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Nitrite toxicity assessment in *Danio rerio* and *Poecilia reticulata*

Petra Doleželová, Stanislava Mácová, Vladimíra Pištěková, Zdeňka Svobodová, Iveta Bedáňová, Eva Voslářová

Department of Veterinary Public Health and Toxicology, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic

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Abstract

Nitrite is a natural component of the nitrogen cycle in the environment. Although it usually occurs in low concentrations, elevated concentrations caused by effluents or affected nitrification process can lead to serious health deterioration of fish. Two aquarium fish zebrafish (*Danio rerio)* and guppy (*Poecilia reticulata)* are recommended to use as model organisms in toxicity tests. However, their sensitivity to nitrite can differ. The aim of this study was to define acute toxicity of nitrite by the semistatic method according to OECD No. 203 (Fish, Acute toxicity test). The series of 4 acute toxicity tests was performed, with 10 fish of both species used for each concentration and for the control. The 96hLC50 NO_2^- value for *D. rerio* and *P. reticulata* was 242.55 ± 15.79 $mg \cdot l^{-1}$ and 30.2 ± 8.74 $mg \cdot l^{-1}$, respectively. We have proved significant difference ($p < 0.05$) in sensitivity between *D. rerio* and *P. reticulata.* The results showed different sensitivities to nitrites in tested fish species, which could be related to species-specific branchial chloride uptake mechanism. This is the first study on this fish species.

Acute toxicity, NO_2^-, comparison of the sensitivity, zebrafish, guppy

Nitrite occurs naturally both in freshwater and saline waters, whereas the concentrations of nitrites up to tenths of $mg \cdot l^{-1}$ have been measured in surface waters in the Czech Republic (Kroupová et al. 2008). Elevated concentrations of nitrite can be found in closed intensive systems where water-recirculating is used (Svobodová et al. 2005). Nitrite is an intermediate product in bacterial nitrification and denitrification processes in natural nitrogen cycle (Pitter 1999). Nitrification is aerobic oxidation of ammonia to nitrite (by *Nitrosomonas* sp.) and of nitrite to nitrate (by *Nitrobacter* sp.). Denitrification is reduction of nitrates to the final nitrogen by a number of facultative anaerobic bacteria (Jensen 2003). This natural process is imitated on biological filters of the water-recirculating systems. However, if the nitrification on these filters is disturbed and the nitrification is retarded, the nitrites are accumulated in the water (Svobodová et al. 2005).

Nitrites are considered to be toxic to fish after acute (Pištěková et al. 2005; Weirich and Riche 2006; Park et al. 2007; Rodrigues et al. 2007; Costa et al. 2008; Ozcan et al. 2010) as well as chronic exposure (Siikavuopio and Saether 2006; Kroupová et al. 2008; Voslářová et al. 2008; Kroupová et al. 2010). The toxicity of nitrites for fish differs in dependence of many internal and external factors such as water quality, fish species, their size and individual sensitivity (Lewis and Morris 1986; Jensen 2003; Voslářová et al. 2006). The most important factor influencing nitrite toxicity is the concentration of chloride ions in water. Many authors proved in their studies the protective impact of chloride ions against intoxication of nitrites (Crawford and Allen 1977; Lewis and Morris 1986; Pištěková et al. 2005; Voslářová et al. 2006; Wang et al. 2006; Weirich and Riche 2006).

Nitrite is actively taken up across the chloride cells on gills. Nitrite in the blood is bound to haemoglobin which is converted to methaemoglobin and the oxygen-carrying capacity of blood is impaired. The increased amount of methaemoglobin in blood is accompanied with brown coloured blood and gills (Cameron 1971).

Address for correspondence:
MVDr. Petra Doleželová, Ph.D.
University of Veterinary and Pharmaceutical Sciences
Palackého 1-3, 612 42 Brno
Czech Republic

Phone: + 420 541 562 789
Fax: +420 541 562 790
E-mail: dolezelovap@vfu.cz
http://www.vfu.cz/acta-vet/actavet.htm

Toxicity tests are often used to determine a possible toxic effect of chemical substances on aquatic organisms. Particularly short-term toxicity tests on fish are suitable for evaluation of new chemical substances, effluents or wastes intended for disposal. The procedure of such tests is established by the Organization for Economical Cooperation and Development (OECD) which also specified recommended organisms used in toxicity tests. The recommended organisms include two aquarium fish species – zebrafish (*Danio rerio*) and guppy (*Poecilia reticulata*).

The aim of this study is to determine the possible difference in sensitivity of these two fish species to nitrites.

Materials and Methods

Acute toxicity tests were carried out on juvenile aquarium fish zebrafish and guppy. Both juvenile zebrafish (aged 2 months, length 30 ± 5 mm, weight 0.3 ± 0.1 g) and juvenile guppies (aged 2 months, length 25 ± 5 mm, weight 0.3 ± 0.1 g) were obtained from a stable local commercial dealer. A total number of 210 fish of each species were used. All fish were kept in glass tanks for a minimum of 7 days for the acclimatization period (water temperature 23 ± 1 °C, with a 12 h/12 h light/dark cycle) during which they were fed commercial fish pellets. Food was withheld 24 h before the start of the test. Experimental procedures were in compliance with the national legislation (Act No. 246/1992 Coll. on the protection of animals against cruelty, as amended by Decree No. 207/2004 Coll. on the protection, breeding and use of experimental animals).

The method followed OECD 203 Guideline (Fish, Acute toxicity test) in the semi-static condition with solution replacement after 24 h to ensure stable concentration of nitrites in tested solutions. Five progressive concentrations of the tested substance were prepared in 5 l tanks by dissolution of nitrite (dosed as $NaNO_2$) in dilution water of the following quality: $ANC_{4.5}$ 1.0–1.2 mmol·l^{-1}; COD_{Mn} 0.8–1.2 mg·l^{-1}; total ammonia below the limit of determination (< 0.04 mg·l^{-1}); NO_3^- 11.2–13.5 mg·l^{-1}; NO_2^- below the limit of determination (< 0.02 mg·l^{-1}); Cl^- 18.5-19.1 mg·l^{-1}; Σ Ca + Mg 14 mg·l^{-1}.

A series of four acute toxicity tests with five ascending concentrations of the tested substance separately for *P. reticulata* (10, 20, 30, 40, and 50 mg·l^{-1}) and for *D. rerio* (70, 100, 200, 300 and 400 mg·l^{-1}) were used. Concurrently, the control test with only dilution water was performed. Ten fish of each species randomly picked from the spare stock were placed into each concentration and control. The total length of acute toxicity tests was 96 h and fish were daily inspected for mortality. During the tests, the temperature, pH, the concentration of oxygen dissolved in test and control tanks and fish mortality rate were recorded. No fish died in the control tank during the experiments.

Temperature of the tested solutions was 24 ± 1 °C, the concentration of dissolved oxygen did not drop below 80–94% and pH was within the range 7.89–8.62.

Using the number of fish dying at individual test concentrations in the time period of 96 h, the medium lethal concentrations (96h LC50) were calculated by applying the probit analysis of the EKO-TOX 5.2 software. The significance of the difference between LC50 values for zebrafish and guppies was calculated using the non-parametric Mann-Whitney test and the Unistat 5.1 software.

Results

On the basis of the results of acute toxicity tests on juvenile *D. rerio*, the lethal concentrations of nitrite in 96 h varied from 223.5 mg·l^{-1} to 256.6 mg·l^{-1} (mean ± SD: 242.6 ± 15.79 mg·l^{-1}), whereas in juvenile *P. reticulata* the lethal concentrations varied from 21.9 mg·l^{-1} to 39.9 mg·l^{-1} (mean ± SD: 30.2 ± 8.74 mg·l^{-1}). In both experiments, the chloride concentration was about 19 mg·l^{-1}. The particular lethal concentrations of nitrite with confidence intervals for each lethal concentration and with mean and standard deviation for zebrafish are shown in the Table 1 and for guppy in Table 2.

Significant difference ($p < 0.05$) between 96hLC50 values for *D. rerio* and *P. reticulata* was recognized.

Discussion

Acute toxicity tests on fish are important for the assessment of impact of some chemical substances on fish. Although nitrites are a natural part of the nitrogen cycle in nature, significant accumulation of nitrites (e.g. surface water pollution with waste water, increased density of fish in the stock or insufficient degradation of nitrites)

Table 1. Calculated 96hLC50 values of nitrites ($mg \cdot l^{-1}$) with 95% confidence intervals for *Danio rerio*.

Test no.	LC50 $mg \cdot l^{-1}$	95% confidence interval
1	235.7	177.2–282.6
2	256.6	170.6–359.1
3	223.5	130.4–361.6
4	254.4	106.3–407.4
Mean LC50	242.6	
Standard deviation	15.79	

Table 2. Calculated 96hLC50 values of nitrites ($mg \cdot l^{-1}$) with 95% confidence intervals for *Poecilia reticulata*.

Test no.	LC50 $mg \cdot l^{-1}$	95% confidence interval
1	21.9	19.2–38.8
2	23.8	20.8–41.9
3	39.9	22.3–47.4
4	35.2	28.6–59.2
Mean LC50	30.2	
Standard deviation	8.74	

usually lead in fish poisoning with signs of asphyxia.

Acute toxicity of nitrites differs according to fish species. In our study we determined the 96hLC50 of NO_2^- in aquarium fish *D. rerio* as 242.6 ± 15.79 $mg \cdot l^{-1}$ (mean ± SD), whereas in *P. reticulata* it was 30.2 ± 8.74 $mg \cdot l^{-1}$. Significant difference ($p < 0.05$) in the sensitivity of these two fish species was found. Many authors investigated the effects of nitrites on different fish species. Lewis and Morris (1982) reported significant differences in interspecies sensitivity of fish to nitrites. Comparing lethal concentrations at the comparable chloride concentration of 20 $mg \cdot l^{-1}$, the most sensitive fish was rainbow trout (*Oncorhynchus mykiss*) with the LC50 of 21.7 $mg \cdot l^{-1}$, whereas in common carp (*Cyprinus carpio*) the LC50 value was 207 $mg \cdot l^{-1}$, in fathead minnow (*Pimephales promelas)* 217 $mg \cdot l^{-1}$ and in bluegill (*Lepomis macrochirus)* 355 $mg \cdot l^{-1}$. Hence it could be concluded that guppy is similarly sensitive to nitrites as rainbow trout and zebrafish is as tolerant as cyprinids, especially carps and fathead minnow. Ozcan et al. (2010) tested acute toxicity in another cyprinid fish, transcaucasian barb (*Capoeta capoeta capoeta*) and genotoxic and histopathologic effects were determined simultaneously. The median lethal concentration of nitrite at 96 h of exposure was 122.8 $mg \cdot l^{-1}$, slightly lower than in previous cyprinid species, hence we can consider differences in tolerance to nitrite in one family.

The differences in tolerance to nitrite ions are connected to the mechanism of action of nitrites. Nitrites have an affinity for branchial Cl^- uptake mechanism and so fish with high branchial Cl^- uptake rates (e.g. trouts) are more sensitive to nitrite than species with low uptake rates (*Cyprinidae*). Other possible explanation includes the difference in chloride cell number or proliferation of chloride cells during nitrite exposure (Jensen 2003).

The high influence on nitrite toxicity is attributed to chloride concentration in water. Wang et al. (2006) investigated the effects of nitrite on tilapia (*Oreochromis niloticus*) at different chloride concentrations. According to his results, the 96hLC50 raised from 28.18 $mg \cdot l^{-1}$ at 35.0 $mg \cdot l^{-1}$ chloride level to 44.67 $mg \cdot l^{-1}$ at 70.0 $mg \cdot l^{-1}$ chloride level. The protective effect of chloride ions against nitrite toxicity was reported in *D. rerio* (Voslářová et al. 2006) or in chinook salmon (*Oncorhynchus tshawytscha*) (Crawford and Allen 1977). Svobodová et al. (2005) have suggested some measures that should be done to prevent nitrite poisoning in fish. Regarding aquaculture facilities, it is important to supply such facilities with fish gradually according to filter capacity and to monitor the indicators of the water. Both in aquaculture facilities and aquariums it is possible to raise chloride concentration when there is an increase of nitrogenous metabolites.

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