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Experimental Factors That May Affect Toxicity of Cadmium to Freshwater Organisms

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Abstract. The effects of exposure duration, test organism, and test endpoint on the toxicity of cadmium to a variety of freshwater species were evaluated. Toxicity of cadmium was assessed by monitoring the survival and reproduction of *Ceriodaphnia dubia* Richard; the survival of *Daphnia magna* Straus; and the survival and growth of *Hyalella azteca* Saussure, *Chironomus tentans* Fabricius, and *Pimephales promelas* Rafinesque. Organisms were exposed in static systems for 48 h, 96 h, 7 d, 10 d, and 14 d to determine acute and chronic toxicity. Relative sensitivities of test organisms exposed to aqueous cadmium varied with test duration and test endpoint. In general, *H. azteca* was the most sensitive organism tested, followed in decreasing sensitivity by *P. promelas*, C. *dubia*, *D. magna*, and *C. tentans*. Mortality of *C. dubia* and *D. magna* was consistent up to 7 d, after which little additional mortality occurred. Effects of test duration on cadmium toxicity were most pronounced for *H. azteca* and *C. tentans*, with mortality and growth becoming increasingly sensitive with increasing test duration.

Assessments of contaminant effects on aquatic organisms typically involve conducting laboratory experiments. The ability of such experiments to detect adverse effects of contaminants depends on several factors, including exposure duration, species selection, and test endpoints. Exposure duration was previously shown to affect the potential for contaminant toxicity. For example, *Hyalella azteca* and *Chironomus tentans* required 10–14 d of exposure to accurately assess the potency of a copper-contaminated sediment (Suedel *et al*. 1996).

Accurate laboratory assessment of the potential potency of a given contaminant also requires selection of appropriate experimental organisms. Species such as *C. tentans* and *Chironomus riparius* have been perceived to be insensitive to various contaminants (Williams *et al*. 1986; Gauss *et al*. 1985), but these observations were based on testing with third to fourth instar larvae, which can be thousands of times less sensitive than first instar larvae (Pascoe *et al*. 1989). Microcrustaceans such as *Daphnia magna* and *Ceriodaphnia dubia* have historically demonstrated sensitivity to a wide variety of toxicants, but more recent studies have shown that other species such as amphipods may be as sensitive or more sensitive than microcrustaceans to various toxicants in chronic and subchronic exposures (Borgmann *et al*. 1989; Suedel *et al*. 1993).

Sensitive test endpoints are also a necessary component of toxicity tests in order to accurately assess contaminant toxicity (Suter *et al*. 1987; Winner 1988; Suedel *et al*. 1996). Test results have indicated a wide range of sensitivity of test endpoints. Change in *H. azteca* body length was less sensitive than percent survival (Ingersoll and Nelson 1990), and *C. tentans* growth as dry weight can be substantially more sensitive than survival (Suedel *et al*. 1996). Test endpoint sensitivity is both chemical- and species-specific, with body length and total number of young produced per female being the most sensitive endpoints for *D. magna* and *C. dubia*, respectively, when exposed to aqueous cadmium (Winner 1988).

Although a substantial toxicological data base exists for cadmium and freshwater biota (US EPA 1980), this study adds data for species not previously reported (*e.g.*, *H. azteca*, *C. tentans*, and *C. dubia*) and allows direct comparisons between aquatic organisms representing a variety of niches and physiological capabilities. Predicting the impact of cadmium on aquatic organisms is complicated by differing chemical forms of cadmium that may exist in freshwaters, *i.e.*, free cadmium ion, organic and inorganic complexes, and insoluble complexes with hydroxide or sulfide ions (US EPA 1980). The aqueous chemistry of cadmium is affected by parameters such as pH, alkalinity, hardness, and organic carbon content (US EPA 1979, 1980). Cadmium is generally more bioavailable as hardness, alkalinity, and dissolved organic content decrease (US EPA 1980). Observed responses of organisms should be dependent on the chemical form of the metal.

Experiments were conducted, and the test species *Chironomus tentans* Fabricius, *Hyalella azteca* Saussure, *Daphnia magna* Straus, *Ceriodaphnia dubia* Richard, and *Pimephales promelas* Rafinesque were used to: 1) determine the relative sensitivities of these organisms to cadmium in aqueous exposures; 2) determine the duration of exposure required to elicit mortality and/or sublethal biological effects (*e.g.*, reduced growth or reproduction); and 3) determine if sublethal test

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endpoints are more sensitive than survival for test organisms used in this study. Understanding the effects of these factors on toxicity will help to reduce uncertainty regarding the determination of toxicant potency of a given test material to aquatic organisms.

Materials and Methods

Test Organism Culture Procedures

All test organisms were cultured in the laboratory at the University of Mississippi Biology Department. *H. azteca* culturing procedures followed the methods of de March (1981). Amphipods used for testing were removed from cultures and gently washed through a 1.0-mm mesh sieve. Organisms that passed through the 1.0-mm sieve but were retained by a 0.6-mm sieve (approximately 2–3 weeks old) were collected and used for testing. *C. tentans* culture methods followed those of Townsend *et al.* (1981). Midges used for testing were second instar larvae (10-d-old). *D. magna* and *C. dubia* culturing procedures followed the methods of Peltier and Weber (1985). Hardness and alkalinity of University of Mississippi Biological Field Station (UMBFS) water were adjusted with (0.1 g/L) $NaHCO_3$ and $CaCl_2$ to a total hardness of 80 mg/L as $CaCO_3$ and alkalinity of 60 mg/L as $CaCO_3$ for *D. magna* cultures. *P. promelas* culturing procedures followed the methods of Peltier and Weber (1985). Fathead minnows used in testing were 2–4-d-old.

Experimental Design

Twenty-five experiments were conducted, one for each species and test duration. All experiments were conducted in temperature and light (1500–1800 lux) controlled incubators under a 16-h light/8-h dark photoperiod. Experiments were started by adding eight *H. azteca*, six *C. tentans*, eight *D. magna*, or eight *P. promelas* to each of four replicate beakers. Experiments with *C. dubia* were started by adding one neonate to each of ten replicate beakers per treatment. Experiments were conducted in 250-ml borosilicate glass beakers with 200 ml of UMBFS water, except for *C. dubia* tests, which were conducted in 50-ml beakers with 40 ml of UMBFS water. UMBFS water was used as a control, except for *D. magna* tests, where adjusted UMBFS pond water (see culture procedures) was used. Glass beads (150–212 µm, Sigma Chemical Co., St. Louis, MO, USA) were used as a substrate in *C. tentans* tests to allow for tube building and to reduce stress (Suedel and Rodgers 1993). In all tests, feeding regimes for each organism were as follows: *D. magna* and *C. dubia*--0.5 and 0.05 ml, respectively, of *S. capricornutum* algae daily; *H. azteca*--0.5 g wet wt. of leached and ground maple leaves at test initiation; *C. tentans*--0.1-ml cerophyll suspension at test initiation and every other day thereafter; *P. promelas*—0 to 20 newly hatched *Artemia* nauplii per fish daily. Dissolved oxygen concentrations did not drop below 40% of saturation in any test; therefore, aeration was not required. Water quality parameters measured are presented in Table 1.

Analytical Procedures

Stock solutions were prepared by dissolving reagent grade cadmium chloride ($CdCl_2$) in Milli-Q water. Water samples for analysis were obtained from sacrificial beakers (one replicate per concentration) at the start of each test. Water samples were filtered through a 0.45-µm filter and acidified with redistilled nitric acid (Aldrich Chemical Co., Milwaukee, WI, USA) to a pH of 1–2 before analysis. Cadmium samples of <30 µg/L were concentrated by boiling before analysis. Cadmium concentrations were determined using a Buck model 200-A flame atomic absorption spectrophotometer. A preliminary fate experiment using water samples taken at 0, 2, 4, 7, 10, and 14 d under the experimental conditions listed above indicated that measured cadmium concentrations were 102.6 ± 16.3% (mean ± S.D., $N = 36$) of nominal concentrations, and measured values obtained at the start of each test were 102.3 ± 3.7%(mean ± S.D.) of nominal concentrations. Hence, nominal values were used for calculations and presentations.

Table 1. Water characteristics measured in experiments conducted with freshwater organisms. D. magna data are presented separately because adjusted UMBFS pond water was used

Parameter	All Other Experiments	D. magna Experiments
Temperature (°C)	20.7– 24.8[a]	23.9– 24.0[a]
	19.2– 22.3[b]	19.6– 21.5[b]
pH	5.5– 7.4[a]	6.9– 8.0[a]
	5.9– 7.7[b]	7.0– 8.3[b]
D.O. (mg/L)	4.2– 9.3	7.7– 9.0
Alkalinity (mg/L as $CaCO_3$)	8 – 18	68 – 70
Hardness (mg/L as $CaCo_3$)	6 – 28	69 – 87
Conductivity (µS/cm)	22 –130	286–312

[a] Range at test initiation.
[b] Range at test termination.

Statistical Analyses

All statistical tests were performed using the TOXSTAT® Version 3.4 statistical computing package (WEST, Inc., and Gulley, Cheyenne, WY, USA). Lethal concentrations (LC_{50}) and 95% confidence intervals (C.I.) for all tests were calculated using the probit method. Tests for normality and homogeneity of variance were performed using Shapiro-Wilk's test and Bartlett's test, respectively. Analysis of variance (ANOVA) was used to test the null hypothesis that all treatment means were equal (except *C. dubia* data), and Bonferroni's *T*-Test was used to detect differences between control and treatment means of survival, growth, and reproductive endpoints and for the determination of no-observable-effects concentrations (NOEC) and lowest-observable-effects concentrations (LOEC). For *C. dubia*, Fisher's Exact test (survival) and Dunnett's Multiple Range Test (reproduction) were used to detect differences between control and treatment means and to calculate NOEC and LOEC values. The 5% alpha level was used in all statistical tests.

Results and Discussion

Survival of control organisms in all experiments ranged from 81 to 100% (Figure 1). As shown in Table 1, low hardness (6–28 mg/L as $CaCO_3$) may have resulted in a ''worst case'' cadmium exposure (except for *D. magna*), as increasing water hardness is thought to reduce the toxicity of cadmium (Wong 1987; Sprague 1987). These conditions also represent the limits of environmental tolerance for these water characteristics for these test species.

Ceriodaphnia dubia

C. dubia responded to cadmium predominantly within the first 96 h of exposure (Figure 1; Table 2). Thereafter, LC_{50} values

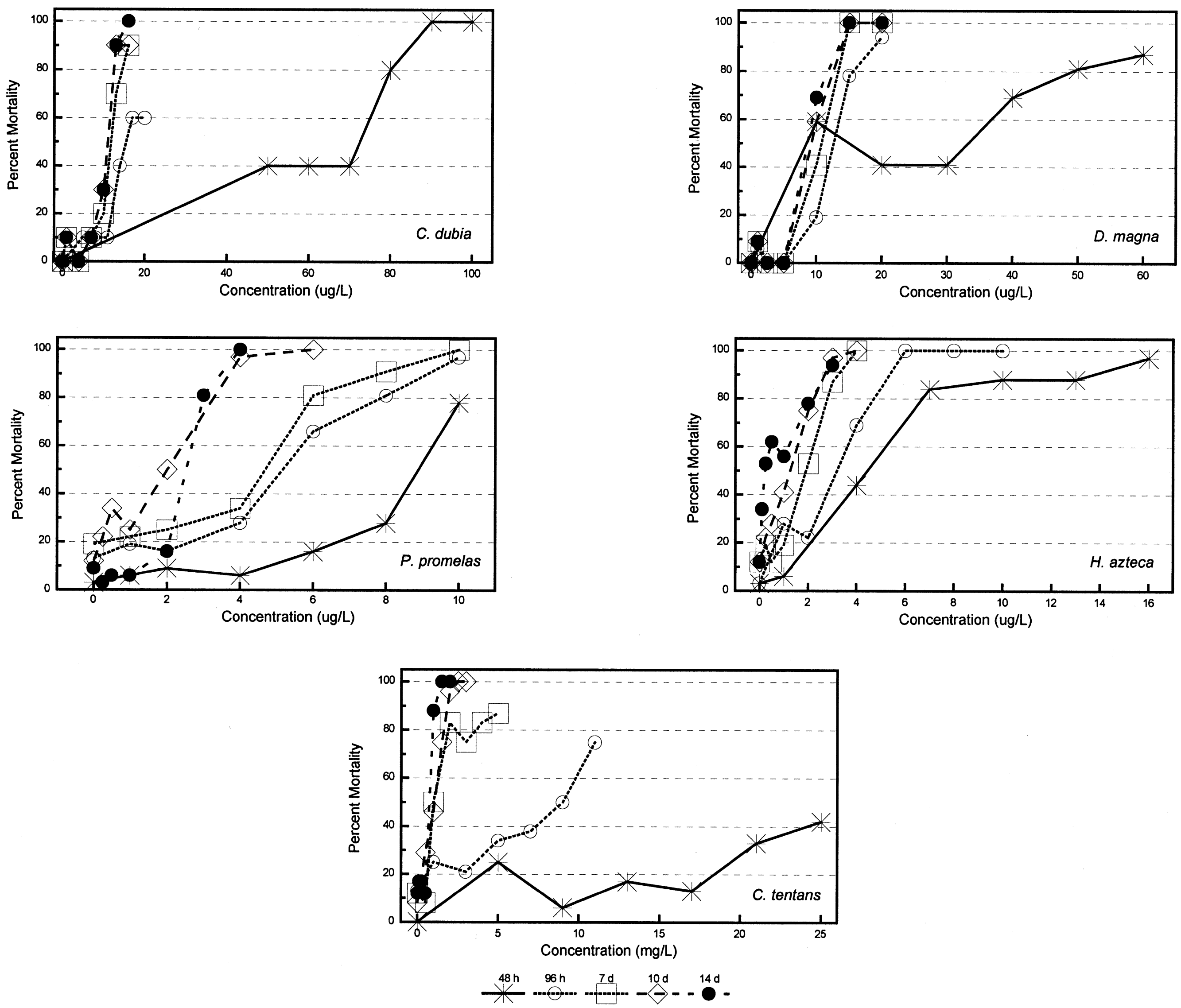

Fig. 1. Exposure-response curves of test species exposed to cadmium

decreased only slightly with increasing test duration and remained nearly constant from 7–14 d of exposure. No-observable-effects concentrations for both survival and reproduction remained constant during the 7-, 10-, and 14-d tests (Table 3). *C. dubia* reproduction, expressed as the total number of offspring per female, was more sensitive than survival in the 7-, 10-, and 14-d tests with NOEC values of 10.0 µg Cd/L for survival and 1.0 µg Cd/L for reproduction. Suedel *et al*. (1996) also observed consistent survival after 96 h for *C. dubia* exposed to copper under comparable test conditions.

In a previous study reporting acute toxicity data for *C. dubia* exposed to cadmium chloride, similar 48-h LC_{50} values (66–110 µg Cd/L; hardness range = 45–200 mg/L as $CaCO_3$) were obtained (Hall *et al*. 1986). Tests exposing *C. dubia* to cadmium sulfate resulted in 7-d NOEC values of 1.0 and 0.50 µg Cd/L based on survival and number of offspring per female, respectively (Winner 1988). Winner (1988) concluded that for *C. dubia* the total number of offspring per female was more sensitive than survival.

Daphnia magna

Length of cadmium exposure had minimal effect on the survival of *D. magna* after 7 d (Figure 1; Tables 2 and 3). LC_{50} values decreased from 26.4 µg Cd/L to 12.7 µg Cd/L from the 48-h test to the 96-h test. Both LC_{50} and NOEC values were similar after 7, 10, or 14 d of exposure, with LC_{50}s of 9.9–8.6 µg Cd/L and an NOEC value of 5.0 µg Cd/L (Tables 2 and 3). The survival pattern exhibited by *D. magna* was also observed following exposure to copper under similar test conditions (Suedel *et al*. 1996).

The 48-h LC_{50} value of 26.4 µg Cd/L reported in Table 2 is within the range of values (6.1–118 µg Cd/L) from other studies reporting 48-h LC_{50} values for *D. magna* exposed to cadmium chloride (Attar and Maly 1982; Schuytema *et al*. 1984; Nebeker *et al*. 1986; Hall *et al*. 1986). The cadmium criteria document presents LC_{50} values for *D. magna* exposed to cadmium chloride of 9.9 to 63 µg Cd/L (no duration given) for waters

Table 2. LC_{50} values (μg/L) for organisms exposed to cadmium

Species	Test Duration	LC_{50}	95% C.I.
C. dubia	48h	63.1	55.5 – 70.6
	96h	16.9	13.8 – 20.0
	7d	11.6	9.7 – 13.5
	10d	10.6	8.9 – 12.3
	14d	10.1	8.5 – 11.7
D. magna	48h	26.4	21.2 – 31.7
	96h	12.7	11.5 – 13.9
	7d	9.9	8.8 – 10.9
	10d	9.0	8.0 – 10.1
	14d	8.6	7.6 – 9.6
P. promelas	48h	8.9	7.7 – 10.1
	96h	4.8	4.1 – 5.5
	7d	4.4	3.7 – 5.2
	10d	1.6	1.3 – 2.0
	14d	2.3	2.1 – 2.6
H. azteca	48h	5.6	4.6 – 6.5
	96h	2.8	2.4 – 3.3
	7h	1.7	1.4 – 2.0
	10d	1.2	0.96– 1.4
	14d	0.65	0.38– 0.92
C. tentans	48h	29,560	21,250 –37,870
	96h	8,000	6,230 – 9,760
	7d	1,700	1,250 – 2,150
	10d	963	786 – 1,140
	14d	635	515 – 755

with hardness values ranging from 51 to 209 mg/L as $CaCO_3$ (US EPA 1980). For *D. magna* exposed to cadmium chloride for 21 d, LC_{50} values ranged from 3.3 to 8.3 μg Cd/L in water with a hardness of 225 mg/L as $CaCO_3$ (Enserink *et al*. 1993). Studies reporting reproductive effects of cadmium chloride on *D. magna* gave NOEC and LOEC values ranging from 0.35 to 2.0 μg Cd/L, values lower than the NOEC and LOEC values reported in Table 3 based on survival (van Leeuwen *et al*. 1985; Biesinger *et al*. 1986; Borgmann *et al*. 1989).

Pimephales promelas

P. promelas' response to cadmium was intermediate compared to other species tested and showed a general decrease in survival with increasing exposure duration (Figure 1; Table 2). LC_{50} values decreased through 10 d of exposure, but increased at 14 d. *P. promelas'* growth (expressed as dry weight) was less sensitive than survival in the 10- and 14-d tests (Table 3). Lower sensitivity of the growth endpoint relative to survival was also observed for 2–4-d-old *P. promelas* exposed for 10 and 14 d to copper-contaminated sediment (Suedel *et al*. 1996).

Acute toxicity values for fathead minnows exposed to cadmium reported in other studies are higher than those in the present study. Hall *et al*. (1986) reported 96-h LC_{50} values ranging from 12 to 150 μg Cd/L from several studies (hardness range = 39–200 mg/L as $CaCO_3$). Differing life stages (*i.e.*, larval to adult) and water hardness values likely account for some of the observed differences in acute toxicity. A 30-d LC_{50} value of 2.6 μg Cd/L (hardness = 50 mg/L as $CaCO_3$) for fathead minnows exposed to cadmium (Sprague 1987) is comparable to the 14-d value of 2.3 μg Cd/L observed in the present study at a hardness of <28 mg/L as $CaCO_3$.

Table 3. NOEC, LOEC, and corresponding effects for five species exposed to cadmium

Species	Test Duration (Endpoint)	NOEC (μg/L) (Value)	LOEC (μg/L) (Value)
C. dubia	7 d (Survival)[a]	10 (80)	13 (30)
	7 d (Reproduction)[b]	1.0 (1.8)	4.0 (0)
	10 d (Survival)	10 (70)	13 (0)
	10 d (Reproduction)	1.0 (3.4)	4.0 (0.5)
	14 d (Survival)	10 (70)	13 (10)
	14 d (Reproduction)	1.0 (6.0)	4.0 (3.2)
D. magna	7 d (Survival)	5.0 (100)	10 (59)
	10 d (Survival)	5.0 (100)	10 (41)
	14 d (Survival)	5.0 (100)	10 (31)
P. promelas	7 d (Survival)	4.0 (66)	6.0 (19)
	10 d (Survival)	1.0 (75)	2.0 (50)
	10 d (Growth)[c]	2.0 (0.156)	>2.0 (—)
	14 d (Survival)	2.0 (84)	3.0 (19)
	14 d (Growth)	3.0 (0.445)	>3.0 (—)
H. azteca	7 d (Survival)	1.0 (81)	2.0 (47)
	10 d (Survival)	1.0 (59)	2.0 (25)
	14 d (Survival)	0.1 (66)	0.25 (47)
	14 d (Growth)	2.0 (0.175)	>2.0 (—)
C. tentans	7 d (Survival)	500 (92)	1,000 (50)
	7 d (Growth)	<500 (—)	500 (0.033)
	10 d (Survival)	500 (71)	1,000 (54)
	10 d (Growth)	<500 (—)	500 (0.036)
	14 d (Survival)	500 (88)	1,000 (13)
	14 d (Growth)	<100 (—)	100 (0.051)

[a] Percent
[b] Total number of offspring per female
[c] mg

Hyalella azteca

H. azteca was the most sensitive species tested, showing decreasing survival with increasing test duration through 14 d of exposure (Figure 1; Table 2). Test durations of at least 14 d may be required to accurately assess the toxicity of cadmium to *H. azteca. H. azteca* survival (0.1 μg Cd/L NOEC) was at least

20 times more sensitive than growth (>2.0 µg Cd/L NOEC) measured in the 14-d experiment (Table 3).

Toxicity data reported herein were slightly lower than data reported in other studies exposing *H. azteca* to cadmium. The 96-h (8 µg Cd/L) and 10-d (<2.8 and 6.0 µg Cd/L) LC_{50} values reported by Nebeker *et al*. (1986) and the 96-h (85 µg Cd/L) LC_{50} value reported by Wong *et al*. (1987) are higher than the 96-h (2.8 µg Cd/L) and 10-d (1.2 µg Cd/L) LC_{50} values reported in Table 2. Collyard *et al*. (1994) reported 96-h LC_{50} values of approximately 6 to 13 µg Cd/L for *H. azteca* exposed to cadmium chloride in water with a hardness of 90 mg/L as $CaCO_3$. The 6-week NOEC reported by Borgmann *et al*. (1989) of 0.57 µg Cd/L based on survival was higher than the 14-d NOEC of 0.1 µg Cd/L based on survival in the present study. Similar to results in this study, Borgmann *et al*. (1989) noted that *H. azteca* growth (expressed as wet weight) was not a sensitive toxicological endpoint in cadmium exposures. Based on water chemistry data for the above studies, lower hardness (6–28 mg/L as $CaCO_3$) and pH values (5.5–7.7) in this study may contribute to the observed differences in cadmium toxicity. The cadmium criteria document lists no data for *H. azteca* (US EPA 1980).

Chironomus tentans

C. tentans survival decreased through time when exposed to cadmium, but *C. tentans* was the least sensitive species tested (Figure 1; Table 2). *C. tentans* growth, expressed as dry weight, was considerably more sensitive than survival in the 7-, 10-, and 14-d tests (Table 3). In this study, no NOEC for growth was achieved for *C. tentans* exposed to cadmium for up to 14 d of exposure. Greater sensitivity of the growth endpoint compared to survival was also observed for *C. tentans* exposed for 10 d to copper-contaminated sediment (Suedel *et al*. 1996). Test durations of at least 14 d may be required to accurately assess the toxicity of cadmium to *C. tentans.* Conducting laboratory evaluations with *C. tentans* with an insufficient test duration (*i.e*., <14 d) or insensitive endpoint (*i.e*., survival) may lead to erroneously concluding that samples containing cadmium are not toxic.

The results for *C. tentans* presented herein were generally similar to results found for other chironomid species. The 48- and 96-h LC_{50} values of 45,000 and 13,000 µg Cd/L (hardness = 100–110 mg/L as $CaCO_3$), respectively, for *C. riparius* (Williams *et al*. 1986) were similar (within a factor of two) to 48- and 96-h LC_{50} values for *C. tentans* of 29,560 and 8,000 µg Cd/L (Table 2). The LOEC of 100 µg Cd/L based on *C. tentans* growth (Table 3) was similar to the 150 µg Cd/L value for *C. riparius* exposed for 17 d (hardness = 98 mg/L as $CaCO_3$) based on emergence and development effects (Pascoe *et al*. 1989). However, the mean (± s.d.) 10-d LC_{50} value of 3.8 ± 3.5 µg Cd/L (hardness = 47 mg/L as $CaCO_3$) for the parthenogenetic chironomid *Tanytarsus dissimilis* was substantially lower than those reported for other chironomid species (Anderson *et al*. 1980). There are no data presented in the cadmium criteria document for *C. tentans* (US EPA 1980).

Relative Sensitivities, Test Duration, and Endpoints

Relative sensitivities of species exposed to cadmium were dependent on test duration and test endpoint (Table 3). *H. azteca* was the most sensitive species tested, with an observed NOEC for survival as low as 0.1 µg Cd/L. *P. promelas* survival was more sensitive than *D. magna* and *C. dubia* survival, with NOECs observed for *P. promelas* at 1.0 µg Cd/L (10–14 d), for *C. dubia* at 10.0 µg Cd/L (7–14 d), and for *D. magna* at 5.0 µg Cd/L (7–14 d).

The effect of test duration and endpoint on test results was species-dependent (Table 3). In general, the cladocerans, *C. dubia* and *D. magna*, responded to cadmium within 7 d, with no appreciable mortality occurring thereafter. *C. dubia* reproduction was ten times more sensitive than survival in 7-, 10-, and 14-d tests. *P. promelas* growth in 10- and 14-d tests was 20–30 times less sensitive than survival, even though tests were started with 2–4-d-old fry. *H. azteca* survival decreased through time, with mortality continuing through 14 d. In this study, *C. tentans* achieved a survival NOEC at 500 µg Cd/L at 7–14 d of exposure, but growth expressed as dry weight was at least five times more sensitive than survival. Tests of at least 10–14-d duration were required to accurately assess the potency of cadmium to *H. azteca* and *C. tentans*.

Organism physiology plays an important role in organisms' sensitivities to cadmium. Chironomid larvae regulate accumulation of copper, nickel, and zinc in their tissues when exposed to these metals in sediments (Krantzberg and Stokes 1989). Metal-binding proteins (*e.g*., metallothioneins) act as sinks or sequester metals such as zinc, copper, cadmium, and mercury in organism tissues (Petering and Fowler 1986; Fowler 1987; Olsson and Haux 1986). This may account for the much higher concentration of cadmium needed to elicit a response compared to other species tested. Increased exposure of *C. tentans* to cadmium resulted in increased mortality and reduced growth, indicating that a minimum exposure of 10–14 d to cadmium is required to manifest an adverse response.

The quantity of metal transferred into biological tissues is influenced by both the physiological state of the organism and by biological factors involved in metal metabolism (Luoma 1983). Surface adsorption is especially important in crustaceans exposed to dissolved metals (Luoma 1983). The cladocerans in this investigation were fed *S. capricornutum* algae daily. The addition of the algae to test chambers may have provided additional organic binding sites for cadmium. The cladocerans' exposure might therefore have included ingested cadmium-bound algae that was less bioavailable than the dissolved fractions of cadmium. *H. azteca* cadmium exposures would have been primarily at critical external binding sites such as gill surfaces, and would be influenced by the rate of water transport across the gills, which may account for the increased sensitivity of this species.

Summary

Effects of experimental conditions including exposure duration, test organism selection, and test endpoint on the observed toxicity of cadmium to five freshwater species were evaluated. Relative sensitivities of test species varied with test duration and test endpoint. In general, *H. azteca* was the most sensitive organism tested, followed in decreasing sensitivity by *P. promelas*, *C. dubia*, *D. magna*, and *C. tentans*. Effects of test duration on cadmium toxicity were most pronounced for *H. azteca* and *C. tentans*, with mortality and growth effects becoming increasingly sensitive with increasing test duration.

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