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ACUTE AND CHRONIC TOXICITY OF NICKEL TO A CLADOCERAN (*CERIODAPHNIA DUBIA*) AND AN AMPHIPOD (*HYALELLA AZTECA*)

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Abstract—This study evaluated acute and chronic nickel (Ni) toxicity to *Ceriodaphnia dubia* and *Hyalella azteca* with the objective of generating information for the development of a biotic ligand model for Ni. Testing with *C. dubia* was used to evaluate the effect of ambient hardness on Ni toxicity, whereas the larger *H. azteca* was used to derive lethal body burden information for Ni toxicity. As was expected, acute *C. dubia* median lethal concentrations (LC50s) for Ni increased with increasing water hardness. The 48-h LC50s were 81, 148, 261, and 400 μg/L at hardnesses of 50, 113, 161, and 253 mg/L (as $CaCO_3$), respectively. *Ceriodaphnia dubia* was found to be significantly more sensitive in chronic exposures than other species tested (including other daphnids such as *Daphnia magna*); chronic toxicity was less dependent on hardness than was acute toxicity. Chronic 20% effective concentrations (EC20s) were estimated at <3.8, 4.7, 4.0, and 6.9 μg/L at hardnesses of 50, 113, 161, and 253 mg/L, respectively. Testing with *H. azteca* resulted in a 96-h LC50 of 3,045 μg/L and a 14-d EC20 of 61 μg/L at a hardness of 98 mg/L (as $CaCO_3$). Survival was more sensitive than was growth in the chronic study with *H. azteca*. The 20% lethal accumulation effect level based on measured Ni body burdens was 247 nmol/g wet weight.

Keywords—Nickel Daphnids Amphipods Biotic ligand model

INTRODUCTION

Relative to other divalent metals, nickel (Ni) has not been well studied in terms of toxicity, mode of action, and bioavailability. When the ambient water-quality criteria for Ni were released [1], only six species had been tested in chronic exposures. Of these studies, three tests were with invertebrates—the cladoceran *Daphnia magna*, the caddisfly *Clistoronia magnifica*, and the snail *Juga plicifera*. No-observed-effect concentrations (NOECs) were relatively high for the latter two species, at 124 and 62 μg/L, respectively [1]. The sensitivity of *D. magna* appears to be strongly dependent on hardness, with NOECs of 10, 101, and 220 μg/L at water hardnesses of 51, 105, and 205 mg/L [1].

Since publication of the ambient water-quality criteria, two other invertebrates have been tested for their chronic sensitivity to Ni. Kszos et al. [2] studied chronic Ni toxicity to the cladoceran *Ceriodaphnia dubia* and Borgmann et al. [3] investigated chronic Ni toxicity in the amphipod *Hyalella azteca*. Kszos et al. [2] estimated an NOEC of 5 μg/L for *C. dubia*, whereas Borgmann et al. [3] estimated a 25% inhibition concentration of 17 μg/L for *H. azteca*. Given the limited chronic toxicity data available for Ni, additional data are needed to better characterize the hardness-dependent relationship between Ni and chronic toxicity, identify the toxic mode of action, and provide a calibration data set for development of the Ni biotic ligand model (BLM) [4]. To help achieve these objectives, we evaluated acute and chronic Ni toxicity to *C. dubia* at several different water hardnesses (nominal of 50, 100, 175, and 250 mg/L as $CaCO_3$). For calibration of the BLM, analyses of dissolved organic carbon (DOC), cations, and anions also were conducted. We also evaluated acute (96-h) and chronic (14-d) Ni toxicity and lethal body burden to the freshwater amphipod *Hyalella azteca*, because its larger size is more suitable for measurement of body burdens. In addition to standard toxicological endpoints, we measured Ni accumulation in *H. azteca* as a function of exposure concentration in the chronic study. We specifically selected a different set of exposure conditions and test duration from previously published studies with *H. azteca* to evaluate whether a similar lethal body burden could be derived. This would substantiate the concept that Ni effects can be predicted as a function of Ni body burden, regardless of exposure condition and duration.

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METHODS AND MATERIALS

Experimental design

Nickel chloride pentahydrate (>97% pure; lot 015298) was obtained from Fisher Scientific (Pittsburgh, PA, USA). Stock solutions were prepared by dissolving the Ni salt in Milli-Q® deionized water (Millipore, Burlington, MA, USA). The dilution water for each test with *C. dubia* was prepared by adding the following reagent-grade salts to deionized water: calcium sulfate (dihydrate, 98%, Aldrich, Milwaukee, WI, USA), magnesium sulfate (All-World Scientific, Lynnwood, WA, USA), sodium bicarbonate (grade, Fisher Scientific), and potassium chloride (Fisher Scientific). Each water was formulated according to standard U.S. Environmental Protection Agency (U.S. EPA) moderately hard water [5] except that Ca and Mg were modified to achieve the desired water hardness. The four nominal hardness levels evaluated in the acute and chronic toxicity tests with *C. dubia* were 50, 100, 175, and 250 mg/L. The dilution water for *H. azteca* testing was natural spring water from Woodinville (WA, USA; hardness 91–98 mg/L).

Dissolved oxygen, pH, and temperature were measured dai-

Table 1. Parameters for gill species in the nickel biotic ligand model

Gill species	Binding constant, log *K*
Ni	4.0
Ca	4.0
H	6.7
Na	3.0
Gill binding site density	1,000 nmol/g wet wt

ly in all tests. Dissolved oxygen was measured with an Orion meter and probe (model 835, Thermo-Orion, Waltham, MA, USA) and pH was measured with a Fisher meter and probe (model AP62, Fisher Scientific). Alkalinity and hardness were measured with Hach titration kits (Hach, Loveland, CO, USA) and were confirmed via analysis of cations and anions. Hardness results reported herein were calculated based upon Ca and Mg analysis.

All tests were conducted in general accordance to U.S. EPA or American Society for Testing and Materials guidelines, or both [5–7]. The different requirements of the two species tested necessitated slightly different experimental designs.

Acute tests with *C. dubia* were conducted according to standard U.S. EPA guidelines for conducting 48-h acute toxicity tests [5]. Organisms were obtained from laboratory cultures maintained at 25°C and 90 mg/L hardness and were <24-h-old at test initiation. Static, nonrenewal tests were conducted at 20°C and organisms were not fed during exposure. For each test, a control and six exposure concentrations were used. Four replicate test chambers (250-ml glass beakers) containing five neonates were prepared for each exposure. Nickel, Ca, Mg, organic carbon, K, Na, sulfate, bicarbonate, carbonate, and chloride were measured at test initiation. All measurements were performed on the dissolved fraction after filtration and samples were preserved after filtration. Survival was monitored at 24 and 48 h and mortality was defined as immobility after gentle prodding with a disposable pipet (i.e., the organism remained immobile after stimulation that would cause an avoidance response in a live organism).

Chronic tests with *C. dubia* were conducted in general accordance with standard U.S. EPA guidelines for conducting 7-d chronic toxicity tests [8]. Organisms were obtained from laboratory cultures maintained at 25°C and 90 mg/L hardness and were <24-h-old at test initiation. Static, daily renewal tests were conducted at 25 to 26°C and organisms were fed a standard mixture of yeast, cerophyll, and trout chow (YCT) and *Selenastrum capricornutum* during exposure [8]. For each test, a control and six or seven exposure concentrations were used. Ten replicate test chambers (25-ml polypropylene cups) containing one neonate were prepared for each exposure. Each test chamber contained 15 ml of test solution. Nickel, Ca, Mg, organic carbon, K, Na, sulfate, bicarbonate, carbonate, and chloride were measured at three times (test initiation, day 3 or 4, and at day 7). All measurements were performed on the dissolved fraction after filtration. Because of laboratory error, the Ni concentrations for the 50-mg/L hardness test were not measured and results are reported as nominal.

Table 3. Dilution water characteristics for toxicity tests with *Hyalella azteca*[a]

Water-quality parameter	Acute	Chronic
pH	7.7–8.0	8.0–8.3
Dissolved oxygen (mg/L)	8.0–8.4	7.2–8.3
Temperature (°C)	23	23–25
Na (mg/L)	30	8
Ca (mg/L)	14	14
Mg (mg/L)	2	<2
Chloride (mg/L)	6.2	6.4
Sulfate (mg/L)	14	12
Hardness (mg/L as $CaCO_3$)	98	91
Alkalinity (mg/L as $CaCO_3$)	64	70
DOC (without food)	0.61	0.66
DOC (with food)	NA	1.4

[a] DOC = dissolved organic carbon; NA = not applicable.

Because food was added to the test system in the chronic exposure, attempts were made to ensure that Ni was in equilibrium between the total and dissolved phases. Dissolved organic carbon complexation kinetics have been shown to be relatively slow for other metals [9], although DOC complexation kinetics have not been studied for Ni. To accomplish this, test concentrations were prepared 24 h before use and YCT were added to test solutions at this time. This was done to ensure that Ni was in equilibrium with DOC associated with the YCT. Although not a specific objective of this study, the equilibration likely also increased any dietary Ni exposure that may occur when the daphnids feed on YCT. In contrast, *S. capricornutum* was added 1 h before use of the test solution because adsorption to algal cells has been shown to be relatively fast for a number of metals [10,11]. Further, addition of *S. capricornutum* only 1 h before use reduced any effects Ni might have on algae quantity between treatment levels. Survival and reproduction were monitored daily and mortality was defined as immobility after gentle prodding.

The acute 96-h test for *H. azteca* followed standard American Society for Testing and Materials guidelines for conducting acute toxicity tests [6]. The 14-d chronic toxicity test followed U.S. EPA guidelines [7]. Test organisms were obtained from Chesapeake Cultures (Hayes, VA, USA). The amphipods were 10- to 12-d-old at test initiation for the 96-h test

Table 2. Water-quality conditions for toxicity tests with *Ceriodaphnia dubia*[a]

Dilution water hardness (mg/L as $CaCO_3$)	pH	DO (mg/L)	Ca (mg/L)	Mg (mg/L)	K (mg/L)	Na (mg/L)	Cl (mg/L)	SO_4 (mg/L)	HCO_3 (mg/L)	CO_3 (mg/L)	Acute DOC (mg/L)	Chronic DOC (mg/L)
50	7.66	8.37	16	2.4	ND	14	1.1	51	31	ND	0.53	1.3
113	7.70	8.34	31	8.7	ND	15	0.85	99	31	ND	0.5	1.3
161	7.61	8.44	43	13	ND	17	2.1	180	29	ND	0.41	1.3
253	7.80	8.63	68	20	ND	16	8.6	226	31	ND	0.43	1.3

[a] DO = dissolved oxygen; DOC = dissolved organic carbon; ND = not detected.

Table 4. Acute toxicity test results for *Ceriodaphnia dubia*[a]

Hardness (mg/L)	LC50 (μg/L)	95% CI (μg/L)
50	81	55–118
113	148	107–192
161	261	212–323
253	400	311–515

[a] LC50 = median lethal concentration; CI = confidence intervals.

and 7- to 8-d-old for the 14-d test. The experimental design for the acute test consisted of four replicate test chambers (300-ml high-form lipless beakers) for each of eight Ni concentrations and a control. The acute test was conducted under static nonrenewal conditions without feeding. The endpoint was mortality, defined as immobility under gentle prodding.

The experimental design for the 14-d test consisted of 10 replicate test chambers for each of seven Ni concentrations and a control. The 14-d test was conducted under static renewal conditions by using a Zumwalt dilutor system with two volume additions per day [12]. To ensure that Ni was in equilibrium between the total and dissolved phases, test concentrations were prepared 24 h before use. As for testing with *C. dubia*, YCT equivalent to 1.0 ml per beaker was added to the test treatments 24 h before use. Each test chamber contained 10 amphipods and artificial substrate consisting of a 3 × 3-cm piece of coarse filter media from Aquatic Eco-Systems (Apopka, FL, USA). The endpoints for this study were mortality and growth (dry wt). Nickel concentrations also were analyzed in tissue of *H. azteca* at termination of the 14-d test. Test temperature was maintained at 23 ± 1°C by using a water bath with submerged aquarium heaters.

Analytical chemistry

Aqueous Ni, Ca, Mg, K, and Na concentrations were analyzed in filtered samples preserved with nitric acid (trace-metals grade, Fisher Scientific) via U.S. EPA Method 6010 (inductively coupled plasma emission spectrometry). The U.S. EPA Method 6020 (inductively coupled plasma–mass spectrometry) was used for Ni when lower detection limits were warranted. Organic carbon was determined by using U.S. EPA Method 9060 and U.S. EPA Method 300A was used for chloride and sulfate. Bicarbonate and carbonate were measured by using Standard Method 2320B. Filtration was performed at the time of sample collection by using 0.45-μm cellulose-nitrate filters (Whatman, Clifton, NJ, USA) for anions and cations. A 0.50-μm glass fiber filter (Gelman Labs/Pall Life Sciences, Ann Arbor, MI, USA) was used for analysis of organic carbon.

Tissue analyses for *H. azteca* were performed by digesting weighed tissue in precleaned vials on a hot plate with 5 ml of concentrated nitric acid (trace-metals grade, Fisher Chemical). A Teflon® conical cap was used to enhance refluxing. After digestion, samples were diluted to 20 ml with Milli-Q reagent water (Millipore). Quality-control samples (preparation blanks, duplicate blank spikes, and certified reference materials) were prepared at the same time as the sample preparation. Nickel was measured using inductively coupled plasma–mass spectrometry with a Perkin-Elmer ELAN 6100 (Norwalk, CT, USA).

Statistical analysis

Toxicity data were statistically analyzed by using the computer software program ToxCalc, Version 5.0 [13]. A number of different endpoints were calculated. Survival, reproduction, growth, and effective body burdens (i.e., 50% and 20% lethal concentrations [LC50s and LC20s, respectively], 20% effective concentrations [EC20s], and 50% and 20% lethal accumulation levels [LA50s and LA20s, respectively]) and corresponding 95% confidence intervals were statistically evaluated by using Probit analysis while the NOEC and lowest-observed effect concentrations (LOECs) were estimated by using Dunnett's test or Steel's many–one rank test.

Biotic ligand model

The BLM has been relatively well developed for Cu and Ag, and is in various phases of development for Cd, Pb, Ni, and Zn [4,14]. Meyer et al. [15] performed the initial studies with fathead minnows (*Pimephales promelas*) to demonstrate that the general principles of the BLM apply to Ni. Specifically, Meyer et al. [15] demonstrated that acute Ni toxicity was variable as a function of water concentration when ambient water-quality conditions (i.e., hardness) were varied, but was constant as a function of gill Ni burden under these same varying ambient water-quality conditions. By using data from this study along with several other studies with *P. promelas* [16,17], an initial calibration of the BLM for Ni has been made.

Using data generated in this study, along with data from a previous study with *D. magna* [18] and *C. dubia* [17], we constructed an initial Ni BLM for daphnids. The same general procedure that has been used with other metals was applied to each of these data sets [4,14]. That is, the binding site density and binding constants (log *K*s) for the biotic ligand were kept the same as for *P. promelas* (Table 1), the organism that provided surrogate accumulation data for the biotic ligand for model calibration purposes, and the LA50 was adjusted to simulate differences in organism sensitivity.

Table 5. Results for chronic tests with *Ceriodaphnia dubia*[a]

	Survival (μg/L)					Reproduction (μg/L)				
Hardness (mg/L)	NOEC	LOEC	Chronic value	LC20	95% CI	NOEC	LOEC	Chronic value	LC20	95% CI
50[b]	<3.8	3.8	<3.8	<3.8	NA	<3.8	3.8	<3.8	<3.8	NA
113	5.3	9.9	7.2	4.8	3.1–6.2	5.3	9.9	7.2	4.7	4.1–5.3
161	15.3	27.5	20.5	11.9	7.7–14.7	3.4	5.3	4.2	4.0	1.8–6.1
253	9.6	17.3	12.9	10.4	6.7–13.3	5.8	9.6	7.5	6.9	4.9–8.4

[a] NOEC = no-observed-effect concentration; LOEC = lowest-observed-effect concentration; LC20 = 20% lethal concentration; CI = confidence interval; EC20 = 20% effective concentration; NA = not applicable.
[b] Results for the chronic test with *C. dubia* at a hardness of 50 mg/L are based on nominal test concentrations.

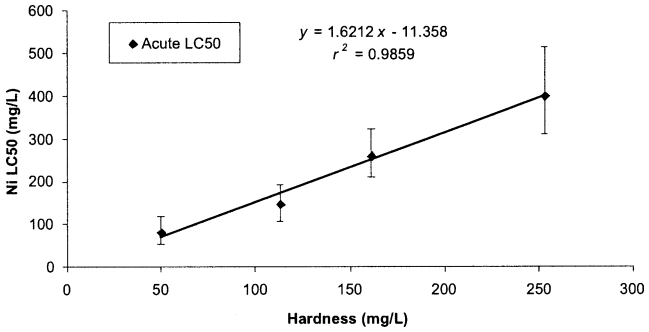

Fig. 1. Acute nickel toxicity to *Ceriodaphnia dubia* as a function of water hardness. Error bars are 95% confidence limits on median lethal concentration (LC50) estimates.

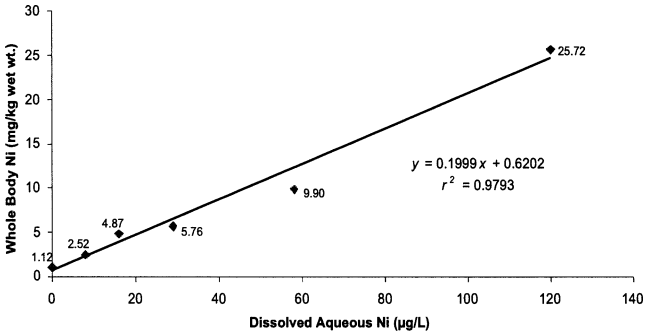

Fig. 3. Nickel body burdens (mg/kg wet wt) of *Hyalella azteca* as a function of waterborne Ni exposure (μg/L) for 14 d.

RESULTS

Water quality and analytical chemistry

Water-quality conditions for all tests are summarized in Tables 2 and 3. Test temperature, dissolved oxygen, and pH all met test acceptability requirements (i.e., per the cited methods). Mean measured dissolved Ni concentrations for each study were comparable to nominal concentrations. Measured concentrations across all tests remained stable for the duration of testing. All statistical analyses were performed based on the mean of the measured dissolved Ni test concentrations except for the acute study with *C. dubia* conducted at 50 mg/L hardness, which is based on nominal test concentrations.

Toxicity testing

The 48-h LC50 for *C. dubia* ranged from 81 to 400 μg/L and, consistent with studies of other organisms, toxicity decreased as water hardness increased from 50 to 253 mg/L (Fig. 1 and Table 4). Chronic results, reported as LC20s for survival and EC20s for reproduction are presented in Figure 2 and Table 5. The chronic hardness-dependent slopes for survival and reproduction were not significantly greater than zero ($p = 0.20$ for survival, $p = 0.14$ for reproduction), suggesting no hardness-dependent effect on the chronic toxicity of Ni to *C. dubia* at the Ca:Mg ratio evaluated in this study.

The 96-h LC50 for *H. azteca* was 3,045 μg/L. In the chronic study, survival was more sensitive than growth. The 14-d EC20 was 61 μg/L with an NOEC of 29 μg/L and an LOEC of 58 μg/L. Both tests had a strong dose–response relationship and <10% mortality in the controls. Nickel bioconcentration, as determined from Ni measured in the whole body tissue of *H. azteca* during the 14-d test, increased with increasing Ni test concentrations (Fig. 3). The LA20 was calculated by using toxicity data and Ni tissue concentrations from the 14-d test. The 14-d LA20 was 247 nmol/g wet weight (Fig. 4).

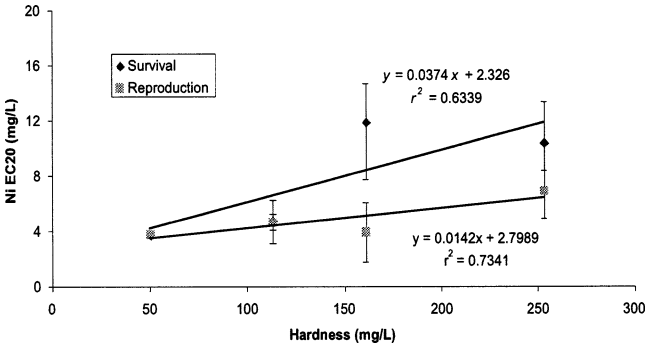

Fig. 2. Chronic nickel toxicity to *Ceriodaphnia dubia* as a function of water hardness. Error bars are 95% confidence limits on 20% effective concentration (EC20) estimates. Note slopes of regression lines are not statistically different from 0 ($p = 0.20$ for survival and $p = 0.14$ for reproduction).

Biotic ligand model

Results for the initial calibration of the acute Ni BLM for daphnids generally are encouraging, with all toxicity values except one being within a factor of two of the line of perfect agreement between measured and predicted LC50s (Fig. 5). The one exception to this result is the acute test with *C. dubia* conducted by Schubauer-Berigan et al. [17] at pH 8.6. To achieve the presented calibration, the two data sets for *C. dubia* required use of different LA50s. In fact, these LA50s are nearly an order of magnitude different at 0.21 and 1.92 nmol/g wet weight. Possible explanations for the pH 8.6 data point and the substantially different LA50s are discussed later.

DISCUSSION

Comparison to other freshwater nickel studies

To compare the results of this study to other published studies, all toxicity data were normalized to a hardness of 50 mg/L (as $CaCO_3$) by using the equation derived by U.S. EPA [19]

$$\text{hardness-normalized LC50} = \exp\{\ln(\text{LC50}) - 0.8460[\ln(\text{test hardness}) - \ln(50)]\}$$

For the acute studies, Schubauer-Berigan et al. [17] measured the acute sensitivity of *C. dubia* and *H. azteca* to Ni at three

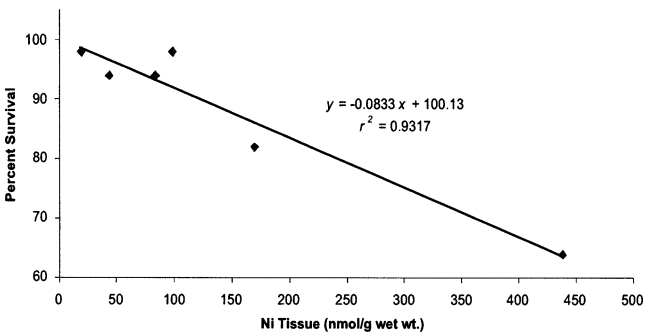

Fig. 4. Survival of *Hyalella azteca* after 14 d of exposure as a function of Ni body burdens (mg/kg wet wt).

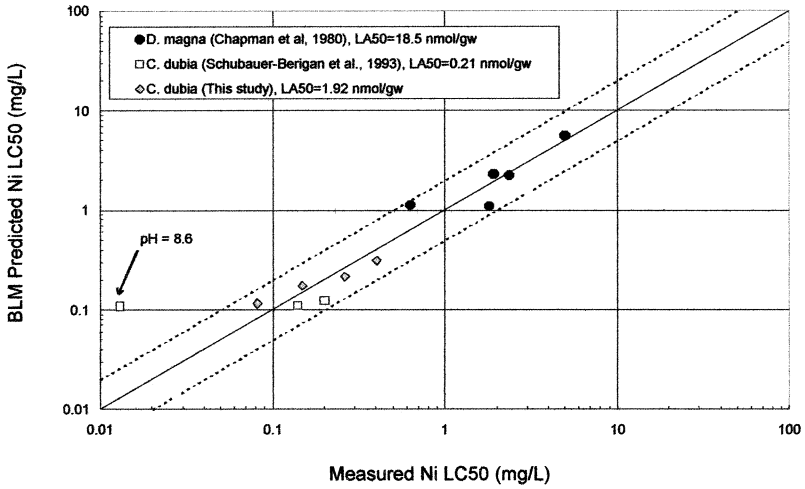

Fig. 5. Results of biotic ligand model predictions for *Daphnia magna* [18] and *Ceriodaphnia dubia* [17]. Binding constant (log *K*) gill-Ni = 4.0, log *K* gill-Ca = 4.0, log *K* gill-H = 6.7, log *K* gill-Na = 3.0. BLM = biotic ligand model; LC50 = median lethal concentration; LA50 = 50% lethal accumulation level.

pH levels. At pH 6.3, 7.1, and 8.6, the hardness-normalized 48-h LC50s for *C. dubia* were 45, 32, and 2.9 μg/L, respectively. The geometric mean LC50 for *C. dubia* from the present study, with a pH range of 7.6 to 7.8, was 89 μg/L. Accordingly, the hardness-normalized mean LC50 from the present study is approximately three times greater than that reported by Schubauer-Berigan et al. [17] at pH 7.1. A similar pattern was observed in the data for *H. azteca*. At pH levels 6.7, 7.5, and 8.5, 96-h LC50s for *H. azteca* were 452, 430, and 201 μg/L, respectively [17]. In the present study, the hardness-normalized LC50 was 1,723 μg/L at a pH of 7.9, or approximately four times greater than that reported by Schubauer-Berigan et al. [17] at pH 7.5. This difference is unlikely to be due to the small difference in pH (0.4 pH units). The basis for the differences between the two studies is unknown, but may be due to variability in the test organisms or slight differences in the test methodology (e.g., test temperature, organism age, or fed vs unfed). This observation is not unique to Ni. Of the other four metals tested by Schubauer-Berigan et al. [17] (Cd, Cu, Pb, and Zn), the hardness-normalized LC50s they reported for *C. dubia* and *H. azteca* were the lowest values reported in the U.S. EPA AQUIRE database (www.epa.gov/ecotox/) for those species (with the exception of acute Pb toxicity to *C. dubia*).

The only chronic study with *C. dubia* on Ni was performed by Kszos et al. [2]. They performed two tests at hardnesses of 42 and 117 mg/L as $CaCO_3$. The first test consisted of a control and two treatments, whereas the second test only had three treatments, making regression analysis of the data difficult. Hypothesis testing indicated chronic values (geometric mean of NOEC and LOEC) of 5.3 and 10.6 μg/L, respectively. These values are reasonably comparable to those observed in the present study where chronic values of Ni at <3.8 and 7.2 μg/L were observed at comparable ambient hardnesses (50 and 100 mg/L, respectively).

The only study comparable to the chronic 14-d test with *H. azteca* was conducted by Borgmann et al. [3]. They conducted a 28-d study that evaluated survival and growth, and also measured Ni accumulation and estimated an LA25. They estimated a hardness-normalized LC25 of Ni at 8 μg/L. This compares with the hardness-normalized LC20 of 37 μg/L obtained in the present study. Hence, the LC20 for the present study was approximately five times higher than the LC25 reported in the 28-d study by Borgmann et al. [3]. This is not surprising given the shorter duration of our study.

In contrast to the water-based comparison of the two studies, comparison of endpoints based on whole-body Ni are much more similar. We estimated an LA20 for the 14-d study of 247 nmol/g wet weight compared with the LA25 of 197 nmol/g wet weight reported in the 28-d study [3]. These results provide a good demonstration of the utility of the BLM approach in which approximately the same effective body burden is estimated for two studies with different exposure durations, aqueous Ni exposure concentrations, and ambient water-quality conditions.

Overall, *C. dubia* and *H. azteca* are relatively sensitive to Ni compared to other test organisms, being the first and third most sensitive genera tested, respectively. Similarly, for chronic toxicity, *C. dubia* and *H. azteca* represent the two most sensitive species tested to date (Fig. 6).

Application to biotic ligand model

The BLM for Ni is in the early stages of development. However, the initial calibration with daphnids shows the approach will be applicable to Ni (Fig. 5). Additionally, the congruence in the LA20 and LA25 between the two available studies on *H. azteca* provides evidence analogous to that de-

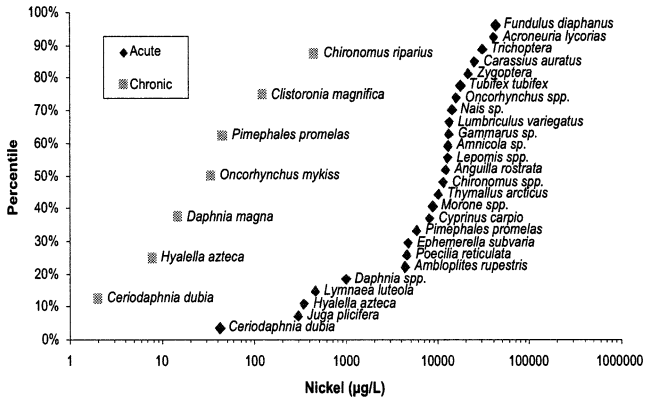

Fig. 6. Genus mean acute and chronic values for Ni. All data were normalized to a hardness of 50 mg/L by using the equation given by the U.S. Environmental Protection Agency [19].

veloped for fish regarding the applicability of a biotic ligand concept for Ni. These studies analyzed in concert with existing data identify several areas where further research is needed.

First, although we were able to develop a BLM including data from the present study and that of Schubauer-Berigan et al. [17], this was only accomplished by estimating significantly different LA50s for *C. dubia* for the two studies. Conceptually, only one LA50 should exist for a given species and life stage. The reason for the substantially different LA50s in this initial calibration is not immediately obvious. Although test conditions differed slightly, the differences are unlikely to account for the order of magnitude differences in LA50s. Some of the BLM water-quality input parameters (e.g., DOC) not available for the data of Schubauer-Berigan et al. [17] had to be estimated based on data from other studies conducted in the same laboratory. However, it again seems unlikely that these uncertainties would account for the significant differences in LA50s. Equally problematic is that the estimated LA50s are quite possibly below background Ni concentrations in most organisms. For example, the estimated LA50 for data from this study is 1.92 nmol/g wet weight, which translates to 0.113 mg/kg wet weight. This compares to a Ni concentration of 0.42 mg/kg wet weight in *D. magna* exposed to an aqueous Ni concentration of just 0.9 μg/L [20]. Clearly, additional study of *C. dubia* is needed to resolve this issue.

The second issue identified in this initial calibration is associated with the pH 8.6 data point from Schubauer-Berigan et al. [17] that is not well predicted by the BLM (Fig. 5). Under the test conditions employed by Schubauer-Berigan et al. [17] at pH 8.6, nickel carbonate ($NiCO_3$) is expected to be the dominant Ni species present. The present form of the Ni BLM assumes that this species is not bioavailable to aquatic organisms. Additional studies measuring Ni accumulation and toxicity at pH $>$ 8.5 are needed to clarify the data of Schubauer-Berigan et al. [17] and the bioavailability of the Ni species forming at higher pH.

CONCLUSION

Ceriodaphnia dubia appears to be the most sensitive species tested with Ni to date in both acute and chronic exposures. Consistent with other species, Ni toxicity values are dependent on hardness for *C. dubia* under acute exposures, and to a lesser extent under chronic exposures. Initial calibration of the acute BLM for Ni and cladocerans is promising. However, further research, particularly with regard to directly measuring lethal accumulation levels in *C. dubia* and the bioavailability of Ni forms at high pH, is needed. Finally, when compared with the study of Borgmann et al. [3], the study with *H. azteca* demonstrates that the underlying principal of the BLM is applicable to Ni, because similar body burdens associated with effects were observed between the two studies despite significantly different water chemistry and exposure durations.

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