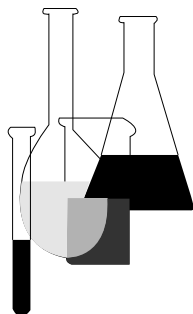




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# Ecological Effects Test Guidelines

## OPPTS 850.1790 Chironomid Sediment Toxicity Test



**“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](http://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](mailto: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](mailto: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](http://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](http://gopher.epa.gov)) under the heading “Environmental Test Methods and Guidelines.”

**OPPTS 850.1790 Chironomid sediment toxicity test.**

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline is 40 CFR 795.135 Chironomid Sediment Toxicity Test (proposed in the FEDERAL REGISTER of June 25, 1991 (56 FR 29149)).

(b) **Purpose.** This guideline may be used to develop data on the toxicity and bioavailability of chemical substances and mixtures (“chemicals”) in sediments subject to environmental effects test regulations under TSCA. This guideline prescribes tests to be used to develop data on the toxicity of chemicals present in sediments to chironomid larvae (midges). The EPA will use data from these tests in assessing the hazard of a chemical to the environment.

(c) **Definitions.** The definitions in section 3 of TSCA and 40 CFR part 792, Good Laboratory Practice Standards (GLPS), apply to this test guideline. In addition, the following definitions also apply:

*Bioconcentration factor* (BCF) is the quotient of the concentration of a test substance in tissues of the chironomids at or over a specific time period of exposure divided by the concentration of test substance in the overlying water, interstitial water, or in the sediments at or during the same time period.

*Cation exchange capacity* (CEC) is the sum total of exchangeable cations that a sediment can absorb. The CEC is expressed in milliequivalents of negative charge per 100 g or milliequivalents of negative charge per gram of sediment (dry weight).

*COD* is chemical oxygen demand.

*EC50* is an experimentally-derived concentration of test substance in the sediment that is calculated to affect 50 percent of a test population during continuous exposure over a specified period of time.

*Flow-through* is a continuous or intermittent passage of dilution water through a test chamber or culture tank with no recycling of water.

*Geometric mean* is the calculated mean between the highest test concentration with no statistically significant effects and the lowest concentration showing significant effects.

*Interstitial water* is liquid which is found in or directly adjacent to sediments and can be extracted from these sediments by several processes.

*Loading* is the ratio of chironomid biomass (grams wet weight) to the volume (liters) of test solution in a test chamber at a specified time or passing through the test chamber during a specific interval.

*Lowest-observed-effect-concentration* (LOEC) is the lowest treatment (i.e., test concentration) of a test substance that is statistically different in adverse effect on a specific population of test organisms from that observed in controls.

*MATC* (maximum acceptable toxicant concentration) is the maximum concentration at which a chemical may be present and not be toxic to the test organism.

*No-observed-effect-concentration* (NOEC) is the highest treatment (i.e., test concentration) of a test substance that shows no statistical difference in adverse effect on a specific population of test organisms from that observed in controls.

*Overlying water* is liquid which is found above or placed over sediments. For purposes of this guideline, overlying water is equivalent to the term *water column*.

*Partial life-cycle toxicity test* is one which uses a sensitive portion of the life of a test organism (second instar of midges) to assess the effects of test substances.

*Redox potential* (Eh) means the oxidizing or reducing intensity or condition of a solution expressed as a current, referenced against a hydrogen electrode. Zero or negative Eh values may exist due to reducing conditions within wet sediments.

*Sediment* is matter that settles to the bottom of a liquid in natural situations or a substrate prepared from a combination of natural sediments and artificial components. *Sediment* is equivalent to the term solid-phase sediments in this guideline.

*Sediment partition coefficient* is the ratio of the concentration of test substance on the sediment to the concentration in the overlying water. For the purposes of this guideline, this term is identical to *soil-water partition coefficient*.

*Spiking* is the addition of a test substance to a negative control and/or reference sediment so that the toxicity of a known quantity of test substance can be determined in a known nontoxic sediment. Often a solvent carrier is needed for low-water soluble test substances.

*Subchronic toxicity test* is a method used to determine the concentration of a test substance in water and for sediment which produces an adverse effect on chironomids over a partially extended period of time. In

this guideline, mortality and growth (expressed as change in wet weight of midges) are the criteria of toxicity.

*TOC* is total organic carbon.

(d) **Test procedures**—(1) **Summary of test.** (i) This flow-through test consists of three parts. Part 1. is a 14-day aqueous exposure test, with minimal sediments, with food, and with the test substance added to the overlying water. Part 2. is a 14-day sediment exposure test, with one or more sediments (4 to 6 cm in thickness) which may have varying amounts of organic carbon, with food, and with the test substance added to sediments. Part 3. is a 14-day interstitial exposure test, with one or more sediments (4 to 6 cm in thickness) which may have varying amounts of organic carbon, with food, and with the test substance added to overlying water. The flow-through test is illustrated in the following Table 1.

Table 1.—Experimental Design for the Chironomid Sediment Flow-Through Toxicity Test

Test system	Test substance concentrations (2 replicates each) <sup>1</sup>	Number of sediments (2 replicates each)	Number of Samples Analyzed (2 replicates each)			
			Overlying water P/C <sup>2</sup>	Interstitial water P/C <sup>2</sup>	Sediments	Midges <sup>3</sup>
<b>Part 1</b>						
14-Day Aqueous Exposure .....	5(10)	na <sup>4</sup>	5 (10)	na	na	5 (10)
Control (2 reps) .....	na	na	1 (2)	na	na	1 (2)
Solvent Control (2 reps) .....	na	na	1 (2)	na	na	1 (2)
<b>Part 2</b>						
14-Day Sediment Exposure .....	5 (10)	1-3 <sup>5</sup> (2-6)	5 (10)	na	5 (10)	5 (10)
Control (2 reps) .....	na	1 (2)	1 (2)	na	1 (2)	1 (2)
Solvent Control (2 reps) .....	na	1 (2)	1 (2)	na	1 (2)	1 (2)
<b>Part 3</b>						
14-Day Interstitial Water/Sediment Exposure .....	5 (10)	1-3 <sup>5</sup> (2-6)	5 (10)	5 (10)	5 (10)	
Control (2 reps) .....	na	1 (2)	1 (2)	1 (2)	1 (2)	1 (2)
Solvent Control (2 reps) .....	na	1 (2)	1 (2)	1 (2)	1 (2)	1 (2)

<sup>1</sup> Test substance concentration in all replicates measured at days 0 and 14 (reps = replicates)

<sup>2</sup> P/C = physical/chemical measurements (dissolved oxygen, temperature (in °C), and pH) on days 0, 4, 7, 10 and 14.

<sup>3</sup> Midges are observed throughout the test, dead chironomids recorded, removed, and weighed on days 4, 7, and 10. At end of each test, remaining midges from each replicate are removed, counted, and weighed.

<sup>4</sup> na = not applicable

<sup>5</sup> Number of sediment types tested will depend on range of TOC content tested; 1 to 3 types (low, medium, and high TOC levels) are recommended.

(ii) The day before the test is to be started, sediments (in treatments, and reference and negative controls) should be screened to remove large particles and endemic animals (especially midge predators) and added to the test chambers. The amount of sediments to be added to each test chamber will depend on the experimental design and test species. Only a minimum amount (to a depth of 2 mm) should be added in the aqueous exposure portion of the test. Each replicate test chamber should contain the same amount of sediments. Overlying water should be added to each test chamber.

(iii) In this flow-through test, the flow of dilution water through each chamber is begun and adjusted to the rate desired. The test substance should be introduced into each test chamber. The addition of test substance in the flow-through system should be done at a rate which is sufficient

to establish and maintain the desired concentration of test substance in the test chamber.

(iv) At the initiation of the test, chironomids which have been cultured or acclimated in accordance with the test design are randomly placed into the test chambers. Midges in the test chambers are observed periodically during the test. Immobile or dead larvae should be counted, removed, and weighed, and the findings recorded. "Floating" larvae are nonviable and should be replaced. Dissolved oxygen (DO) concentration, pH, temperature, the concentration (measured) of test substance, and other water quality parameters should be measured at specified intervals in selected test chambers, during all three parts of this test. (See Table 1 under paragraph (d)(1)(i) of this guideline.) Data should be collected during the test to determine any significant differences ( $P < 0.05$ ) in mortality and growth as compared to the controls. BCFs should be calculated at the end of the test based on route of exposure.

(2) **Range-finding test.** (i) A range-finding test should be conducted prior to beginning each of the three parts of the test to establish test solution concentrations for the three definitive parts of the test.

(ii) The chironomids should be exposed to a series of widely spaced concentrations of the test substance (e.g., 1, 10, 100 mg/L).

(iii) A minimum of 10 chironomids should be exposed to each concentration of test substance for a period of time which allows estimation of appropriate test concentrations. No replicates are required and nominal concentrations of the chemical are acceptable.

(3) **Definitive test.** (i) The purpose of the definitive portion of the test is to determine concentration-response curves, EC50 values, effects of a chemical on mortality and growth, and the determination of BCFs during subchronic exposure.

(ii) A minimum of 30 midges per concentration (15 midges per replicate test chamber) should be exposed in each part of the test to five or more concentrations of the test substance chosen in a geometric series in which the ratio is between 1.5 and 2.0 (e.g., 2, 4, 8, 16, 32, 64 mg/L). An equal number of chironomids should be placed in two replicates. The concentration ranges should be selected to determine the concentration-response curves, EC50 values, and MATC. Solutions should be analyzed for chemical concentration prior to use and at designated times during the test.

(iii) Each test should include controls consisting of the same dilution water, sediments, conditions, procedures, and midges from the same population (same egg mass in culture container), except that none of the test substance is added.

(iv) The test duration is 14 days for each of the three parts of the test. The test is unacceptable if more than 20 percent of the control organisms die or are stressed or diseased during the test. A test period longer than 14 days may be necessary for high log  $K_{ow}$  chemicals.

(v) The number of dead chironomids in each test chamber should be recorded on days 4, 7, 10, and 14 of the test. At the end of the test, surviving midges are removed from the test chambers and weighed after being blotted dry. Concentration-response curves, EC50 values, and associated 95 percent confidence limits for mortality should be determined for days 4, 7, 10, and 14 in the aqueous exposure portion of the test. MATC, NOEC, and LOEC values should be determined for midge survival and growth.

(vi) In addition to survival and growth, any abnormal behavior or appearance of the chironomids should be reported.

(vii) Distribution of midges among the test chambers should be randomized. In addition, test chambers within the testing area should be positioned in a random manner or in a way that appropriate statistical analyses can be used to determine variation due to placement.

(viii) A control sediment and/or a reference sediment should be used in each part of this test. Use of these controls/references will help determine if the test is acceptable, serve to monitor the health of the chironomids used in the testing and the quality and suitability of test conditions, parameters and procedures, and aid in analyzing data obtained from this test. A negative control should be run in the test, using a sediment known to be nontoxic to the midges. A reference sediment can be run in the test in addition to or in place of the negative control. The reference sediment should be obtained from an area that is known to have low levels of chemical contamination and which is similar to or identical to the test sediments in physical and chemical characteristics.

(ix) In the first part of this test, the aqueous exposure, a minimal amount of sediment (<2mm) is placed in the test chambers. The presence of sediment is necessary to allow the midges to construct tubes, to reduce stress to the chironomids, and to reduce cannibalism.

(x) BCFs should be calculated at the end of each part of the test.

(4) **Analytical measurements**—(i) **Water quality analysis.** (A) The hardness, acidity, alkalinity, conductivity, TOC or COD, and particulate matter of the dilution water serving as the source of overlying water should be measured on days 0 and 14. The month-to-month variation of these values should be less than 10 percent and the pH should vary less than 0.4 units.

(B) During all three parts of the flow-through test, DO, temperature, and pH should be measured in each chamber on days 0, 4, 7, 10, and 14.

(ii) **Analysis of test substance.** (A) Deionized water should be used in making stock solutions of the test substance. Standard analytical methods should be used whenever available in performing the analyses of water and sediments. Radiolabeling of the test substance (e.g., by use of  $^{14}\text{C}$ ) may be necessary to measure quantities present in the sediments accurately. The analytical method used to measure the amount of test substance in the sample should be validated by appropriate laboratory practices before beginning the test. An analytical method is not acceptable if likely degradation products of the test substance, such as hydrolysis and oxidation products, give positive or negative interference which cannot be systematically identified and corrected mathematically. When radiolabeled test substances are used, total radioactivity should be measured in all samples. At the end of the test, water, sediments, and tissue samples should be analyzed using appropriate methodology to identify and estimate any major (at least 10 percent of the parent compound) degradation products or metabolites that may be present.

(B) The overlying water from each test chamber should be sampled for the test substance on days 0, 7, and 14 for all three aqueous exposure parts of this test.

(C) For the nonaqueous exposure parts of the test, the interstitial water from each test chamber should be analyzed for the test substance on days 0, 7, and 14. Interstitial water can be sampled by using a variety of methods, such as removal of overlying water and centrifugation, filtration of sediments, pressing the sediments, or using an interstitial water sample. Care should be taken during these measurements to prevent the biodegradation, transformation, or volatilization of the test substance.

(D) For the nonaqueous exposure portion of the test, the sediments from each test chamber should be analyzed for the test substance on days 0, 7, and 14.

(E) The sediment partition coefficient or soil-water partition coefficient is determined by dividing the average test substance concentration in sediment by the respective average concentration in the water column. Concentrations of test substance in the sediments to be used in this test can be chosen by measuring these partition coefficients. This sediment partition coefficient should be determined in triplicate by placing a quantity of a sediment with a known TOC content and spiked with the radiolabeled test substance into a quantity of dilution water. The ratio of sediment to dilution water should simulate the ratio present in the test. The sediment/dilution water mixture is shaken periodically, and the radiolabeled test sub-



stance measured. This shaking and sampling procedure is repeated until equilibrium is reached, as defined by the stage on the desorption curve.

(F) Overlying water samples should be filtered through a 0.45 µm filter to determine the concentration of dissolved test substance.

(G) BCFs should be calculated by determining the amount of test substance in the midge tissue and dividing by the concentration of test substance in the water column, interstitial water, and sediments. At test termination, the midges remaining in each test concentration are analyzed for test substance. Suitable methods are available, such as radiolabeling (<sup>14</sup>C) the test substance, combusting the midges, trapping and counting the resulting radioactivity and the BCF calculated. If insufficient chironomid biomass is present at the conclusion of the test replicates may be pooled. BCFs cannot be calculated if after pooling there is insufficient biomass or if the accumulated test substance concentration is lower than the detection limit for the test substance.

(iii) **Numerical.** (A) The number of dead midge second instars should be counted during each definitive test. Appropriate statistical analyses should provide a goodness-of-fit determination for mortality concentration-response curves calculated on days 4, 7, 10, and 14. A 4-, 7-, 10-, and 14-day LC50 value based on second instar mortality, and with corresponding 95 percent confidence intervals, should be calculated. The methods recommended for calculating EC50s include probit, logit, binomial, and moving average.

(B) Appropriate statistical tests (e.g., analysis of variance and mean separation tests) should be used to test for significant chemical effects on growth (measured as wet weights) on days 4, 7, and 14. An MATC should be calculated using these test criteria.

(C) In no case should any analytical measurements be pooled except when calculating BCFs when there is insufficient biomass available for individual measurements as described under paragraph (d)(4)(ii)(G) of this guideline.

(e) **Test conditions—(1) Test species—(i) Selection.** (A) The midge, *Chironomus tentans* or *C. riparius*, should be used in this test. Both species are widely distributed throughout the United States, and the larvae and adult flies can be cultured in the laboratory. The larval portion life cycles of both species is spent in a tunnel or case within the upper layers of benthic sediments of lakes, rivers, and estuaries. Feeding habits of both species include both filter feeding and ingesting sediment particles.

(B) Second instar chironomids (<10 days) of the same age and size are to be used in this test. Third and fourth instar are less desirable, as some evidence indicates they are less sensitive, at least to copper. Each instar is 4 to 7 days in duration.

(ii) **Acquisition.** (A) Chironomids to be used in this test should be cultured at the test facility. Adult flies are collected from the chironomid cultures and allowed to mate and lay egg masses. Two egg masses are collected and allowed to hatch. The larvae are fed daily. When the second instar stage (about 10 days after hatching) is reached, larvae are removed and placed in the test chambers. Records should be kept regarding the source of the initial stock and culturing techniques. All organisms used for a particular test should have originated from the same population (culture container) and be the same age and size.

(B) Chironomids should not be used in a test if:

(1) During the final 48 hours of midge holding, obvious mortality is observed.

(2) The larvae are not in the second instar.

(iii) **Feeding.** (A) During the test, the chironomids should be fed the same diet at the same frequency as that used for culturing and acclimation. All treatments and controls should receive, as near as reasonably possible, the same amount of food on a per-animal basis.

(B) The food concentration depends on the type used and the nutritional requirements of the midges. The latter in turn is dependent upon their developmental stage.

(iv) **Loading.** The number of test organisms placed in a test chamber should not affect the test results. Loading should not exceed 30 chironomids per liter per 24 hours in the flow-through test. Loading should not affect test concentrations or cause the DO concentration to fall below the recommended level.

(v) **Care and handling of test organisms.** (A) Chironomids should be cultured in dilution water under similar environmental conditions as those in the test. Food such as Tetra Conditioning Food has been demonstrated to be adequate for chironomid cultures.

(B) Organisms should be handled as little as possible. When handling is necessary, it should be done as gently, carefully, and as quickly as possible. During culturing and acclimation, midges should be observed for any signs of stress, physical damage, and mortality. Dead and abnormal individuals should be discarded. Organisms that are damaged or dropped during handling should be discarded.

(C) Wide-bore, smooth glass tubes or pipets equipped with a rubber bulb can be used for transferring midges.

(vi) **Acclimation.** (A) Midges should be maintained in 100 percent dilution water at the test temperature for at least 4 days prior to the start of the test. This is easily accomplished by culturing them in the dilution

water at the test temperature. Chironomids should be fed the same food during the test as is used for culturing and acclimation.

(B) Midges should be maintained in facilities similar to those of the testing area during culturing and acclimation to the dilution water.

(2) **Test system**—(i) **General.** (A) Facilities needed to perform this test include:

(1) Containers for culturing and acclimating the chironomids.

(2) A mechanism for controlling and maintaining the water temperature during the culturing, acclimation, and test periods.

(3) Apparatus for straining particulate matter, removing gas bubbles, or aerating the water as necessary to ensure that the test solution flows regularly into and out of the test chamber.

(4) Test chambers can be small aquaria capable of holding 3 L of water or test solution, 5.7-L clear glass battery jars, or 1-L beakers made of borosilicate glass. Each chamber should be equipped with screened overflow holes, standpipes, or U-shaped notches covered with Nitex screen.

(B) Construction materials and commercially purchased equipment that may contact dilution water should not contain substances that can be leaked or dissolved into aqueous solutions in quantities that can alter the test results. Materials and equipment that contact test solutions should be chosen to minimize sorption of test substances.

(C) Test chambers should be loosely covered to reduce the loss of test solution or dilution water by evaporation, and to minimize the entry of dust or other particulates into the solutions.

(ii) **Test substance delivery.** (A) In the flow-through test, proportional diluters, metering pump systems, or other suitable systems should be used to deliver the test substance to the test chambers.

(B) The delivery system should be calibrated before and after each test. Calibration includes determining the flow rate through each chamber and the concentration of the test substance in each chamber. The general operation of the test substance delivery system should be checked twice daily during the test. The 24-h flow rate through a test chamber should be equal to at least 5× the volume of the test chamber. During a test, the flow rates should not vary more than 10 percent from any one test chamber to another or from one time to any other.

(iii) **Cleaning of test system.** All test equipment and test chambers should be cleaned before each test following standard laboratory proce-

dures. Cleaning of test chambers may be necessary during the testing period.

(iv) **Dilution water.** (A) Surface or ground water, reconstituted water, or dechlorinated tap water are acceptable as dilution water if chironomids will survive in it for the duration of the culturing, acclimation, and testing periods without showing signs of stress. The quality of the dilution water should be constant and should meet the specifications in the following Table 2.:

Table 2.—Specifications For Dilution Water

Substance	Maximum Concentration
Particulate matter .....	20 mg/L
TOC or COD .....	2 mg/L or 5 mg/L, respectively
Boron, fluoride .....	100 µg/L
Un-ionized ammonia .....	10 µg/L
Aluminum, arsenic, chromium, cobalt, copper, iron, lead, nickel, zinc..	1 µg/L
Residual chlorine .....	3 µg/L
Cadmium, mercury, silver .....	100 ng/L
Total organophosphorus pesticides. ....	50 ng/L
Total organochlorine pesticides and polychlorinated biphenyls (PCBs) or organic chlorine..	50 ng/L or 25 ng/L respectively

(B) The water quality characteristics listed in Table 2. should be measured at least twice a year or when it is suspected that these characteristics may have changed significantly. If dechlorinated tap water is used, daily chlorine analysis should be performed.

(C) If the diluent water is from a ground or surface water source, conductivity, hardness, alkalinity, pH, acidity, particulate matter, TOC or COD, and particulate matter should be measured. Reconstituted water can be made by adding specific amounts of reagent-grade chemicals to deionized or distilled water. Glass distilled or carbon filtered deionized water with conductivity of less than 1 µohm/cm is acceptable as the diluent for making reconstituted water.

(D) If the test substance is not soluble in water, an appropriate carrier such as triethylene glycol (CAS No. 112-27-6), dimethylformamide (CAS No. 68-12-2), or acetone (CAS No. 67-64-1) should be used. The concentration of such carriers should not exceed 0.1 mL/L.

(v) **Sediments.** (A) **Preparation and source.** (1) Sediments used in this test may contain low (<1 percent) to high (>15 percent) amounts of organic carbon because they are derived from variable natural sediments. Prior to use, the sediments should be sieved to remove larger particles. The should be characterized for particle size distribution (sand, silt, clay percentages), percent water holding capacity, total organic and inorganic carbon, total volatile solids, COD, BOD, cation exchange capacity, redox

potential (Eh), oils and greases, petroleum hydrocarbons, organophosphate pesticide concentrations, organochlorine pesticide and polychlorinated biphenyl (PCB) concentrations, toxic metal concentrations, and pH.

(2) The source of the sediments used in this test should be known and the characteristics listed above should be measured every time additional sediments are obtained. The sediments should not contain any endemic organisms, as these may be chironomid predators.

(3) Sediments should not be resuspended during the test.

(3) **Test parameters.** (i) Environmental conditions of the water contained in test chambers should be maintained as specified below:

(A) Temperature of  $20 \pm 1$  °C for *C. tentans* and  $22 \pm 1$  °C for *C. riparius*.

(B) DO concentration of the dilution water should be 90 percent of saturation or greater. The DO concentrations of the test solutions should be 60 percent or greater of saturation throughout the test. Aeration may be necessary, and if this is done, all treatment and control chambers should be given the same aeration treatment.

(C) A photoperiod of 16 h light/and 8 h dark with a 15– to 30–minute transition period.

(ii) Additional measurements include:

(A) The concentration of dissolved test substance (that which passes through a 0.45  $\mu\text{m}$  filter) in the chambers should be measured during the test.

(B) At a minimum, the concentration of test substance should be measured as follows:

(1) In each chamber before the test.

(2) In each chamber on days 7 and 14 of the test.

(3) In at least one appropriate chamber whenever a malfunction is detected in any part of the test substance delivery system.

(C) Among replicate test chambers of a treatment concentration, the measured concentration of the test substance should not vary by more than 20 percent at any time or 30 percent during the test.

(D) The dissolved oxygen concentration, temperature, and pH should be measured at the beginning of the test and on days 7 and 14 in each chamber.

(f) **Calculated values**—(1) **Sediment partition coefficient.** (A) The sediment or soil-water partition coefficient ( $K_p$ ) is defined as the ratio of

the concentration of the test substance in the sediment ( $C_s$ ) to the concentration in the water or interstitial water ( $C_w$ ) as given in the following expression:

$$K_p = C_s/C_w$$

The resultant  $K_p$  values for the sediment or sediments tested are used to select test substance concentrations for the sediment test.

(B) The  $K_p$  value is equivalent or related to the sediment organic carbon sorption coefficient multiplied by the percent organic carbon content of the sediment.

(C) The sediment partition coefficient should be determined in triplicate for each sediment type at equilibrium by spiking with the radiolabeled test substance and shaking. The test substance concentration in the water is measured radiometrically at intervals and the data used to create a desorption curve. The process is repeated until an equilibrium is reached, as defined by the shape of the curve.

(2) **BCFs.** BCFs should be calculated for each part of the test. These values are computed as the amount of test substance present in the midge tissues divided by test substance concentrations in the water column, interstitial water, and sediments. At test termination, the chironomids remaining in each test concentration are analyzed for radiolabeled test substance.

(g) **Reporting.** The sponsor should submit all data developed by the test that are suggestive and predictive of toxicity and all associated toxicologic manifestations to the Agency. In addition to the reporting requirements prescribed in the GLPS, the reporting of test data should include the following:

(1) The name of the test, sponsor, testing laboratory, study director, principal investigator, and dates of testing.

(2) A detailed description of the test substance including its source, lot number, composition (identity and concentration of major ingredients and major impurities), known physical and chemical properties, and any carriers or other additives used and their concentrations.

(3) The source of the dilution water, its chemical characteristics (e.g., conductivity, hardness, pH, TOC or COD, and particulate matter) and a description of any pretreatment.

(4) The source of the sediment, its physical and chemical characteristics (e.g., particle size distribution, TOC, pesticide and metal concentrations), and a description of any pretreatment.

(5) Detailed information about the chironomids used as a stock, including the scientific name and method of verification, age, source, treat-

ments, feeding history, acclimation procedures, and culture methods. The age (in days) and instar stage of the midges used in the test should be reported.

(6) A description of the test chambers, the volume of solution in the chambers, and the way the test was begun (e.g., conditioning and test substance additions). The number of test organisms per test chamber, the number of replicates per treatment, the lighting, the test substance delivery system, flow rates expressed as volume additions per 24 hours for the flow-through subchronic test, the method of feeding (manual or continuous), and type and amount of food.

(7) The concentration of the test substance in the water, interstitial water, and sediments in test chambers at times designated in the flow-through tests.

(8) The number and percentage of organisms that show any adverse effect in each test chamber at each observation period, and wet weights of midges in each test chamber at days 7 and 14.

(9) BCFs for all three parts of the test (i.e., overlying water or water column, sediment, and interstitial water modes of exposure).

(10) All chemical analyses of water quality and test substance concentrations, including methods, method validations, and reagent blanks.

(11) The data records of the culture, acclimation, and test temperatures. Information relating to calculation of sediment (or soil-water) partition coefficients ( $K_p$ ).

(12) Any deviation from this test guideline, and anything unusual about the test (e.g., diluter failure and temperature fluctuations).

(13) An LC50 value based on mortality and an EC50 value based on adverse effects on growth (wet weights), with corresponding 95 percent confidence limits, when sufficient data are present for days 4, 7, and 14. These calculations should be made using the average measured concentration of the test substance.

(14) Concentration-response curves utilizing the average measured test substance concentration should be fitted to both number of midges that show adverse effects (mortality) and effects on growth or wet weights of midges at days 4, 7, and 14. A statistical test of goodness-of-fit should be performed and the results reported.

(15) The MATC to be reported is calculated as the geometric mean between the lowest measured test substance concentration that had significant ( $P < 0.05$ ) effect and the highest measured test substance concentration that had no significant ( $P > 0.05$ ) effect on days 4, 7, and 14 of the test. The criterion selected for MATC computation is the one which exhibits

an effect (a statistically significant difference between treatment and control groups ( $P < 0.05$ ) at the lowest test substance concentration for the shortest period of exposure. Appropriate statistical tests (analysis of variance and mean separation tests should be used to test for significant test substance effects. The statistical tests employed and the results of these tests should be reported.

(h) **References.** The following references should be consulted for further background information on this test guideline.

(1) Adams, W.J. et al. *Aquatic safety assessment of chemicals sorbed to sediments*. R.D. Cardwell, R. Purdy, and R.C. Bahner, eds. In: *Aquatic Toxicology and Hazard Assessment*. ASTM STP 854. American Society for Testing and Materials, Philadelphia, PA (1985).

(2) Nebeker, A.V. et al. Relative sensitivity of *Chironomus tentans* life stages to copper. *Environmental Toxicology and Chemistry* 3:151–158. (1984).

(3) Nebeker, A.V. et al. Biological methods for determining toxicity of contaminated freshwater sediments to invertebrates. *Environmental Toxicology and Chemistry* 3:617–630. (1984).