Polish J. of Environ. Stud. Vol. 19, No. 2 (2010), 453-456

Short Communication

Acute Toxicity of Nickel to Five Species of Freshwater Fish

Gintaras Svecevičius*

Institute of Ecology of Vilnius University, Akademijos 2, LT-08412, Vilnius-21, Lithuania

Received: 10 August 2009
Accepted: 2 November 2009

Abstract

Flow-through toxicity tests were conducted on common freshwater fishes: rainbow trout (*Oncorhynchus mykiss*), three-spined stickleback (*Gasterosteus aculeatus*), roach (*Rutilus rutilus*), perch (*Perca fluviatilis*) and dace (*Leuciscus leuciscus*), to estimate their sensitivity to acute toxicity of nickel. The 96-hour median lethal concentration (96-hour LC50) values obtained from the tests ranged from 19.3 to 61.2 mg Ni/l. According to nickel sensitivity, the species tested may be arranged in the following sensitivity order: rainbow trout > three-spined stickleback > perch = roach > dace. Obtained data could be successfully applied in solving theoretical and practical goals of aquatic toxicology.

Keywords: freshwater fish, acute toxicity, nickel

Introduction

Nickel is widely used in industry and is a common aquatic pollutant [1]. In natural waters Ni^{2+} is the dominant chemical species [2-4]. In aquatic ecosystems nickel interacts with numerous inorganic and organic compounds and occurs as soluble salts adsorbed onto substances of different chemical origin [2-4]. Some of these interactions are additive or synergistic in producing adverse effects, and some are antagonistic [4].

The presence of four heavy metals (copper, zinc, nickel, and hexavalent chromium) has been identified as indicators of poor water quality [5]. The toxicity of nickel to aquatic life was intensively investigated during previous decades, and a considerable amount of experimental data has been compiled and reviewed [2-4]. Data on nickel toxicity to fish are still scarce and most are from North American fishes often using a single species. Moreover, data on sensitivity of European fish species to nickel are almost totally lacking.

*e-mail: Gintaras.Svecevicius@takas.lt

Toxicity testing with a single fish species is inadequate for evaluation pollutant hazard to the environment and does not allow for identification of pollutant selective toxicity. In order to obtain information on pollutant toxicity range, species with generally different susceptibilities or metabolic activities should be used, including those easily available and common in the area where the toxicant may occur. Therefore, comparative toxicity studies should be developed to identify species that produce results suitable to the evaluation of ecotoxicity of the pollutant under study [6]. Furthermore, the same fish species in distinct geographical regions may show different sensitivity to the same pollutant. That is why data obtained in different regions can hardly be extrapolated to local conditions.

Several physico-chemical factors of water are known that modify nickel toxicity to fish. Acute lethality of nickel increases with decreasing water pH and decreases as hardness, alkalinity and total suspended solids increases [7, 8].

The purpose of this study is by means of acute lethality tests to estimate the comparative sensitivity to nickel of five common freshwater fish species.

Table 1. Length and weight of test fishes*

Fish species	Total length (mm)		Weight (g)	
	Range	Mean ± SEM	Range	Mean ± SEM
Rainbow trout	70 - 90	82 ± 0.69	3.8 – 9.4	6.0 ± 0.18
Three-spined stickleback	45 - 57	51 ± 0.30	0.8 – 1.7	1.2 ± 0.03
Roach	70 - 90	79 ± 0.60	2.7 – 6.5	4.3 ± 0.12
Perch	55 - 75	64 ± 0.69	1.7 – 3.5	2.5 ± 0.06
Dace	52 - 62	57 ± 0.33	1.1 – 1.9	1.4 ± 0.03

*Number of fish for each species = 120.

Experimental Procedures

Toxicity tests were conducted on the following fish species: rainbow trout *Oncorhynchus mykiss* (Walbaum), a species commonly used for aquatic toxicity testing; three-spined stickleback *Gasterosteus aculeatus* L.; roach *Rutilus rutilus* (L.); perch *Perca fluviatilis* L. and dace *Leuciscus leuciscus* (L.).

Species were chosen because they are common in the European region, and represent systematically and ecologically different fish groups.

Rainbow trout were obtained from Žeimena Hatchery (Švenčionys District, Lithuania). The other species were collected in reference sites of the Neris River, using a drag-net of 6 mm mesh. Length and weight data of fishes used in toxicity tests are presented in Table 1.

The test fish were acclimated to laboratory conditions for one week prior to testing. The fish were kept in flow-through holding tanks supplied with aerated deep-well water (minimum flow rate 1 l per 1 g of their wet body mass per day), under natural illumination and were fed live feed (*Chironomus* sp.) daily in the morning; the total amount was about 1% of their wet body mass per day. The day before and during the tests the fish were not fed.

Reagent grade nickel sulphate ($NiSO_4 \cdot 7H_2O$) (REAKHIM Company, Russia) was used as the toxicant. Stock solution was prepared by dissolving a necessary amount of nickel sulphate in distilled water, the final concentration being recalculated according to the amount of heavy metal ion.

Deep-well water was used for dilution. Its chemical characteristics are given in Table 2.

The tests were conducted under flow-through conditions. The system included 6 tanks of 30-liter volume and the same number of proportional diluters according to the Mount and Brungs [9] design. Test fishes were exposed to a series of nominal concentrations ranging from 18.0 to 100 mg/l of nickel with five treatments and one control. The factor of dilution was 0.75, and 400 ml of the solution was added to each tank every 3 minutes. The testing was started in clean water, 10 fish being placed into each tank for acclimation. In order not to stress the fish, the concentration of toxicant in tanks was increased gradually, 50% test concentration being reached within 3.8 hours and full toxicant concentration within 18.8 hours. Each test had two replicates.

Table 2. Chemical and physical characteristics of the dilution water (All values are in mg/l, unless otherwise noted).

pH	8.0 (7.9 – 8.1)
Dissolved oxygen	9.0 (8.0 – 10.0)
Temperature	11.0 (10.5 – 11.5)
Hardness (as $CaCO_3$)	284 (271 – 296)
Alkalinity (as HCO_3^-)	244 (232 – 256)
Suspended solids	2.7
BOD	0.000
Mg^{2+}	16.0
Ca^{2+}	64.0
SO_4^{2-}	15.0
NO_2^-	0.000
NO_3^-	0.000
PO_4^{3-}	0.032
Cl^-	1.9
Phenols	0.000
Oil hydrocarbons	0.000
Fe	0.56
Mn	43.7
Zn	0.0101
Cu	0.00252
Cr	0.00056
Ni	0.00072
Cd	0.00004
Pb	0.00000

Mortality observations were made at 24-hour intervals. Dead fish were removed, weighed individually (after being lightly blotted dry), and measured (total length) at the time of mortality observation. Fish still living at the conclusion of a test were sacrificed, weighed and measured at that time. No mortality was observed among control fish.

Table 3. Nickel concentration scale (mg/l) used in toxicity tests.

Nominal concentration	18	24	32	42	56	75	100
Measured concentration*	17.19 ± 0.51	22.96 ± 0.55	30.72 ± 0.82	39.90 ± 0.95	58.52 ± 1.45	72.01 ± 1.87	104.82 ± 2.31

*Mean ± SEM; number of measurements = 10.

The amount of oxygen in the tanks as well as temperature and pH were measured every 24 hours with a handheld multi-meter (WTW Multi 340i/SET, Germany).

At the end of each test water samples were taken from the tanks, acidified with nitric acid, and total amount of nickel was measured with an atomic absorption spectrophotometer (SHIMADZU AA-6800, Japan), either with the flame (Detection lower limit ≤ 0.006 ppm Cu) or graphite furnace (Detection lower limit ≤ 0.03 ppb Mn) techniques using proprietary software. Each sample was analyzed 3 times. Mean measured concentrations were within 5% of target (Table 3).

Median-Lethal-Concentration (LC50) values and their 95% confidence intervals were estimated using the trimmed Spearman-Karber method [10].

Results and Discussion

The data obtained (Table 4) showed that the calculated 96-hour LC50 values of the five fish species tested vary within the range of 19.3-61.2 mg Ni/l. No sharp boundary between lethal and non-lethal nickel concentrations has been found. The lowest 96-hour LC50 was found for rainbow trout and the highest one for dace. The 95% confidence intervals of 96-hour LC50 for perch and for roach were close and overlapped. Rainbow trout was from 1.75 to 3.17 times more sensitive to nickel than the other test species based on 96-hour LC50 data.

According to sensitivity to nickel, the species tested may be arranged in the following sensitivity order: rainbow trout > three-spined stickleback > perch = roach > dace.

Exposure duration evidently influenced the value of LC50 only in rainbow trout. The greatest differences were observed between 48-hour and 96-hour values and amounted to 2.4, while in other species this ratio ranged from 1.2 in perch to 1.4 in three-spined stickleback. 72-hour and 96-hour values were quite close. Their ratio varied within a very small range, from 1.1 to 1.2, while this ratio in rainbow trout was highest at 1.5.

The 96-hour LC50 reported here are similar to previously published data on other fish species. US EPA [3] reported Species Mean Acute Value (SMAV) of nickel to fish ranging from 12.2 mg/l for American eel (*Anguilla rostrata*) to 43.3 mg/l for banded killifish (*Fundulus diaphaus*), and of 13.4 mg/l for rainbow trout at the estimated water hardness correction of 50 mg/l as $CaCO_3$. Rainbow trout in this study were shown to be less sensitive than those in previous studies. For example, Nebeker et al. [11] reported a 96-hour LC50 of 10.0 mg Ni/l for larval rainbow trout in water with a hardness of 33.0 mg/l as $CaCO_3$. In contrast, Brix et al. [12] recently reported a 96-hour LC50 value of 20.8 mg Ni/l for juvenile rainbow trout in water with a hardness of 91.0 mg/l as $CaCO_3$ and dissolved organic carbon of 0.8 mg/l.

Table 4. Calculated LC50 as total nickel values for fish species tested in toxicity tests.

Exposure duration (hours)	LC50 (mg Ni/l)	95% confidence interval (mg Ni/l)
Rainbow trout		
24	> 56	–
48	46.4	30.3 – 71.1
72	28.0	24.6 – 31.8
96	19.3	15.0 – 24.9
Three-spined stickleback		
24	> 75	–
48	45.7	42.3 – 49.5
72	37.7	35.3 – 40.3
96	33.7	31.3 – 36.3
Perch		
24	64.8	59.8 – 70.2
48	56.2	51.8 – 70.0
72	52.4	47.7 – 57.5
96	48.1	43.4 – 53.2
Roach		
24	> 100	–
48	67.6	60.7 – 75.3
72	56.1	50.5 – 62.4
96	48.7	43.3 – 54.9
Dace		
24	> 100	–
48	77.2	71.6 – 83.1
72	68.7	63.2 – 74.7
96	61.2	56.1 – 66.8

The mechanism of the toxic effect of nickel is partly understood. Eisler [4] summarized data on the toxic mode of action of nickel and concluded that toxic and carcinogenic effects of nickel compounds are associated with nickel-mediated oxidative damage to DNA and proteins and to inhibition of cellular antioxidant defenses. Signs of nickel poisoning in fishes include surfacing, rapid mouth and opercular movements and, prior to death, convulsions and loss of equilibrium. Destruction of the gill lamellae by ionic nickel decreases the ventilation rate and may cause blood hypoxia and death. Other signs of nickel poisoning in fishes include decreased concentrations of glycogen in muscle and liver with simultaneous increases in levels of lactic acid and glucose in blood, depressed hydrogen peroxide production in tissues and a reduction in superoxide dismutase, and contractions of vascular smooth muscle signs similar to those associated with hypertension in mammals. Recent investigations confirmed that toxic effect of nickel is related to effects on respiration rather than ionoregulatory disruption [13, 12].

In a previous study perch demonstrated the same sensitivity to hexavalent chromium as rainbow trout [14]. It was suggested that perch is a promising species for which further research into its sensitivity to pollutants is needed, as rainbow trout could be replaced by it in standard toxicity tests. In this study rainbow trout sensitivity to nickel was highest and significantly differed from other test species, while perch dropped to the third-fourth place of the sensitivity order.

Different tolerances may be due to physiologic differences and species-specific effects in salmonids. This once more confirms the suggestion about how important it is to use species with generally different susceptibilities or metabolic activities in toxicity tests simultaneously in order to get information on pollutant-selective toxicity, the range of pollutant toxicity, and, finally, to evaluate the hazard of pollutant to environment.

In this study only fish mortality as a biological endpoint of interest was investigated. Those are basic data which could be useful in further studies into nickel sublethal effects to fish or in determining or revising water-quality guidelines for the protection of aquatic biota, as well as in solving practical problems of aquatic toxicology, e. g. in choosing the most suitable test-objects for bioassay testing of waters polluted with nickel and other heavy metals.

References

1. MOORE J.W. Inorganic contaminants of surface water: research and monitoring priorities. Springer-Verlag, New York, Heidelberg, Berlin, **1991**.
2. US EPA. Ambient water quality criteria for nickel. EPA-440/5-80-060. Office of Water Regulations and Standards. Washington, DC, **1980**.
3. US EPA. Ambient water quality criteria for nickel – 1986. EPA-440/5-86-004. Office of Water Regulations and Standards. Washington, DC, **1986**.
4. EISLER R. Nickel hazards to fish, wildlife and invertebrates: a synoptic review. Biological Science Report USGS/BRD/BSR-1998-0001, Laurel, MD, **1998**.
5. SCORECARD. Water quality indicators. http://www.scorecard.org. **2005**.
6. VITTOZI L., DE ANGELIS G. A critical review of comparative acute toxicity data on freshwater fish. Aquat. Toxicol. **19**, 167, **1991**.
7. PYLE G.G., SWANSON S.M., LEHMKUHL D.M. The influence of water hardness, pH, and suspended solids on nickel toxicity to larval fathead minnows (*Pimephales promelas*). Water, Air, and Soil Pollution. **133**, 215, **2002**.
8. HOANG T.C., TOMASSO J.R., KLAINE S.J. Influence of water quality and age on nickel toxicity to fathead minnows (*Pimephales promelas*). Environ. Toxicol. Chem. **23**, 86, **2004**.
9. MOUNT D.I., BRUNGS W.A. A simplified dosing apparatus for fish toxicity studies. Water Res. **1**, 21, **1967**.
10. HAMILTON M.A., RUSSO R.C., THURSTON R.V. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ Sci Technol **11**, 714, **1977**.
11. NEBEKER A.V., SAVONEN C., STEVENS D.G. Sensitivity of rainbow trout early life stages to nickel chloride. Environ. Toxicol. Chem. **4**, 233, **1985**.
12. BRIX K.V., KEITHLY J., DEFOREST D.K., LAUGHLIN J. Acute and chronic toxicity of nickel to rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. **23**, 2221, **2004**.
13. PANE E.F., RICHARDS J.G., WOOD C.M. Acute waterborne nickel toxicity in the rainbow trout (*Oncorhynchus mykiss*) occurs by a respiratory rather than ionoregulatory mechanism. Aquat. Toxicol. **63**, 65, **2003**.
14. SVECEVIČIUS G. Acute toxicity of hexavalent chromium to European freshwater fish. Bull. Environ. Contam. Toxicol. **77**, 741, **2006**.