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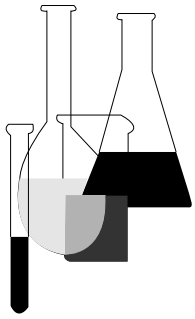
Ecological Effects Test Guidelines

OPPTS 850.1735 Whole Sediment Acute Toxicity Invertebrates, Freshwater



“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 850.1735 Whole sediment acute toxicity invertebrates, freshwater.

(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 sediments in freshwater invertebrates. Natural sediment is spiked with different concentrations of test chemical 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 may 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.

ppt. ppt is parts per thousand.

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 the amphipod, *Hyalella azteca* and the midge, *Chironomus tentans*. Duration of whole sediment tests is 10 to 28 days and is accomplished in 300–mL test chambers containing 100 mL of sediment and 175 mL of overlying water. The overlying water may be renewed daily or a flow-through system may be used. Test organisms are fed during the toxicity test. The endpoint for *H. azteca* is survival, and for *C. tentans*, survival, growth and/or emergence.

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

(e) **Water, formulated sediment, reagents, and standards**—(1) **Water.** (i) Testing and culture water must be of uniform quality, and is acceptable if it allows satisfactory survival, growth, and reproduction of the test organisms. Disease or apparent stress (e.g. discoloration, unusual behavior) should not be prevalent. If problems occur during testing or culturing, water characteristics should be analyzed.

(ii) Natural water is considered to be of uniform quality if the ranges of hardness, alkalinity, and specific conductance are within 10 percent of the respective averages. The monthly pH range should be < 0.4 units. Sources of natural water should be uncontaminated well or spring or surface water. Special considerations for surface water include minimizing quality and contamination variables, maximizing the levels of DO, and confirming that sulfides and iron levels are low. Chlorinated water should not be used for testing or culturing because chlorine-produced oxidants and residual chlorine are toxic to aquatic organisms. Tap water is acceptable if it is dechlorinated, deionized, and carbon filtered, but its use is not encouraged.

(iii) If source water is contaminated with facultative pathogens, it should be UV-irradiated using intensity meters and flow-controls, or filtered through 0.45 μm pore size.

(iv) The DO concentration of source water should be between 90 and 100 percent saturation. In some cases aeration may be required using air stones, surface aerators, or column aerators.

(v) High-purity distilled or deionized water may be reconstituted by adding specified amounts of reagent grade chemicals. The deionization

system should produce water with a resistance of 1 MΩ. For each batch of reconstituted water, the following parameters should be measured: Conductivity, pH, hardness, DO, and alkalinity. Aeration should be employed to maintain acceptable levels of pH and DO.

(vi) The preparation of 100 L of reconstituted water was developed at the USEPA EMSL-Cincinnati and has been tested with *H. azteca*, *C. tentans*, and *Chironomus riparius* in round-robin tests and is given as follows:

(A) Add approximately 75 L of deionized water to a properly cleaned container capable of holding 100 L.

(B) Add 5 g of $CaSO_4$ and 5 g of $CaCl_2$ to a 2–L aliquot of deionized water and mix (e.g., on a stir plate) for 30 min or until the salts dissolve.

(C) Add 3 g of $MgSO_4$, 9.6 g $NaHCO_3$, and 0.4 g KCl to a second 2-L aliquot of deionized water and mix on a stir plate for 30 min.

(D) Pour the two 2-L aliquots containing the dissolved salts into the 75 L of deionized water and fill the carboy to 100 L with deionized water.

(E) Aerate the mixture for at least 24 h before use.

(F) The water quality of the reconstituted water should be approximately the following: Hardness, 90 to 100 mg/L as $CaCO_3$, alkalinity 50 to 60 mg/L as $CaCO_3$, conductivity 330 to 360 μS/cm, and pH 7.8 to 8.2.

(vii) Synthetic seawater may be prepared by adding commercial sea salts to deionized water. *H. azteca* may be cultured or tested at salinities up to 15 ppt.

(2) **Artificial sediment.** Artificial sediments consist of mixtures of materials designed to mimic natural sediments. Because artificial sediments have not been used routinely to assess the toxicity of contaminants in sediment, the use of uncontaminated natural sediment is recommended. If the use of artificial sediment is necessary, detailed information may be found in paragraph (l)(1) of this guideline.

(3) **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.

(4) **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 contaminants 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 microorganisms, 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 as well as other macroorganisms, rocks, wood, and the like 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 artificial sediments. Additional research is needed before artificial sediments may be used routinely. The responses of spiked sediment may be affected by mixing time and aging. Spiked sediment may be aged for at least 1 month to achieve equilibrium with the spiked chemicals, if the chemical is known to be persistent. Sediments spiked with industrial chemicals should be used as soon as possible. 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 includes 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 the 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. and should not be used.

(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. Precision, accuracy, and bias should be determined in sediment, water, and tissue for each analytical method. Analysis should include analytical standards and reagent blanks as well as recovery calculations.

(iii) Concentrations of spiked chemicals may be measured in sediment, interstitial water, and overlying water at the beginning and at the end of the test if so required. Measurement of degradation products may also be required. 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, culture, and maintainence of test organisms**—(1) **Hyalella azteca**—(i) **Life history.** (A) *H. azteca* are found throughout North and South America in permanent lakes, ponds, and streams. They are commonly found in mesotrophic or eutrophic lakes that are capable of supporting aquatic plants and that remain warm (20 to 30 °C) for most of the summer months. Densities may exceed 10,000 M^2 in optimal habitats. *H. azteca* are epibenthic detritivores that burrow into the sediment. They may be found in saline waters up to 29 percent, but are sensitive to hardness (e.g. they are not found in waters with calcium at < 7 mg/L and DO at < 2 mg/L).

(B) *H. azteca* reproduce sexually, averaging 18 eggs per brood and approximately 15 broods every 152 days. Hatching occurs approximately 5 to 10 days after fertilization at 24 to 28 °C. They proceed through a minimum of 9 instars, which are separated into 5 to 8 prereproductive instars and an indefinite number of postreproductive instars. Instars 1 through 5 form the juvenile life stage, instars 6 and 7 form the adolescent stage of development, instar 8 is the nuptial life stage, and later instars form the adult stages of the amphipod.

(C) *H. azteca* may be cultured under illumination of 500–1,000 lx. They feed during daylight and avoid bright light by hiding under litter.

(D) *H. azteca* is tolerable of a wide range of temperatures (0–33 °C), but are immobile at temperatures < 10 °C and die at temperatures > 33 °C. Reproduction can occur at temperatures of 10–18 °C, but the highest rate of reproduction occurs at temperatures between 26 and 28 °C.

(E) *H. azteca* can tolerate a wide range of substrates. Survival and growth of have not been shown to be negatively affected by either particle size (> 90 percent silt and clay particles to 100 percent sand-sized particles) or grain size and organic matter in 10–day tests. In tests where organisms were not fed, survival decreased.

(ii) **Culturing procedures.** (A) To start a sediment test, 7– to 14–day–old amphipods must be produced. If growth is an endpoint, a narrower range, such as 1– to 2–day–old amphipods should be used. Details and further discussion of acceptable culture procedures for *H. azteca* are presented in paragraph (l)(1) of this guideline.

(B) *H. azteca* should be held and fed under the same conditions as the mass culture for at least 2 days prior to test initiation.

(2) **Chironomus tentans**—(i) **Life history.** (A) *C. tentans* are found in eutrophic ponds and lakes. In soft bottoms, approximately 95 percent of chironomid larvae are found in the upper 10 cm. Chironomid larvae are generally not found in sediments with hydrogen sulfide concentrations > 0.3 mg/L.

(B) The aquatic phases of *C. tentans* include the larval and pupal stages. Female chironomids can oviposit eggs within 24 h of emergence, releasing a single gelatinous egg mass containing roughly 2,300 eggs. Hatch occurs in 2 to 4 days at 23 °C. The emergence of pupae as adults occurs after 21 days at 23 °C.

(C) *C. tentans* are able to tolerate a wide range of grain sizes and percentage organic matter. However, low percentage organic matter in conjunction with no feeding may result in decreased survival. Survival is best above pH 6.5. Poor control survival occurs at pH < 6.5. Growth may also be impacted by coarser sediment.

(ii) **Culturing procedures.** (A) The third instar chironomids must be used to start a sediment test. Larvae should develop to the third instar within 9 to 11 days at a temperature of 23 °C. The instar stage of midges must be confirmed by head capsule width (~0.38 mm). Weight and height of midges should be monitored at the beginning of a sediment test. Details and further discussion of acceptable culture procedures are presented in paragraph (l)(1) of this guideline.

(B) The time to first emergence and the success of emergence should be recorded for all culture chambers. Growth may be monitored by periodically measuring the midge head capsule width.

(h) **Test method: Hyalella azteca 10– to 28–day sediment toxicity test**—(1) **Test conditions.** General test conditions required for a 10–day sediment toxicity test with *H. azteca* are presented in the following table XX. The 10–day sediment toxicity test must be conducted at 23 °C with a 16light:8dar photoperiod. Illumination should be approximately 500 to 1,000 lx. The recommended test chambers are 300–mL high-form beakers without lips containing 100 mL of sediment and 175 mL of overlying water. The test is started using 10 7– to 14–day–old amphipods. Eight replicates/treatment are recommended for routine testing. Because of potential impacts on study results, feed added to the test chamber should be kept to a minimum. Thoroughly mix food prior to removing aliquots. In order to prevent bacterial and fungal growth, feeding should be suspended for 1 to 2 days if food collects on sediment. Feeding should also be suspended if DO falls below 40 percent of saturation. When feeding is suspended in one treatment it should be suspended in all treatments. Feeding rates and appearance of sediment surface should be observed daily

and detailed records manitained. Each chamber should receive 2 volume additions per day or flow-through of overlying water. Sources of overlying water can be culture water, well water, surface water, site water, or reconstituted water.

Table XX.—General Test Conditions for 10-day Sediment Toxicity with *H. azteca*

Parameter	Conditions
1. Test type	Whole-sediment toxicity test with renewal of overlying water
2. Temperature	23± 1 °C
3. Light quality	Wide-spectrum fluorescent lights
4. Illuminance	500 to 1000 Lux
5. Photoperiod	16L:8D
6. Test chamber	300-mL high-form lipless beaker
7. Sediment volume	100 mL
8. Overlying water volume	175 mL
9. Renewal of overlying water	2 volume additions/d
10. Age of organisms	7- to 14- d old at start of test
11. Number of organisms/chamber	10
12. Number or replicate chambers/ treatment.	8
13. Feeding	Feed 1.5 mL daily to each test chamber
14. Aeration	None (unless D.O. drops below 40% of saturation)
15. Overlying water	Culture water, well water, surface water, site water or reconstituted water
16. Test chamber cleaning	Gently brush outside of screen when clogged
17. Overlying water quality	Hardness, alkalinity, conductivity, pH, and ammonia at beginning and end of test; temperature and D.O. daily
18. Test duration	10 - 28 d
19. Endpoints	Survival (growth optional)
20. Test acceptability	Minimum mean control survival of 80% and above conditions

(2) **Sediment into test chambers.** (i) Sediment should be thoroughly mixed and added to test chambers the day before (day – –1) the start of the test. The degree of homogeneity should be inspected visually. Homogeneity may be quantified by taking replicate subsamples and analyzing for TOC, chemical concentration, and particle size.

(ii) Equal amounts of sediments should be added to each test chamber on the basis of volume or dry weight. To minimize disturbance of sediment, overlying water should be poured gently along the sides of the test chambers or poured over a Teflon baffle (with handle) positioned above the sediment. The renewal of overlying water should commence on day – 1. The test begins once organisms are added to the test chambers (day – 0).

(3) **Renewal of overlying water.** Renewal or flow-through of overlying water is recommended during a test. Flow rates through any two test chambers should not differ by more than 10 percent at any time during the test. Each water-delivery system should be calibrated prior to test initiation to verify that the system is functioning properly. Renewal of overlying water is started on day – –1 before the addition of test organisms or food on day – 0.

(4) **Acclimation.** Test organisms must be cultured and tested at 23 °C. The same water used for culture should be used for testing. Acclimation of test organisms to the test water is not required.

(5) **Placement of organisms in test chambers.** Handle test organisms as little as possible. Amphipods may be placed into test chambers by pipetting the organisms directly into the overlying water just below the air-water interface or by placing the organisms into 30–mL counting cups and floating them in the test chamber for 15 min prior to placement into the overlying water. Measurements of length or weight should be made on a subset of 20 organisms prior to test initiation.

(6) **Monitoring a test.** All test chambers should be checked daily. Test organisms should be observed for abnormal behavior, such as sediment avoidance. The exposure system should also be monitored daily to assure proper operation.

(7) **Measurement of overlying water-quality characteristics.** (i) Conductivity, hardness, pH, alkalinity, and ammonia should be measured in all treatments at the beginning and end of a test, and during any test should not vary more than 50 percent. Samples should be removed with a pipet from 1 to 2 cm above the sediment surface without disturbance. Caution is required to avoid removing test organisms when sampling.

(ii) DO should be measured daily, and should be maintained between 40 percent and 100 percent saturation. Both DO and pH may be measured in overlying water using a probe.

(iii) Temperature should be measured daily in one test chamber from each treatment. The mean and instantaneous temperatures should not vary from the desired temperature by more than 1 °C and 3 °C, respectively.

(8) **Feeding.** *H. azteca* may be fed with a mixture of yeast, Cerophyl, and trout chow (YCT) at a rate of 1.5 mL daily per test chamber. Food is required for proper maintenance of the test organisms but should be kept to a minimum to prevent alteration of contaminant availability or the growth of microbials such as fungus and bacteria. Collection of food on the bottom of the test chamber or reduced concentration of DO are indicators of possible overfeeding. Should either of the above conditions occur, feeding should be suspended in all test chambers until conditions have readjusted. Detailed records and observations should be made daily.

(9) **Ending a test.** Surviving amphipods may be pipetted from the test chamber prior to sieving the sediment. Immobile organisms isolated from either sediment or sieved material are considered dead. Sediment may be sieved by pouring one-half of the overlying water volume followed by one-half of the sediment through a #50 sieve (300 μm) into an examination pan. The coarser sediment remaining in the test chamber should be washed through a #40 (425 μm) sieve into a second examination pan.

Surviving organisms should be isolated and preserved (e.g. 8 percent sugar formalin) and measured for growth. The amount of time taken to recover test organisms should be consistent (e.g. 10 min per replicate). A recovery rate of 90 percent of organisms from the sediment is acceptable.

(10) **Test data.** (i) The primary endpoint for 10–day sediment toxicity test with *H. azteca* is survival.

(ii) Amphipod body length should be measured from the base of the first of antenna to the tip of the third uropod along the curve of the dorsal surface.

(iii) To determine dry weight of surviving amphipods:

(A) Pool all surviving organisms from a replicate.

(B) Dry the sample to constant weight at 60 to 90 °C.

(C) Bring sample to room temperature in a desiccator.

(D) Weigh the sample of organisms to the nearest 0.01 mg. This measure will give the mean weight of surviving organisms per replicate.

(11) **Interpretation of results**—(i) **Age sensitivity.** The relative sensitivity of *H. azteca* is comparable up to 24– to 26–day–old organisms. Amphipods 7– to 14–day–old represent sensitivity of *H. azteca* up to adult life stage.

(ii) **Grain size.** *H. azteca* tolerate a wide range of substrates. Neither grain size nor TOC correlate with the toxic response in sediment toxicity tests.

(iii) **Isolating organisms at the end of a test.** Quantitative recovery of amphipods < 7–days–old is difficult. Starting testing with 7–day–old amphipods facilitates recovery.

(iv) **Influence of indigenous organisms.** The presence of oligochaetes does not reduce the survivability of amphipods in 28–day sediment tests. However, high density of oligochaetes does reduce the growth of amphipods. The number of oligochaetes and presence of predators in test sediment should be determined to improve the interpretation of growth data.

(i) **Interferences.** (1) Interferences are defined as those characteristics of sediment or sediment test systems that are unrelated to sediment-associated contaminants, but have the potential to affect the survival of test organisms. Interferences may lead to both Type I (false-positive) and Type II (false-negative) errors.

(2) Interferences may result from sediment characteristics that affect survival independently of chemical concentration, altered bioavailability

(e.g. sediment manipulation, storage, etc.), or when indigenous species are present.

(3) Test procedures and organism selection criteria were designed to minimize impacts due to interferences, and are suitable for providing direct measure of contaminant effects on benthic organisms.

(4) Several noncontaminant factors have the potential to affect sediment toxicity. These factors include but are not limited to avoidance, lighting, and geomorphological and physicochemical characteristics. Although laboratory sediment toxicity tests results may be used to predict effects in the field, extrapolations to the field may not prove valid in cases where motile organisms are able to avoid exposure.

(5) Toxicological responses of some chemicals may be altered by UV radiation contained in natural sunlight. Sediment testing with some chemicals, which are photoinduced by UV light, may not provide results useful for predicting field effects, because typical lighting (i.e. fluorescent) does not emit UV radiation.

(6) Natural geomorphological and physicochemical characteristics of sediment should be within the tolerance limits of the test organism. Factors such as texture, grain size, and organic carbon may influence the toxic response of the test organism.

(7) Sediment toxicity tests were designed to predict anticipated contaminant-related effects in the field or under natural conditions. However, sediment toxicity is related to bioavailability, which can be altered by physical manipulation, temperature, adjuncts, and organism uptake.

(8) In some cases bioavailability may differ between the laboratory and in situ. Sediment collection, handling, and storage are critical to preserving the integrity of contaminant equilibrium. The manipulation of sediment may disrupt the equilibrium with organic carbon and the pore water/particle system, resulting in the increased availability of organic compounds.

(9) The testing temperature is important to bioavailability. Temperature affects contaminant solubility, the partitioning coefficient, as well as the physical and chemical characteristics of sediment. Bioavailability may also be altered by interactions between sediment and overlying water.

(10) Adjuncts such as food, water, or solvents may alter bioavailability and promote the growth of microorganisms. While food addition is necessary, the quantity and composition of food added must be carefully considered.

(11) Uptake of contaminants by the test organisms or test chambers may influence bioavailability. Test organisms are sinks for contaminants, but to a lesser degree than sediments.

(12) The routes of exposure for sediment contaminants are not always known. In some cases, it may desirable to normalize sediment concentrations of contaminants to factors other than dry weight, such as organic-carbon for nonionic organic compounds or acid volatile sulfides for certain metals.

(13) The Agency recommends using natural sediments for spiking in sediment toxicity tests. However, indigenous species sometimes exist in field-collected sediments and their presence could negatively effect the growth rates of test organisms. Biological activity may be inhibited by gamma radiation, heat, sieving, mercuric chloride, or antibiotics, and their impact on sediment characteristics must be determined prior to the commencement of testing.

(j) **Test method—Chironomus tentans 10–day survival and growth test for sediments**—(1) **Test conditions.** The 10–day sediment toxicity test with *C. tentans* should be conducted at a temperature of 23 °C and photoperiod of 16 h light:8 h dark at 500 to 1,000 lx. The recommended test chambers are 300–mL high-form beakers without lips containing 100 mL of sediment and 175 mL of overlying water. Each test chamber is filled with 10 third-instar midges to begin the test. All organisms must be third-instar (50 percent of organisms) or younger. For routine testing, eight replicates are recommended. Midges should be fed 1.5 mL of a 4 g/L suspension of Tetrafin daily. Overlying water in each test chamber should receive two volume changes per day and can be culture water, well water, surface water, site water, or reconstituted water.

(2) **Sediment into test chambers.** Test sediment should be mixed thoroughly and placed into test chambers one day (day – –1) before commencement of the test. Sediment should be checked for homogeneity visually and quantitatively by analyzing TOC, chemical concentrations, and particle size. Equal volumes of sediment should be added to each test chamber, and on day – 1 overlying water should be added by pouring water along a baffle to avoid any disturbance of the sediment. The test begins once the test organisms are added to the test chambers (day – 0).

(3) **Renewal of overlaying water.** The renewal of overlying water is required and should be conducted on day – –1 prior to the addition of test organisms or food on day – 0. Flow rates should not vary by more than 10 percent between any two test chambers at any time during the test. Proper system operation should be verified by calibration prior to initiation of the test.

(4) **Acclimation.** The required culture and testing temperature is 23 °C. The test organisms should be cultured in the same water to be used for testing. Acclimation of the test organisms to the test water is not required.

(5) **Placing organisms in test chambers.** Handle test organisms as little as possible. Midges may be placed into test chambers by pipetting the organisms directly into the overlying water just below the air-water interface or by placing the organisms into 30–mL counting cups and floating them in the test chamber for 15 min prior to placement into the overlying water. Measurements of length or weight should be made on a subset of 20 organisms prior to test initiation. Head capsule widths should be measured on midges to determine the instar used at test initiation.

(6) **Monitoring a test.** All test chambers should be checked daily. Test organisms should be observed for abnormal behavior, such as sediment avoidance. The exposure system should also be monitored daily to assure proper operation.

(7) **Measurement of overlying water-quality characteristics.** (i) Conductivity, hardness, pH, alkalinity, and ammonia concentration should be measured in all treatments at the beginning and end of a test, and during any test should not vary more than 50 percent. Samples should be removed with a pipet from 1 to 2 cm above the sediment surface without disturbance. Caution is required to prevent removing test organisms when sampling.

(ii) DO should be measured daily, and should be maintained between 40 and 100 percent saturation. Both DO and pH may be measured in overlying water using a probe.

(iii) Temperature should be measured in one test chamber from each treatment daily. The mean and instantaneous temperatures should not vary from the desired temperature by more than 1 and 3 °C, respectively.

(8) **Feeding.** Food is required for proper maintenance of the test organisms but should be kept to a minimum to prevent alteration of contaminant availability or the growth of microbials such as fungus and bacteria. Collection of food on the bottom of the test chamber or reduced concentration of DO are indicators of possible overfeeding. Should either of the these conditions occur, feeding should be suspended in all test chambers until conditions have readjusted. Detailed records and observations should be made daily.

(9) **Ending a test.** Surviving amphipods may be pipetted from the test chamber prior to sieving the sediment. Immobile organisms isolated from either sediment or sieved material are considered dead. Surviving organisms should be preserved (e.g. 8 percent sugar-formalin) and measured for growth. Specific sieving instruction may be found in paragraph (l)(1) of this guideline.

(10) **Test data.** (i) The endpoints measured in 10–day sediment tests with *C. tentans* are dry weight and survival. At the end of the test, *C. tentans* in control sediment should have an average size of 0.6 mg. Head

capsule width should be measured prior to dry weight. To determine dry weight of surviving midges:

(A) pool all surviving organisms from a replicate.

(B) Dry the sample at 60 to 90 °C to constant weight.

(C) Bring sample to room temperature in a desiccator.

(D) Weigh the sample of organisms to the nearest 0.01 mg. This measure will give the mean weight of surviving organisms per replicate.

(iv) Pupae and adults should be excluded from dry weight determinations. Length measurement is optional, but measurements should be from the anterior of the labrum to the posterior of the last abdominal segment.

(11) **Interpretation of results**—(i) **Age sensitivity.** First and second instar midges are more sensitive than third and fourth instar midges. Sediment tests should be initiated with midges of uniform size and age to avoid changes in sensitivity. Sediment tests are conducted with the third-instar midges because the greater size facilitates handling and isolation from sediment at test termination.

(ii) **Grain size.** *C. tentans* are tolerant of a wide range of substrates. The sensitivity of midges does not correlate with TOC or grain size. However, sensitivity may be influenced by artificial sediment when test organisms are not fed during the test.

(iii) **Isolating organisms at the end of a test.** Isolation and recovery of midges at the end of the test is not difficult. The midges are typically red and greater 5–mm in length.

(iv) **Influence of indigenous organisms.** There are no reports on the influence of indigenous organisms on *C. tentans* survival and response in sediment toxicity tests. However, survival of a congener, *Chironomus riparius*, was not reduced in the presence of oligochaetes, but growth was reduced in the presence of high numbers of oligochaetes. The number of oligochaetes and presence of predators in test sediment should be determined to improve the interpretation of growth data.

(k) **Reporting.** In addition to information meeting general reporting requirements, a report of the results of a whole sediment toxicity test should also 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 ingredient 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 and number of test organisms/treatment, date and time test started and ended, 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 LC50 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.

(14) Published reports should contain enough information to clearly identify the methodology used and the quality of the results.

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

(1) U.S. Environmental Protection Agency. Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates. EPA 600/R–94/024 (1994).

(2) [Reserved]