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Acute toxicity of lanthanum to fish *Danio rerio* and *Poecilia reticulata*

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Abstract

Lanthanides are widely used in industry as well as medicine, where serve as contrast agents in imaging methods and techniques. Due to these facts, lanthanides can become significant pollutants of living environment. In our work, we were focused in investigation of toxicity of lanthanum on juvenile stages of two fish species- *Danio rerio* and *Poecilia reticulata* and embryonic stages of *D. rerio*. The 96hLC50 values were 156.33 ± 5.59 mg.l^{-1} for juvenile *D. rerio* and 128.38 ± 5.29 mg.l^{-1} for juvenile *P. reticulata*. The 144hLC50 for embryonic stages of *D. rerio* was 152.98 ± 8.06 mg.l^{-1}. Results of toxicity tests indicate possible toxicity of lanthanum in the case of presence in aquatic environment.

Keywords: Zebrafish, guppy, embryonic stages, juvenile stages

Introduction

Lanthanides include group of elements from lanthanum to lutetium, which are widely used in industry. Lanthanides (III) ions have many biological properties, which are based especially on similarity with calcium ions. These properties include blocking of calcium channels, which results in inhibition of contraction of skeletal, smooth and cardiac muscles, replacing of calcium(II) ions is structure of many proteins, which means that these proteins can lose their functions, or, contrariwise, in some cases their function can be activated or increased. Possibility of interactions with nucleic acids was also demonstrated (Kohoutkova et al. 2009). Some sources indicate that lanthanides are non–toxic after oral application because of inability to cross bell biomembranes, but these results are contradictory to results of other works (Fricker et al. 2006). Lanthanides are used as contrast agents, but some complexes find utilization in therapy of cancer, because of their significant cytostatics properties (Kynast et al. 2004; Heffeter et al. 2004). Due to these facts, it is evident that lanthanides represent potential source of living environment contamination. Our work was focused on testing of toxicity of lanthanum(III) ions on juvenile stages of *Danio rerio* and *Poecilia reticulata* and embryonic stages of *D. rerio*.

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Materials and methods

Parameters of used tap water

The basic physical and chemical parameters of the dilution water used in toxicity tests on embryonic and juvenile stages were: $ANC_{4.5}$ (acid neutralization capacity) 3.6–3.7 mmol.l^{-1}; COD_{Mn} (chemical oxygen demand) 1.4–1.9 mg.l^{-1}; total ammonia below the limit of determination (< 0.04 mg.l^{-1}); NO_3^- 24.5–31.4 mg.l^{-1}; NO_2^- below the limit of determination (< 0.02 mg.l^{-1}); Cl^- 18.9–19.1 mg.l^{-1}; Σ Ca ± Mg 3.1 mmol.l^{-1}. Test solutions were made from a stock solution by using $LaCl_3$.

Acute toxicity tests

Acute toxicity tests on juvenile stages of zebrafish (*D. rerio*) and guppy (*P. reticulata*) (2–3 months old) were performed according to the OECD method No. 203 (Fish, acute toxicity test). Fish were acclimatized 72 hours before the tests in the tap dilution water under the standard conditions. Used concentrations of lanthanum were 75, 100, 125, 150, and 175 mg.l^{-1}. In each concentration and control were placed 10 fish which were randomly picked from the spare stock. Six series were made with *D. rerio* and 4 series with *P. reticulata*. The tests were made using a semi–static method with the solution replacement after 24 hours. During the tests, records of the water temperature, pH, the concentration of oxygen dissolved in test tanks and fish mortality were noted. Duration of each test was 96 hours. The temperature of the experimental bath was 23.0 ± 1.5 °C, pH was between 7–8, and dissolved oxygen concentration did not fall below 60%. No fish died in the control tanks during the experiments.

Embryo toxicity tests

Embryo toxicity tests were made according to the method OECD No. 212 (Fish, short–term toxicity test on embryo and sac–fry stages). The fertilized eggs of *D. rerio* were placed in Petri dish within 8 hours at the latest after fertilization. Five series, each with five concentrations of tested substance (100, 125, 150, 175, 200; 50, 100, 150, 200, 250 mg.l^{-1}) were used. Twenty embryos in Petri dish were tested at each concentration and in control. The semi–static method with the replacement of the tested solution after 24 hours was used. The tests were terminated after hatching of all individuals in the control Petri dish which was 144 h after the start of the test. Hatching and survival of embryos were recorded during the tests in 24 h intervals. Test bath temperatures were between 24.5 and 25.5°C. The mortality rate of the control embryos did not exceed 20%.

Results processing

The results of the toxicity tests (the number of dead individuals at particular test concentrations) were subjected to a probit analysis using an EKO–TOX 5.2 programme to determine the LC50 values of lanthanum. The statistical significance of the difference between LC50 values for the juvenile and the embryonic stages of *D. rerio* and the juvenile stages of *P. reticulata* was calculated using the non–parametric Mann–Whitney test and the Statistica v8.0 for Windows (StatSoft, USA).

Results and Discussion

We determined that lanthanum toxicity depends on used fish species; average LC50 after 96–hour exposition was 156.33 ± 5.59 mg.l^{-1} for *D. rerio* and 128.38 ± 5.29 mg.l^{-1} for *P. reticulata*. In the case of embryo toxicity tests on *D. rerio*, LC50 value 152.98 ± 8.06 mg.l^{-1}after 144–hour expositions was determined. The sensitivity of juvenile and embryonic stages of *D. rerio* was comparable. The highest sensitivity was found for juvenile stages of *P. reticulata* in comparison of juvenile stages of *D. rerio* (p<0.01) (Fig. 1).

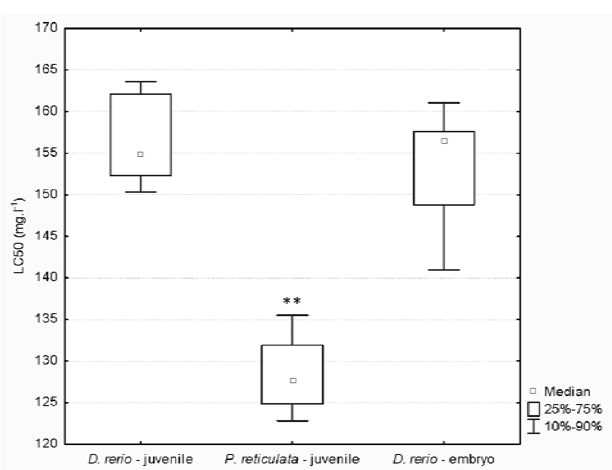

Figure 1: 96hLC50 values of juvenile stages of *D. rerio* and *P. reticulata* and 144hLC50 values of embryonic stages of *D. rerio*

Different results were presented by Zhang (2008) who stated 96hLC50 value 11.49 mg.l^{-1} for *Cyprinus carpio*. Chronic toxicity exposition (4 months) of lanthanides(III) ions of carp caused anomalies of erythrocytes (Zhang, 2008). Toxicity of lanthanides was demonstrated also for *Daphnia carinata* and *Artemia salina* (Barry and Meehan 2000; Cunqi 1998). Barry and Meehan (2000) assessed acute and chronic toxicity of lanthanum for *D. carinata* in three different media. The 48h EC50 to *Daphnia* was 43 µg.l^{-1} in soft tap water, 1180 µg.l^{-1} in ASTM hard water and 49 µg.l^{-1} in the daphnid growth medium, based on diluted sea water. The chronic toxicity was measured in the daphnid growth medium and ASTM medium. There was found 100% mortality at concentration ≥ 80 µg.l^{-1} by after six days of the experiment in daphnid growth medium. In ASTM media was observed significant mortality at concentrations ≥ 39 µg.l^{-1}. Lower acute toxicity (66.47 mg.l^{-1}) of $La(NO_3)_3 \cdot 6H_2O$ was observed for *A. salina* after 96 hours exposure (Cunqi 1998). These results indicate possible toxicity of lanthanum ions in the case of presence in aquatic ecosystem.

Conclusion

In our study, lanthanum demonstrated toxicity on two fish species- *D. rerio* and *P. reticulata* and two developmental stages of *D. rerio*. There are some important questions, which must be solved in future; these questions include especially mechanism of toxic action of lanthanum ions including histochemical evaluation of fish tissues and mechanism of transport of lanthanum ions through biomembranes.

Acknowledgement

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