC50 where "concentration" refers to dry or wet weight concentration in sediment. Results of a field survey with single samples to determine a spatial or temporal distribution of sediment toxicity may be reported in terms of percent mortality (see Section 16). Field surveys can be designed to provide either a *qualitative* reconnaissance of the distribution of sediment toxicity or a *quantitative* statistical comparison of toxicity among stations.

1.7This guide is arranged as follows: has two sublethal endpoints: growth and reproduction. These sublethal endpoints have potential to exhibit a toxic response from chemicals that otherwise might not cause acute effects or significant mortality in a test. Sublethal response to chronic exposure is also valuable for population modeling of contaminant effects. These data can be used for population-level risk assessments of benthic pollutant effects.

1.12.2 An evaluation of the distribution of *L. plumulosus* in Chesapeake Bay indicates that its distribution is negatively correlated with the degree of sediment contamination (Pfitzenmeyer, 1975 **(54)**; Reinharz, 1981 **(55)**). A field validation study of the 10- and 28-d *L. plumulosus* tests by McGee and Fisher (1999 **(42)**) in Baltimore Harbor, indicated good agreement between acute toxicity, sediment associated contaminants and responses of the *in situ* benthic community. In this study, the chronic 28-d test was less sensitive to sediment contamination than the acute 10-d test and therefore had a poorer association between sediment contaminants and benthic community health. It should be noted that the feeding regime used in this evaluation is different than the one currently recommended in Annex A2 and may have influenced the test results. Field validation studies with the revised 28-d test have not been conducted.

1.13 *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.

1.14 This standard is arranged as follows:

~~1.8The values stated in SI units are to be regarded as standard.~~

~~1.9*This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* While some safety considerations are presented in this guide, it is beyond the scope of this guide to enc~~

~~1.15*This all safety requirements pass and be aware of the inherent toxicity of* Specific hazard statements are given in Section~~

*of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*Specific hazard statements are given in Section 8.

TABLE 1 Reconstituted Salt Water (14) f

A "+" or M "–" Rartine ag Ind Esicatues a Positive or Negatinve C Attribustaceans

AddCrithe followriong r	Ampeliscagent gr abde (13)ita e	Eohemicalus inthe amoriunts estuand order liust	Ledpto 890 mL of water. Each chemicarus plumulost bus	Rhe dpoxyniussolved abefore theonextius added.A
	Chemical	Amount		
Relative sensitivity toxicity data base	+	Amount		
	NaF	3 mg		
+	+	+		
	$SrCl_2 \cdot 6H_2O$	20 mg		
Round-robin studies conducted	+	20 mg		
	H_3BO_3	30 mg		
+	+	+		
	KBr	100 mg		
Contact with sediment	+	100 mg		
	KCl	700 mg		
+	+	+		
	$CaCl_2 \cdot 2H_2O$	1.47 g		
Laboratory culture	+/-	1.47 g		
	Na_2SO_4	4.00 g		
-	+	-		
	$MgCl_2 \cdot 6H_2O$	+	+	+
Taxonomic identification	+	+	+	+
10.78gical importance	+	+	+	+
Ecological importance	+	+	+	+
	NaCl	PAC	ATL	PAC
Geographical distribution	ATL, PAC, GOM	PAC	ATL	PAC
23.50 gSediment physicochemical tolerance	+	+	+	+
Sediment physicochemical tolerance	+	+	+	+
	Na_{2+}	$+_A SiO_3 \cdot 9H_2O$	20 mg+	+
Response confirmed with benthos populations	+	$+^A$	+	+
	$NaHCO_3$	+	+	+
Peer reviewed	+	+	+	+
200 mgonitored	Survival	Survival, reburial	Survival	Survival, reburial
Endpoints monitored	Survival	Survival, reburial	Survival	Survival, reburial

A If th Anderesulting solution is dilut edto 1 L, the s alinity should be 34 ± 0.5 g/kg and the pH 8. (20 ±0.2. The desired test salinity is attained by dilution at time of use. The reconstituted salt water should be stripped of trace metals1 (1540)).

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

2. Referenced Documents

2.1 *ASTM Standards:* [3]

D 1129 Terminology Relating to Water D3976Practice for Preparation of Sediment Samples for Chemical Analysis

D 4447 Guide for the Disposal of Laboratory Chemicals and Samples

E380Practice for Use of the International System of Units (SI) (the Modernized Metric System) 29 Practice for Using Significant Digits in Test Data to Determine Conformance with Specifications

E729Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians[6] 105 Practice for Probability Sampling Of Materials

E943Terminology Relating to Biological Effects and Environmental Fate[6] 122 Practice for Calculating Sample Size to Estimate, With Specified Precision, the Average for a Characteristic of a Lot or Process

E1023Guide for Assessing the Hazard of a Material to Aquatic Organisms and Their Uses[6] 141 Practice for Acceptance of Evidence Based on the Results of Probability Sampling

E 177 Practice for Use of the Terms Precision and Bias in ASTM Test Methods

E 178 Practice for Dealing With Outlying Observations

E 456 Terminology Relating to Quality and Statistics

E 691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

E 729 Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians

E 943 Terminology Relating to Biological Effects and Environmental Fate

E 1241 Guide for Conducting Early Life-Stage Toxicity Tests with Fishes

E 1325 Terminology Relating to Design of Experiments

E 1391 Guide for Collection, Storage, Characterization, and Manipulation of Sediments for Toxicological Testing and for Selection of Samplers Used to Collect Benthic Invertebrates

E 1402 Terminology Relating to Sampling

E 1525 Guide for Designing Biological Tests with Sediments

[3] This guide is based largely on Guide E729 and Ref (3).

[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.

E 1611 Guide for Conducting Sediment Toxicity Tests with Polychaetous Annelids
E 1688 Guide for Determination of the Bioaccumulation of Sediment-Associated Contaminants by Benthic Invertebrates
E 1706 Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates
E 1847 Practice for Statistical Analysis of Toxicity Tests Conducted Under ASTM Guidelines
E 1850 Guide for Selection of Resident Species as Test Organisms for Aquatic and Sediment Toxicity Tests
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 of Terms Specific to This Standard:*~~

~~3.1.1The term "*sediment*" is used here to denote a naturally occurring particulate material that has been transported and deposited at the bottom of a body of water. The procedures described can also be applied using an experimentally prepared substrate within which the amphipods can burrow.~~

~~3.1.1.1*clean sediment*— denotes sediment that does not contain concentrations of toxicants that cause apparent stress to the test organisms or reduce their survival.~~

~~3.1.1.2*solid-phase sediment*—distinguished from elutriates and resuspended sediments in that the whole, intact sediment is used to expose the organisms, not a form or derivative of the sediment.~~

~~3.1.2*toxicity*—the property of a material or combination of materials, to adversely affect organisms (see Terminology E943~~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 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 E 729 and E 1241 and Terminology E 943 and D 1129. For an explanation of units and symbols, refer to IEEE/ASTM SI 10.

3.3 *Definitions of Terms Specific to This Standard:*

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

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

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

3.3.4 *control sediment*, *n*—a sediment that is essentially free of contaminants and is used routinely to assess the acceptability of a test. Any contaminants in control sediment may originate from the global spread of pollutants and does not reflect any substantial input from local or non-point sources. Comparing test sediments to control sediments is a measure of the toxicity of a test sediment beyond inevitable background contamination.

3.3.5 *EC50*, *n*—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*, *n*—mixtures of materials used to mimic the physical components of a natural sediment.

3.3.7 *IC50*, *n*—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*, *n*— water occupying space between sediment or soil particles.

3.3.9 *LC50*, *n*—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)*, *n*—in a toxicity test, the lowest tested concentration of a material at which organisms were adversely affected compared to control organisms as determined by statistical hypothesis tests-should be accompanied by a description of the statistical tests and alternative hypotheses, levels of significance, and measures of performance, for example, survival, growth, reproduction, or development-and must be above any other concentration not producing statistically significant adverse effects.

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

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

3.3.13 *reference sediment*, *n*—a whole sediment near an area of concern used to assess sediment conditions exclusive of

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

3.3.14 *reference-toxicity test*, *n*—a test conducted with reagent-grade reference chemical to assess the sensitivity of the test organisms. Deviations outside an established normal range may indicate a change in the sensitivity of the test organism population. Reference-toxicity tests are most often performed in the absence of sediment.

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

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

4. Summary of Standard

4.1 *Method Description*—Procedures are described for testing estuarine or marine amphipod crustaceans in the 10-d laboratory exposures to evaluate the toxicity of contaminants associated with whole sediments. 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 10 and 11). 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.

4.2 A procedure is also described for determining the chronic toxicity of contaminants associated with whole sediments with the amphipod *Leptocheirus plumulosus* in laboratory exposures (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 seawater. Four hundred millilitres of overlying water is renewed three times per week, at which time test organisms are fed. Tests are initiated with neonate amphipods that mature and reproduce during the 28-d test period. The endpoints in the 28-d toxicity test are survival, growth rate, and reproduction of amphipods. Survival is calculated as the percentage of newly born (neonate) amphipods at test initiation that survive as adults at test termination. Growth rate is calculated as the mean dry weight gain per day per adult amphipod surviving at test termination. Reproduction is calculated as the number of offspring per surviving adult. This test is applicable for use with sediment having pore-water salinity ranging from 1 ‰ to 35 ‰ . Typically, endpoint selection for new toxicity tests is generally guided by methodologies for related toxicity tests (Gray et al., 1998 **(56)**). Sediment toxicity tests using macroinvertebrates often incorporate survival and growth endpoints (Ingersoll, 1995 **(57)**). Gray et al. (1998 **(56)**) recommend optimal endpoint measures for the *L. plumulosus* sediment toxicity test based on four criteria: relevance of each measure to its respective endpoint; signal-to-noise ratio (the ratio between the response to stressor and the normal variation in the response variable); redundancy to other measures of the same endpoint; and cost of labor, training, and equipment. Signal-to-noise ratios are independent of experiment design considerations (that is, Type I and Type II errors, and sample size) and are positively correlated with power (Gray et al., 1998 **(56)**).

~~3.1.3*exposure*—contact with a chemical or physical agent (see Terminology E943~~

4.3 *Experimental Design*—The following section is a general summary of experimental design. See Section 13 for additional detail.

4.3.1 *Control and Reference Sediment* :

4.3.1.1 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 Ankley and Thomas, 1992 **(58)**. Comparing test sediments to control sediments is a measure of the toxicity of a test sediment beyond inevitable background contamination and organism health Ankley and Thomas, 1992 **(58)**. 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.

4.3.1.2 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.

4.3.1.3 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 reasonable 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.

4.3.1.4 Natural physico-chemical characteristics such as sediment texture may influence the response of test organisms **(59)**. 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 section 12.1 and Annex A1 and Annex A2 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 Dewitt et al. 1988, **(59)** , Ankley et al., 1994**(60)**. The use of formulated sediment can also be used to evaluate physico-chemical characteristics of sediment on test organisms Walsh et al., 1991 **(61)** Suedel and Rodgers, 1994, **(64)** Kembel et al.,**(63)** USEPA, 2000,**(62)**, section 7.2 and Guide E 1391).

~~3.1.4*interstitial water*—the water within a wet sediment that surrounds the sediment particles. The amount of interstitial water in sediment is expressed as the percent ratio of the weight of the water in the sediment to that of the wet sediment.~~

~~3.1.5*overlying water*—the water that is added to the test chamber over the solid phase of the sediment in a toxicity test.~~

~~3.1.6 *spiking of sediment*, refers to the experimental addition of a test material such as a chemical or mixture of chemicals, sewage sludge, oil, particulate matter, or highly contaminated sediment to a clean negative control or reference sediment to determine the toxicity of the material added. After the test material is added, sometimes with a solvent carrier, the sediment is thoroughly mixed to evenly distribute the test material throughout the sediment.~~

~~3.1.7The LC50 is the statistically or graphically derived best estimate of the concentration of test material added to or contained in sediment that is expected to be lethal to 50% of the test organisms under specified conditions within the test period (see Terminology E943~~

4.3.2 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 Green, 1979 **(65)**. See Section 13 for additional guidance on experimental design and statistics.

4.3.2.1 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 (Table A1.3 in Annex A1 and Table A2.3 in Annex A2) and might include a control sediment or a formulated sediment (section 7.2). 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 (that is, grain size, organic carbon) may be useful in these evaluations. A summary of field sampling design is presented by Green, 1979 **(65)**. See Section 13 for additional guidance on experimental design and statistics.

4.3.2.2 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.

4.3.2.3 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 13). 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.

4.3.2.4 Sediments often exhibit high spatial and temporal variability **(66)**. 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.

4.3.2.5 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 may include compositing cores collected to project depth from a specified dredged material management area.

4.3.2.6 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 E 1241). 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 (Guide E 729). 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 13).

3.1.8The EC50 is the statistically or graphically estimated concentration of test material in sediment that is expected to cause a measured sublethal effect (for example the inability of amphipods to rebury in clean sediment at the end of the test period), in 50% of the test organisms under specified conditions (see Terminology E943).

3.1.9The words "must," "should," "may," "can," and "might" have very specific meanings in this guide.

3.1.9.1"Must" is used to express an absolute requirement, that is, to state that the test ought to be designed to satisfy the specified condition, unless the purpose of the test requires a different design. "Must" is only used in connection with factors that directly relate to the acceptability of the test (see Section 15).

3.1.9.2"Should" is used to state that the specified condition is recommended and ought to be met if possible. Although 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.

3.1.9.3"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."

3.2For definitions of other terms used in this guide, refer to Terminology D1129, Guide E729, Terminology E943, and Guide E1023. For an explanation of units and symbols, refer to Practice E380.

4.Summary of Guide

4.1The relative toxicity of marine or estuarine sediments can be determined through a 10-day static test with solid phase sediment and overlying water in aerated 1-L glass test chambers. Mortality and sublethal effects such as emergence from sediment and inability to bury in clean sediment are determined after exposure of a specific number (usually 20) of amphipods to a quantity of test sediment. Response of the amphipods to the test sediment is compared with response in control sediment. A negative control or reference sediment is used to provide (*a*) a measure of the acceptability of the test by providing evidence of the health and relative quality of the test organisms, and the suitability of the overlying water, test conditions and handling procedures, etc., and (*b*) the basis for interpreting data obtained from the test sediments.

4.1.1The toxicity of field collected sediment is indicated by the percent mortality of amphipods exposed to that sediment compared to those exposed to control sediment. The toxicity of field sediments may also be assessed by testing dilutions of a highly toxic test sediment with clean sediment to obtain information on the toxicity of proportions of that sediment.

4.1.2The toxicity of a toxicant experimentally added to sediments can be expressed by analyzing the mortality and reburial data to determine an LC50 and an EC50 for the toxicant for the duration of exposure.

5. Significance and Use

5.1The test procedure in this guide is not intended to exactly simulate the exposure of benthic amphipods to contaminants 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.

5.2Protection 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. Protection of the most sensitive species within a community will theoretically protect the community as a whole.

5.3Amphipods are an abundant component of the soft bottom marine and estuarine benthic community. They are a principal prey of many fish, birds, and larger invertebrate species. Some species are predators of smaller benthic invertebrates. Others ingest sediment particles and thus are directly exposed to contaminants. Amphipods are among the first taxa to disappear from benthic communities impacted by pollution, and have been shown to be more sensitive to contaminated sediments than several other major taxa (4**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; Stephan et al.(67). The ecological importance of amphipods, their wide geographical distribution, ease of handling in the laboratory, and their sensitivity to contaminated sediments make them appropriate species for sediment toxicity testing.

5.4An acute toxicity test is conducted to obtain information concerning the immediate effects on test organisms of a short-term exposure to a test material under specific experimental conditions. An acute toxicity test does not necessarily provide information about whether delayed effects will occur, although a post exposure observation period, with appropriate feeding if necessary, could provide such information.

5.5Results of acute sediment toxicity tests can be used to predict acute effects likely to occur on aquatic organisms in field situations as a result of exposure under comparable conditions, except that (*a*) motile organisms might avoid exposure when possible and (*b*) toxicity to benthic organisms can be dependent on sediment characteristics, dynamics of equilibrium partitioning, and the route of exposure to the benthic organisms.

5.6The amphipod sediment toxicity test might be used to determine the temporal or spatial distribution of sediment toxicity. Test methods can be used to detect horizontal and vertical gradients in toxicity. Mortality data can be used to indicate the relative toxicity of field collected sediments.

5.7Results of acute tests with toxicants experimentally added to sediments can be used to compare the acute sensitivities of different species and the acute toxicities of different test materials, and to define the effects of various environmental factors on results of such tests.

5.8Results of acute sediment toxicity tests are useful for studying biological availability of, and structure-activity relationships between, test materials in sediment.

5.9Results of acute sediment toxicity tests might be an important consideration when assessing the hazards of materials to aquatic organisms (see Guide E1023) or when deriving sediment quality criteria for aquatic organisms (5) **are not exceeded, yet organisms in or near sediments are adversely affected Chapman, 1989 (68). Sediment toxicity tests might be useful in making decisions regarding the extent of remedial action needed for contaminated sites.**

6.Interferences

6.1Due to the limited time sediment toxicity tests have been practiced, the methodology continues to develop and evolve with time and research needs. Because of the developmental nature of sediment toxicity testing, there are limitations to the methods described in this guide.

6.2Results of acute sediment toxicity tests will depend, in part, on the temperature, water quality, physical and chemical properties of the test sediment, condition of the test organisms, exposure technique, and other factors. Factors potentially affecting results from static sediment toxicity tests might include:

6.2.1Alteration of field sediments in preparation for laboratory testing.

6.2.1.1Maintaining the integrity of the sediment environment during its removal, transport, and testing in the laboratory is extremely difficult. The sediment environment is composed of a myriad of microenvironments, redox gradients, and other interacting physiochemical and biological processes. Many of these characteristics influence sediment toxicity and bioavailability to benthic and planktonic organisms, microbial degradation, and chemical sorption. Any disruption of this environment complicates interpretations of treatment effects, causative factors, and in situ comparisons.

6.2.1.2Testing of sediments at temperatures or salinities other than those at which they were collected might affect contaminant solubility, partitioning coefficients, and other physical and chemical characteristics.

6.2.2Interactions between the sediment particles, overlying water, interstitial water, and humic substances, and the sediment to overlying water ratio.

6.2.3Interactions among chemicals that might be present in test sediment.

6.2.4Realism of using spiked sediment (that is, whether the spiked sediment is at equilibrium and evenly mixed).

6.2.5Photolysis and other processes degrading test chemicals.

6.2.6Maintaining acceptable quality of overlying water.

6.2.7Excess food might change sediment partitioning and water quality parameters.

6.2.8Resuspension of sediment during the toxicity test.

6.2.9Limited opportunity for biological observations during the test because organisms bury in test sediment.

6.2.10Natural geochemical properties of test sediment collected from the field that might not be within the tolerance limits of the test organisms.

6.2.11Recovery of test organisms from the test system.

6.2.12Endemic organisms which might be present in field collected sediments including (*a*) predators, (*b*) species that might be the same as or closely related to the test species, (*c*) microorganisms (for example, bacteria, molds), and algae colonizing sediment and test chamber surfaces.

6.3Static tests might not be applicable to materials that are highly volatile or are rapidly biologically or chemically transformed. Furthermore, the overlying water quality might change considerably from the initial overlying water. Because the experimental chambers are aerated, the procedures can usually be applied to materials that have a high oxygen demand. Materials dissolved in interstitial waters might be removed from solution in substantial quantities by adsorption to sediment particles and to the test

chamber during the test. The dynamics of contaminant partitioning between solid and dissolved phases at the initiation of the test should therefore be considered, especially in relation to assumptions of chemical equilibrium.

7.Apparatus

7.1*Facilities*—Flow-through troughs or aquaria containing either clean (uncontaminated) natural sea water or reconstituted sea water should be used for holding amphipods after field collection and prior to a test. The holding tanks and any areas used for manipulating live amphipods should be located in a room or space separate from that in which toxicity tests are to be conducted, stock solutions or test materials are prepared, or equipment is cleaned. The water supply system should be equipped with salinity and temperature control and aeration.

7.1.1Test chambers containing sediment should be held in a well-lighted (at least 100 l× at the test sediment surface), constant temperature room, incubator, or recirculating water bath to maintain the experimental temperature. Air used for aeration should be free of fumes, oil, and water; filters to remove oil and water are desirable. The area containing the test chambers must be well ventilated and free of fumes, both to prevent contamination of test materials and to protect researchers from exposure to toxic volatile materials that might be released from the test sediments. Enclosures may be needed to ventilate the area surrounding test chambers.

7.1.2The exposure room should be equipped with a timing device for photoperiod control. If a photoperiod other than continuous light is used, it might be desirable to incorporate a 15 to 30-min transition period when lights go on or off to reduce stress to the organisms from sudden large changes in light intensity **(10. 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, whole sediment concentrations have not been strongly correlated to bioavailability Burton, 1991 (69). It is also desirable to have the room temperature and light controls and the aeration on emergency power to protect the experiment in case of a power failure.**

7.2*Construction Materials*—Equipment and facilities that contact stock solutions, test solutions, or any water or sediment into which test organisms will be placed should not contain substances that can be leached or dissolved by aqueous solutions in amounts that adversely affect test organisms. In addition, equipment and facilities that contact stock or test solutions or sediment should be chosen to minimize sorption of test materials from water. Glass, Type 316 stainless steel, nylon, high-density polyethylene, polycarbonate and fluorocarbon plastics should be used whenever possible to minimize dissolution, leaching, and sorption, except that stainless steel should not be used in tests on metals in salt water. Concrete and rigid plastics may be used for holding tanks and in the water-supply system, but they should be soaked, preferably in flowing sea water, for a week or more before use **(11. 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 Di Toro et al. 1991(70) . Brass, copper, lead, cast iron pipe, galvanized metal, and natural rubber should not contact test sea water, stock solutions, or test sediment before or during the test. Tubing used in making up test sea water and in aerating the test chambers should be nontoxic vinyl. New tubing should be aged at least one week prior to use. Separate sieves, dishes, containers, and other equipment should be used to handle test sediment or other toxic materials and these should be kept and stored separately from those used to handle live animals prior to testing.**

7.3*Test Chambers*—Species specific information on test chambers is given in Annex A2-Annex A5. The test chambers should be placed in water bath to minimize temperature fluctuations, and should be aerated. Aeration can be provided as in 13.1Giesy et al. 1988 **(71)**. 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 Kemp et al. 1988, **(72)**. 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 (Test Method E 1706, Guide E 1525, Guide E 1850; Annex A1, Annex A2; USEPA, 2000 **(73)**, EPA 1994b, **(74)**, Environment Canada 1997a, **(75)**, Enviroment Canada 1997b,**(76)**). 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 that 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 USEPA, 2000 **(73)**; Sibley et al.1996, **(77)**; Sibley et al.1997a, **(78)**; Sibley et al.1997b, **(79)**; Benoit et al.1997, **(80)**; Ingersoll et al.1998, **(81)**). Sublethal endpoints in sediment tests might also prove to be better estimates of responses of benthic communities to contaminants in the field, Kembel et al. 1994 **(82)** . Insufficient information is available to determine if the long-term test conducted with *Leptocheirus plumulosus* (Annex A2) is more sensitive than 10-d toxicity tests conducted with this or other species.