Acute Toxicity of the Preparation PAX-18 for Juvenile and Embryonic Stages of Zebrafish (*Danio rerio*)

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Abstract

The preparation PAX-18 is a coagulation agent, which is used in water and wastewater treatment facilities and for the treatment of natural waters. The active compound is polyaluminium chloride (9% of Al). The application to the water environment could present a potential risk to different developmental stages of fish. The aim of this study was to assess and compare the toxicity of the preparation PAX-18 for embryonic and juvenile developmental stages of zebrafish (*Danio rerio*). The acute toxicity tests with juvenile fish aged 2–3 months were conducted according to the method OECD No. 203 in 5 series. For embryo toxicity test the method OECD No. 212 was used in 5 series. The semistatic methods were selected. The results of toxicity tests (the number of dead individuals at particular test concentrations) were subjected to probit analysis using the EKO-TOX 5.2 programme to determine LC50 values of PAX. The LC50 mean value of PAX for juvenile *D. rerio* was 749.7 ± 30.6 $mg \cdot l^{-1}$ (67.5 ± 2.8 $mg \cdot l^{-1}$ of Al) and 731.5 ± 94.1 $mg \cdot l^{-1}$ (65.8 ± 8.5 $mg \cdot l^{-1}$ of Al) for embryonic stages of *D. rerio*. The sensitivity of juvenile and embryonic stages to PAX were comparable ($p > 0.01$). The acute toxicity values of PAX-18 found in tests on *D. rerio* were 6–13 × higher than the concentration which is usually applied to waters (5–10 $mg \cdot l^{-1}$ of Al). Therefore, the acute toxicity effect on fish can be considered minimal.

Polyaluminium chloride, zebrafish, danio, developmental stages

The preparation PAX-18 is used as one of the possible methods in the restoration of eutrophic lakes. High loads of nutrients, mainly phosphorus, that enter surface waters, cause the eutrophication and intensive development of the phytoplankton with a negative effect on the water quality and species diversity (Holz and Hoagland 1999; Klouček and Vaverová 2005). The active compound of the preparation PAX-18 is polyaluminium chloride (9% of Al). Polyaluminium chloride is one of the most effective coagulant agents in water and wastewater treatment facilities and for the treatment of natural waters. It is used as an eliminator of colloids and suspended particles, organic matter, metal ions and phosphates (Pitter 2009; Zouboulis and Tzoupanos 2010).

Aluminium treatment was found to be effective in lowering phosphorus concentration in water. Aluminium binds phosphorus and forms complexes that permanently attach this element to the bottom sediments. Furthermore, during the sedimentation of these complexes, floccules remove plankton from the water column and the water transparency improves (Holz and Hoagland 1999; Klouček and Vaverová 2005). Direct application of PAX-18 into the water (usually at doses of 5–10 mg of Al per one litre) or to the sediments (in tens of grams per 1 m^2 of surface of the bottom sediments) is possible (Pitter 2009).

The application of PAX-18 to the natural waters could present a potential danger to fish and other water non target organisms due to the presence and accumulation of aluminium.

Aluminium is a ubiquitous element in natural waters, where it can be found in a number of chemical forms. The chemical form of aluminium is highly dependent on water pH and influences the toxicity of aluminium (Freeman and Everhart 1971; Baker and Schofield 1982; Cleveland et al. 1986; Buckler et al. 1995; Poléo et al. 1997; Pitter

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2009). The solubility of Al increases with the increasing acidity or alkalinity of the water (Driscoll and Postek 1996). In most of the natural waters, pH values range between 6 and 8 (Miller et al. 1984) and therefore the concentration of free aluminium ions in the natural water is extremely low ($c < 0.1$ mg·l^{-1}). For the safe application of aluminium coagulant to the surface waters it is necessary to keep the range of pH in the interval of 5.5–9 (Klouček and Vaverová 2005).

Toxicity of aluminium is affected beside pH by water temperature, chemical properties of water like amount of organic material such as humic acid and is dependent on fish species, developmental stages or size (Baker and Schofield 1982; Howells et al. 1990; Poléo 1991, 1995; Buckler et al. 1995). The organ most affected by Al contaminated water is gills (Dietrich and Schlatter 1989; Exley et al. 1996). Aluminium has two types of toxic effect on fish: 1) interference with the ion-regulatory system and 2) interference with the respiratory system, due to the changes on gill surface (Dietrich and Schlatter 1989; Gensemer and Playle 1999). Generally, salmonid fish are the most sensitive freshwater fish species to aqueous aluminium (Howells et al. 1990). The aquarium fish *Danio rerio*, which was chosen for the toxicity tests performed during our study, is often used for the assessment of the toxic effects of chemicals (Jurčíková et al. 2007; Voslářová et al. 2008; Mikula et al. 2009).

The preparation PAX-18 is a liquid of acidic character thus it could influence water pH after the application into the waters. In water with a naturally low acid-neutralizing capacity or water that is poorly buffered there is a higher risk of fish damage due to the decrease in the pH (Baker and Schofield 1982). PAX-18 should be applied only to selected water areas that match the requirements for its application, namely higher pH and alkalinity.

The aim of this study was to assess and compare acute toxicity of the preparation PAX-18 for embryonic and juvenile developmental stages of zebrafish (*Danio rerio*).

Materials and Methods

PAX-18

PAX-18 is a yellowish liquid of acidic character, freely miscible with water. Its active compound is polyaluminium chloride $Al_n(OH)_mCl_{3n-m}$ in water solution. The content of Al is 9.0 ± 0.3%, pH 1.0 ± 0.5. The preparation was obtained from Kemwater ProChemie (Czech Republic).

Acute toxicity tests

Acute toxicity tests on juvenile stages of *D. rerio* (2–3 months old) were performed according to the OECD method No. 203 (Fish, acute toxicity test). Fish were acclimatized for 72 h before the tests in the tap dilution water under the standard conditions. Five series of 5 ascending concentrations of PAX-18 (700, 750, 800, 850, 900 mg·l^{-1}) were used. Ten fish randomly picked from the spare stock were placed into each concentration and control. The tests were made using a semi-static method with the solution replacement after 48 h. During the tests, records of water temperature, pH, the concentration of oxygen dissolved in test tanks and fish mortality were noted. Duration of each test was 96 h.

The temperature of the experimental bath was 23.5 ± 1.5 °C, pH was between 7.5 and 8.5 in control, but lower in testing concentrations. The pH decrease depended on increasing concentration of PAX-18, in the highest concentration was pH 4.5. Dissolved oxygen concentration did not fall below 60% (70-80%). 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 h at the latest after fertilization. Five series, each with 4 concentrations of the tested substance (200, 600, 1000, 1400 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 h was used. The tests were terminated after the hatching of all individuals in the control Petri dish which was 120 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, pH in control tank and tested concentration tanks was between 7.5–8.5 and 4.5–7.4, respectively. The mortality rate of the control embryos did not exceed 20%.

Diluting water

The basic physical and chemical properties 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 14 mg·l^{-1}.

Statistical analysis

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 PAX-18. The statistical significance of the difference between LC50 values for the juvenile and the embryonic stages of *D. rerio* was calculated using the non-parametric Mann-Whitney test and the Unistat 5.1 software.

Results

The lethal concentration of PAX-18 for the juvenile stages of zebrafish expressed as the 96hLC50 value was determined in range 727.3–783.2 mg·l^{-1} (mean 96hLC50 = 749.7 ± 30.6 mg·l^{-1}). LC50 value for the embryonic life stage of *D. rerio* expressed as the 120hLC50 was determined to be between 645.0 and 889.1 mg·l^{-1} (mean 120hLC50 = 731.5 ± 94.1 mg·l^{-1}). The mean cumulative mortality of *D. rerio* in five embryo toxicity test (i.e. 100 eggs for each concentration) is presented in Fig. 1. No significant difference was found between the sensitivity of juvenile and embryonic developmental stages ($p > 0.01$). The LC50 values expressed for aluminium content were 67.5 ± 2.8 mg·l^{-1} of Al for the juvenile and 65.8 ± 8.5 mg·l^{-1} of Al for the embryonic developmental stage.

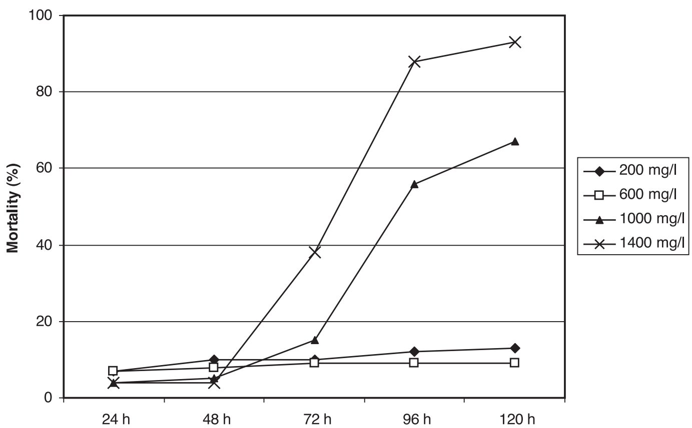

Fig. 1. Mean cumulative mortality of *D. rerio* in five embryo toxicity test

Discussion

A considerable decrease of pH due to the acidic character of the preparation PAX-18 was noted during the test. The pH was considerably lowered (4.5–5.2) especially at the highest concentration tested (900 mg·l^{-1}). The optimum pH for fish is in the range 6.5–8.5. For cyprinid fish, pH lower than 5.0 is considered to be lethal (Svobodová et al. 2007). The lethal effects of PAX-18 at the tested concentrations were probably the result of a combinatory effect of the low pH and elevated concentration of aluminium. Many studies proved that Al potentiates the effects of acidic pH on fish (Baker and Schofield 1982; Cleveland et al. 1986; Buckler et al. 1995; Poléo et al. 1997).

In embryo toxicity tests on *D. rerio*, pH of the tested solution was also lowered in dependence on PAX-18 concentration. The pH at the highest concentration (1800 mg·l^{-1}) was approximately 4.5. Based on the results of our previous unpublished embryo-toxicity tests on *D. rerio* with lower pH it seems that embryonic life stages of this species are not very sensitive to acidic conditions. The pH 4 did not affect the survival and hatching of embryos during embryo-toxicity test (according OECD 212 methodology). Dave (1984) studied the influence of pH 4 to 9 on the hatching of *D. rerio* with or without the presence of aluminium. Hatching was stimulated when pH was lowered from 7 to 6 but further lowering of pH retarded hatching (in absence of Al). Aluminium was more toxic at high pH levels. Oyen et al. (1991) observed higher mortality of common carp eggs and a delay in the rate of embryonic development and hatching in the pH range between 4.75 and 5.2. Buckler et al. (1995) exposed eggs of Atlantic salmon (*Salmo salar*) to pH ranging from 4.5 to 7.2 and did not find any adverse effect on the ability of eggs to hatch, but larvae that hatched in the pH 4.5 and 5 did not completely shed the chorionic membrane. They also stated that hatching in the presence of Al ranging from 33 to 264 $\mu g \cdot l^{-1}$ was not affected at pH 5.5. The decreased hatching success for Atlantic salmon eggs at pH 4.5 and 5.0 has been reported by Cleveland et al. (1986).

The LC50 values of PAX-18 which were evaluated during toxicity tests on juvenile and embryonic developmental stages of *D. rerio* are comparable with the results of acute toxicity tests obtained for juvenile common carp (*Cyprinus carpio*) (Mácová et al. 2009). Acute toxicity value for carp expressed as 96hLC50 was 753.1 ± 24.3 $mg \cdot l^{-1}$ (67.8 $mg \cdot l^{-1}$ Al). The toxic effect of aluminium on *D. rerio* was assessed by Anadhan and Hemaltha (2009). The acute toxicity of aluminium expressed as 96hLC50 was 56.92 $mg \cdot l^{-1}$; pH within the test was 6.8. This value is comparable with our results. Poléo et al. (1995) stated that the acute toxicity of aluminium seems to be highest when pH is between 5.0 and 6.0. The relative sensitivity in seven freshwater fish species to the acute aluminium toxicity was documented by Poléo et al. (1997). The most sensitive species were *Salmo salar* followed by *Rutilus rutilus*, intermediate sensitivity was detected in *Phoxinus phoxinus*, *Perca fluviatilis*, *Thymallus thymallus* and *Salmo trutta* and a species highly tolerant was *Salvelinus alpinus*. Fish were exposed to the water with 402 ± 6 $\mu g \cdot l^{-1}$ of Al at pH 4.97–5.32. *S. salar, R. rutilus* and *P. phoxinus* showed almost 100% mortality (LT50 was 85; 97 and 331 h), only *S. alpinus* showed lower mortality than 50%.

It is known that Al-sensitivity can differ not only among fish species but also among life developmental stages (Howells et al. 1990). Poléo et al. (1997) observed higher sensitivity for larger mature fish (> 1+) *Rutilus rutilus* in comparison to smaller fry (0+). However, these experiments were conducted at a slightly different water temperature, which influences Al toxicity in fish (Poléo et al. 1991). In the present study, the sensitivity of juvenile and embryonic developmental stages of *D. rerio* to the toxicity of polyaluminium chloride was comparable.

The application of PAX-18 to eutrophic waters at commonly used concentrations (5-10 $mg \cdot l^{-1}$ Al) is safe according to the results of the presented acute toxicity tests on *D. rerio*. This concentration is 6–13 times lower compared to the lethal concentration of PAX-18 found for juvenile and embryonic developmental stage of *D. rerio*. Therefore, the acute toxicity effect of PAX-18 on fish during the cyanobacteria removal can be considered as minimal. Furthermore, the safety of the preparation is potentiated by the fact that the concentration of active compound drops rapidly after the application to the natural water because of its reactions with phosphorus etc. and fish are exposed to the applied dose only for a short time period.

Akutní toxicita přípravku PAX-18 pro juvenilní a embryonální stádium ryb *Danio rerio*

Cílem práce bylo zhodnotit a porovnat toxicitu přípravku PAX-18 pro embryonální a juvenilní stádia ryb danio pruhované (*Danio rerio*). PAX-18 je koagulační činidlo používané pro úpravu vod, v čističkách odpadních vod i přímo aplikované do vodního prostředí. Účinnou látkou přípravku je polyaluminium chlorid (9 % Al). Akutní testy

toxicity na juvenilních rybách ve věku 2–3 měsíců byly prováděny v pěti sériích dle metodiky OECD 203 a embryonální testy toxicity v pěti sériích dle metodiky OECD 212. Byla použita semistatická metoda. Výsledky testů toxicity byly vyhodnoceny pomocí programu EKO-TOX 5.2, kde byla stanovena hodnota LC50. Zjištěná hodnota LC50 pro juvenilní ryby *D. rerio* byla 749,7 ± 30,6 $mg \cdot l^{-1}$ (67,5 ± 2,8 $mg \cdot l^{-1}$ of Al) a pro embryonální stádium 731,5 ± 94,1 $mg \cdot l^{-1}$ (65,8 ± 8,5 $mg \cdot l^{-1}$ of Al). Citlivost juvenilního a embryonálního stádia byla srovnatelná ($p > 0,01$). Hodnoty akutní toxicity zjištěné v těchto testech byly 6–13 krát vyšší než koncentrace obvykle aplikované do vody (5–10 $mg \cdot l^{-1}$ Al), proto lze považovat akutní toxický účinek aplikovaného přípravku za minimální.

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