ORIGINAL ARTICLE

Polyaluminium chloride (PAX-18) - acute toxicity and toxicity for early development stages of common carp *(Cyprinus carpio)*

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Abstract

OBJECTIVES: The aim of this study is to assess the toxicity of PAX-18 in different developmental stages of common carp (*Cyprinus carpio*). The preparation PAX-18, with its active ingredient polyaluminium chloride (9% of Al), is a coagulation agent that is used mainly to precipitate phosphates, to prevent surface water eutrophication and incidences of cyanobacteria. It is applied to the water environment and thus could present a potential risk to fish.

DESIGN: The toxicity tests were performed on common carp according to OECD (203, 210) methodologies. The acute toxic effect was evaluated for juveniles and the early development stage effect was observed in embryo-larval toxicity tests. 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.

RESULTS: Acute toxicity value expressed as 96hLC50 was 753.1 ± 24.3 $mg.l^{-1}$ (67.8 $mg.l^{-1}$ Al). Effect on early development stage expressed as the no observed effect concentration was 10 $mg.l^{-1}$ (0.9 $mg.l^{-1}$ Al), the lowest observed effect concentration was 50 $mg.l^{-1}$ (4.5 $mg.l^{-1}$ Al). No significant effects of the preparation PAX-18 in concentrations of 50 $mg.l^{-1}$ of PAX-18 and lower were found on hatching, length and weight parameters, morphology and histopathology.

CONCLUSION: The lethal concentration of PAX-18 found in acute toxicity tests on common carp was 7–14 times higher than the concentration which is usually applied to water (5–10 $mg.l^{-1}$ Al). Moreover, fish in eutrophicated water sources are exposed to PAX-18 concentrations corresponding with the lowest observed effect concentration only for a short time, therefore the effect on them can be considered as minimal.

Abbreviations & units:
NOEC – no observed effect concentration
LOEC – lowest observed effect concentration
LC – lethal concentration
ANOVA – analysis of variance
$ANC_{4.5}$ – acid neutralization capacity
COD_{Mn} – chemical oxygen demand
FWC – Fulton's weight condition factor

INTRODUCTION

Polyaluminium chloride, which is the active compound of the preparation PAX-18, is used as a coagulant for the precipitation of phosphorus in water treatment and wastewater treatment plants and also for the treatment of natural waters (Pitter, 2009). In natural waters the use of PAX-18 is one of the possible methods in the restoration of eutrophic lakes. Due to intensive industry and agriculture, high loads of nutrients, mainly phosphorus, enter surface waters and cause the eutrophication. High level of phosphorus causes an intensive development of phytoplankton having a negative effect on water quality and species diversity, i.e. high algal bloom, incidences of toxic cyanobacteria, bad odour, loss of water transparency, low oxygen content and fish mortality (Falconer, 2005; Holz & Hoagland, 1999; Kloucek & Vaverova, 2005). Aluminium treatment was found to be effective in lowering phosphorus in waters and in reducing the release of this element from the sediments (Wauer *et al.* 2004). It is possible to apply PAX-18 directly into the water (usually in a dose of 5–10 mg Al per one litre) or to the sediment (in tens of gram per 1 m^2) of surface of the bottom sediments) (Pitter, 2009). Every application of PAX-18 has to be performed according to the valid legislation. After the application of PAX-18, the phosphorus binds with the aluminium and forms complexes and these are then permanently attached to the bottom sediment. During the sedimentation, floccules remove plankton from the water column, which then sinks to the bottom, and the water transparency improves temporarily (Holz & Hoagland, 1999; Kloucek & Vaverova, 2005; Wauer *et al.* 2004).

One possible problem in the water environment could be the presence and the accumulation of aluminium. Aluminium represents the third most prevalent metal (8%) of the earth's crust. Aluminium occurs ubiquitously in natural waters (Pitter, 2009). Occurrence of aluminium forms found in natural waters is highly dependent on pH. Aluminium is relatively insoluble at pH 6 to 8. The toxicity of aluminium is highly dependent on water pH (Baker & Schofield, 1982; Buckler *et al.* 1995; Cleveland *et al.* 1986; Freeman & Everhart, 1971; Hyne & Wilson, 1997; Poleo *et al.* 1997). The solubility of Al increases under more acidic and more alkaline conditions (Driscoll & Postek, 1996). Al^{3+} ions prevail in acidic environment (pH < 4.5), anion hydrocomplexes prevail at pH value over 8.5 (Pitter, 2009; Svobodova *et al.* 2007).

In most of the natural waters, pH values range between 6 and 8 (Miller *et al.* 1984) and therefore the concentration of the aluminium ions in the natural water is extremely low ($c < 0.1 mg.l^{-1}$). Aluminium is responsible for fish mortality in acidified water (Poleo *et al.* 1997). For the safe application of aluminium coagulants to surface water, it is necessary for the pH to be within a range of 5.5–9.

Aluminium has two types of toxic effect on fish: 1) interference with the ion-regulatory system, involving the concentration of plasma chlorides (Dietrich & Schlatter, 1989; Gensemer & Playle, 1999) and 2) interference with the respiratory system, due to the changes on gill surface (Dietrich & Schlatter, 1989; Gensemer & Playle, 1999). Ion-regulatory effects are commonly explained as the same mechanism as H^+ toxicity, it is, the displacement of Ca from tight junctions of the gill membrane and interference with gill Na/K-ATPase (Gensemer & Playle, 1999). The cause of respiratory malfunction is the precipitation or polymerization of Al dissolved in acidic water in the more basic gill microenvironment (Gensemer & Playle, 1999; Poleo, 1995). The ion-regulatory effects are dominant in water with low pH, and respiratory effects prevail in only moderately acidic pH. Aluminium content in the diet has no significant adverse effects to fish (Poston, 1991).

The aim of this study was to evaluate the acute toxicity concentration of PAX-18 with its active compound polyaluminium chloride, and its toxicity for early development stages of common carp and to compare these concentrations with the concentrations of PAX-18 applied to natural water. Common carp is the most often bred fish in the Czech Republic. That is why it is often used for the toxicity tests of chemicals and for the contamination monitoring of surface waters (Modra *et al.* 2008; Dobsikova *et al.* 2006).

MATERIAL AND METHODS

PAX-18. PAX-18 is a yellowish liquid with acidic characteristics, and is freely miscible with water. Its active compound is polyaluminium chloride $Al_n(OH)_mCl_{3n-m}$ in a water solution. The Al content is 9.0 ± 0.3%, pH 1.0 ± 0.5. It is produced by Kemwater ProChemie.

Diluting water. The basic physical and chemical parameters of diluting tap water used in toxicity tests were: $ANC_{4.5}$ (acid neutralization capacity) 1.0–1.2 $mmol.l^{-1}$; COD_{Mn} (chemical oxygen demand) 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^- 10.2–12.5 $mg.l^{-1}$; Σ Ca ± Mg 14 $mg.l^{-1}$.

Acute toxicity tests. Acute toxicity tests on carp were conducted according to OECD methodology No. 203 (Fish Acute Toxicity Test). For the tests juvenile fish age 2–3 months with a mean weight of 7.6 ± 1.6 g and a mean body length of 68 ± 11 mm were used. Fish were acclimatized 72h before the tests under the standard

conditions in dilution water. Ten fish from the spare stock were then randomly placed into the aquarium with the testing solutions and also to the control aquarium with dilution water. A semi-static method with solution replacement after 48 hours was used in the tests. Three series of acute toxicity tests were made. In the first series concentrations of 300, 400, 500, 600, 700, 800, 900 mg.l^{-1} of PAX-18 were tested, and in next two series concentrations of 700, 740, 780, 820, 860 and 900 mg.l^{-1} of PAX-18 were used. The duration of each test was 96 hours. The results of the acute 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 temperature during all tests was 22.5 ± 1.5 °C; the pH in the control aquarium was between 7.5 and 8.5, in tested solutions 300, 400, 500, 600, 700, 800, 900 mg.l^{-1}; 700, 740, 780, 820, 860 and 900 mg.l^{-1} it reached lower values than in the control (mean pH 7.5; 7.4; 7.3; 7.0; 6.7; 6.1; 4.4 and 6.8; 6.5; 4.9; 4.8; 4.6; 4.5, respectively), the dissolved oxygen concentration did not fall below 60% and ranged between 70 and 90%. No fish died in the control tanks during the experiments.

Embryo-larval toxicity tests. These tests were carried out using a modified test according to the OECD guideline 210 (Fish, Early-life stage toxicity test). Fertilized eggs of common carp were obtained from the Breeding Station of the Department of Fish Genetics and Breeding of the Research Institute of Fish Culture and Hydrobiology in Vodnany, University of South Bohemia. Eggs were produced according to standard methods of artificial reproduction as described by Kocour *et al.* (2005).

The fertilized eggs were separated from those unfertilized 24h after fertilization and 100 eggs were randomly transferred into the each of the crystallization dishes containing tested solutions of PAX-18 and also into the control dish. Four ascending concentrations of tested solutions and control were used, each with 100 fertilized eggs in triplicate groups. The concentrations were marked as follows:

Group-1: 5 mg.l^{-1} of PAX-18 (0.45 mg.l^{-1} Al)
Group-2: 10 mg.l^{-1} of PAX-18 (0.9 mg.l^{-1} Al)
Group-3: 50 mg.l^{-1} of PAX-18 (4.5 mg.l^{-1} Al)
Group-4: 75 mg.l^{-1} of PAX-18 (6.75 mg.l^{-1} Al)
Control: PAX-18 free dilution water.

The semistatic method was used. The bath replacement was made twice a day. Observations of hatching and survival were made twice a day and dead embryos and larvae were collected. During the test, larvae were fed *ad libitum* twice a day prior to the bath exchange with freshly hatched *Artemia salina*. The temperature, pH and oxygen saturation were noted daily.

During the test, the temperature was in the range of 19.5–24.0 °C; pH was between 7.54–7.94 in the control group 7.44–7.97 in group-1, 7.57–7.92 in group-2, 7.52–7.81 in group-3, 4.9–5.2 in group-4, and the dissolved oxygen did not drop bellow 60%.

The beginning of the test is labelled as day 1 (one day after fertilization). Hatching was completely finished by day 6, and on day 7 the feeding with *A. salina* began.

The test was concluded after 33 days when the majority of the fish in the control basins reached the juvenile stage.

During the test, as well as at the end, embryos and larvae were sampled to monitor the stages of development; length, weight, FWC and morphological anomalies. Samples from each concentration and from the control group in number of 15 (5 fish from each replicate group) were collected at day 7 (before the feeding began), day 13 and day 19 of the experiment and in number of 30 (10 fish from each replicate group) at day 28 and day 33 of the experiment, i.e. at the end of the test. Samples were fixed in a 4% formalin solution.

The developmental stages were determined according to Penaz *et al.* (1983) who have described nine embryonic (E1-E9), six larval (L1-L6) and two juvenile (J1-J2) developmental stages of common carp. Total length (TL) was measured under a stereomicroscope, and using a micrometer to 0.01 mm, weight (W) was measured at 0.1 mg.

Fulton's weight condition factor was calculated for each experimental group at every sampling time.

$$FWC = \frac{W.10^5}{L^3} \quad \text{[W (g), L=TL (mm)]}$$

Statistical analysis. Results were analysed using the statistical package Unistat 5.1. (Unistat Ltd., GB). Data (after testing for normality and homogeneity of variances) were subjected to one-way ANOVA with the concentration of PAX-18 as a main effect and subsequently to the Student-Newman-Keuls test (Zar, 1999) for multiple comparisons in order to assess the statistical significance of differences between means in all possible pairs of groups (control, group-1, group-2, group-3).

Histopathological examination. Fish were fixed in a 10% neutral formalin solution and processed using conventional paraffin techniques. Tissue sections were stained with haematoxylin and eosin. Histological changes in samples of skin, gills and liver were examined by light microscopy.

RESULTS

Acute toxicity tests. The LC50 of PAX-18 for juvenile common carp was 775.4; 756.8 and 727.2 mg.l^{-1} (mean 96hLC50 = 753.1 ± 24.3 mg.l^{-1}).

Embryolarval toxicity tests.

Hatching. PAX-18 in concentrations of 5, 10 and 50 mg.l^{-1} did not affect the hatching as a whole. The hatching had started on day 4, and by day 5 the majority of

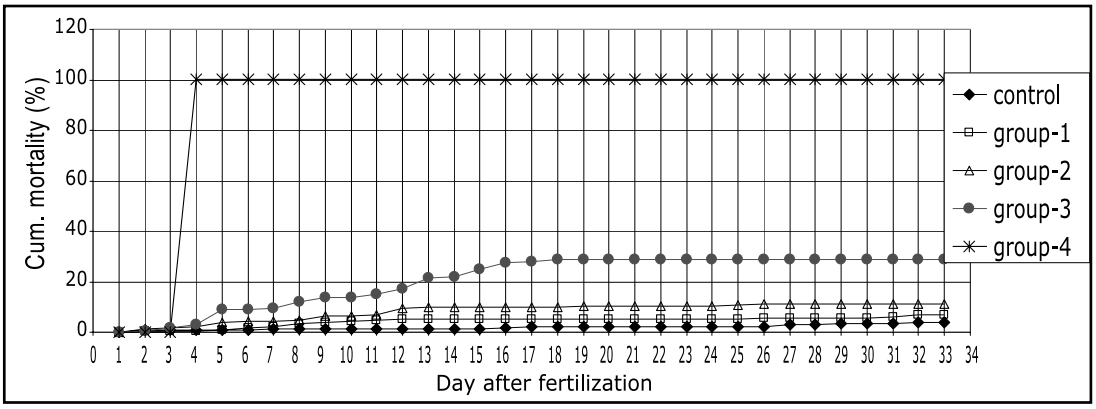

Figure 1. Cumulative mortality of common carp in embryolarval toxicity test.

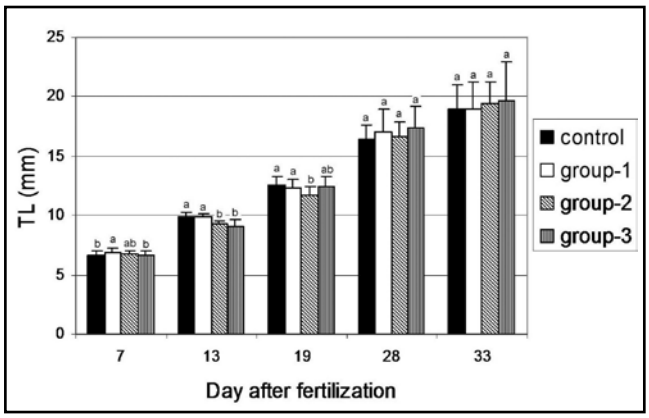

Figure 2. Length growth of common carp during the embryolarval toxicicty test (TL – total length).

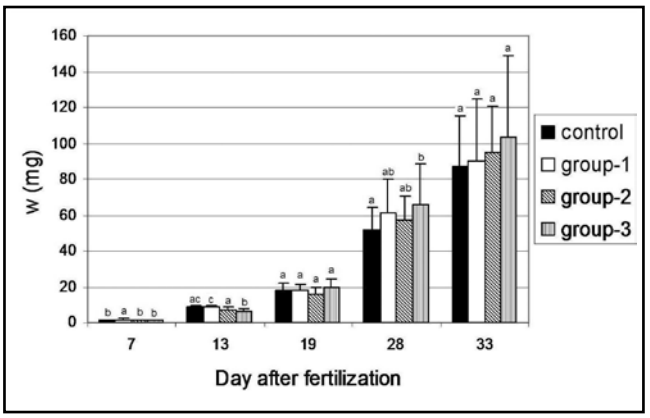

Figure 3. Weight growth of common carp during the embryolarval toxicity test.

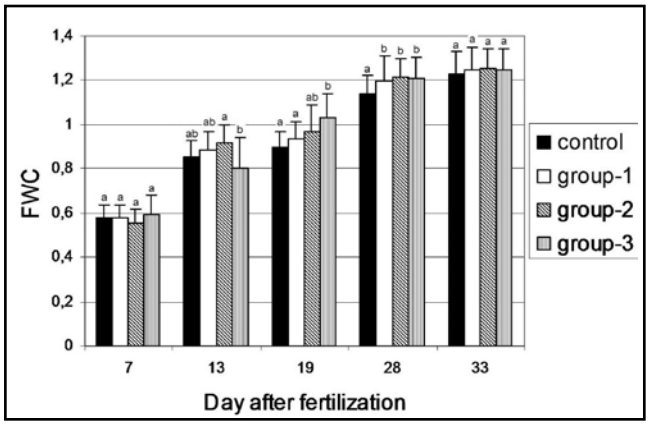

Figure 4. Fultons weight condition factor of common carp during the embryolarval toxicity test.

the embryos hatched. The hatching was completed by day 6 in all of the dishes. The highest tested concentration of PAX-18 caused the mortality of organisms before the hatching.

Mortality. The cumulative mortality of the common carp embryos and larvae during the test is depicted in ***Figure 1***. In the highest concentration (group-4), 100% mortality occurred on the 4th day of the test.

The cumulative mortality in the control group, group-1 and group-2 did not exceed 25% (3.7; 6.7; and 11.4%, respectively) which is the mortality of control organisms allowed for a valid test control according to the methodology OECD 210. The average cumulative mortality in group-3 was 29.0%, but the data varied significantly - in two of the triplicates of group-3 the mortality rate was below 25% (18.0 and 22.0%) while it was high in the last one (43%).

On the basis of cumulative mortality in all replications, NOEC (No Observed Effect Concentration) = 10 $mg.l^{-1}$ (0.9 $mg.l^{-1}$ Al) and LOEC (Lowest Observed Effect Concentration) = 50 $mg.l^{-1}$ (4.5 $mg.l^{-1}$ Al) were calculated.

Length and weight parameters. Length and weight parameters are shown in ***Figure 2 and 3***. In general, length and weight values among all groups were comparable during the experiment. Statistically significant differences ($p<0.05$) were found only in some cases. At the end of the test there were again found comparable values of length and weight in all experimental and control groups.

The changes of Fulton's weight condition factor during the embryo-larval toxicity test are displayed in ***Figure 4***. FWC values of all groups were comparable during the experiment. Statistically significant differences ($p<0.05$) were found only in some cases. By day

Table 1. Characterization of common carp ontogeny by using developmental stages during the embryolarval toxicity test

	Day	control	group-1	group-2	group-3
Developmental stages	7	E9	E9	E9	E9
	13	L4	L4	L4	L3-L4
	19	L4-L5	L5	L4-L5	L5
	28	L5-L6	L5-J1	L5-J1	L5-J1
	33	L6-J1	L5-J1	L5-J1	L5-J1

33 there were no statistically significant differences between FWC values.

Developmental stages. The developmental stages in each sampling time are listed in ***Table 1***. From the 28th day there were higher differences in fish developmental stages in experimental groups compared to the control group.

Morphological anomalies. Morphological anomalies were found only in fish within the experimental groups, and not in the control group. The total incidence of these anomalies was very low, i.e. 1.1%, which could be considered as a spontaneous appearance. However, on day 19, a dilatation of intestines was observed which evocated a moderate swelling of the abdominal wall in all individuals in the experimental group-3. These changes are not included in the percentage of morphological anomalies.

Histopathology. No histopathology anomalies were found on the skin, gills or livers in the experimental groups when compared to the control group.

DISCUSSION

Acute toxicity tests. The mean 96hLC50 value found in acute toxicity tests was 753.1 ± 24.3 mg.l^{-1}. The toxic effects of this preparation in acute toxicity tests on common carp were probably the result of an interaction between low pH (4.5 in the highest tested concentrations) and elevated aluminium concentrations. The optimum pH for fish is in the range from 6.5 to 8.5 and the mortality of cyprinid fish occurs in pH levels higher than 10.8 and lower than 5.0 (Svobodova *et al.* 2007). The toxicity of aluminium in water is highly dependent on pH, and Al potentiates the effects of acidic pH on fish (Baker & Schofield, 1982; Buckler *et al.* 1995; Freeman & Everhart, 1971; Hyne & Wilson, 1997; Poleo *et al.* 1997). The results of Poleo *et al.* (1997) confirm that acidic water at a pH of 5.0 is not toxic or at least much less toxic to the seven fish species (*Salmo salar*, *S. trutta*, *Rutilus rutilus*, *Phoxinus phoxinus*, *Perca fluviatilis*, *Salvelinus alpinus*, *Thymallus thymallus*) than acidic Al-containing water of the same pH.

Embryolarval toxicity tests. The subchronic toxic effect of PAX-18 on embryonic and larval developmental stages expressed as the LOEC value was 50 mg.l^{-1} of PAX-18 (4.5 mg.l^{-1} of Al) and the NOEC value was 10 mg.l^{-1} of PAX-18 (0.9 mg.l^{-1} of Al). Freeman & Everhart (1971) stated that concentrations of either dissolved or suspended aluminium below 0.5 mg.l^{-1} were safe for fish which is well in line with the results of the presented study. Concentrations of aluminium over 1.5 mg.l^{-1} caused marked physiological and behavioural changes and acute mortality.

The mortality of 100% was observed in the highest PAX-18 concentration (75 mg.l^{-1} of PAX-18; 6.75 mg.l^{-1} of Al) shortly after beginning of the test, before the time of hatching. The fish mortality rate of the experimental group 3 was higher in days 5–16 compared to the rest days of the experiment. The critical periods were soon after the beginning of the test, at the time of hatching, and within 10 days following after the hatching had finished. Our results indicate that these periods were critical, because they included hatching, and the end of the endogenous nutrition coupled with the start of exogenous nutrition. After 18 days there was only accidental mortality observed which indicated higher tolerance of the older larvae to the substance tested. The similar results were found out by Cleveland *et al.* (1986). Embryos and fish of brook trout (*Salvelinus fontinalis*), older than 36 days were more tolerant to acidic conditions and aluminium than were larvae less than 15 days old. Buckler *et al.* (1995) studied early life stages (from eyed eggs stage until 60 days after hatching) of Atlantic salmon (*Salmo salar*). They stated that the damage of the gills induced by the synergistic effect of Al and acidic pH impaired the early life stages of fish when they became more active and more reliant on the gill respiration than epidermal exchange as a source of oxygen. The period in which the embryonic respiratory organs of common carp have the vital importance for the respiration is relatively short. This interval lasts predominantly to the end of embryonic developmental period. At the beginning of the larval developmental period within the first larval stage (mixed nutrition), the gill organs already take the main respiratory function (Penaz *et al.* 1983) which happened during the day 6 in the case of our experiment. In compliance with the results of Buckler *et al.* (1995) it can be stated that during the initial stages of the embryonic developmental period, when the epidermal respiration prevails, the

mortality detected was low and comparable with the control group. Higher mortality was observed from the day 5 or 6 of the experiment. The importance of the epidermal respiration is already insignificant in this period because the gills take over this function.

The effects of the toxicant in tested concentrations on the length and weight of fish could not be clearly specified. At the day 13 of the experiment PAX seemed to retard growth in higher concentrations, but this effect was not evident afterwards. The significant effect on growth parameters was on the other hand observed by Buckler *et al.* (1995) in *Salmo salar.* At day 60 the fish exposed to pH 5.5 and 71 $\mu g.l^{-1}$ of Al were significantly smaller compared to those in the control group in the experiment made by Buckler *et al.* (1995). However, the duration of this test was twice longer than in our case and the water pH 5.5 suggests higher occurrence of ionic (toxic) form of aluminium. Fish species used for the experiment should be considered an important factor, salmonid fish are in general more sensitive to negative effects of the environment in comparison to the cyprinid fish.

Even so, some conclusions could be estimated from the FWC values. Beginning with the day 19, lower values of this parameter were found in the control group compared with those of the test groups, although this difference was not always statistically significant. These changes could be explained due to a slightly higher retention of food in the intestine in test groups. The assumption that food is retained in the intestine is confirmed by the observed dilatation of the intestine and moderate swelling of the abdominal wall in the concentration 50 $mg.l^{-1}$ on the 19th day of the experiment.

Histological investigation at the conclusion of the embryo-larval test on carp did not show any observable histological changes on the gill surface of the surviving fish. The toxic effect of aluminium is caused by changes on the gill surface due to the precipitation or polymerization of Al (Gensemer & Playle, 1999; Poleo, 1995). The occurrence of the alteration on the gill surface is dependent on water pH. Poleo (1995) stated that the polymerization of aluminium on the gill surface did not occur if the pH increased to 6.0. The pH level in the experimental groups during our embryo-larval test was 7.4 to 7.9. Exley *et al.* (1996) studied acute aluminium toxicity in rainbow trout (*Onchorhynchus mykiss)* at pH 6.5 and stated that the mechanism of the toxic effect was probably asphyxiation caused by aluminium-induced changes in the rheological and diffusional properties of the mucus on the surface of the gill epithelium. In natural conditions it is possible that the changes on the gill surface could be caused by other factors like poor buffering capacity of the water, stress of fish evocated by suboptimal water chemistry, recent decline in water temperature or the action of infectious agents. For example, Stephens & Ingram (2006) described a case study of the toxic effects of aluminium and pH (0.22 $mg.l^{-1}$ Al; pH 5.4) where histological changes on gills as diffuse hypertrophy and hyperplasia of gill epithelium, fusion of secondary lamellae, evidence of aggregations of fungal hyphae and bacteria between secondary lamellae were detected.

The concentration which is usually applied into the natural waters is 5–10 $mg.l^{-1}$ of Al i.e. 45–90 $mg.l^{-1}$ of the preparation PAX-18. The concentration used is 7-14 times lower compared to the lethal concentration of PAX-18 found for common carp. The concentration of the available active compound drops rapidly after application because of its reactions in the water (e.g. aluminium with phosphorus). Therefore, water organisms are not exposed to the applied dose for an extended period of time. However, higher risk of fish damage due to the decrease in the pH value and elevated aluminium toxicity occurs in waters with a naturally low acid-neutralizing capacity or water that is poorly buffered (Baker & Schofield, 1982). PAX-18 should be applied only to selected water areas which match the requirements for its application, namely higher pH and alkalinity. In such areas the risk of PAX-18 adverse effects on fish populations can be considered as minimal.

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