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ACUTE AND CHRONIC TOXICITY OF NICKEL TO RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

KEVIN V. BRIX,*† JAMES KEITHLY,‡ DAVID K. DEFOREST,‡ and JIM LAUGHLIN‡
†EcoTox, 590 Ocean Drive, 2C, Key Biscayne, Florida 33149, USA
‡Parametrix, 5808 Lake Washington Boulevard NE, Suite 200, Kirkland, Washington 98033, USA

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Abstract—Of the fish species tested in chronic Ni exposures, rainbow trout (*Oncorhynchus mykiss*) is the most sensitive. To develop additional Ni toxicity data and to investigate the toxic mode of action for Ni, we conducted acute (96-h) and chronic (85-d early life-stage) flow-through studies using rainbow trout. In addition to standard toxicological endpoints, we investigated the effects of Ni on ionoregulatory physiology (Na, Ca, and Mg). The acute median lethal concentration for Ni was 20.8 mg/L, and the 24-h gill median lethal accumulation was 666 nmol/g wet weight. No effects on plasma Ca, Mg, or Na were observed during acute exposure. In the chronic study, no significant effects on embryo survival, swim-up, hatching, or fingerling survival or growth were observed at dissolved Ni concentrations up to 466 μg/L, the highest concentration tested. This concentration is considerably higher than the only other reported chronic no-observed-effect concentration (<33 μg/L) for rainbow trout. Accumulation of Ni in trout eggs indicates the chorion is only a partial barrier with 36%, 63%, and 1% of total accumulated Ni associated with the chorion, yolk, and embryo, respectively. Whole-egg ion concentrations were reduced by Ni exposure. However, most of this reduction occurred in the chorion rather than in the embryos, and no effects on hatching success or larval survival were observed as a result. Plasma ion concentrations measured in swim-up fingerlings at the end of the chronic-exposure period were not significantly reduced by exposure to Ni. Nickel accumulated on the gill in an exponential manner but plateaued in trout plasma at waterborne Ni concentrations of 118 μg/L or greater. Consistent with previous studies, Ni did not appear to disrupt ionoregulation in acute exposures of rainbow trout. Our results also suggest that Ni is not an ionoregulatory toxicant in long-term exposures, but the lack of effects in the highest Ni treatment precludes a definitive conclusion.

Keywords—Nickel Rainbow trout Ionoregulation Biotic ligand model

INTRODUCTION

Nickel is a common metal in most surface waters, with both natural sources (e.g., weathering of rocks) and anthropogenic ones (e.g., industrial discharges from electroplating and smelting) [1]. In general, world consumption of refined Ni has declined, but aqueous Ni concentrations may be elevated near natural deposits, Ni mining and refining operations, and other industrial emissions [2]. Relative to other divalent metals, Ni has not been well studied in terms of toxicity to different species and mode of action. Thirteen fish species have been tested in acute (96-h) exposures, but only two species, *Oncorhynchus mykiss* (rainbow trout) and *Pimephales promelas* (fathead minnow), have been tested in chronic exposures that meet U.S. Environmental Protection Agency (U.S. EPA) guidelines for ambient water-quality criterion (AWQC) development. Based on previous studies, *O. mykiss* is relatively insensitive to acute Ni exposures, with a geometric mean median lethal concentration (LC50) of 13 mg/L [1]. However, the available data indicate this species is quite sensitive to chronic exposures, with a chronic value (geometric mean of no-observable-effect concentration [NOEC] and lowest-observable-effect concentration [LOEC]) of less than 35 μg/L [3]. In comparison, *P. promelas* is considerably less sensitive to Ni, with a chronic value of 217 μg/L when tested at a similar water hardness [4]. In 1986, when the Ni AWQC were published, *O. mykiss* was the most sensitive species in chronic tests with Ni, although two invertebrate species have subsequently been found to be more sensitive [5,6]. Given that *O. mykiss* is the most sensitive fish species tested to date for Ni, we selected it as an ideal model organism for further evaluating the metal's acute and chronic modes of toxic action.

Several other mono- and divalent metals (e.g., Ag, Cd, Cu, Pb, and Zn) have been shown to be ionoregulatory toxicants in acute exposures. These metals disrupt either Na or Ca balance at the gill, triggering a cascade of physiological dysfunctions that eventually cause mortality [7]. This specific mode of action and the fact that it is a function of metal accumulation on the gill have led to the development of the biotic ligand model for predicting acute toxicity [8,9]. Although Ni has not been tested with the same rigor as the other metals mentioned above, the limited data currently available suggest the biotic ligand model will be applicable to Ni as well. For example, Meyer et al. [10] demonstrated that acute Ni toxicity could be predicted as a function of gill Ni burden for *P. promelas* when tested at various levels of water hardness. For invertebrates, Borgmann et al. [5] and Keithly et al. [6] estimated very similar Ni body-burden effect levels for the amphipod *Hyalella azteca* under different exposure conditions and durations.

For the reasons noted, we hypothesized that Ni was an ionoregulatory toxicant for *O. mykiss* and that, with collection of the appropriate data, a biotic ligand model for Ni using this species could be developed. To test this hypothesis, we conducted two experiments. The first experiment was an acute (96-h) study in which mortality was the biological endpoint of interest. We also collected data regarding Ni accumulation at the gill over time and measured Ca, Mg, and Na levels in

* To whom correspondence may be addressed (kubrix@aol.com).

plasma over the course of the exposure period. The second experiment was a chronic early life-stage study beginning with newly fertilized embryos. Standard biological endpoints (i.e., embryo survival, hatchability, swim-up, fingerling survival, and growth) were measured over the course of the 85-d experiment. Additionally, Ni accumulation along with Ca, Mg, and Na levels in various tissues were measured at key developmental stages.

METHODS AND MATERIALS

Experimental design

Nickel chloride (purity, >97%; lot no. 015298) was obtained from Fisher Scientific (Pittsburgh, PA, USA). Stock solutions were prepared by dissolving the Ni salt in Milli-Q (Millipore, Billerica, MA, USA) deionized water. Dilution water was a natural spring water collected from Woodinville (WA, USA). Dilution water was collected as needed and transported to a storage tank at the laboratory, where it was circulated continuously, aerated, and passed through a 5-μm, carbon-core filter and ultraviolet sterilizer. The dilution water was characterized periodically for pH, hardness, alkalinity, ionic composition, total suspended solids, and dissolved organic carbon.

Dissolved oxygen, pH, and temperature were measured daily in both tests. Dissolved oxygen was measured using an Orion meter and probe (Model 835; Thermo-Orion, Waltham, MA, USA), and pH was measured using a Fisher meter and probe (Model AP62; Fisher Scientific). Alkalinity was measured using a Hach titration kit (Hach, Loveland, CO, USA). Hardness was calculated from measured concentrations of Ca and Mg (see *Analytical chemistry*).

Both tests were conducted in general accordance with American Society for Testing and Materials guidelines [11,12]. The only departure from these guidelines was in the chronic study, in which test organisms were not thinned at hatching, as is normally done. We did not thin test organisms to maximize the number available for physiological analyses. Fish in the acute study were obtained from Nisqually Trout Farm (Nisqually, WA, USA). They were 18-d postswim-up and weighed, on average, 1.6 g wet weight at test initiation. The chronic study was initiated with embryos, also obtained from Nisqually Trout Farm, less than 4 h after fertilization.

The test system was a proportional diluter as described by the U.S. EPA [13]. A calibrated laboratory pump (Fluid Metering, Oyster Bay, NY, USA) was used to inject Ni stock solution into the proportional diluter system. The physical system consisted of an enclosed box housing the test chambers and controlled lighting. Water temperature in the test chambers was maintained using a temperature-controlled water bath.

For both the acute and chronic studies, the test design consisted of five concentrations and a dilution-water control. Each concentration and control was assessed using four replicate, randomly positioned, 5.7-L test chambers. Two replicates from each treatment were used for physiology measurements. The proportional diluter delivered 0.5 L of test solution to each test chamber every 2 h, replacing approximately one tank volume per day.

Lighting consisted of fluorescent lights (100–150 foot candles) operated on a 16:8-h light:dark photoperiod. Before hatching in the chronic study, subdued lighting (≤20 foot candles) was maintained in the test system. To maintain dissolved oxygen levels and provide water circulation, gentle aeration was applied to each test chamber (<100 bubbles/min) throughout the tests.

Physiological measurements

In the acute study, gill and plasma samples were collected for analysis from each treatment at 24 and 96 h. Gill and plasma samples also were collected from a random sample of the test population just before introducing trout to the exposure chambers to provide baseline data. Gill samples were collected by immobilizing the fish with a quick blow to the head, then excising both branchial arches. Excised gills were rinsed in control water for 5 s and blotted dry before being placed in digestion vials. Gills from 10 fish were sampled at each time interval and pooled for analysis of Ni. Plasma samples were obtained by stunning fish with a quick blow to the head, removing the caudal fin, and extracting whole blood into a capillary tube. Whole-blood samples were centrifuged at room temperature for 2 min at approximately 5,000 *g* to separate plasma from other blood components. Centrifuged samples were then frozen until analysis for Ni (chronic study only), Na, Mg, and Ca.

In the chronic study, whole eggs, chorion, and embryos (with yolk sac removed) were sampled 28 d postfertilization. Gill and blood plasma were sampled at the end of the test in the same manner as described for the acute study, with plasma Ni added as an additional parameter.

All tissue samples were based on pooled tissue from the two replicates ($n = 1$). This effectively only allows for observation of trends in the resulting data, but it precludes tests for statistically significant differences.

Analytical chemistry

Aqueous concentrations of Ni, Ca, Mg, K, and Na were analyzed in filtered samples preserved with nitric acid (trace-metals grade; Fisher Scientific) using U.S. EPA Method 6020 (inductively coupled plasma-emission spectrometry) [14]. Filtration was performed using cellulose-nitrate filters (pore size, 0.45 μm; Whatman, Clifton, NJ, USA). A glass-fiber filter (pore size, 0.50 μm; Gelman Labs/Pall Life Sciences, Ann Arbor, MI, USA) was used for analysis of dissolved organic carbon. Organic carbon was determined using U.S. EPA Method 9060 (U.S. EPA 1986, No. 2565), and U.S. EPA Method 300A was used for chloride and sulfate (U.S. EPA 1993, No. 2566). Bicarbonate and carbonate were analyzed using Standard Method 2320B (American Public Health Association 1995, No. 2567).

Gill and egg samples were digested in precleaned vials at 120°C with 5 ml of concentrated nitric acid (trace-metals grade; Fisher Scientific) for 3 h. A Teflon® conical cap was used to encourage refluxing. Following digestion, samples were diluted to between 5 and 20 ml, depending on the mass of the sample collected, with Milli-Q reagent water. Quality-control samples (preparation blanks, duplicate blank spikes, and certified reference materials) were prepared at the same time as the sample using a subsample of the same digestion vials. Samples were analyzed for Na, Mg, Ca, and Ni by inductively coupled plasma–mass spectrometry with a Perkin-Elmer ELAN 6100 DRC inductively coupled plasma–mass spectrometer (Perkin-Elmer, Wellesley, MA, USA).

Statistical analysis

Toxicity data were analyzed statistically using ToxCalc software (version 5.0) [15]. Survival, reproduction, growth, lethal accumulation (i.e., LC50, 20% effect concentration, and median lethal accumulation concentration [LA50]), and corre-

Table 1. Water-quality conditions for acute and chronic toxicity tests (mean ± standard deviation)

Parameter	Acute[a]	Chronic
Temperature (°C)	10.0 ± 0.0	10.2 ± 0.4
pH (standard unit)	8.0 ± 0.1	7.9 ± 0.1
Dissolved oxygen (mg/L)	10.0 ± 0.7	10.4 ± 0.5
Ca^{2+} (mg/L)	15.5	15.9 ± 1.2
Mg^{2+} (mg/L)	12.7	13.3 ± 1.0
Na^{+} (mg/L)	7.8	7.5 ± 0.4
Cl^{-} (mg/L)	9.2	9.2 ± 3.6
SO_4^{2-} (mg/L)	10.9	11.0 ± 0.4
HCO_3^{-} (mg/L)	75	73.3 ± 3.8
Hardness (mg/L)	91	89 ± 2
Alkalinity (mg/L)	74	74 ± 4
Dissolved organic carbon (mg/L)	0.8	1.1 ± 0.3

[a] Parameters with no standard deviation were measured only once.

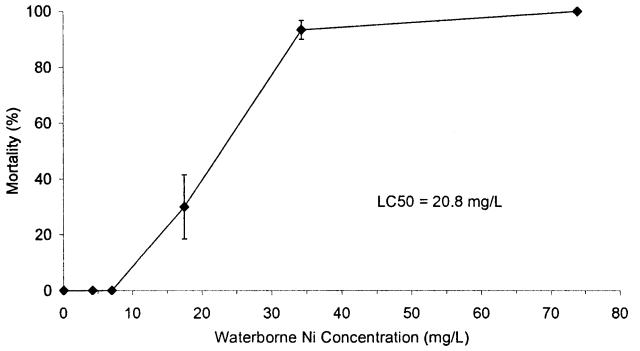

Fig. 1. Acute (96-h) toxicity of nickel to rainbow trout. Error bars represent one standard error. LC50 = median lethal concentration.

sponding 95% confidence intervals were estimated using linear interpolation, probit, or Spearman-Karber analysis. Both NOEC and LOEC values were estimated using Dunnett's multiple-comparison test [16] after checking for data normality and homogeneity of variance.

RESULTS

Water quality and analytical chemistry

Water-quality conditions for both tests are summarized in Table 1. Test temperature, dissolved oxygen, and pH all met requirements for test acceptability. Mean measured concentrations of dissolved Ni for both studies were comparable to nominal concentrations (Table 2). All statistical analyses were performed based on the means of the measured concentrations of dissolved Ni.

Toxicity test results

A clear exposure–response relationship was observed in the 96-h acute toxicity test, with an estimated LC50 of 20.8 mg/L (Fig. 1). In the chronic study, no statistically significant effects were observed for any of the endpoints evaluated (hatchability, swim-up, survival, length, and weight). Thus, the NOEC was 466 μg/L, and the LOEC was greater than 466 μg/L (Fig. 2).

Physiological results

In the acute study, Ni accumulated at the trout gills in an exposure-dependent manner but, apparently, had not reached steady state at 24 h (Fig. 3a), the time period typically used to estimate a gill LA50 [8]. When mortality was plotted as a function of the amount of Ni accumulated at the gill, the resulting estimated LA50 values were 666 (95% confidence interval, 607–752) and 2,079 (95% confidence interval, 1,764–2,450) nmol/g wet weight at 24 and 96 h, respectively (Fig. 3b). When Ni accumulation at the gill was plotted for each waterborne-exposure concentration, distinctly different accumulation patterns are observed. Nickel accumulated at the gill reaches a plateau after 24 h for the two lower waterborne Ni exposures but continued to increase through 96 h for the two higher concentrations, for which both 24- and 96-h gill Ni data were available (Fig. 3c). A slight reduction (15%) in plasma Na may have been observed at 24 h in the highest treatment, but no effect on plasma Ca or Mg was observed (data not shown). An insufficient number of fish was available to analyze plasma ion concentrations at 96 h.

In the chronic study, measurements on day 28 showed whole-egg Ni accumulated in an exposure-dependent manner, with the highest whole-egg Ni concentration being associated with the chorion (Fig. 4a). Measurement of Na, Ca, and Mg in whole egg, embryo, and chorion resulted in consistent trends, with the whole-egg measurements indicating no ionoregulatory disruption. Measurements of Na, Ca, and Mg in the embryo suggested, perhaps, a minor effect, and ion balance at the chorion apparently was significantly disrupted (Fig. 4b–d). Relative to the control, Na, Ca, and Mg concentrations in embryos from the highest Ni treatment were reduced by 19%, 25%, and 29%, respectively. For the chorion, Na, Ca, and Mg were reduced by 64%, 29%, and 34%, respectively, relative to the control at the lowest Ni concentration tested (29 μg/L) and by 81%, 47%, and 54%, respectively, relative to the control at the highest Ni exposure concentration (466 μg/L).

At test termination on day 85, Na, Ca, and Mg concentrations in the plasma did not appear to be affected by Ni exposure (Fig. 5). Measurement of Ni accumulation at trout gills and in plasma both showed strong exposure-dependent responses (Fig. 6). Plasma Ni increased steadily until the 118-μg/L treatment, where it appeared to plateau (Fig. 6a). In contrast, gill Ni increased in an exponential fashion up to the maximum exposure concentration. The resulting maximum Ni concentration at the gill was 125 nmol/g wet weight (Fig. 6b).

Table 2. Measured concentrations of dissolved Ni in acute and chronic toxicity tests (mean ± standard deviation)

Test	Control	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Acute (mg/L)[a]	0.003	4.2	7.0	17.5	34.3	73.9
Chronic (μg/L)	1 ± 0	29 ± 4	52 ± 9	118 ± 20	233 ± 27	466 ± 59

[a] Mean of two measurements.

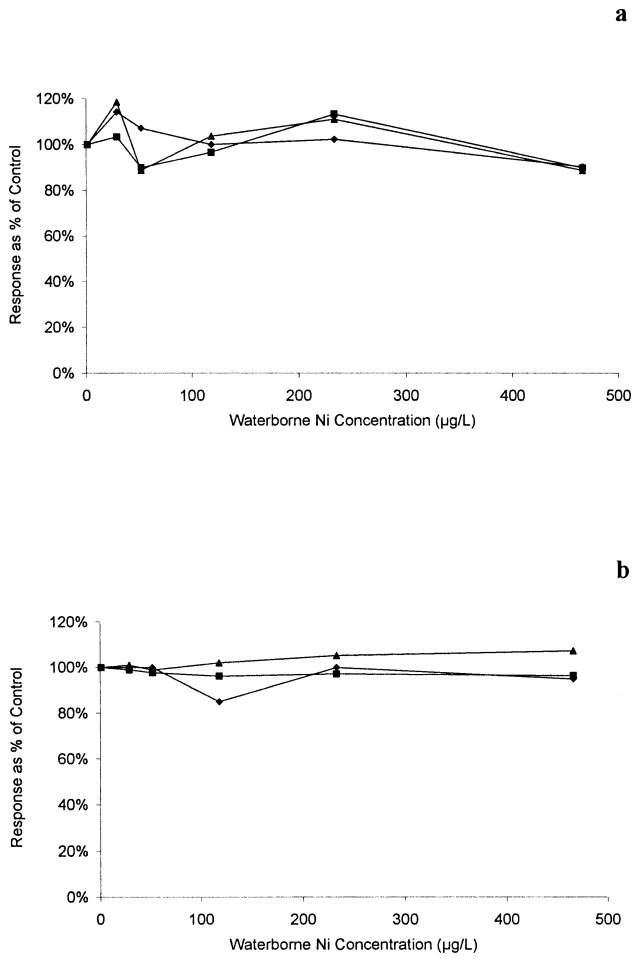

Fig. 2. Chronic toxicity of Ni to rainbow trout. (**a**) Embryo survival and hatchability. ♦ = egg survival, day 28; ■ = hatch, day 44; ▲ = swim-up, day 55. (**b**) Larval survival and growth. ♦ = survival; ■ = length; ▲ = weight.

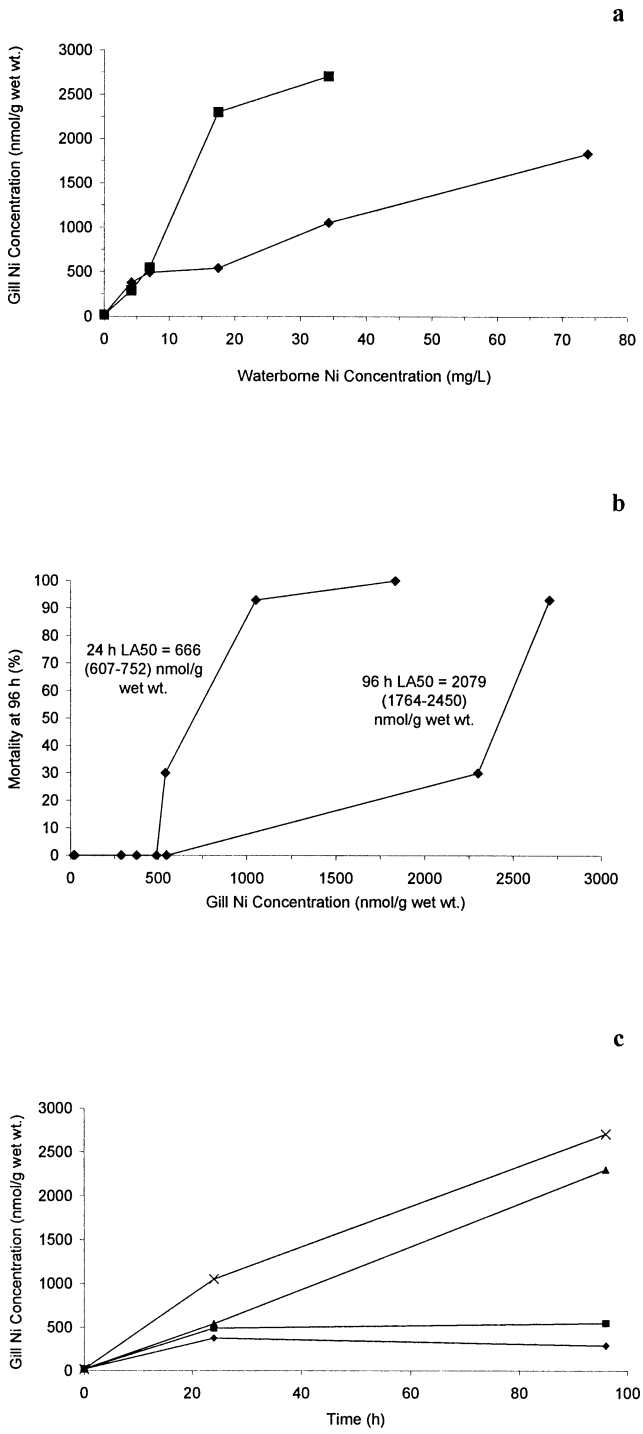

Fig. 3. Nickel accumulation on trout gills during acute (96-h) exposure. (**a**) Gill Ni as a function of waterborne Ni exposure. ♦ = 24 h; ■ = 96 h. (**b**) 96-h trout mortality as a function of gill Ni measured at 24 and 96 h. ♦ = 24-h gill and 96-h mortality (24-h median lethal accumulation [LA50] = 666 nmol/g wet wt); ■ = 96-h gill and 96-h mortality (96-h LA50 = 2,079 nmol/g wet wt). (**c**) Ni accumulation at the gill over time at different waterborne Ni concentrations. ♦ = 4.2 mg/L; ■ = 7 mg/L; ▲ = 17.5 mg/L; × = 34.3 mg/L.

DISCUSSION

Acute toxicity of nickel

The estimated 96-h LC50 in the acute study of 20.8 mg/L dissolved Ni is consistent with the results of previous acute studies involving *O. mykiss*. For example, Nebeker et al. [3] estimated a 96-h LC50 of 10.0 mg/L in soft water (hardness, 33 mg/L). Normalizing these two LC50 values to a hardness of 50 mg/L using the U.S. EPA hardness correction [1] resulted in similar LC50 values of 12.8 and 14.2 mg/L, respectively. In contrast, Pane et al. [17] recently estimated a 96-h LC50 of 15.3 mg/L at a water hardness of 140 mg/L, which, when normalized to a hardness of 50 mg/L, results in an estimated LC50 of 6.4 mg/L.

Nickel accumulation at the trout gill in the acute study resulted in a 24-h LA50 of 666 nmol/g wet weight. To our knowledge, the only other published LA50 was estimated by Meyer et al. [10] for the fathead minnow (*P. promelas*). Those authors conducted tests at four water-hardness levels and estimated 24-h LA50 values ranging from 150 to 300 nmol/g wet weight; the LA50 generally increased with increasing hardness. The corresponding 96-h LC50 values ranged from 8.8 to 88 mg/L of total Ni.

Meyer et al. justified 24 h as an appropriate time to measure LA50s based on preliminary studies indicating that 85% of the estimated asymptotic gill Ni accumulation had occurred within 24 h. In contrast, our results for rainbow trout indicated that this was true only at a relatively low concentration of waterborne Ni (<7 mg/L). At higher concentrations, Ni appeared to continue accumulating on the gill through 96 h (Fig. 3a). For example, in the 17.5-mg/L treatment, Ni accumulation at the gill was 541 and 2,300 nmol/g wet weight at 24 and 96

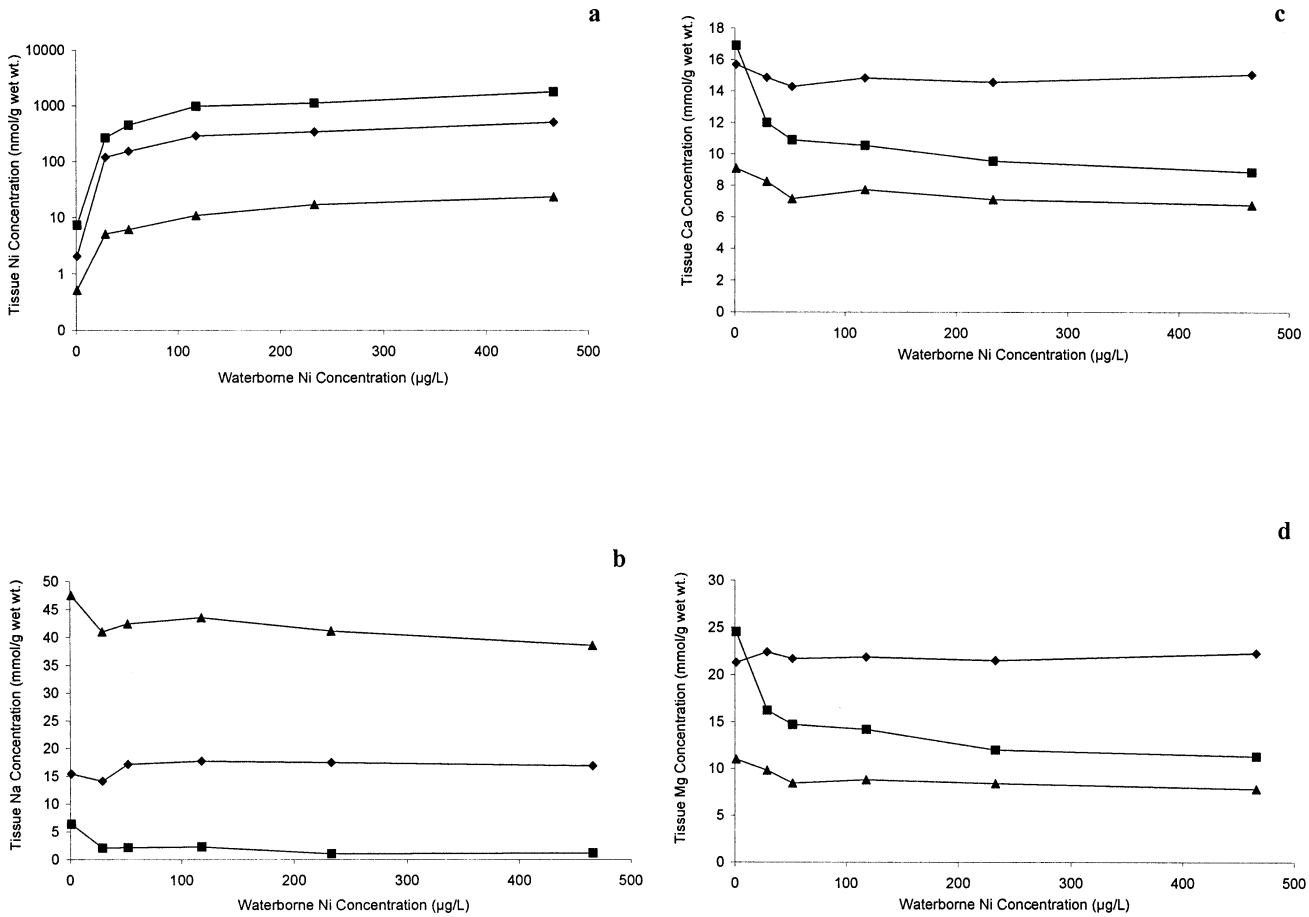

Fig. 4. Nickel, sodium, calcium, and magnesium concentrations in trout eggs after 28 d of Ni exposure. (**a**) Nickel; (**b**) sodium; (**c**) calcium; and (**d**) magnesium. ♦ = Whole egg; ■ = chorion; ▲ = embryo.

h, respectively. As a result of this continuing accumulation, a significantly higher LA50 was estimated using 96-h accumulation data (2,079 nmol/g wet wt).

When the acute-exposure gill data were plotted over time for individual concentrations of waterborne Ni, results suggest two separate binding sites: A relatively low-capacity site that is saturable, and a higher-capacity site that did not saturate at the concentrations tested. This pattern has been observed for other metals. For example, Taylor et al. [18] demonstrated a high-affinity, low-capacity site and a low-affinity, high-capacity site for Cu binding to rainbow trout gills. The data generated in our study suggest that a similar set of sites might operate for Ni, but we believe this is not the case.

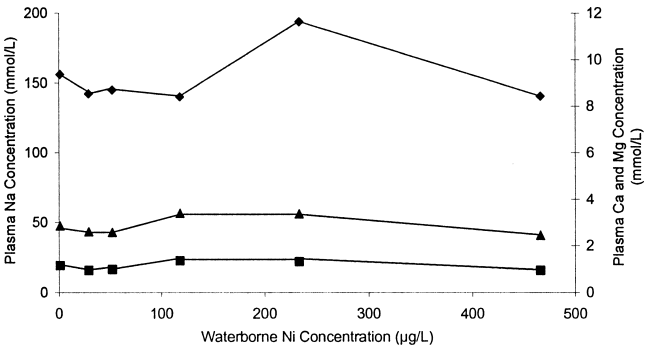

Fig. 5. Plasma Na, Ca, and Mg in rainbow trout on day 85 of the chronic Ni study. ♦ = Na; ■ = Mg; ▲ = Ca.

Unlike the Cu study by Taylor et al., the acute Ni exposure in the present study was conducted at very high (mg/L) concentrations. At the higher concentrations tested in our study, Pane et al. [17] observed gross physiological damage to the gill and significant production of mucus. We suggest that the apparent high capacity–binding site observed in the present study is actually a result of mucus production rather than a true binding site for Ni at the gill. Hence, the additional accumulation observed at the gill at 96 h in the present study might not be toxicologically relevant. Further study is required to test this hypothesis, but we suggest that the 24-h gill accumulation data and corresponding LA50 value are most appropriate for development of a biotic ligand model.

Physiological measurements indicate that Ni is not an ionoregulatory toxicant, at least in acute exposure scenarios. No effect was observed on plasma concentrations of Ca and Mg as a function of Ni exposure concentration, whereas only a slight reduction (15%) in plasma Na concentration may have been observed at the highest Ni concentration tested. Given that an approximate 35 to 40% reduction in plasma Na concentration is normally required to elicit mortality in rainbow trout [7], the Na reduction we observed seems unlikely to have caused the observed mortality. Closer examination of the dose–response relationship supports this hypothesis. Specifically, the 15% reduction of plasma Na in the highest Ni treatment was associated with 100% mortality at 96 h. In contrast, plasma Na was 13% higher in the second-highest Ni treatment (34 mg/L), but this treatment still suffered 93% mortality at 96 h.

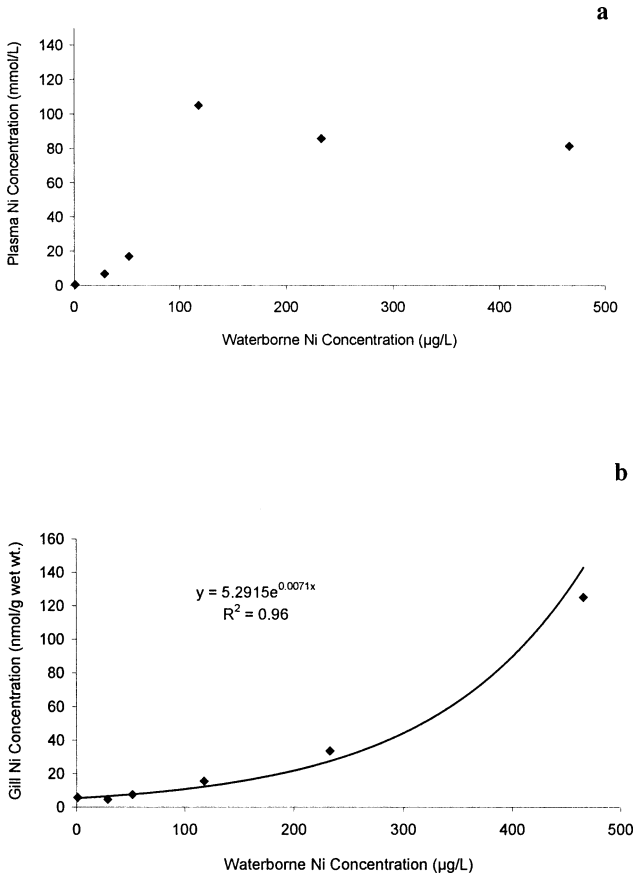

Fig. 6. Plasma (**a**) and gill (**b**) Ni as a function of waterborne nickel exposure on day 85 of the chronic Ni study. Exponential model in (**b**) derived using the Excel® regression tool (Microsoft, Redmond, WA, USA).

Given the lack of replicate data for these measurements, these values may simply represent within-test variation. Consistent with these results, Pane et al. [17] found no significant effects on plasma ions when rainbow trout were exposed to 11.3 mg/L of Ni for 117 h.

Chronic toxicity of nickel

To our knowledge, Nebeker et al. [3] conducted the only other published chronic study of Ni with rainbow trout. They estimated a LOEC of 35 µg/L and a NOEC of less than 35 µg/L (the lowest concentration tested) based on statistically significant effects on trout growth (both fork length and wet wt). The dose–response relationship for length and weight were both relatively flat. For fork length, a 7% ($p < 0.05$) reduction relative to the control was observed at 35 µg/L, whereas at 700 µg/L, this reduction was only 11%. Greater effects were observed for wet weight: A 13% reduction in weight occurred at 35 µg/L. However, only a marginal increase in effect (21% relative to the control) was observed at 700 µg/L. Not until 1,100 µg/L was a large increase in the effects of Ni on growth observed, with 28% and 65% reductions in fork length and wet weight, respectively.

The relatively flat exposure–response relationship reported by Nebeker et al., and the corresponding uncertainty as to where the true effect threshold occurred, prompted us to conduct the chronic study. Our results indicate a substantially higher effect threshold than that observed by Nebeker et al., with a NOEC of 466 µg/L and a LOEC of greater than 466

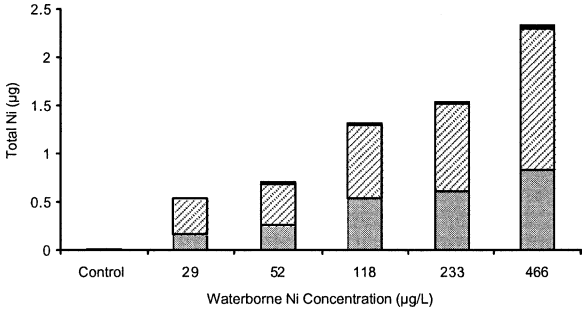

Fig. 7. Total Ni (mass) in trout eggs after 28 d of Ni exposure. ■ = embryos; ▨ = yolk; □ = chorion.

µg/L for all endpoints. Because we used two replicates from each treatment for physiological measurements, the present study had lower statistical power than that of Nebeker et al. However, close inspection of the data indicates that observed discrepancies likely are not a function of differences in statistical power between the two studies. For example, Nebeker et al. observed a 65% reduction in hatching success at 431 µg/L, whereas we observed only a 10% reduction at 466 µg/L (Fig. 2a). Nebeker et al. also observed a 24% reduction in trout growth (wet wt) at 431 µg/L, whereas growth in the present study was actually 7% higher in the 466-µg/L treatment than in the controls. Furthermore, growth in the controls for the two studies was similar (0.96 and 0.97 g wet wt/fish, respectively).

The substantial differences in results between the two studies are not easily explained. Both studies were initiated with the same life stage and used similar exposure durations. Some differences in water-quality parameters were evident. Test temperature was 12°C in the Nebeker et al. study and 10°C in the present study. Water hardness and pH were 53 mg/L and 7.0, respectively, in the Nebeker et al. study and 89 mg/L and 7.9, respectively, in the present study. All the water-quality parameters in the Nebeker et al. study would tend to increase the bioavailability of Ni relative to the conditions in the present study.

To investigate this further, we input the water-quality conditions and estimated NOEC values from these two studies into the geochemical speciation model CHESS [19] to evaluate the relative fractions of free Ni ions present at the NOEC. The speciation model predicted that 54% of dissolved Ni was in the free ionic form in the Nebeker et al. [3] study, compared with only 25% in the free ionic form in the present study. Additionally, competitive interactions between Ni and other divalent cations (e.g., Ca and Mg) that are likely to occur at the gill, but that cannot currently be quantified for Ni, would also be higher in the present study than in the study by Nebeker et al. Hence, differences in overall bioavailability likely are even greater than those estimated by simply considering the fraction of free Ni ion. Overall, the differences in Ni bioavailability between the two studies likely account for some, but not all, of the discrepancies in toxicological results.

Despite the lack of effects on standard toxicological endpoints in the chronic study, several interesting observations can be made regarding Ni accumulation and potential ionoregulatory disturbance. First, the chorion proved to be only a partial barrier to Ni exposure for the developing trout embryos. Figure 7 shows the relative proportions of total Ni (µg) in whole eggs. Nickel mass associated with embryos and chorions was determined by direct measurement, whereas yolk Ni was

estimated as the difference between measured whole-egg Ni and the combined measured embryonic and chorionic Ni. These results indicate a relatively consistent distribution in each of the Ni treatments (excluding the control), with 1.0 ± 0.1%, 63 ± 5%, and 36 ± 5% of Ni associated with the embryo, yolk, and chorion, respectively. The distribution may have been slightly different in the control (1 μg/L), with 5%, 44%, and 51% of Ni mass associated with the embryo, yolk, and chorion, respectively. The ultimate fate of the Ni in the yolk is unknown. It is possible it would be absorbed by the developing embryo, providing an additional Ni dose. However, if this occurred, it resulted in no obvious toxicological effect in the present study.

In comparison to the present study with Ni, Guadagnolo et al. [20] found approximately 80 to 85% of Ag mass to be associated with rainbow trout chorion at elevated concentrations of waterborne Ag (1.2 and 13.5 μg/L) on day 28 in an experiment of similar design. They hypothesized that the chorion may have prevented most of the Ag from reaching the embryo by binding to sulfhydryl groups associated with cysteine, a significant component of chorionic protein [21]. Our results are consistent with this hypothesis: Ni has a considerably lower binding affinity for sulfhydryl groups compared with Ag, so a greater fraction of Ni would be expected to pass through the chorion.

Large changes in ion concentrations were observed in the chorion, but these changes did not fully translate to the embryo or the whole egg. For example, when comparing the control to the highest Ni treatment, Na concentrations in the chorion were reduced by 81%, but only by 19% in the embryo (Fig. 4b). Similarly, for Ca and Mg, reductions of 47% and 54%, respectively, occurred in the chorion, compared with only 25% and 29%, respectively, in the embryo (Fig. 5c and d). The ionoregulatory effects of metals on trout embryos (and ionoregulation of trout embryos in general) have not been extensively studied, so it is unclear whether the observed reductions of ion concentrations in embryos were approaching toxicologically significant levels.

In the chronic study, Ni concentrations in plasma appeared to plateau at 80 to 100 μmol/L (Fig. 6a). Whether the observed plateau is a function of saturation or active regulation is unclear. Pane et al. [17] measured a plasma Ni concentration of 107 μmol/L in a 99-h exposure to 11 mg/L of dissolved Ni, and they observed continually increasing Ni plasma over the time course of this exposure, with no indication of saturation. This suggests that the plateau observed in the present study might be the result of active regulation, but this suggestion remains to be confirmed. Regardless, these concentrations are remarkably high compared with those of other metals (e.g., Ag, Cd, Cu, and Zn), for which measured plasma metal concentrations on the order of 2 to 25 μmol/L have been measured [22–25].

CONCLUSION

The acute toxicity results from the present study are generally consistent with those of previous acute studies involving rainbow trout. In contrast, the chronic study suggests a substantially higher effects threshold for rainbow trout than reported in the single previous study. Some of this difference may be accounted for by differences in bioavailability between the two studies, but a real discrepancy also appears to exist.

Overall, we conclude that acute Ni toxicity is not related to the disruption of ion regulation. This is consistent with a recently published study by Pane et al. [17], who concluded that acute Ni toxicity is related to effects on respiration. Our results also suggest that ionoregulatory disruption is not the mode of action for chronic toxicity, although the absence of significant effects at even the highest concentration tested does not allow us to conclusively rule it out.

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