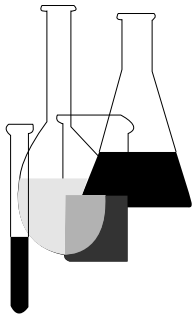
United States
Environmental Protection
Agency

Prevention, Pesticides
and Toxic Substances
(7101)

EPA 712–C–96–355
April 1996

Ecological Effects Test Guidelines

OPPTS 850.1740 Whole Sediment Acute Toxicity Invertebrates, Marine



“Public Draft”

INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Public Draft Access Information: This draft guideline is part of a series of related harmonized guidelines that need to be considered as a unit. *For copies:* These guidelines are available electronically from the EPA Public Access Gopher (gopher.epa.gov) under the heading ‘‘Environmental Test Methods and Guidelines’’ or in paper by contacting the OPP Public Docket at (703) 305–5805 or by e-mail: guidelines@epamail.epa.gov.

To Submit Comments: Interested persons are invited to submit comments. By mail: Public Docket and Freedom of Information Section, Office of Pesticide Programs, Field Operations Division (7506C), Environmental Protection Agency, 401 M St. SW., Washington, DC 20460. In person: bring to: Rm. 1132, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA. Comments may also be submitted electronically by sending electronic mail (e-mail) to: guidelines@epamail.epa.gov.

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on *The Federal Bulletin Board*. By modem dial 202–512–1387, telnet and ftp: fedbbs.access.gpo.gov (IP 162.140.64.19), or call 202–512–0135 for disks or paper copies. This guideline is also available electronically in ASCII and PDF (portable document format) from the EPA Public Access Gopher (gopher.epa.gov) under the heading ‘‘Environmental Test Methods and Guidelines.’’

OPPTS 850.1740 Whole sediment acute toxicity invertebrates, marine.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*).

(2) [Reserved]

(b) **Objective.** This guideline may be used to determine the toxicity and bioaccumulation potential of chemicals in estuarine or marine sediments in marine invertebrates. Natural sediment is spiked with different concentrations of pesticide or contaminant and the results from the sediment toxicity tests can be used to determine causal relationships between the chemical and biological response. Reported endpoints from whole sediment toxicity tests include the LC50 (median lethal concentration), EC50 (median effective concentration), NOEC (no-observable-effect-concentration), or the LOEC (lowest-observable-effect-concentration).

(c) **Definitions.**

Clean. Clean denotes a sediment or water that does not contain concentrations of test materials which cause apparent stress to the test organisms or reduce their survival.

Concentration. Concentration is the ratio of weight or volume of test material(s) to the weight or volume of sediment.

Contaminated sediment. Contaminated sediment is sediment containing chemical substances at concentrations that pose a known or suspected threat to environmental or human health.

Control sediment. Control sediment is 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.

Effect concentration (EC). Effect concentration is the toxicant concentration that would cause an effect in a given percent of the test population. Identical to LC when the observable adverse effect is death. For example, the EC50 is the concentration of toxicant that would cause death in 50% of the test population.

Inhibition concentration (IC). Inhibition concentration is the toxicant concentration that would cause a given percent reduction in a non-quantal measurement for the test population. For example, the IC25 is the concentration of toxicant that would cause a 25% reduction in growth for

the test population and the IC50 is the concentration of toxicant that would cause a 50% reduction.

Interstitial water or pore water. Interstitial water or pore water is water occupying space between sediment or soil particles.

Lethal concentration (LC). Lethal concentration is the toxicant concentration that would cause death in a given percent of the test population. Identical to EC when the observable adverse effect is death. For example, the LC50 is the concentration of toxicant that would cause death in 50% of the test population.

Lowest observable effect concentration (LOEC). Lowest observable effect concentration is the lowest concentration of a toxicant to which organisms are exposed in a test which causes an adverse effect on the test organisms (i.e., where the value for the observed response is statistically significant different from the controls).

No observable effect concentration (NOEC). No observable effect concentration is the highest concentration of a toxicant to which organisms are exposed in a test that causes no observable adverse effect on the test organisms (i.e., the highest concentration of a toxicant in which the value for the observed response is not statistically significant different from the controls).

Overlying water. Overlying water is the water placed over sediment in a test chamber during a test.

Reference sediment. Reference sediment is 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.

Reference-toxicity test. Reference-toxicity test is a test conducted in conjunction with sediment tests to determine possible changes in condition of the test organisms. Deviations outside an established normal range indicate a change in the condition of the test organism population. Reference-toxicity tests are most often performed in the absence of sediment.

Sediment. Sediment is particulate material that usually lies below water. Formulated particulate material that is intended to lie below water in a test.

Spiked sediment. Spiked sediment is a sediment to which a material has been added for experimental purposes.

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

(d) **Test method.** (1) Whole sediment toxicity tests are outlined for four species of estuarine/marine amphipods, *Ampelisca abdita*, *Eohaustorius estuarius*, *Rhepoxynius abronius*, and *Leptocheirus plumulosus*. Whole sediment tests last 10 or more days, and are conducted in in 1–L test chambers containing 175 mL (2 cm) of sediment and 800 mL of overlying water. The overlying water does not have to be renewed and test organisms do not have to be fed during the toxicity test. The endpoint is survival, but reburial for *E. estuarius*, *L. plumulosus*, and *R. abronius* is optional.

(2) A range-finding test to establish a suitable range of test concentrations is recommended. If no toxicity is observed at concentrations of 100 mg/kg dry weight of sediment, a definitive test is not required.

(e) **Water, reagents, and standards**—(1) **Water.** (i) Sea water used in sediment toxicity test should be of uniform quality and allow satisfactory survival, growth, or reproduction of the test organisms. Organisms cultured and tested in the selected sea water should not show signs of disease or stress.

(ii) Natural sea water should be from uncontaminated surface-water upstream of known discharges. Sea water should be collected at slack high tide or within 1 h of high tide. Full strength sea water should be collected from areas with salinities of 28 ppt. Sea water for estuarine test may be collected from areas with salinities close to the test salinity or diluted with freshwater. Water prepared from natural sea water should be covered, maintained at 4 °C, and used with 2 days.

(iii) Although natural sea water is preferable, reconstituted water is acceptable. Reagent grade chemicals should be added to high-purity distilled or deionized water (1 MΩ). Each batch of reconstituted water should be measured for salinity, pH, and dissolved oxygen (DO). Suspended particles should be removed by filtration (≤5 μm) from reconstituted water at least 24 h before use.

(2) **Reagents.** All reagents and chemicals purchased from supply houses should be accompanied by appropriate data sheets. All test materials should be reagent grade. However, if specified as necessary, commercial product, technical-grade, or use-grade materials may be used. Dates for receipt, opening, and shelf-life should be logged and maintained for all chemicals and reagents. Do not use reagents beyond shelf-life dates.

(3) **Standards.** Acceptable standard methods for chemical and physical analyses should be used. When appropriate standard methods are not

available or lack the required sensitivity, other sources should be consulted for reliable methods.

(f) **Sample collection, storage, manipulation, and characterization**—(1) **Sample collection.** (i) Procedures for handling natural sediments should be established prior to collection. Pertinent data such as location, time, core depth, water depth, and collection equipment should be recorded.

(ii) Replicate sampling should be used for the collection of natural sediment to determine the variance in sediment characteristics. While some disruption of the sediment is inevitable regardless of the sampling equipment used, disruption of sediment should be kept to a minimum. Several devices are available for collecting sediment, but benthic grab or core samplers are recommended. The depth of sediment collected should reflect the expected exposure. During sediment collection, exposure to direct sunlight should be kept to a minimum. Cooling of sediment to 4 °C is recommended.

(2) **Storage.** Storage of sediment may affect bioavailability and toxicity. Although nonionic and nonvolatile organic contaminates in sediment may not result in substantive changes, metals and metalloids may affect redox, oxidation, or microbial metabolism in sediment. It is best to hold sediments at 4 °C in the dark and test within 2 to 8 weeks after collection. Long storage may result in changes of sediment properties. Sediment tests, and especially pore water tests, should be performed within 2 weeks of collection to minimize property changes in the sediment.

(3) **Manipulation.** (i) During homogenization, water above sediment that may have settled during shipment should be mixed back into the sediment. Sieving should not be used to remove indigenous organisms, unless an excessive number of oligochaetes are present. Because oligochaetes may inhibit the growth of the test organisms, it may be advantageous to remove them by sieving. If sieving is used, sediment samples should be analyzed before and after sieving to document the influence of sieving on sediment characteristics. Sediments collected from multiple locations or sites may be pooled and mixed using suitable apparatus (e.g. stirring, rolling mill, feed mixer, etc.).

(ii) The preparation of test sediment may be accomplished by the spiking of natural or formulated sediments. Additional research is needed before formulated sediments may be used routinely. The responses of spiked sediment may be affected by mixing time and aging. Spiked sediment should be used immediately. Point estimates of toxicity or minimum concentrations at which toxic effects are observed may be determined by spiking natural sediments with a range of chemical concentrations. The test material should be reagent grade unless there is a specific need to use commercial product, technical-grade, or use-grade material. Specific

information required for all test materials include but is not limited to the following:

(A) Identity and concentration of major ingredients and impurities.

(B) Solubility in test water.

(C) Estimated toxicity to the test organism and to humans.

(D) When measured test concentrations are required, the precision and bias of analytical method at the planned concentrations of test material.

(E) Recommended handling and disposal procedures.

(iii) Organic solvents should not be added to the sediment mixture because they may affect the concentration of dissolved organic carbon in pore water.

(4) **Characterization.** (i) The characteristics of all sediment should be determined, and at a minimum the following factors should be measured: pH and ammonia concentration of pore water, organic carbon content (total organic carbon, TOC), particle size distribution (percent sand, silt, clay), and percent water content. Additional analyses are suggested and include biological oxygen demand, chemical oxygen demand, cation exchange capacity, Eh, total inorganic carbon, total volatile solids, acid volatile sulfides, metals, synthetic organic compounds, oil and grease, and petroleum hydrocarbons. Various physicochemical parameters should also be determined for interstitial water. Sediment characterization should also include qualitative parameters such as color, texture, and the presence of macrophytes or animals.

(ii) Standard analytical methods should be used to determine chemical and physical data. For each analytical method, precision, accuracy, and bias should be determined in sediment, water, and tissue. Analysis should include analytical standards and reagent blanks as well as recovery calculations.

(iii) Concentrations of spiked chemicals should be measured in sediment, interstitial water, and overlying water at the beginning and at the end of the test. Degradation products should also be measured where appropriate. Sediment chemistry should be monitored during and at the end of a test. Separate replicates resembling the biological replicates and containing organisms should be specified for chemical sampling. The concentration of test material in water is measured by pipetting water samples from 1 to 2 cm above the sediment surface. Caution should be used to eliminate the presence of any surface debris, material from the sides of the chamber, or sediment in the overlying water sample. At the end of the test, the test material may be removed for chemical analysis by siphoning (without disturbing sediment) the overlying water. Appropriate samples

of sediment can then be removed for chemical analysis. The suggested method for isolation of interstitial water is by centrifugation without filtration.

(g) **Collection and maintainence of test organisms.** (1) The methods for collection of test organisms are species-specific. Subtidal species, such as *A. abdita* and *L. plumulosus*, may be collected with a small dredge or grab and may also be collected by skimming the sediment surface with a long-handled, fine-mesh net. *E. estuarius* and *R. abronius* are found both subtidally and intertidally. The aforementioned methods are suitable for subtidal populations. Intertidal populations may be collected using a shovel. Approximately one-third more organisms should be collected than are required for testing.

(2) All collecting, sieving, and transporting equipment should be clean and constructed of nontoxic material and clearly marked ‘‘live only’’. All apparatus should be cleaned and rinsed with an appropriate water source before use.

(3) Collected organisms should be handled gently, carefully, quickly, and no more than necessary. The sieving operation should be conducted by slowly immersing the sieve into collection site water. Sieved test organisms should be kept submerged in ambient collection water at all times. Direct exposure to sunlight of amphipods out of sediment must be avoided.

(4) *A. abdita* and *L. plumulosus* should be isolated from collection site sediment by using a 0.5 mm mesh sieve. *A. abdita* which remain in tubes must be left undisturbed for 20 to 30 min to allow for natural exit of the organisms. A 1.0 mm sieve should be used to isolate *E. estuarius* and *R. abronius*.

(5) *A. abdita* and *L. plumulosus* may be collected with a small dredge or grab apparatus (e.g. PONAR, Van Veen, etc.) and *E. estuarius* and *R. abronius* may be collected with a shovel. Collected amphipods should be sieved in the field by slow immersion in collection site water. Sieved amphipods should be separated from detritus and predators and transferred gently to transport containers containing 2 cm of collection site sediment. Mesh sizes of 0.5 to 1.0 mm should be utilized. Salinity and temperature of collection site sediment should be recorded surface and bottom locations. Amphipods should be transported in coolers with ice packs and held in the collection-site sediment at or below the temperature at the collection site. Aeration is recommended for transport times exceeding 1 h. Collection site sediment should be used as holding sediment in the laboratory and as control test sediment.

(h) **Ampelisca abdita, Eohaustorius estuarius, Leptocheirus plumulosus, or Rhepoxynius abronius 10– to 28–Day Survival Test For Sediments**—(1) **Recommended test method.** The recommended test temperatures for conducting sediment toxicity tests with *E. estuarius* and *R.*

abronius, *A. abdita*, and *L. plumulosus* are 15, 20, and 25 °C, respectively. *E. estuarius* and *L. plumulosus* should be tested at a salinity of 20 ppt and *A. abdita* and *R. abronius* at a salinity of 28 ppt. The recommended photoperiod is 24 h of light with illumination of 500 to 1,000 lx. Sediment (175 mL) and 800 mL of overlying seawater are placed in 1–L glass test chambers. Twenty organisms are placed in each test chamber to begin the test. Five replicates per treatment are recommended, however, this number may vary depending upon need. The size and life stage of amphipod required for testing varies from 2–4 mm for *L. plumulosus* to 3–5 mm for the three remaining species. Additionally, no mature male or female *A. abdita* or *L. plumulosus* should be used for testing. Overlying water does not have to be renewed and test organisms do not have to be fed during the test.

(2) **General procedure**—(i) **Introduction of sediment.** The test sediment should be homogenized one day before the test is to commence (day −−1) using a rolling mill, feed mixer, or other suitable apparatus. The sediment should be observed for homogeneity visually and quantitatively by measuring TOC, chemical concentrations, and particle size. A 175–mL aliquot of homogenized sediment should be added to each test chamber, and settled with the use of a nylon, fluorocarbon, or polyethylene spatula.

(ii) **Addition of overlying water.** A turbulence reducer (a disk cut from polyethylene, nylon, or Teflon, or a glass Petri dish attached to a glass pipet) should be used when adding overlying water. Turbulence reducers should be rinsed with seawater between replicates, and individual turbulence reducers used between treatments. Test chambers should be covered, immersed in a temperature bath, and gently aerated. The test commences once the test organisms are added to the test chambers (day − 0).

(iii) **Addition of amphipods.** Twenty amphipods are randomly added to each test chamber in batches of 5 to 10 on day −−0 following the addition of sediment and overlying water. One-third more amphipods than necessary are sieved from culture or control sediment and transferred to sorting trays. Recommended sieve sizes are 0.5 mm for *A. abdita* and *L. plumulosus* and 1.0 mm for *E. estuarius* and *R. abronius*. Isolated amphipods are transferred from the sorting tray to 150 mL of test sea water using pipets. The test organisms are observed for injury or stress after addition. If any *E. estuarius*, *L. plumulosus*, and *R. abronius* have not burrowed within 5 to 10 min, they should be replaced. *A. abdita* that have not burrowed within 1 h should also be replaced. Organisms expressing sediment avoidance, should be removed, recorded, but not replaced.

(3) **Test conditions**—(i) **Aeration.** Overlying sea water should be continuously aerated from day − 1 to day − 10 except when test organisms are being added. DO should be maintained at approximately 90 percent

saturation using gentle aeration without disturbing the sediment. Results are unacceptable if DO falls to below 60 percent saturation.

(ii) **Lighting.** Lights must be left on for the duration of the 10–day testing period. The constant light assures that the test organisms to remain burrowed during the test.

(iii) **Feeding.** None of the four test species need to be fed during the 10–day testing period.

(iv) **Water temperature.** The respective selected test temperatures are representative of the summertime thermal maximum for each species. *E. estuarius* and *R. abronius* (Pacific Coast amphipods) must be tested at 15 °C. *A. abdita* and *L. plumulosus* must be tested at 20 °C and 25 °C, respectively.

(v) **Salinity.** Overlying water salinity should be 28 ppt for *A. abdita* and *R. abronius* and 20 ppt for *E. estuarius* and *L. plumulosus*. Pore water salinity must be measured prior to the start of the test.

(4) **Measurements and observations.** (i) Temperature should be measured daily from at least one replicate from each treatment. Temperature of the water bath or exposure chamber must be monitored continuously.

(ii) Salinity, DO, and pH should be measured in overlying water daily in one test chamber in each treatment. These parameters should be measured in all test chambers at the beginning of the test and at termination.

(iii) Ammonia concentration should be measured near day – 2 and day – 8 during the 10–day test period. Ammonia concentration measurements should be accompanied by pH and temperature measurements. pH, temperature, and ammonia concentration should be measured in pore water at the beginning of the test.

(iv) Air-flow to overlying sea water must be monitored daily. Any test organisms trapped in air-water interface must be gently pushed back down using a glass rod or pipet.

(5) **Ending a test.** (i) Recovery of organisms from control sediment should equal or exceed 90 percent in a 10–day test or 80 percent in a 28–day test.

(ii) Test animals are isolated from the test chambers by sieving with sea water. Sieves should not exceed 0.5 mm. Test organisms should be washed into sorting trays containing sea water. Caution should be taken that no tube-dwelling organisms remain trapped on the sieve. Slapping the sieve forcefully against the surface of the water should successfully dislodge all *A. abdita*. The remaining species should be easily separated by the sieving process.

(iii) Small portions of material should be washed into sorting trays and should be examined carefully. The tubes of *A. abdita* should be teased apart under a dissecting microscope to ensure that all organisms are accounted for. The numbers of living, missing, or dead amphipods should be observed and recorded for all test chambers. Missing animals and all observed animals failing to respond to gentle prodding (i.e. neuromuscular twitch of pleopods or antennae) are recorded as dead.

(6) **Test data.** The primary endpoint for the 10–day sediment test is survival. Effective mortality (the sum of dead animals plus survivors that fail to rebury) may also be determined. To determine reburial, *E. estuarius*, *L. plumulosus*, and *R. abronius* should be transferred to a 2–cm layer of 0.5 mm sieved control sediment and overlying test sea water (2 cm).

(7) **Interpretation of results**—(i) **Influence of indigenous organisms.** Because test sediments collected from the field may contain indigenous species, data interpretation may be complicated by the presence of organisms similar to the test organism or predatory organisms.

(ii) **Effect of grain size.** While the four estuarine/marine test species are generally tolerable of a wide range of sediment types, grain size may adversely affect some species of amphipod. When this possibility exists, a clean control/reference sediment should be incorporated into the test design to facilitate distinction of contaminant effects versus particle size effects. Species-specific ranges of grain sizes are as follows.

(A) *A. abdita*: Survival may be impacted in sediments containing 95 percent or more sand. Test sediment should contain less than 95 percent sand.

(B) *L. plumulosus*: Survival should not be impacted in clean sediments containing 100 percent sand to 100 percent sand + clay.

(C) *E. estuarius*: Survival is unaffected by clean sediments containing 0.6 to 100 percent sand. However increased mortality may be associated with increased proportions of fine-grained sediment. In these cases an appropriate control/reference should be included.

(D) *R. abronius:* Very fine grains, particularly silts and clays, may reduce survival of this species. When test sediments contain silts and clays, the use of control/reference groups with particle sizes characteristic of the test sediment is recommended.

(iii) **Effects of pore water salinity.** The range of salinity tolerance is variable for the four amphipod species. For sediment testing, two scenarios for test salinity are acceptable given that appropriate conditions are met:

(A) *Salinity tolerance range* is the range of salinity in which a given species can survive for 10 days when the overlying water salinity is matched to that of the pore water salinity. In laboratory sediment testing, the overlying water salinity can be based on the standard salinity for each test species, or adjusted to match the salinity of pore water. It is critical that pore water salinity be measured prior to test initiation and that the appropriate species be used. Salinity tolerance ranges for *A. abdita*, *E. estuarius*, *L. plumulosus*, and *R. abronius* are 20–32 ppt, 2–34 ppt, 1.5–32 ppt and 25–32 ppt, respectively.

(B) *Salinity application range* is the range of pore water salinities in which a given species can survive for 10 days when using the species-specific overlying water salinity. Salinity application ranges for *A. abdita* with overlying water salinity of 28 to 32 ppt, *E. estuarius* with overlying water salinity of 20 ppt, *L. plumulosus* with overlying salinity of 20 ppt, and *R. abronius* with overlying water salinity of 28 to 32 ppt are 0 to 34, ≤2 to 34, ≤1.5 to 32, and 25 to 34, respectively.

(iv) **Effects of sediment-associated ammonia.** Ammonia concentrations in field-collected sediments may be toxic to amphipods. When ammonia concentration exceeds the water column no-effect levels, any mortality observed during the 10–day sediment test may be due to the ammonia. Ammonia levels should be measured approximately 1 cm above the sediment surface on day – 0 and, if necessary, reduced prior to the addition of test organisms by flushing the overlying water for up to two consecutive 24–h periods (six volume replacements per hour). Following flushing, the overlaying water ammonia concentration should be remeasured. If ammonia is at acceptable levels testing may be initiated but flushing at a rate of sic volume changes per 24–h period must be maintained throughout the test. Ammonia concentrations in overlaying water should be measured again on day – 10. If ammonia is not at acceptable levels, 24–h flushings must continue at the six–volume change per 24 h rate and ammonia concentration measured every 24 h.

(i) **Interferences.** (1) Interferences are characteristics of sediment or the sediment test system with potential to affect survival of test organisms independent of sediment-associated contaminant affects. Interferences are categorized into three categories: Noncontaminant factors causing reduced survival, changes in bioavailability due to manipulation or storage, and the presence of indigenous species. Noncontaminant factors can make extrapolation of laboratory test results to the field difficult. Specifically, the motility of organisms (i.e. escapism) and photoinduced toxicity due to UV light in from the sun (e.g. UV light absent from fluorescent light) may be markedly different between laboratory conditions and the natural environment. Other noncontaminant factors include sediment particle size, pore water salinity, and pore water ammonia concentration. The test conditions must be matched appropriately with the tolerance limits of the four amphipod test species (see paragraph (k)(1) of this guideline).

(2) Bioavailability of sediment-associated contaminants can be altered by collection, handling, and storage. The handling, storage, and preparation of test sediment should be as consistent as possible. Test sediments should be presieved and rehomogenized prior to introduction into the test chambers. Bioavailability may also be affected by temperature, salinity, the ratio of sediment to overlying water, and the depletion of contaminant due to organismal uptake. In some cases it is advantageous to normalize sediment concentrations to dry weight, organic-carbon content, or acid volatile sulfides.

(3) Test sediment collected from the field may contain indigenous organisms, and can potentially make interpretation of treatment effects difficult. If the presence of indigenous or predatory organisms is suspected, the test sediment should be press-sieved prior to test initiation.

(j) **Reporting.** In addition to information meeting the general reporting requirements of a toxicity test, a report of the results of a sediment toxicity test for estuarine and marine amphipods should include the following:

(1) Name of test and investigators, name and location of laboratory, and dates of start and end of test.

(2) Source of control or test sediment, method for collection, handling, shipping, storage, and disposal of sediment.

(3) Source of test material, lot number if applicable, composition (identities and concentrations of major ingredients and impurities if known), known chemical and physical properties, and the identity and concentrations of any solvent used.

(4) Source and characteristics of overlying water, description of any pretreatment, and results of any demonstration of the ability of an organism to survive or grow in the water.

(5) Source, history, and age of test organisms; source, history and age of brood stock, culture procedures; and source and date of collection of the test organisms, scientific name, name of person who identified the organisms and the taxonomic key used, age or life stage, means and ranges of weight or length, observed diseases or unusual appearance, treatments, holding procedures.

(6) Source and composition of food, concentrations of test material and other contaminants, procedure used to prepare food, feeding methods, frequency and ration.

(7) Description of the experimental design and test chambers, the depth and volume of sediment and overlying water in the chambers, lighting, number of test chambers an number of test organisms/treatment, date and time test starts and ends, temperature measurements, DO concentration

(as percent saturation) and any aeration used before starting a test and during the conduct of a test.

(8) Methods used for physical and chemical characterization of sediment.

(9) Definitions of the effects used to calculate LC50s or EC50s, biological endpoints for tests, and a summary of general observations of other effects.

(10) A table of the biological data for each test chamber for each treatment including the controls in sufficient detail to allow independent statistical analysis.

(11) Methods used for statistical analyses of data.

(12) Summary of general observations on other effects or symptoms.

(13) Anything unusual about the test, any deviation from these procedures, and any other relevant information.

(k) **References.** The following references should be consulted for additional background material on this test guideline.

(1) U.S. Environmental Protection Agency. Methods for Assessing the Toxicity of Sediment-Associated Contaminants with Estuarine and Marine Amphipods. EPA 600/R–94/025 (1994).

(2) [Reserved]