Bull. Environ. Contam. Toxicol. (1988) 41:574–581

Environmental Contamination and Toxicology

# Chronic Effects of Contaminated Sediment on *Daphnia magna* and *Chironomus tentans*

Alan V. Nebeker, Samuel T. Onjukka, and Michael A. Cairns

U.S. Environmental Protection Agency, Corvallis Environmental Research Laboratory, Corvallis, Oregon 97333

Chronic tests were conducted with *Daphnia magna* (cladoceran) and *Chironomus tentans* (midge) to determine their usefulness as test organisms for chronic sediment assays, and to estimate the potential long-term impact of contaminated freshwater sediments and contaminated Superfund site soils on freshwater invertebrates. These two species have been used successfully in acute sediment tests (Adams et al. 1985; Malueg et al. 1983; Nebeker et al. 1984a; Cairns et al. 1984) and have been shown to be useful in chronic tests in water-only bioassays (Leversee et al. 1982; Nebeker et al. 1984b; Nebeker et al. 1986).

## MATERIALS AND METHODS

*Daphnia magna* is maintained in continuous culture (20°C; 16 h light; well water: pH 7.8, alkalinity 160 mg/L, hardness 195 mg/L) at the EPA Western Fish Toxicology Station at Corvallis, Oregon, where all testing was completed (Nebeker et al. 1984a). To obtain 5- and 6-day-old *Daphnia* for testing, adults and young in 4-L rearing jars were poured onto a 10 x 15-cm, 1.5-mm mesh screen to retain adults only, which were returned to the rearing jars. An 11 x 16-cm fine mesh screen (< 0.2 mm) with upturned edges was placed under the 1.5 mm mesh "adult" screen to retain young, which were then discarded. The same procedure was repeated the next day to recover the < 24-h-old young released overnight. The young were placed in 4-L rearing jars and fed and reared for 5 or 6 days before testing (Nebeker et al. 1986).

Three types of *Daphnia* chronic tests were conducted. The first type was a 10-day test at 20°C starting with 5-day-old animals in 4-L jars (containing 2.5 L water and 500 mL test sediment). Sediment was collected with Ekman and Ponar dredges by experienced state personnel from several U.S. localities, shipped to Corvallis, and held at 4°C until used (Table 1). It was thoroughly mixed and screened through one or more standard sieve screens (e.g., 5 cm, 1 cm) to remove large particles. Non-toxic control sediment was collected from Soap Creek Pond #7 and Porter Lake near Corvallis and

Send reprint requests to A. V. Nebeker at the above address.

Table 1. Sediments used in chronic toxicity tests.

| Sediment | Location | Percent Organic | Percent Sand | Silt | Clay |
|---|---|---|---|---|---|
| Soap Cr Pond | Oregon, Soap Cr. Pond #7, 12 km N. of Corvallis | 5.2 | 15 | 29 | 56 |
| Porter Lake | Oregon, 13 km S. of Corvallis | 5.8 | 20 | 34 | 46 |
| Marys River | Oregon, at Corvallis | --- | -- | -- | -- |
| Green Bay | Wisconsin, 1.9-6.0 km NE of Fox River in Green Bay | 2.6-3.7 | 51-71 | 24-41 | 5-11 |
| Elk Lake 1 | Wisconsin, at Phillips, near metal plating outfall | 26.7 | 24 | 50 | 26 |
| Elk Lake 2 | Wisconsin, at Phillips | --- | -- | -- | -- |
| Wilson Lake | Wisconsin, at Phillips | 53.8 | 25 | 52 | 23 |
| Keweenaw | Upper Michigan, Keweenaw Peninsula north of Portage Lake | 2.4 | 15 | 66 | 19 |
| Dolly Creek | California, Plumas Co., west slope Sierra Mts., 0.3 mi above Walker copper mine | 12.7 | 50 | 40 | 10 |
| Little Grizzly Creek | California, receives drainage from Walker mine | 0.8 | 96 | 3 | 1 |
| Indian Creek | California, above confluence with Little Grizzly Creek | 0.3 | 98 | 1 | 1 |
| McClaren Tailings | Superfund site, Cooke City, Montana | --- | -- | -- | -- |
| Douglassville Disposal | Superfund site, Union, Pennsylvania | --- | -- | -- | -- |

treated similarly. Sediment was added to test beakers and allowed to settle overnight. Aeration was initiated in the beakers 30 min before addition of test animals. The 2nd and 3rd test types were conducted for 7-days at 20 or 22°C starting with 6-day-old animals in 1-L beakers. The two types of *Daphnia* tests in 1-L beakers were:

(1) those with 200 mL sediment and 800 mL well water; and (2) those with 1000 mL eluate only with no sediment. The water-only eluate is prepared by extracting Superfund site soils with water (1 part soil:4 parts water), and centrifuging (15,000 RPM) and filtering (0.45 um) the extract (Joe Greene and Cathy Bartels, personal communication; Friedman 1985, Nebeker et al. 1984a). At the start of each test the 5- or 6-day-old *Daphnia* were counted and transferred to test containers with a 5-mm ID glass pipette. The *Daphnia* were exposed for 7 or 10 days through maturation and release of young (2 or 3 broods). The test was then terminated and adults and young were counted. Number of surviving adults and young, compared with controls, were the endpoints for establishing toxicity. The tests were started by adding *Daphnia*, 10 for the 7-day beaker tests, or 20 for the 10-day jar tests, to each container. Food for the 10-day jar tests was fed at the rate of 2 mg/L solids (1.0 mg/L algae, *Selenastrum capricornutum*, plus 1 mg/L solids from a fish food-yeast mixture: 12 g pelleted fish food and 3 g yeast, blended in 1000 mL water) (Nebeker et al. 1984a; ASTM 1982). Algae only was fed in the 7-day beaker tests at the rate of 3-7 mg/L solids. Approximately 10 mL *Selenastrum* culture per beaker will turn the test water a very light but distinct green and will approximate the feeding rate as outlined above. *Daphnia* in each jar were fed the same amount of food once every other day during the 10-day tests and daily during the 7-day tests. The containers were gently aerated with a glass-tipped plastic air line (tip 4 cm below the water surface). At the end of the 10-day sediment tests, water and fine suspended solids, but not the bulk of the sediment, were poured through a 10 x 15 cm (0.5-mm mesh) screen to retain surviving adults and young *Daphnia*. They were gently rinsed from the screen into a beaker of clean water for counting. At the end of the 7- and 10-day beaker tests the animals were collected directly from the test beakers with a 5 mm ID glass pipette.

Two types of *Chironomus tentans* tests were conducted, a 25-day adult emergence test and a 15-day larval growth test. *C. tentans* was maintained in culture at Western Fish Toxicology Station (Nebeker et al. 1984a). The tests were started with 10-day-old 2nd-instar larvae. The endpoints were a count of emerged adults or number of surviving larvae with their length (mm) and weight (mg).

The sediment was placed in two types of containers for the adult emergence tests, either 20-L aquaria or 4-L jars and covered by screen to retain adults. The sediment layer was 3 cm deep, overlain by 15 cm of gently aerated water. At the start of the test up to 100 larvae were added to the test containers (transferred with 5 mm ID pipette). A food mixture of 600 mg Cerophyl (1.5 mL dry volume powdered rye grass leaves) and 100 mg (0.3 mL) finely crushed Tetra flakes was thoroughly wetted with distilled water in a small beaker and fed (dispersed over the water surface) to the animals at the start of the test and again on day 8. On day 14 they were fed 800 mg (2.0 mL) Cerophyl and 100 mg (0.3 mL) Tetra, and on day 18 they were fed 1000 mg (2.5 mL) Cerophyl and 100 mg (0.3 mL) Tetra. When

Table 2. *Daphnia magna* 10-day chronic tests at 20°C in 4-L jars (20 5-day-old *Daphnia*/jar).

| Sediment[a] | Jar[b] | Percent Adult Survival | Total Number Young | pH[c] | Alka-[c] linity (mg/L) | Hard-[c] ness (mg/L) |
|---|---|---|---|---|---|---|
| Indian Creek | 1 | 90 | 201 | 8.0 | 89 | 123 |
| | 2 | 100 | 255 | | | |
| Green Bay | 1 | 100 | 202 | 8.1 | 115 | 160 |
| | 2 | 95 | 213 | | | |
| Elk Lake 1 | 1 | 100 | 173 | 7.6 | 45 | 86 |
| | 2 | 100 | 124 | | | |
| Little Grizzly Creek | 1 | 0 | 0[d] | 8.2 | 94 | 131 |
| | 2 | 0 | 0 | | | |
| Wilson Lake | 1 | 95 | 132 | 7.4 | 38 | 77 |
| | 2 | 100 | 164 | | | |
| Keweenaw | 1 | 60 | 29[d] | --- | --- | --- |
| Porter Lake Control | 1 | 95 | 227 | 7.7 | 55 | 94 |
| | 2 | 100 | 230 | | | |
| Elk Lake 2 | 1 | 100 | 228 | 7.9 | 52 | 86 |
| | 2 | 100 | 258 | | | |
| Well Water | 1 | 100 | 272 | 8.1 | 98 | 113 |
| | 2 | 100 | 260 | | | |

a - Sediment was screened through 4.7-mm mesh. b - Continuously aerated, dissolved $O_2$ 8.6-8.8 mg/L. c - Analyzed at end of test. d - Toxic due to heavy metal contamination.

adults began emerging (after 20 days) the test was continued for another 5 days to count all emerging adults and to observe any delayed development. A small vacuum pump with a 10-mm diameter glass-tube-tipped plastic line running through an Erlenmeyer flask trap was used to collect adults and make daily counts: the screen cover was slowly lifted from the container and the adults were "vacuumed" from the screen and the inside walls of the container.

Procedures for the *Chironomus* larval survival test were the same as those for the adult emergence test, but larval survival and growth (length and weight) after 15 days were the endpoints. At the end of the test, animals were screened (0.5 mm mesh) from the test water and sediment and counted. Food for *Chironomus*, in addition to that obtained from the sediment, was Cerophyl and Tetra. The Cerophyl-Tetra food mixture (600 mg Cerophyl, 1.5 mL dry volume, and 100 mg powdered Tetra, 0.3 mL) was mixed with 100 mL distilled water in a small beaker and dispersed over the water surface of the aquaria (we recommend powdered pelleted rabbit food for future tests).

Table 3. *Daphnia magna* 7-day and 10-day chronic tests at 22°C in 1000-mL beakers (10 6-day-old *Daphnia*/beaker).

| Test Material | Beaker | Percent Adult Survival | Total Number Young | pH | Hardness (mg/L) |
|---|---|---|---|---|---|
| Soap Cr Pond | 1 | 90 | 78 | 7.3 | 158 |
| Sediment and water | 2 | 80 | 70 | 7.4 | 164 |
| (7-day test) | 3 | 80 | 59 | 7.4 | 167 |
| | 4 | 70 | 36 | 7.4 | 166 |
| Soap Cr Pond | 1 | 100 | 175 | --- | --- |
| Sediment and water | 2 | 90 | 147 | --- | --- |
| (10-day test) | 3 | 100 | 178 | --- | --- |
| | 4 | 100 | 226 | --- | --- |
| Eluate from | 1 | 100 | 244 | 7.8 | 148 |
| McClaren | 2 | 100 | 196 | 7.8 | 148 |
| Tailings site | 3 | 100 | 225 | 7.0 | 410 |
| (7-day test) | 4 | 100 | 230 | 7.0 | 410 |
| Eluate from | 1 | 100 | 186 | 6.3 | --- |
| Douglassville | 2 | 100 | 171 | 6.3 | --- |
| Disposal site | 3 | 100 | 201 | 6.4 | 92 |
| (7-day test) | 4 | 100 | 159 | 6.4 | 92 |
| Eluate from | 1 | 100 | 31[a] | 6.8 | --- |
| Douglassville | 2 | 100 | 34[a] | 6.8 | --- |
| Disposal site (7-day test) | | | | | |
| Water only controls | 1 | 100 | 167 | 8.3 | 195 |
| (7-day test) | 2 | 100 | 153 | 8.2 | 195 |

a - Toxic sample, no second brood produced.

Routine chemical analyses, dissolved oxygen, pH, total hardness, and total alkalinity (APHA 1980) were conducted to characterize the test water. Analyses such as particle size, organic content, total carbon, heavy metals, ammonia, turbidity, and organics were used to characterize the sediment and overlying water.

## RESULTS AND DISCUSSION

*Daphnia* 10-day chronic tests at 20°C, starting with 5-day old animals, were successfully completed with nine sediment samples (Table 2). Counting the total adults and young exposed to uncontaminated sediments after 10 days gave an average of 97% survival of adult *Daphnia*; an average of 210 young were recovered from each test jar. The copper-contaminated sample from Little Grizzly Creek killed all animals, and the copper-contaminated Keweenaw sample caused mortality and decreased young production. The Elk Lake 1 sediment from near the metal-plating discharge possibly caused a

Table 4. *Chironomus tentans* 15-day larval survival tests.

| Sediment | Test container | No.[a] midges used | Percent larval survival | Water[b] hardness (mg/L) | pH[c] | Alka-[c] linity (mg/L) | Hard-[c] ness (mg/L) |
|---|---|---|---|---|---|---|---|
| Soap Cr | 1[d] | 50 | 76 | 32 | 7.1 | 43 | 44 |
| Pond Control | 2 | 50 | 84 | | 7.2 | 46 | 45 |
| Little | 1[d] | 50 | 0[f] | 32 | 7.7 | 78 | 77 |
| Grizzly Creek | 2 | 50 | 0 | | 7.7 | 83 | 81 |
| Marys River | 1[e] | 65 | 86 | 28 | 7.6 | 139 | 108 |
| | 2 | 65 | 69 | | | | |
| Wilson Lake | 1[e] | 65 | 68 | 28 | 7.2 | 70 | 30 |
| | 2 | 65 | 68 | | | | |
| Marys River | 1[d] | 100 | 81 | 27 | 7.9 | 82 | 76 |
| | 2 | 100 | 92 | | | | |
| Porter Lake | 1[d] | 100 | 92 | 27 | 7.5 | 54 | 44 |
| Control | 2 | 100 | 89 | | | | |

a - Fed twice a week, 0.5 mL tetra and 1.5 mL cerophyl (dry volume) per container. b - Before addition to sediment. c - After sediment-water contact. d - 20-L aquaria (dissolved $O_2$ = 6.6-7.5 mg/L). e - 4-L jars. f - Copper mine contamination.

reduction in young *Daphnia*. The tests were relatively easy to set up, and recovery of the large 15-day old *Daphnia* and the older (> 2 day) young with 0.5 mm mesh screen was satisfactory. The amount of food used could be reduced by half as reproduction was higher than necessary to give adequate numbers of young to define effect levels, and it would reduce the interaction with food and toxic chemicals.

*Daphnia* 7-day chronic tests at 22°C, starting with 6-day old animals, were successfully completed with samples from Soap Creek Pond, two Superfund sites, and water-only controls (Table 3). This procedure is preferred over the 10-day tests because it takes less time and produces the same type of data. Two broods normally hatch during the 7-day period, and are easily separated by size when counted. In the toxic sample from the Douglassville site, no adult mortality occurred, but little growth occurred during the 7-day test period, production of first brood was limited (4/10 adults in Beaker 1 and 5/10 in Beaker 2 still retained first brood eggs in their brood pouch), and no 2nd brood eggs developed in those animals that successfully released the first brood.

Six *Chironomus* larval survival tests were conducted, with larval recoveries ranging from 68-92% (Table 4). Two adult tests starting with 2nd-instar larvae (Table 5), and one starting with 3rd-instar larvae were completed. Adult emergence ranged from 42 to 74%. Results were similar between 20-L aquaria and 4-L jars. Sediment

Table 5. *Chironomus tentans* 25-day adult emergence tests.

| Sediment | Test[a] container | No. of adults emerged males | females | Percent adult emergence | pH | Test water[b] alka-linity | hard-ness |
|---|---|---|---|---|---|---|---|
| Soap Cr Pond | 1[c] | 19 | 9 | 56 | 7.4 | 26 | 28 |
| Control | 2 | 42 | 32 | 74 | | | |
| Soap Cr Pond | 1[c] | 28 | 20 | 48 | 7.1 | 42 | 46 |
| Control | 2 | 31 | 11 | 42 | | | |
| Green Bay | 1[c] | 34 | 25 | 59 | - | - | - |
| | 2 | 28 | 15 | 43 | | | |
| Elk Lake 2 | 1[c] | 41 | 29 | 70 | - | - | - |
| | 2 | 44 | 26 | 70 | | | |
| Indian Creek | 1[d] | 17 | 28 | 45 | 7.1 | 30 | 33 |
| | 2 | 17 | 33 | 50 | | | |
| Green Bay | 1[d] | 23 | 20 | 43 | - | - | - |
| | 2 | 32 | 31 | 63 | | | |

a - 100 midges/container, except 50 in Soap Creek Pond. Fed twice a week, 1 mL tetra and 1 mL Cerophyl (dry volume) per container. b - Before addition to sediment. c - 20-L aquaria. d - 4-L jars.

from Little Grizzly Creek, containing high concentrations of copper from mine tailings, killed all larvae; no other sediment was so obviously toxic to *Chironomus*.

Acknowledgments. We wish to thank J. Gakstatter, A. Knight, K. Malueg, and G. Schuytema for furnishing test sediments, J. Greene and C. Bartels for providing Superfund site eluate, D. Krawczyk for chemical analyses of sediment, T. Stutzman for bioassay aid, S. Dominguez for providing algae, and N. Lanpheare for manuscript preparation.

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Received August 3, 1987; Accepted November 19, 1987.