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Acute Toxicity of Waterborne Cd, Cu, Pb, Ni, and Zn to First-Instar *Chironomus riparius* Larvae

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Abstract The acute toxicities of waterborne Cd, Cu, Pb, Ni, and Zn were determined in the first-instar larvae (generally considered to be the most sensitive) of *Chironomus riparius*, under standardized conditions. Toxicity tests were conducted in soft water (hardness, 8 mg/L as $CaCO_3$ equivalents) in the absence of food and were limited to 24 h to avoid control mortality associated with food deprivation. For each metal, a logarithmic range of concentrations was tested between 0 and 25 mg/L. First-instar *C. riparius* are most sensitive to Pb, with a 24-h LC50 of 0.61 mg/L (CI of 0.26–1.15 mg/L), and to Cu, with a 24-h LC50 of 2.09 mg/L (CI of 1.57–2.96 mg/L). The LC50 for Cd was 9.38 mg/L, while the LC50s for Zn and Ni were both higher than the highest tested concentration of 25 mg metal/L. Our results found that even first-instar chironomid larvae are well protected by both the current CCME Canadian water quality guidelines for the protection of aquatic life and the USEPA Water Quality Criteria, as LC50s were at least 25 times higher than the guideline concentrations.

Keywords Chironomids · Toxicity · LC50 · Cd · Cu · Pb · Ni · Zn

Assessing the toxicity of metals on aquatic life has been a long-standing practice. Metals such as cadmium (Cd), copper (Cu), lead (Pb), nickel (Ni), and zinc (Zn) have all been found in waterways, particularly from sources such as municipal waste water effluents, industrial point source effluents (e.g., mining spoil runoff), urban runoff, landfill leachates, natural sources, and soil/substrate disturbances (Environment Canada 2001). Toxicity tests serve to quantify how poisonous or lethal various metals are to aquatic life. Both acute and chronic studies are necessary; however, acute exposure data are useful because they help explain toxic effects (Watts and Pascoe 2000). Many benthic freshwater invertebrates have been used for these toxicity tests, among them amphipods, oligochetes, and chironomid larvae (USEPA 1994).

In general, chironomids appear to be exceptionally tolerant to metals. Indeed, it has been shown that chironomids are able to both acclimate (Miller and Hendricks 1996) and adapt (Groenendijk et al. 2002) to metals in their environment. In this way, they have proven themselves to be (a) gradually adapting to metal exposure (Groenendijk et al. 2002), (b) efficient at storing metal, for example, in the form of metallothionein-like proteins (Gillis et al. 2002), and (c) efficient at excreting (Postma et al. 1996) or shedding (Groenendijk et al. 1999) metal.

It is generally agreed that of the four larval instars of chironomids, the first and youngest instar is the most sensitive to waterborne toxicants (Gauss et al. 1985; Nebeker et al. 1984; Pascoe et al. 1989; Williams et al. 1986). Therefore the first instar should be the most appropriate life-stage for toxicity tests aimed at environmental protection. However, Larrain et al. (1997) argue that an older instar may be viewed as more appropriate when background mortality is taken into consideration. Most likely this is because the ability of first instars to survive without food for extended periods of time is relatively limited. Food deprivation is a necessity in acute waterborne tests

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with metals, because the presence of organic matter, such as food, will complex the metal and reduce its toxicity (De Schamphelaere et al. 2004).

Although many studies have examined the toxicity of metals to chironomids (Milani et al. 2003; Phipps et al. 1995; Rao and Saxena 1981), a direct comparison of the toxicity of a suite of metals to the most sensitive life stage of a single chironomid species is lacking. The goal of our study was to compare the acute waterborne toxicity of Cd, Cu, Pb, Ni, and Zn to first-instar *C. riparius* under standardized conditions. The exposures were conducted in a simplified laboratory soft water to minimize the protective effects of water chemistry. To avoid the probable background mortality problem caused by food deprivation, we limited our tests to 24 h.

Materials and Methods

The *C. riparius* culture was initiated with egg masses acquired from Environment Canada (Burlington, Ontario, Canada). The cultures (and exposures) were held under a 16 h:8 h light:dark cycle. *C. riparius* egg masses were collected from culture tanks and placed in glass petri dishes containing aerated, dechlorinated, moderately hard Hamilton tap water from Lake Ontario held at a temperature of 20° ± 1°C, hereafter referred to as culture water. The ionic composition of the Hamilton city tap water (mM) was $[Na^+] = 0.6$, $[Cl^-] = 0.8$, $[Ca^{2+}] = 1.8$, $[K^+] = 0.4$, $[Mg^{2+}] = 0.5$, pH 8.05, 140 mg/L hardness as $CaCO_3$. In order to ensure that only newly hatched, first-instar larvae were used in the toxicity tests, 48 h after collection egg ropes were transferred to a fresh petri dish containing culture water. Toxicity tests were conducted in synthetic soft water (52.9 μM/L Ca^{2+}, 22.9 μM/L Mg^{2+}, 112.6 μM/L Na^+, 6.3 μM/L K^+, pH 6.52, 8 mg/L hardness as $CaCO_3$). Individual metal stock solutions were made from metal salts, Cd from $Cd(NO_3)_2*4H_2O$ (Fisher Scientific), Cu from $CuSO_4*5H_2O$ (BDH Inc), Ni from $NiSO_4*6H_2O$ (Sigma Aldrich), Pb from $Pb(NO_3)_2$ (Sigma Aldrich), and Zn from $ZnCl_2$ (Sigma Aldrich). All chemicals used in this study were analytical grade. The test solutions ranged logarithmically from 0 to 25 mg metal/L soft water.

Two hours after the final egg mass transfer (i.e., larvae were <2 h old), 10 first-instar chironomids were added, under a dissecting microscope, to each well of a multiwell plate containing 2 ml of exposure solution. Staggered start times by concentration were employed in order to keep 24-h exposure consistent across concentrations. Each exposure concentration had two or three replicates (each of 10 larvae,) dependent on the number of newly hatched first-instar larvae available. After 24 h, percentage survival was determined in each replicate well. Preliminary experiments found that if exposures were extended beyond 24 h, significant control mortality would result, likely due to lack of food.

The larvae were assumed to be dead if they did not move in reaction to flickering light and gentle prodding. Experiments were considered valid only if control survival met a minimum of 80%. Repeat experiments were done in the same way for all metals tested: Cd, Cu, Pb, Ni, and Zn. Water samples (1.5 ml) were taken from each well at the end of each experiment and were filtered (through an Acrodisk 0.45-μm in-line-syringe-tip filter) and analyzed for metal concentration using a GTA 110 Varian Graphite Furnace Atomic Absorption Spectrometer (AA; Varian Techtron, Mulgrave, Victoria, Australia). Measured water metal concentrations were all within ±20% of nominal values, and the recovery of each metal was ±15% as determined from the Analytical Reference Material TM15 (Environment Canada, National Water Research Institute). Subsequent Probit analysis yielded 24-h median lethal concentrations with 95% confidence intervals (CIs), where possible, for each metal.

Results

Percentage survival data of first-instar *C. riparius* after 24 h in exposure solutions of Cd, Cu, Pb, Ni, and Zn at each concentration are given in Figs. 1a–e. Estimated LC50 values calculated for these data are also presented in Table 1. Note that 95% CIs could not be estimated for the Cd LC50 due to heterogeneity of the data. LC50s for Zn and Ni were greater than the highest concentration tested of 25 mg metal/L.

The LC50 for first-instar LC50s are compared to the CCME (2006) and USEPA (1996) Criterion Maximum Concentrations (CMCs) in Table 1. Note that, although the USEPA CMC values in Table 1 are calculated for a hardness of 20 mg/L $CaCO_3$ equivalents (the lowest recommended extrapolation), this hardness level (20mg/L) is still higher than the laboratory water used in this experiment (hardness, 8 mg/L).

Discussion

The present LC50 data for first-instar *C. riparius* are compared with relevant literature data in Table 2.

Cadmium

Our 24-h first-instar LC50 of 9.38 mg Cd/L is comparable to the 24-h LC50 of 2.1 mg Cd/L for the same instar found

Fig. 1 Percentage survival (24 h) of first-instar *C. riparius* larvae at various dissolved water concentrations of (**a**) Cd, (**b**) Cu, (**c**) Pb, (**d**) Ni, and (**e**) Zn. Note logarithmic scale for water metal concentrations. The dotted lines indicate the LC50 values. For Cd and Cu $n = 5$ replicates within two experiments, for Pb and Ni $n = 6$ replicates within two experiments, and for Zn $n = 8$ replicates within three experiments (each with 10 larvae per replicate). A different symbol is used in each experiment. Error bars represent standard error

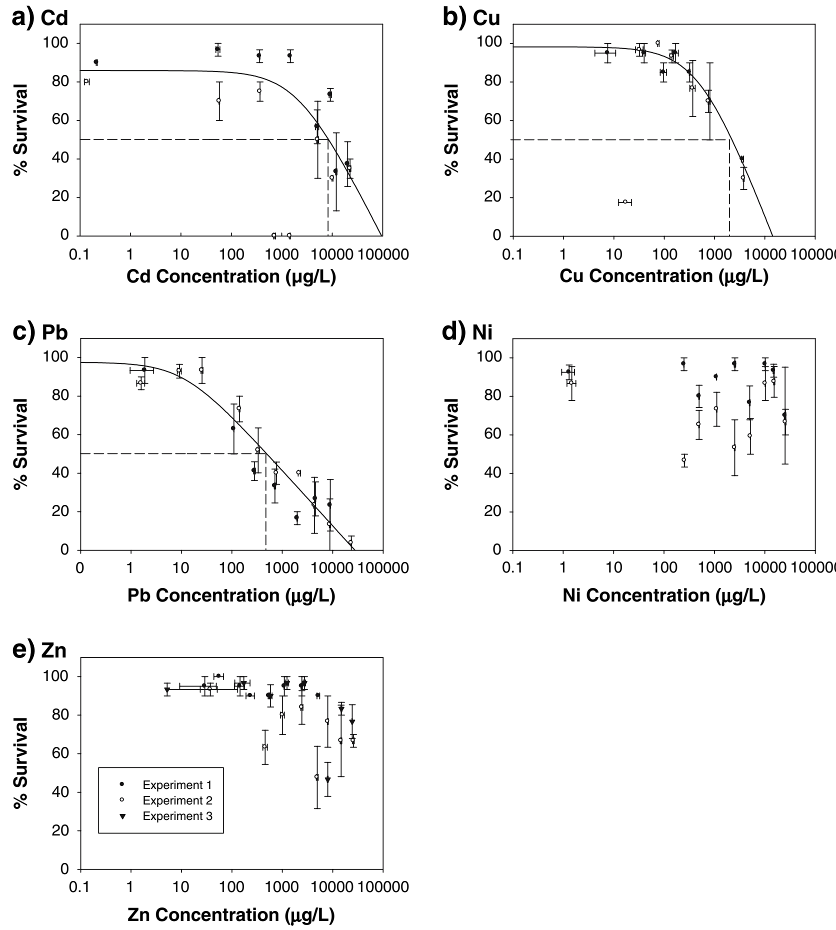

Table 1 The criterion maximum concentrations (CMC) of Cd, Cu, Pb, Ni, and Zn adjusted for water hardness (20 mg/L $CaCO_3$ equivalents) expressed as dissolved metal in the water column, as determined by the CCME (2006) and the USEPA (1996) compared to first-instar *C. riparius* LC50s with 95% CIs determined in the present study at a water hardness of 8 mg/L $CaCO_3$ equivalents

Metal	CCME (µg/L)	CMC USEPA (µg/L)	1st-instar LC50, µg/L (upper–lower 95% CI)
Cadmium (Cd)	0.017	0.4	9380
Copper (Cu)	2	2.9	2,090 (1,570–2,960)
Lead (Pb)	1	10.8	610 (260–1150)
Nickel (Ni)	25	120.0	>25,000
Zinc (Zn)	30	30	>25000

by Williams et al. (1986) but is much higher than the 0.021 mg/L that Milani et al. (2003) reported for a first-instar, 96-h exposure in hard water. Differences in LC50 values not only are observed with first-instar chironomids but also are seen with older instars and may be related to differences in test conditions. Fed third- and fourth-instar *C. riparius* 48-h LC50s have been noted at 72 and 725 mg/L Cd, respectively (Williams et al. 1986), while Rao and Saxena (1981) reported a 48-h LC50 of 50 mg/L using third- to fourth-instar *C. tendipes* larvae, both in hard water.

Copper

We obtained a 24-h first-instar LC50 of 2.09 mg Cu/L for *C. riparius.* In contrast, acute toxicity data for *C. tentans* in a soft water (43 mg/L $CaCO_3$ equivalents) indicate that unfed first instars are sensitive to copper, with a 96-h EC50 of 16.7 µg/L Cu (Gauss et al. 1985). First-instar *C. riparius* are similarly sensitive to Cu, with a 96-h LC50 of 43 µg/L in hard water (Milani et al. 2003). Likewise, Nebeker et al. (1984) found that first-instar C. tentans are sensitive to copper with a 96-h LC50 of 298 µg/L. Although data from some studies (Gauss et al. 1985; Milani et al. 2003; Nebeker et al. 1984) place chironomids in the microgram per liter range for sensitivity, others indicate somewhat greater tolerance including Taylor et al. (1991), who found a 48-h LC50 of 1.2 mg Cu/L for second-instar *C. riparius*, and the present study, with a first-instar *C. riparius* 24-h LC50 for Cu in the mg/L range (2.09 mg/L). These differences in chironomid sensitivity may be explained by the

Table 2 A comparison of LC50 values for chironomids exposed to the metals Cd, Cu, Pb, Ni, and Zn under various conditions

Metal	Species	Instar	Duration (h)	LC50 (mg/L)	Substrate for building tubes	Fed	Water hardness (mg/L)	Study
Cd	*riparius*	1st	24	2.1	Food	Y	100–110	Williams et al. (1986)
	riparius	1st	96	0.021	Silica sand	Y	120–140	Milani et al. (2003)
	riparius	1st	24	9.381	N	N	8	Present study
	riparius	2nd	48	2.620	Filter paper	Y	114	Watts and Pascoe (2000)
	tendipes	3rd & 4th	48	25	—	—	—	Rao and Saxena (1981)
	riparius	3rd	24	500	Food	Y	100–110	Williams et al. (1986)
Cu	*tentans*	1st	96	0.0167*	N	N	43	Gauss et al. (1985)
	tentans	1st	96	0.298	Food	Y	71–84	Nebeker et al. (1984)
	riparius	1st	96	0.043	Silica sand	Y	120–140	Milani et al. (2003)
	riparius	1st	24	2.093	N	N	8	Present study
	riparius	2nd	48	1.2	Cellulose mulch	Y	142–160	Taylor et al. (1991)
Pb	*riparius*	1st	24	0.613	N	N	8	Present study
	Chironomid sp.	—	24	350	—	—	224	Qureshi et al. (1980)
	Chironomid sp.	—	48	220	—	—	224	"
	tendipes	3rd & 4th	48	50.0	—	—	—	Rao and Saxena (1981)
Ni	*riparius*	1st	48	79.5	N	N	"Hard water"	Powlesland and George (1986)
	riparius	1st	96	5.25	Silica sand	Y	120–140	Milani et al. (2003)
	riparius	1st	24	>25	N	N	8	Present study
Zn	*riparius*	1st	24	>>25	N	N	8	Present study
	tendipes	3rd & 4th	48	62.5	—	—	—	Rao and Saxena (1981)
	tentans	—	240	1.125	Sand	Y	"Hard water"	Phipps et al. (1995)

duration of the exposure, since shorter "acute" exposures are associated with higher LC50s, while longer, more "chronic" exposures are associated with lower LC50s (Watts and Pascoe 2000; Williams et al. 1986).

Lead

Of the five metals tested in this study, Pb was the most toxic to first-instar *C. riparius*. The 24-h LC50 value for first-instar *C. riparius* was 0.61 mg/L Pb. Other values in the literature are substantially higher. Rao and Saxena (1981) found a 48-h LC50 of 50 mg/L using third- to fourth-instar *C. tendipes* larvae in hard water, while Qureshi et al. (1980) measured a 24-h LC50 of 350 mg/L and a 48-h LC50 of 220 mg/L with fourth-instar chironomid larvae (species unidentified) in hard water. Similar sensitivity differences have been noted in comparing literature LC50s for *C. riparius* with other metals (Milani et al. 2003).

Nickel

The 24-h Ni LC50 for first-instar *C. riparius* obtained in this study (>25 mg/L) in soft water is comparable to the 48-h LC50 of 79.5 mg/L obtained with first-instar *C. riparius* in hard water (Powlesland and George 1986). In contrast, Milani et al. (2003) reported a first-instar, fed, 96-h LC50 of 5.25 mg/L in hard water. This difference could be due to length of exposure (Milani et al. used 96 h), as LC50s usually decrease with increased duration of exposure (Watts and Pascoe 2000; Williams et al. 1986).

Zinc

The present study found that the 24-h LC50 value for Zn in first-instar *C. riparius* was higher than the highest concentration tested (25 mg/L) in soft water. In comparison, Rao and Saxena (1981) found an acute 48-h LC50 of 62.5 mg/L with third- to fourth-instar *C. tendipes* larvae in hard water. Chronic toxicity data for *C. tentans* indicated a 10-day Zn LC50 of 1.125 mg/L when chironomids were fed and given a monolayer of sand substrate in natural soft Lake Superior water (Phipps et al. 1995).

Metals in the Environment

In this study we analyzed the toxicity of Cd, Cu, Pb, Ni, and Zn separately. However, in the natural environment, aquatic organisms are often exposed to multiple metals simultaneously. Therefore under natural conditions, there is the potential that these metals may act in an additive or even synergistic manner, although the sensitivity of chironomid larvae to multiple metals cannot be predicted by the individual metal sensitivities generated in this study.

Summary and Conclusions

Of the metals tested, first-instar *C. riparius* larvae were the most sensitive to Pb, followed by Cu, Cd, then Ni, then Zn. The relatively high tolerance of chironomids to Ni and Zn is not surprising given that Zn and Ni are essential elements (Gough 1993), which likely can be regulated. A surprising finding is that Cu, which is essential, was more toxic to *C. riparius* than Cd, which is not essential. Generally, Cd is more toxic to organisms than Cu. Recent studies by Gillis and Wood (2007) suggest that Cd tolerance in late-instar *C. riparius* is at least in part due to their ability to maintain internal calcium balance even with continued Cd exposure. Based on the CCME and the USEPA CMCs (Table 1), we would expect Cd, followed by Cu, then Pb, to be the most toxic; however, in this study, Pb was found to be the most toxic, followed by Cu, then Cd. In general, this study has found *C. riparius* to be more metal tolerant than indicated by other studies, although there is significant variation in the LC50s within the published literature.

It should be noted that due to the rapid transfer of chironomid larvae from hard to soft water, LC50s may be underestimates. This is because the permeability of ions (particularly Ca^{2+}) decreases at the onset of soft water acclimation, i.e., to prevent ion loss in low-ionic water (McDonald and Rogano 1986). There is also reason to believe that LC50s could be exaggerated since soft water tolerance is associated with higher uptake capacities and affinities for ions (at least in fish) (Boisen et al. 2003). This may increase the apical entry of metals through ion channels, thereby elevating the toxicity of the metal to the chironomid larvae. Also, the stress resulting from rapid transfer from hard to soft water in conjunction with the stress of metal exposure may have a synergistic effect, which would result in lower-than-actual LC50s. We surmise that each of these effects due to rapid transfer from hard to soft water may be occurring to a small extent but can ot have affected the main results of our study.

The LC50 values for each metal are all orders of magnitude above the USEPA water quality criteria for the same metals adjusted for water hardness (20 mg/L $CaCO_3$ equivalents; Table 1). Thus, chironomids are well protected by the current USEPA water quality guidelines, and even better protected by the current CCME guidelines.

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