TOXIC EFFECTS OF ALGEXIT AND BLUE EXIT AGENTS ON AQUATIC ORGANISMS

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ABSTRACT

The fish acute toxicity test is a mandatory constituent in the basic testing set for ecotoxicity requirements. Zebra fish (*Danio rerio*) is the most common type of aquarium fish used for toxicity testing. The aim of this study was to determine toxic effects of ALGEXIT and BLUE EXIT agents on fish and algae. Green alga *Pseudokirchneriella subcapitata* and cyanobacteria *Anabaena* sp. were tested in laboratory under the constant conditions. ALGEXIT agent concentrations 0.02 and 0.1 $ml.l^{-1}$ and BLUE EXIT agent concentrations 0.025 and 0.125 $ml.l^{-1}$ were chosen for the inhibitory test with green alga *Pseudokirchneriella subcapitata*. ALGEXIT agent concentration 0.1 $ml.l^{-1}$ and BLUE EXIT agent concentration 0.125 $ml.l^{-1}$ were chosen for the inhibitory test with cyanobacteria *Anabaena* sp. Applied concentrations were used according to agent producers recommendation. Also short-term acute toxicity test on Zebra fish (*Danio rerio*) was conducted. ALGEXIT agent concentrations 0.1, 0.2, 1 and 10 $ml.l^{-1}$ and BLUE EXIT agent concentrations 0.126, 0.25, 1.25 and 12.5 $ml.l^{-1}$ were chosen. Mentioned concentrations are one, twice, ten-times and one-hundred times higher than recommended dose for cyanobacteria and algae extermination. Percentage inhibition of *Pseudokirchneriella subcapitata* cells in 96 hours with ALGEXIT concentration of 0.1 $ml.l^{-1}$ was 71.23% and with BLUE EXIT in concentration of 0.125 $ml.l^{-1}$ was 66.98%. Percentage inhibition of *Anabaena* sp. cells in 96 hours with ALGEXIT in concentration of 0.1 $ml.l^{-1}$ was 43.53%**.** Middle lethal concentration while experimenting on fishes with the BLUE EXIT moves beyond the range of a hundredfold concentration, because no fish died. With using of ALGEXIT 7 fish died after 48 hours, and to determine the LC_{50} further tests with a narrower range of concentration of the product has to be performed.

Key words: algicide, cyanobacteria, green algae, inhibition, toxicity test, Zebra fish (*Danio rerio*),

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INTRODUCTION

Tests of toxicity on organisms of water environment have their irreplaceable role in the evaluation of newly developed and into practice loaded chemicals (Lammer et al., 2009). The toxicity is adverse and sometimes even lethal effect of substances, preparations and wastewaters on organisms. In mild form it is expressed by some physiological functions disorders. Strong effects of toxicity are accompanied by mortality of organism. The toxicity is established using toxicological tests. Toxicological tests on water organisms can be carried out on three levels: on the level of cells and tissues, on the level of organism (individuals) and on biocenosis level (Svobodová, 1987). Acute toxicity of chemicals depends on many factors, such as the species, age, body weight, feeding, conditions of metabolism, temperature, dissolved oxygen concentration in water etc. *Danio rerio* belongs to recommended species by directive OECD for testing of chemicals and it is also species often used in toxicology (Plhalová, 2010).

Cyanobacteria are photosynthetic prokaryotes with wide geographical extension. They produce secondary metabolites called cyanotoxins (Ferrão-Filho & Kozlowsky-Suzuki, 2011). The algae and cyanobacteria are common testing organisms sensitive to many chemicals, and therefore they are widely used in toxicity tests (Zhang et al., 2012). The algae are key functional organisms because they are dominant primary producers and therefore they represent basic segment in aquatic food chains (Machad & Soares, 2012). Freshwater planktonic algae *Pseudokirchneriella subcapitata* is standard species of toxicity tests (Zhang et al., 2012).

MATERIAL AND METHODS

We determined toxic effects of ALGEXIT and BLUE EXIT agents on the fish *(Danio rerio)*, algae (*Pseudokirchneriella subcapitata)* and on the cyanobacteria of genus *Anabaena* sp. The effective substance of ALGEXIT was salicylate. The producer of BLUE EXIT does not specify the effective substance. Tested fish were exposed for 96 hours to effect of various concentrations of testing substances dissolved in standardly prepared diluted water. Aquarium serving as a control contained fish and water free from any testing substance. ALGEXIT agent concentration 0.1; 0.2; 1; 10 $ml.l^{-1}$ and BLUE EXIT concentration 0.126; 0.25; 1.25; 12.5 $ml.l^{-1}$ were chosen for short-term tests of immediate toxicity. Mentioned concentrations are one, twice, ten-times and one-hundred times higher than recommended dose for cyanobacteria and algae extermination. Zebra fish were 3 – 4 months old and they were 15 – 20 mm long. Individual fish were chosen randomly and were not fed during the test. Fish behaviour was observed during the test, dead fish were removed from tank. Values of test were recorded during 24 – 96 hours. In this time, conductivity, pH, temperature, content of dissolved oxygen and death of individuals in tanks was observed. In tested tanks there were 10 fishes in 3000 ml of tested solution with no aeration.

Diluted water was prepared according to ISO 6341 from stock solutions in amount of 11.76 g $CaCl_2.2H_2O$, 4.93 g $MgSO_4.7H_2O$, 2.59 $NaHCO_3$ and 0.23 g KCl (Svobodová, 2000). This prepared diluted water was aerated by airy oxygen (aeration) for 24 hours at first, then left to stand for 24 hours. Final measured pH was 8.

We observed percentage of inhibition of tested substances ALGEXIT and BLUE EXIT. Tests were carried out under the laboratory conditions for 96 hours in constant conditions in Erlenmayers flasks on green alga *Pseudokirchneriella subcapitata* and on the cyanobacteria of genus *Anabaena* sp. For inhibitory tests with green alga *Pseudokirchneriella subcapitata* we chose concentrations of ALGEXIT 0.02; 0.1 $ml.l^{-1}$ and BLUE EXIT 0.025; 0.125 $ml.l^{-1}$. The concentration of ALGEXIT for cyanobacteria of genus *Anabaena* sp. was 0.1 $ml.l^{-1}$and the concentration of BLUE EXIT was 0.125 $ml.l^{-1}$. Applied concentrations were used according to agent producers recommendation. In Erlenmayers flasks there were green algae and cyanobacteria without additional solution, which served as control sample. Test containers were closed to prevent airy contamination and to lower

evaporation of water. Erlenmayers flasks were closed with absorbent cotton wool because of permitting of admittance of CO_2 into containers (ČSN EN ISO 8692).

Before start of the test growth medium for test samples of green algae *Pseudokirchneriella subcapitata* and cyanobacteria of genus *Anabaena* sp. according to standard ČSN EN ISO 8692 were prepared. Per 500 ml of water 10 ml of stock solution 1, 1 ml of stock solution 2, 1 ml of stock solution 3 and 1 ml of stock solution 4 were added. Container was fulfilled to 1000 ml contain by additional water. For reaching equilibrium (ČSN EN ISO 8692) medium was left in contact with air over the night. Quantitative method of cells counting in Bürkers chamber for finding out the inhibitory or stimulatory effects of tested preparations was used. The principle of method of counting according to Bürker is based on counting in a chamber covered by cover glass under the microscope with fluorescence (Svobodová, 2000).

RESULT AND DISCUSSION

We chose two algicidal agents - ALGEXIT and BLUE EXIT – for tests of acute toxicity on fish. Measured temperature in aquariums during 96 hours ranged from 21.6 to 22.8°C. The amount of oxygen ranged from 54.9 to 89.8 %, except one aquarium with concentration of ALGEXIT 10 ml.l^{-1} in which the oxygen amount lowered after 48 hours to 24.8 % (2.09 mg.l^{-1}). The value of pH in nine aquariums was slightly alkaline, meanwhile in one aquarium value of pH during the first day of test was 6.61 with the ALGEXIT agent with concentration 10 ml.l^{-1}. The conductivity ranged from 326 to 1103 µS.cm^{-1}. The mortality occurred only in one aquarium with concentration of ALGEXIT 10 ml.l^{-1}, where after 48 hours 7 fish died. After 96 hours in the same aquarium one fish died. For finding out of LC_{50} we have to do other tests with narrower range of concentration of agents. Other concentrations of ALGEXIT and all tested concentrations of BLUE EXIT do not cause any deaths. The median lethal concentration of BLUE EXIT in tests on fish fluctuated above the limit of 12.5 ml.l^{-1}, which is one-hundred times higher than recommended dose stated by the producer.

In tables 1, 2 and 3 there are the average amount of cells of *Pseudokirchneriella subcapitata* and *Anabaena* sp. in 1 ml during tests presented. Percentage cell inhibition of *Pseudokirchneriella subcapitata* in 96 hours with ALGEXIT in concentration 0.1 ml.l^{-1} was 71.23% and with BLUE EXIT in concentration 0.125 ml.l^{-1} was 66.98%. Percentage cell inhibition of *Anabaena* sp. in 96 hours with ALGEXIT in concentration 0.1ml.l^{-1} was 43.53% and with BLUE EXIT in concentration 0.125 ml.l^{-1} was 90.64%. Vaněk (2012) tested preparations for cyanobacteria and algae extermination in concentration 0.01 ml.l^{-1} of solution contain 1% PHMG, PAHCL + vitriol and 1% PHMG + 0.1% terbutryn, the inhibition in 96 hours was one hundred percent. In comparison with our tests, preparations in Vaněk (2012) tests had stronger effect in concentrations about one order lower. In acute toxicity tests on fishes Vaněk (2012) tested three algicidal substances, pelargonic acid, Guanicid and 1% PHMG. In comparison with allowance of ALGEXIT with concentration of 1 ml.l^{-1} and BLUE EXIT with concentration of 12.5 ml.l^{-1} no fish died. In tests Vaněk (2012) using pelargonic acid, Guanicid and 1% PHMG in concentration of 0.9 ml.l^{-1} the mortality was hundred percent in 24 hours. According to this comparison can be said that ALGEXIT and BLUE EXIT are safe for fish.

Table 1. Preparation ALGEXIT – average cell amount and ±SD Pseudokirchneriella subcapitata in 1 ml

		Control	±SD	0.02 ml.l-1	±SD	0.1 ml.l-1	± SD
23.9.2013	0	889 583	43 750	-	-	-	-
24.9.2013	24	788 510	13 860	454 167	91 667	359 917	6 750
25.9.2013	48	925 000	66 667	1 035 417	14 584	668 055	43 877
26.9.2013	72	1 363 889	136 987	1 479 167	112 500	335 417	6 250
27.9.2013	96	1 993 750	189 583	362 500	20 694	573 611	50 955

Table 2. Preparation BLUE EXIT - average cell amount and ±SD Pseudokirchneriella subcapitata in 1 ml

		Control	±SD	0.025 ml.l-1	± SD	0.125 ml.l-1	± SD
23.9.2013	0	889 583	43 750	-	-	-	-
24.9.2013	24	788 510	13 860	627 083	31 250	53 333	235 285
25.9.2013	48	925 000	66 667	754 167	0	845 834	16 667
26.9.2013	72	1 363 889	136 987	1 089 584	2 084	732 639	179 250
27.9.2013	96	1 993 750	189 583	327 084	10 417	658 334	29 167

Table 3. Average cell amount and ±SD Anabaena sp. in 1 ml

				ALGEXIT		BLUE EXIT	
		Control	±SD	0,1 ml.l-1	±SD	0,125 ml.l-1	±SD
23.9.2013	0	818 750	2 083	-	-	-	-
24.9.2013	24	190 972	62 564	274 306	100 695	145 370	9 594
25.9.2013	48	243 087	42 330	648 611	280 714	180 208	3 125
26.9.2013	72	647 569	352 431	559 722	11 111	37 500	4 167
27.9.2013	96	129 775	43 837	73 289	4 837	12 153	1 736

CONCLUSIONS

Inhibition tests were carried out with chosen preparations on cultures of green alga *Pseudokirchneriella subcapitata* and cyanobacteria *Anabaena* sp. and acute toxicity tests on fish (*Danio rerio).* Effective concentrations for cyanobacteria and algae extermination and toxic effects on fish were found out. The efficiency of preparation by measuring of density in Bürker chamber under the microscope with fluorescent was controlled. Percentage inhibition with both preparations during 96 hours was counted. At acute toxicity tests on (*Danio rerio)* we found out, that using of BLUE EXIT is completely safe, because even with hundredfold concentration no fish died. With ALGEXIT there were 7 dead fish after 48 hours in the highest concentration and the value of LC_{50} ranged from 1 to 10 $ml.l^{-1}$. For value determination of LC_{50} is necessary to carry out further tests with narrower range of concentration of given preparation.

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