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Reproductive impacts and physiological adaptations of zebrafish to elevated dietary nickel

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ABSTRACT

Nickel (Ni) concentrations in the environment can rise due to human industrial activities. The toxicity of waterborne Ni to aquatic animals has been examined in a number of previous studies; however, little is known about the impacts of elevated dietary Ni. In the present study, zebrafish were chronically fed diets containing two concentrations of Ni [3.7 (control) and 116 μg Ni/g diet]. Ni-exposed males, but not females, were significantly smaller (26%) compared to controls at 80 days. In addition, total egg production was decreased by 65% in the Ni treatment at 75–78 days of the experiment. Ni was ubiquitously distributed in control animals (similar to previous studies), and concentrations varied between tissues by 15-fold. Ni exposure resulted in modest but significant Ni accumulation in some tissues (increases were highest in brain, vertebrae and gut; 44%, 34% and 25%, respectively), an effect observed only at 80 days. The limited Ni accumulation may be due to (1) the lack of an acidified stomach in zebrafish and/or (2) the efficient upregulation of Ni transport and excretion mechanisms, as indicated by the 4.5-fold increase in waterborne ^{63}Ni uptake by Ni-exposed fish. Eggs from Ni-exposed adults had Ni concentrations that were 5.2-fold higher than controls. However, by 4 days post fertilization, larvae had similar Ni concentrations as controls, demonstrating a capacity for rapid Ni depuration. Larvae from Ni-exposed adults were also more resistant to waterborne Ni (35% increase in the 96-h LC50 over controls). In conclusion, elevated dietary Ni significantly affected zebrafish reproduction despite only modest tissue Ni accumulation. There were also indications of adaptation, including increased Ni uptake rates and increased Ni tolerance of offspring from Ni-exposed adults. Ni concentrations were particularly elevated in the brain with exposure; possible relations to growth and reproductive impacts require further study.

1. Introduction

Nickel (Ni) is a Group III transition metal of commercial importance that is relatively abundant in the earth's crust (Cempel and Nikel, 2006; Reck et al., 2008). Ni is essential in plants and bacteria (Hänsch and Mendel, 2009; Higgins et al., 2012; Pyle and Couture, 2012); in fish, there is circumstantial evidence that this is also the case, although it has not been proven (Muyssen et al., 2004; Pyle and Couture, 2012). The mining and processing of Ni as well as other anthropogenic activities are primarily responsible for elevated Ni levels in the environment (Eisler, 1998; Cempel and Nikel, 2006; Reck et al., 2008; Pyle and Couture, 2012). Indeed, waters surrounding Ni mining areas have increased concentrations of Ni (Chau and Kulikovsky-Cordeiro, 1995; Eisler, 1998; Couture and Rajotte, 2003; Couture et al., 2008; Pierron et al., 2009; Pyle and Couture, 2012), and this is problematic due to the toxicity that elevated Ni concentrations present to many aquatic animals (Schubauer-Berigan et al., 1993). In rainbow trout, acute waterborne Ni toxicity was associated with disruption of gill respiratory function (Pane et al., 2004a), while Na^+ loss appeared to be the acute toxic mechanism in zebrafish (Alsop and Wood, 2011). A number of chronic effects of elevated waterborne Ni on fish have also been documented, including impacts on survival (Hunt et al., 2002; Deleebeeck et al., 2007), behavior (Giatina et al., 1982; Leonard et al., in press), decreased swimming capacity (Pane et al., 2004b), delayed embryo hatching (Dave and Xiu, 1991) and histopathological changes in a number of organs (Athikesavan et al., 2006).

In contrast to waterborne Ni, there is far less known about the effects of elevated dietary Ni intake of fish. One study fed lake whitefish (*Coregonus clupeaformis*) Ni-supplemented diets (0, 10, 100 and 1000 μg Ni/g diet) for up to 104 days (Ptashynski and Klaverkamp, 2002; Ptashynski et al., 2002). Ni accumulation occurred in a variety of tissues including different parts of the gastrointestinal tract, kidney, scales and others (Ptashynski and Klaverkamp, 2002). Although growth and hematological parameters were not affected, histopathological impacts in kidney and liver were observed (Ptashynski et al., 2002). Ni transport in the gastrointestinal tract has been examined in rainbow

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trout, where one study infused a dose of radiolabeled ^{63}Ni into the stomach and followed the radiolabeled Ni over time (Chowdhury et al., 2008). After 24 h, 3.7% of the Ni had crossed the gut and entered the internal organs, while 2.3% was in the gut tissue itself (Chowdhury et al., 2008). Interestingly, fish pre-exposed to waterborne Ni for 45 days took up less Ni through the gut, indicating integrative homeostatic regulation (Chowdhury et al., 2008). Another study in trout determined that 50% of the Ni present in ingested food (normal commercial trout pellets containing 25 μg Ni/g diet) was absorbed across the gut over 72 h (Leonard et al., 2009). The stomach proved to be the main site of Ni uptake, while the anterior intestine was involved in Ni secretion (Leonard et al., 2009).

The objectives of the present study were to determine the bioaccumulation and effects of elevated dietary Ni in zebrafish, specifically examining a variety of reproductive and physiological end points. We fed zebrafish a control diet (3.7 μg Ni/g diet) or an elevated Ni diet (116 μg Ni/g diet). The exposure concentration was chosen based on the previous studies of Ptashynski et al. (2002) and Ptashynski and Klaverkamp (2002) as well as levels in plants and animals that are found in Ni-polluted environments (Eisler, 1998). Fish were fed the diets for 80 days, during which time growth, tissue Ni concentrations and reproductive capacity were monitored. After 80 days, endocrine status, metabolism and branchial Ni transport were tested. In addition, the offspring of the adults from the two treatments were examined: the Ni content of the embryos and larvae as well as the acute sensitivity of larvae to waterborne Ni. The latter tests were prompted by a report that the offspring of *Daphnia magna* chronically exposed to elevated waterborne Ni exhibited greater resistance to Ni challenge (Pane et al., 2004c).

2. Materials and methods

2.1. Animals and housing

Zebrafish (*Danio rerio*; 0.2 to 0.3 g) were purchased from a commercial supplier and held in six 40-L tanks (3 control and 3 Ni-exposed), each containing 40 fish. Each tank was equipped with aeration, a heater (set to 28 °C), and a recirculating charcoal filter, while photoperiod was maintained at 12 h light/12 h dark. Water was moderately hard, dechlorinated City of Hamilton tap water, from Lake Ontario (hardness = 141 mg $CaCO_3$/L, pH 7.8, Na^+ = 700 μM, K^+ = 38 μM, Ca^{2+} = 1350 μM, Mg^{2+} = 336 μM, Cl^- = 950 μM, dissolved organic carbon = 3.0 mg/L, Ni = 1.7 μg/L). Fish were fed to satiation two times per day. Uneaten food and feces were siphoned out of the tank daily. In addition, every 2–3 days, 75% of the water was removed and replaced with fresh water. Water samples were taken periodically from all tanks to measure waterborne Ni concentrations, in order to determine if there was significant leaching of Ni from the diet, fish or feces. Over the course of the experiment, the Ni concentrations in the water from tanks of fish fed the control diet were 2.61 ± 0.20 μg Ni/L (N = 18), while in the tanks of fish fed the elevated Ni diet, waterborne Ni concentrations were 5.27 ± 0.69 μg Ni/L (N = 18). These waterborne Ni concentrations are well below the Canadian Water Quality Guidelines. At the water hardness of the present experiment (141 mg $CaCO_3$/L), the guideline limit would be 110 μg Ni/L (CCME, 2007).

2.2. Ni measurements in the diets and tissues

Two experimental diets containing different concentrations of $NiSO_4.6H_2O$ (Sigma-Aldrich, Oakville, ON, Canada) were formulated (Table 1). The basic feed formulation was based on previous zebrafish dietary studies (Karanth et al., 2009), and the National Research Council's nutrient requirement recommendations for warm-water fishes (NRC, 1993). Analyzed Ni concentrations in the formulated zebrafish diets were 3.66 ± 0.30 μg Ni/g diet (N = 5) for the control treatment and 115.8 ± 11.3 μg Ni/g diet (N = 5) for the elevated dietary Ni treatment (see below for Ni measurement methods).

Fish were sampled on days 0, 5, 20 and 80 of the exposure to determine fish weights and tissue Ni concentrations. Fish were not fed for 18 h prior to sampling. Fish were first terminally anesthetized with an overdose of MS-222 (neutralized; 0.25 g/L) (Sigma–Aldrich, St. Louis MO, USA), weighed and decapitated. Tissues (blood, brain, eyes, gills, G.I. tract, liver, ovaries, muscle, vertebrae and remaining carcass) were dissected, placed in pre-weighed tubes and weighed. Samples were then snap frozen in liquid nitrogen and stored at −70 °C.

For Ni concentration measurements, tissues were thawed and 10× volume of 33% trace metal grade HNO_3 (Sigma–Aldrich) was added to each diet or fish sample. Tissues were then digested for 48 h at 60 °C. Samples were diluted approximately 5-fold (depending on the tissue) with nanopure water (Sybron/Barnstead 16508 megohm-cm), and Ni was measured by graphite furnace atomic absorbance spectroscopy (Spectra AA 220Z; Varian, Palo Alto, CA, USA). Ni recovery was 99.4 ± 1.8%, as determined with certified reference water for trace elements (TM-15; National Water Research Institute, Environment Canada, Burlington, ON, Canada). Ni concentrations were not corrected for recovery. A certified reference tissue material was not used. Blanks and the TM-15 reference standard were reanalyzed every 30 measurements.

2.3. Reproductive capacity

From 75 to 78 days of the exposure, reproductive performance was evaluated by collecting, counting, incubating and analyzing eggs for four consecutive days. Eggs were acquired with capturing trays placed in each tank just prior to the end of the light period. Trays consisted of a plastic container 7 cm in height by 30 cm in width by 45 cm in length. The container lid was replaced with plastic mesh that allowed the eggs to fall through and prevented fish from consuming them. Plastic plants were glued to the mesh. In the morning, 2 h after the commencement of the light period, the trays were removed from the tanks, and eggs were collected and sorted into the following: (1) groups of 25 eggs that were placed into tubes and snap frozen for Ni analysis, (2) eggs that were incubated in 120-mL beakers with 50 mL of water (50 eggs per beaker) at 28.5 °C. These embryos were raised to 4 days post

Table 1
Composition of experimental diets containing different supplemental quantities of nickel.

Ingredient	Weight (g)
Vitamin-free casein[a]	330
Wheat gluten meal[b]	100
Gelatin[a]	40
Corn oil[c]	40
Fish oil[d]	40
Corn starch (pre-gel)[e]	330
Celufil[a]	81
Vitamin mix[f]	12
Mineral mix[g]	10
Betaine[h]	15
L-Methionine[a]	2
$NiSO_4 \cdot 6H_2O$[i]	Control: 0.013 Ni-supplemented: 0.447
Total	1000

[a] US Biochemical (Cleveland, OH, USA).
[b] Dover Mills Ltd. (Halifax, NS, Canada).
[c] Corey Feed Mills Limited (Fredericton, NB, Canada).
[d] Obtained from the local market.
[e] National Starch and Chemical Co. (Bridgewater, NJ, USA).
[f] Vitamin added to supply the following (per kg diet): vitamin A, 8000 IU; vitamin D_3, 4000 IU; vitamin E, 300 IU; vitamin K_3, 40 mg; thiamine HCl, 50 mg; riboflavin, 70 mg; d-Ca pantothenate, 200 mg; biotin, 1.5 mg; folic acid, 20 mg; vitamin B_{12}, 0.15 mg; niacin, 300 mg; pyridoxine HCl, 20 mg; ascorbic acid, 300 mg; inositol, 400 mg; choline chloride, 2000 mg; butylated hydroxy toluene, 15 mg; butylated hydroxy anisole, 15 mg.
[g] Mineral added to supply the following (per kg diet): manganous sulphate (32.5% Mn), 40 mg; ferrous sulphate (20.1% Fe), 30 mg; copper sulphate (25.4% Cu), 5 mg; zinc sulphate (22.7% Zn), 75 mg; sodium selenite (45.6% Se), 1 mg; cobalt chloride (24.8% Co), 2.5 mg; sodium fluoride (42.5% F), 4 mg.
[h] Betaine anhydrous (96% feed grade) (Finnfeeds, Finland).
[i] Sigma-Aldrich (Oakville, ON, Canada).

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