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Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates

Second Edition

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Section 12
Test Method 100.2
Chironomus tentans 10-d Survival and Growth Test for Sediments

12.1 Introduction

12.1.1 *Chironomus tentans* (Fabricius) have many desirable characteristics of an ideal sediment toxicity testing organism including relative sensitivity to contaminants associated with sediment, contact with sediment, ease of culture in the laboratory, tolerance to varying physicochemical characteristics of sediment, and short generation time. Their response has been evaluated in interlaboratory studies and has been confirmed with natural benthic populations. Many investigators have successfully used *C. tentans* to evaluate the toxicity of freshwater sediments (e.g., Wentsel et al., 1977; Nebeker et al., 1984a; Nebeker et al., 1988; Adams et al., 1985; Giesy et al., 1988; Hoke et al., 1990; West et al., 1993; Ankley et al., 1993; Ankley et al., 1994a; Ankley et al.,1994b). *C. tentans* has been used for a variety of sediment assessments (West et al., 1993; Hoke et al., 1994, 1995; West et al., 1994; Ankley et al., 1994c). Endpoints typically monitored in 10-d sediment toxicity tests with *C. tentans* include survival and growth (ASTM, 1999a).

12.1.2 A specific test method for conducting a 10-d sediment toxicity test is described in Section 12.2 for *C. tentans*. Methods outlined in Appendix A of USEPA (1994a) and in Section 12.1.1 were used for developing test method 100.2. Results of tests using procedures different from the procedures described in Section 12.2 may not be comparable and these different procedures may alter contaminant bioavailability. Comparison of results obtained using modified versions of these procedures might provide useful information concerning new concepts and procedures for conducting sediment tests with aquatic organisms. If tests are conducted with procedures different from the procedures described in this manual, additional tests are required to determine comparability of results (Section 1.3).

12.2 Recommended Test Method for Conducting a 10-d Sediment Toxicity Test with *Chironomus tentans*

12.2.1 Recommended conditions for conducting a 10-d sediment toxicity test with *C. tentans* are summarized in Table 12.1. A general activity schedule is outlined in Table 12.2. Decisions concerning the various aspects of experimental design, such as the number of treatments, number of test chambers/treatment, and water-quality characteristics should be based on the purpose of the test and the methods of data analysis (Section 16). The number of replicates and concentrations tested depends in part on the significance level selected and the type of statistical analysis. When variability remains constant, the sensitivity of a test increases as the number of replicates increases.

12.2.2 The recommended 10-d sediment toxicity test with *C. tentans* must be conducted at 23°C with a 16L:8D photoperiod at an illuminance of about 100 to 1000 lux (Table 12.1). Test chambers are 300-mL high-form lipless beakers containing 100 mL of sediment and 175 mL of overlying water. Ten second- to third-instar midges (about 10-d old) are used to start a test (Section 10.4.1). The number of replicates/treatment depends on the objective of the test. Eight replicates are recommended for routine testing (see Section 16). Midges in each test chamber are fed 1.5 mL of a 4-g/L Tetrafin® suspension daily. Each test chamber receives 2 volume additions/d of overlying water. Water renewals may be manual or automated. Appendix A describes water-renewal systems that can be used to deliver overlying water. Overlying water can be culture water, well water, surface water, site water, or reconstituted water. For site-specific evaluations, the characteristics of the overlying water should be as similar as possible to the site where sediment is collected. Requirements for test acceptability are summarized in Table 12.3.

12.3 General Procedures

12.3.1 Sediment into Test Chambers

The day before the sediment test is started (Day -1) each sediment should be thoroughly homogenized and added to the test chambers (Section 8.3.1). Sediment should be visually inspected to judge the extent of homogeneity. Excess water on the surface of the sediment can indicate separation of solid and liquid components. If a quantitative measure of homogeneity is required, replicate subsamples should be taken from the sediment batch and analyzed for TOC, chemical concentrations, and particle size.

12.3.1.1 Each test chamber should contain the same amount of sediment, determined either by volume or by weight. Overlying water is added to the chambers in a manner that minimizes suspension of sediment. This can be accomplished by gently pouring water along the sides

Table 12.1 Recommended Test Conditions for Conducting a 10-d Sediment Toxicity Test with *Chironomus tentans*

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:	About 100 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 (Appendix A); continuous or intermittent (e.g., one volume addition every 12 h)
10. Age of organisms:	Second- to third-instar larvae (about 10-d-old larvae; all organisms must be third instar or younger with at least 50% of the organisms at third instar; Section 10.4.1)
11. Number of organisms/chamber:	10
12. Number of replicate chambers/treatment:	Depends on the objective of the test. Eight replicates are recommended for routine testing (see Section 16).
13. Feeding:	Tetrafin® goldfish food, fed 1.5 mL daily to each test chamber (1.5 mL contains 6.0 mg of dry solids)
14. Aeration:	None, unless dissolved oxygen in overlying water drops below 2.5 mg/L.
15. Overlying water:	Culture water, well water, surface water, site water, or reconstituted water
16. Test chamber cleaning:	If screens become clogged during a test, gently brush the *outside* of the screen (Appendix A).
17. Overlying water quality:	Hardness, alkalinity, conductivity, pH, and ammonia at the beginning and end of a test. Temperature and dissolved oxygen daily.
18. Test duration:	10 d
19. Endpoints:	Survival and growth (ash-free dry weight, AFDW)
20. Test acceptability:	Minimum mean control survival must be 70%, with minimum mean weight/ surviving control organism of 0.48 mg AFDW. Performance-based criteria specifications are outlined in Table 12.3.

of the chambers or by pouring water onto a baffle (e.g., a circular piece of Teflon with a handle attached) placed above the sediment to dissipate the force of the water. Renewal of overlying water is started on Day -1. A test begins when the organisms are added to the test chambers (Day 0).

12.3.2 Renewal of Overlying Water

12.3.2.1 Renewal of overlying water is required during a test. At any particular time during the test, flow rates through any two test chambers should not differ by more than 10%. Hardness, alkalinity and ammonia concentrations in the water above the sediment, within a treatment, typically should not vary by more than 50% during the test. Mount and Brungs (1967) diluters have been modified for sediment testing, and other automated water-delivery systems have also been used (Maki, 1977; Ingersoll and Nelson, 1990; Benoit et al., 1993; Zumwalt et al., 1994; Brunson et al., 1998; Wall et al., 1998; Leppanen and Maier, 1998). Each water-delivery system should be calibrated before a test is started 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. Appendix A describes water-renewal systems that can be used for conducting sediment tests.

12.3.2.2 In water-renewal tests with one to four volume additions of overlying water/d, water-quality characteristics generally remain similar to the inflowing water (Ingersoll and Nelson, 1990; Ankley et al., 1993); however, in static tests, water quality may change profoundly during the exposure (Shuba et al., 1978). For example, in static whole-sediment tests, the alkalinity, hardness, and conductivity of overlying water more than doubled in several treatments during a four-week exposure (Ingersoll and Nelson, 1990). Additionally, concentrations of metabolic products (e.g., ammonia) may also increase during static exposures, and these compounds can either be directly toxic to the test organisms or may contribute to the toxicity of the contaminants in the sediment. Furthermore, changes in water-quality characteristics such as hardness may influence the toxicity of many inorganic (Gauss et

Table 12.2 General Activity Schedule for Conducting a 10-d Sediment Toxicity Test with *Chironomus tentans* [1]

Day	Activity
-14	Isolate adults for production of egg cases.
-13	Place newly deposited egg cases into hatching dishes.
-12	Prepare a larval rearing chamber with new substrate.
-11	Examine egg cases for hatching success. If egg cases have hatched, transfer first-instar larvae and any remaining unhatched embryos from the crystallizing dishes into the larval rearing chamber. Feed organisms.
-10	Same as Day -11.
-9 to -2	Feed and observe midges (Section 10.4). Measure water quality (e.g., temperature and dissolved oxygen).
-1	Add food to each larval rearing chamber and measure temperature and dissolved oxygen. Add sediment into each test chamber, place chamber into exposure system, and start renewing overlying water.
0	Measure total water quality (temperature, pH, hardness, alkalinity, dissolved oxygen, conductivity, ammonia). Remove third-instar larvae from the culture chamber substrate. Add 1.5 mL of Tetrafin® (4.0 g/L) into each test chamber. Transfer 10 larvae into each test chamber. Release organisms under the surface of the water. Archive 20 test organisms for instar determination and weight or length determination. Observe behavior of test organisms.
1 to 8	Add 1.5 mL of food to each test chamber. Measure temperature and dissolved oxygen. Observe behavior of test organisms.
9	Measure total water quality.
10	Measure temperature and dissolved oxygen. End the test by collecting the midges with a sieve. Measure weight or length of surviving larvae.

[1] Modified from Call et al., 1994

al., 1985) and organic (Mayer and Ellersieck, 1986) contaminants. Although contaminant concentrations are reduced in the overlying water in water-renewal tests, organisms in direct contact with sediment generally receive a substantial proportion of a contaminant dose directly from either the whole sediment or from the interstitial water.

12.3.3 Acclimation

12.3.3.1 Test organisms must be cultured and tested at 23°C. Ideally, test organisms should be cultured in the same water that will be used in testing. However, acclimation of test organisms to the test water is not required.

12.3.3.2 Culturing of organisms and toxicity assessment are typically conducted at 23°C. However, occasionally there is a need to perform evaluations at temperatures different than that recommended. Under these circumstances, it may be necessary to acclimate organisms to the desired test temperature to prevent thermal shock when moving immediately from the culture temperature to the test temperature (ASTM, 1999a). Acclimation can be achieved by exposing organisms to a gradual change in temperature; however, the rate of change should be relatively slow to prevent thermal shock. A change in temperature of 1°C every 1 to 2 h has been used successfully in some studies (P.K. Sibley, University of Guelph, Guelph, Ontario, personal communication; APHA, 1989). Testing at temperatures other than 23°C needs to be preceded by studies to determine expected performance under alternate conditions.

12.3.4 Placing Organisms in Test Chambers

12.3.4.1 Test organisms should be handled as little as possible. Midges should be introduced into the overlying water below the air-water interface. Test organisms can be pipetted directly into overlying water. Developmental stage of the test organisms should be documented from a subset of at least 20 organisms used to start the test (Section 10.4.1). Developmental stage can be determined from head capsule width (Table 10.2), length (4 to 6 mm), or dry weight (0.08 to 0.23 mg/individual). It is desirable to measure size at test initiation using the same measure as will be used to assess growth at the end of the test.

12.3.5 Feeding

12.3.5.1 For each beaker, 1.5 mL of Tetrafin® is fed from Day 0 to Day 9. Without addition of food, the test organisms may starve during exposures. However, the addition of the food may alter the availability of the contaminants in the sediment (Wiederholm et al., 1987; Harkey et al., 1994). Furthermore, if too much food is added to the test chamber or if the mortality of test organisms is high, fungal or bacterial growth may develop on the sediment surface. Therefore, the amount of food added to the test chambers is kept to a minimum.

12.3.5.2 Suspensions of food should be thoroughly mixed before aliquots are taken. If excess food collects on the sediment, a fungal or bacterial growth may develop on the sediment surface, in which case feeding should be suspended for one or more days. A drop in dissolved oxygen below 2.5 mg/L during a test may indicate that the food

Table 12.3 Test Acceptability Requirements for a 10-d Sediment Toxicity Test with *Chironomus tentans*

A. It is recommended for conducting a 10-d test with *C. tentans* that the following performance criteria be met:

1. Tests must be started with second- to third-instar larvae (about 10-d-old larvae; see Section 10.4.1).
2. Average survival of *C. tentans* in the control sediment must be greater than or equal to 70% at the end of the test.
3. Average size of *C. tentans* in the control sediment must be at least 0.48 mg AFDW at the end of the test.
4. Hardness, alkalinity, and ammonia in the overlying water typically should not vary by more than 50% during the test, and dissolved oxygen should be maintained above 2.5 mg/L in the overlying water.

B. Performance-based criteria for culturing *C. tentans* include the following:

1. It may be desirable for laboratories to periodically perform 96-h water-only reference-toxicity tests to assess the sensitivity of culture organisms (Section 9.16.2). Data from these reference-toxicity tests could be used to assess genetic strain or life-stage sensitivity of test organisms to select chemicals.
2. Laboratories should keep a record of time to first emergence for each culture and record this information using control charts. Records should also be kept on the frequency of restarting cultures.
3. Laboratories should record the following water-quality characteristics of the cultures at least quarterly: pH, hardness, alkalinity, and ammonia. Dissolved oxygen in the cultures should be measured weekly. Temperature of the cultures should be recorded daily. If static cultures are used, it may be desirable to measure water quality more frequently.
4. Laboratories should characterize and monitor background contamination and nutrient quality of food if problems are observed in culturing or testing organisms.
5. Physiological measurements such as lipid content might provide useful information regarding the health of the cultures.

C. Additional requirements:

1. All organisms in a test must be from the same source.
2. Storage of sediments collected from the field should follow guidance outlined in Section 8.2.
3. All test chambers (and compartments) should be identical and should contain the same amount of sediment and overlying water.
4. Negative-control sediment and appropriate solvent controls must be included in a test. The concentration of solvent used must not adversely affect test organisms.
5. Test organisms must be cultured and tested at 23°C (±1°C).
6. The daily mean test temperature must be within ±1°C of 23°C. The instantaneous temperature must always be within ±3°C of 23°C.
7. Natural physico-chemical characteristics of test sediment collected from the field should be within the tolerance limits of the test organisms.

added is not being consumed. Feeding should be suspended for the amount of time necessary to increase the dissolved oxygen concentration (ASTM, 1999a). If feeding is suspended in one treatment, it should be suspended in all treatments. Detailed records of feeding rates and the appearance of the sediment surface should be made daily.

12.3.6 Monitoring a Test

12.3.6.1 All chambers should be checked daily and observations made to assess test organism behavior such as sediment avoidance. However, monitoring effects on burrowing activity of test organisms may be difficult because the test organisms are often not visible during the exposure. The operation of the exposure system should be monitored daily.

12.3.6.2 Measurement of Overlying Water-Quality Characteristics

12.3.6.2.1 Conductivity, hardness, pH, alkalinity, and ammonia should be measured in all treatments at the beginning and end of a test. Overlying water should be sampled just before water renewal from about 1 to 2 cm above the sediment surface using a pipet. It may be necessary to composite water samples from individual replicates. The pipet should be checked to make sure no organisms are removed during sampling of overlying water. Water quality should be measured on each batch of water prepared for the test.

12.3.6.2.2 Water-only exposures evaluating the tolerance of *C. tentans* larvae to depressed DO have indicated that significant reductions in weight occurred after 10-d exposure to 1.1 mg/L DO, but not at 1.5 mg/L (V. Mattson, USEPA, Duluth, MN, personal communication). This finding concurs with the observations during method development at the USEPA laboratory in Duluth that excursions of DO as low as 1.5 mg/L did not seem to have an effect on midge survival and development (P.K. Sibley, University of Guelph, Guelph, Ontario, personal communication). Based on these findings, it appears that periodic depressions of DO below 2.5 mg/L (but not below 1.5 mg/L) are not likely to adversely affect test results, and thus should not be a reason to discard test data. Nonetheless, tests should be managed toward a goal of DO > 2.5 mg/L to insure satisfactory performance. If the

DO level of the water falls below 2.5 mg/L for any one treatment, aeration is encouraged and should be done in all replicates for the duration of the test. Occasional brushing of screens on outside of beakers will help maintain the exchange of water during renewals using the exposure system described by Benoit et al. (1993). If a probe is used to measure DO in overlying water, it should be thoroughly inspected between samples to make sure that organisms are not attached and should be rinsed between samples to minimize cross contamination. Aeration can be used to maintain dissolved oxygen in the overlying water above 2.5 mg/L (i.e., about 1 bubble/ second in the overlying water).

12.3.6.2.3 Temperature should be measured at least daily in at least one test chamber from each treatment. The temperature of the water bath or the exposure chamber should be continuously monitored. The daily mean test temperature must be within ±1°C of 23°C. The instantaneous temperature must always be within ±3°C of 23°C.

12.3.7 Ending a Test

12.3.7.1 Immobile organisms isolated from the sediment surface or from sieved material should be considered dead. A #40 sieve (425-µm mesh) can be used to remove midges from sediment. Alternatively, Kemble et al. (1994) suggest sieving of sediment using the following procedure: (1) pour about half of the overlying water through a #50- (300-µm) U.S. standard mesh sieve, (2) pour about half of the sediment through the #50-mesh sieve and wash the contents of the sieve into an examination pan, (3) rinse the coarser sediment remaining in the test chamber through a #40- (425-µm) mesh sieve and wash the contents of this second sieve into a second examination pan. Surviving midges can then be isolated from these pans. See Section 12.3.8.1 and 12.3.8.2 for the procedures for measuring weight or length of midges.

12.3.7.2 A consistent amount of time should be taken to examine sieved material for recovery of test organisms (e.g., 5 min/replicate). Laboratories should demonstrate that their personnel are able to recover an average of at least 90% of the organisms from whole sediment. For example, test organisms could be added to control sediment and recovery could be determined after 1 h (Tomasovic et al., 1994).

12.3.8 Test Data

12.3.8.1 Ash-free dry weight (AFDW) and survival are the endpoints measured at the end of the 10-d sediment toxicity test with *C. tentans*. The 10-d method for *C. tentans* in the first edition of this manual (USEPA, 1994a), as well as most previous research, has used dry weight as a measure of growth. However, Sibley et al. (1997b) found that the grain size of sediments influences the amount of sediment that *C. tentans* larvae ingest and retain in their gut. As a result, in finer-grain sediments, a substantial portion of the measured dry weight may be comprised of sediment rather than tissue. While this may not represent a strong bias in tests with identical grain size distributions in all treatments, most field assessments are likely to have varying grain size among sites. This will likely create differences in dry weight among treatments that are not reflective of true somatic growth. For this reason, weight of midges should be measured as ash-free dry weight (AFDW) instead of dry weight. AFDW will more directly reflect actual differences in tissue weight by reducing the influence of sediment in the gut. The duration of the 10-d test starting with third-instar larvae is not long enough to determine emergence of adults. Average size of *C. tentans* in the control sediment must be at least 0.6 mg at the end of the test (0.48 mg AFDW) (Ankley et al., 1993; ASTM, 1999a; Section 17.5). If test organisms are to be used for an evaluation of bioaccumulation, it is not advisable to dry the sample before conducting the residue analysis. If conversion from wet weight to dry weight is necessary, aliquots of organisms can be weighed to establish wet to dry weight conversion factors. A consistent procedure should be used to remove the excess water from the organisms before measuring wet weight.

12.3.8.2 For determination of AFDW, first pool all living larvae in each replicate and dry the sample to a constant weight (e.g., 60°C for 24 h). Note that the weigh boats should be ashed before use to eliminate weighing errors due to the pan oxidizing during ashing. The sample is brought to room temperature in a dessicator and weighed to the nearest 0.01 mg to obtain mean weights per surviving organism per replicate. The dried larvae in the pan are then ashed at 550°C for 2 h. The pan with the ashed larvae is then reweighed and the tissue mass of the larvae is determined as the difference between the weight of the dried larvae plus pan and the weight of the ashed larvae plus pan. In rare instances where preservation is required, an 8% sugar formalin solution can be used to preserve samples (USEPA, 1994a), but the effects of preservation on the weights and lengths of the midges have not been sufficiently studied. Pupae or adult organisms must not be included in the sample to estimate ash-free dry weight. If head capsule width is to be measured, it should be measured on surviving midges at the end of the test before ash-free dry weight is determined.

12.3.8.3 Measurement of length is optional. Separate replicate beakers should be set up to sample lengths of midges at the end of an exposure. An 8% sugar formalin solution can be used to preserve samples for length measurements (Ingersoll and Nelson, 1990). The sugar formalin solution is prepared by adding 120 g of sucrose to 80 mL of formalin, which is then brought to a volume of 1 L using deionized water. This stock solution is mixed with an equal volume of deionized water when used to preserve organisms. NoTox® (Earth Safe Industries, Belle Mead, NJ) can be used as a substitute for formalin (Unger et al., 1993). Midge body length (±0.1 mm) can be measured from the anterior of the labrum to the posterior of the last abdominal segment (Smock, 1980). Kemble et al. (1994) photographed midges at magnification of 3.5X and measured the images using a computer-interfaced digitizing tablet. A digitizing system and microscope can

also be used to measure length (Ingersoll and Nelson, 1990).

12.4 Interpretation of Results

12.4.1 Section 16 describes general information for interpretation of test results. The following sections describe species-specific information that is useful in helping to interpret the results of sediment toxicity tests with *C. tentans*.

12.4.2 Age Sensitivity

12.4.2.1 Midges are perceived to be relatively insensitive organisms in toxicity assessments (Ingersoll, 1995). This conclusion is based on measuring survival of fourth-instar larvae in short-term water-only exposures, a procedure that may underestimate the sensitivity of midges to toxicants. The first and second instars of chironomids are more sensitive to contaminants than the third or fourth instars. For example, first-instar *C. tentans* larvae were 6 to 27 times more sensitive than fourth-instar larvae to acute copper exposure (Nebeker et al., 1984b; Gauss et al., 1985; Figure 12.1) and first-instar *C. riparius* larvae were 127 times more sensitive than second-instar larvae to acute cadmium exposure (Williams et al., 1986b; Figure 12.1). In chronic tests with first-instar larvae, midges were often as sensitive as daphnids to inorganic and organic compounds (Ingersoll et al., 1990). Sediment tests should be started with uniform age and size midges because of the dramatic differences in sensitivity of midges by age. Whereas third-instar midges are not as sensitive as younger organisms, the larger larvae are easier to handle and isolate from sediment at the end of a test.

12.4.2.2 DeFoe and Ankley (1998) studied a variety of contaminated sediments and showed that the sensitivity of *C. tentans* 10-d tests is greatly increased by measurement of growth in addition to survival. Growth of midges in 10-d sediment tests was found to be a more sensitive endpoint than survival of *Hyalella azteca* (DeFoe and Ankley, 1998). In cases where sensitivity of organisms before the third instar is of interest, the long-term sediment exposures can be used, since they begin with newly hatched larvae (Section 15).

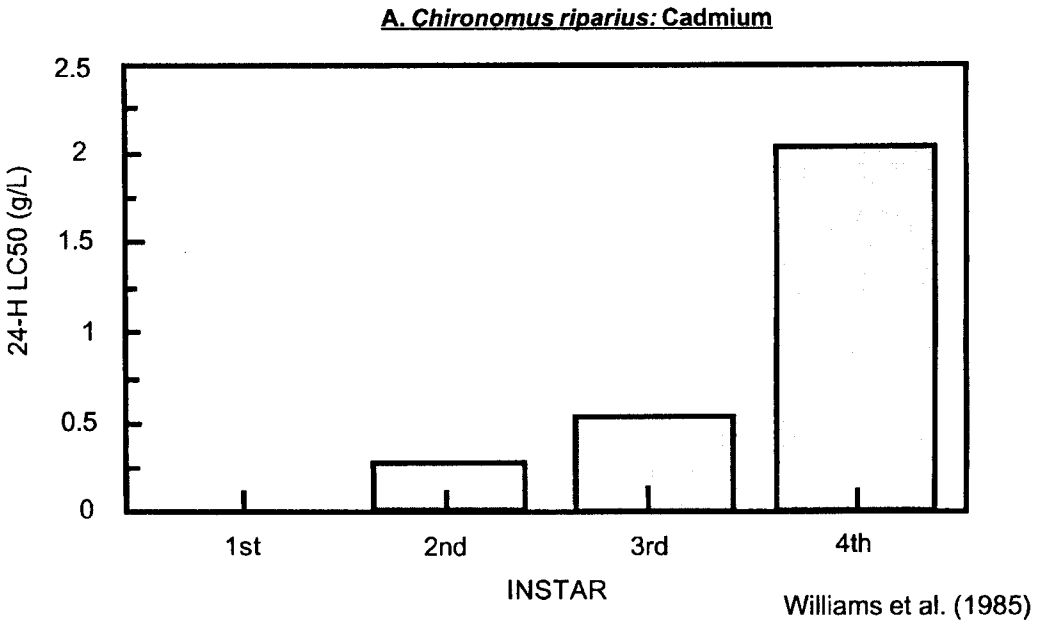
12.4.3 Physical characteristics of sediment

12.4.3.1 Grain Size

12.4.3.1.1 Larvae of *C. tentans* appear to be tolerant of a wide range of particle size conditions in substrates. Several studies have shown that survival is not affected by particle size in natural sediments, sand substrates, or formulated sediments in both 10-d and long-term exposures (Ankley et al., 1994; Suedel and Rodgers, 1994; Sibley et al., 1997b, 1998). Ankley et al. (1994a) found that growth of *C. tentans* larvae was weakly correlated with sediment grain size composition, but not organic carbon, in 10-d tests using 50 natural sediments from the Great Lakes. However, Sibley et al. (1997b) found that the correlation between grain size and larval growth disappeared after accounting for inorganic material contained within larval guts and concluded that growth of *C. tentans* was not related to grain size composition in either natural sediments or sand substrates. Avoiding confounding influences of gut contents on weight is the impetus for recommending ash-free dry weight (instead of dry weight) as the index of growth in the 10-day and long-term *C. tentans* tests. Failing to do so could lead to erroneous conclusions regarding the toxicity of the test sediment (Sibley et al., 1997b). Procedures for correcting for gut contents are described in Section 12.3.8. Emergence, reproduction (mean eggs/female), and hatch success were also not affected by the particle size composition of substrates in long-term tests with *C. tentans* (Sibley et al., 1998; Section 15).

12.4.3.2 Organic Matter

12.4.3.2.1 Based on 10-d tests, the content of organic matter in sediments does not appear to affect survival of *C. tentans* larvae in natural and formulated sediments, but may be important with respect to larval growth. Ankley et al. (1994a) found no relationship between sediment organic content and survival or growth in 10-d bioassays with *C. tentans* in natural sediments. Suedel and Rodgers (1994) observed reduced survival in 10-d tests with a formulated sediment when organic matter was <0.91%; however, supplemental food was not supplied in this study, which may influence these results relative to the 10-d test procedures described in this manual. Lacey et al. (1999) found that survival of *C. tentans* larvae was generally not affected in 10-d tests by either the quality or quantity of synthetic (alpha-cellulose) or naturally derived (peat, maple leaves) organic material spiked into a formulated sediment, although a slight reduction in survival below the acceptability criterion (70%) was observed in a natural sediment diluted with formulated sediment at an organic matter content of 6%. In terms of larval growth, Lacey et al. (1999) did not observe any systematic relationship between the level of organic material (e.g., food quantity) and larval growth for each carbon source. Although a significant reduction in growth was observed at the highest concentration (10%) of the leaf treatment in the food quantity study, significantly higher larval growth was observed in this treatment when the different carbon sources were compared at about equal concentrations (effect of food quality). In the latter study, the following gradient of larval growth was established in relation to the source of organic carbon: peat < natural sediment < alpha-cellulose < leaves. Since all of the treatments received a supplemental source of food, these data suggest that both the quality and quantity of organic carbon in natural and formulated sediments may represent an important confounding factor for the growth endpoint in tests with *C. tentans* (Lacey et al., 1999). However, it is important to note that these data are based on 10-d tests; the applicability of these data to long-term testing has not been evaluated (Section 15).



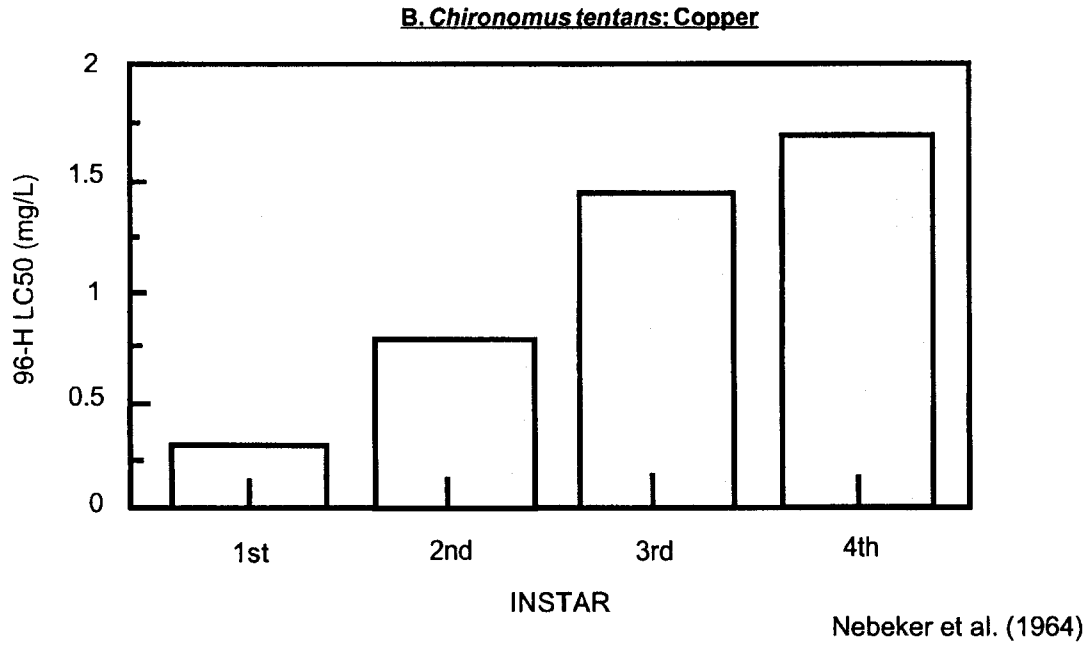


Figure 12.1 Lifestage sensitivity of chironomids.

12.4.4 Isolating Organisms at the End of a Test

12.4.4.1 Quantitative recovery of larvae at the end of a 10-d sediment test should not be a problem. The larvae are red and typically greater than 5 mm long.

12.4.5 Influence of Indigenous Organisms

12.4.5.1 The influence of indigenous organisms on the response of *C. tentans* in sediment tests has not been reported. Survival of a closely related species, *C. riparius* was not reduced in the presence of oligochaetes in sediment samples (Reynoldson et al., 1994). However, growth of *C. riparius* was reduced when high numbers of oligochaetes were placed in a sample. Therefore, it is important to determine the number and biomass of indigenous organisms in field-collected sediment in order to better interpret growth data (Reynoldson et al., 1994; DeFoe and Ankley, 1998). Furthermore, presence of predators may also influence the response of test organisms in sediment (Ingersoll and Nelson, 1990).

12.4.6. Sexual Dimorphism

12.4.6.1 Differences in size between males and females of a closely related midge species (*Chironomus riparius*) had little effect on interpretation of growth-related effects in sediment tests (<3% probability of making a Type I error [nontoxic sample classified as toxic] due to sexual dimorphism; Day et al., 1994). Therefore, sexual dimorphism will probably not be a confounding factor when interpreting growth results measured in sediment tests with *C. tentans*.

12.4.7 Ammonia Toxicity

12.4.7.1 Section 1.3.7.5 addresses interpretative guidance for evaluating toxicity associated with ammonia in sediment.