TOXIC EFFECT OF FLUORESCENCE PIGMENT ON ZEBRA FISH (DANIO RERIO)

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Abstract: The aim of the study was to determine the toxic effects of organic pigments Alizarin Red S and Alizarin Complexone on zebra fish (Danio rerio). For short-term acute toxicity tests on zebra fish concentration of 150; 300 and 600 mg/L were chosen for both dyes. Toxic effect of dyes was observed even in the variant with 10 g/L of sodium chloride. Addition of sodium chloride increases the deposition of dyes in the bone structures of the fish. LC50 values were analyzed graphically by using probit analysis. There was no mortality during the acute toxicity test with Alizarin Red S even at the highest concentration. Toxicity value (72hLC50) for zebra fish with a combination of Alizarin Red S + 10 g/L of sodium chloride is 546.42 mg/L. Mortality for Alizarin Complexone was 100% in 24 hours at concentrations of 150 and 600 mg/L, with concentrations of 150 mg/L there was no mortality. In Alizarin Complexone supplemented with 10 g/L of sodium chloride was 100% mortality at all concentrations up to 72 hours.

Key Words: Alizarin Red S, Alizarin Complexone, LC50, fish, marking

INTRODUCTION

Toxicity tests are used to detect or estimate the potential toxic effects of tested compounds on living organisms (Kočí 2006). Using of model fish species Zebra fish (Danio rerio) is recommended for testing of chemicals in toxicology. It is possible to use other types of freshwater, marine or brackish fish, provided that the appropriate adjustments are made, for example the quality of the dilution water and temperature conditions during test (ÚNMZ 1999).

Marking of fish (individual, group) is a normal part of scientific work or breeding handling (Rodina and Flajšhans 2008). In order to recognize in the water tanks fish from natural spawning from fish planted, marking of planted fish should be carried. When choosing a method of marking it is necessary to take into account a variety of circumstances and requirements. Especially the number of marked fish, durability of marking, laboriousness of application, reading of marks, the possibility to automate these activities, the price and availability of marks and ultimately the rate or risk of damage to fish during application, mark reading and handling with fish (Rodina and Flajšhans 2008). Fish in the early stage are not easy to mark using an external mark (Baer and Rösch 2009). Additionally, handling with fish during the marking can cause large losses due to stress. One way of marking a group of fish is the use of fluorescent dyes that can produce detectable marks in otoliths and other skeletal structures (Liu et al. 2009). Marking using fluorescent dyes has many advantages, marked may be a large number of individuals in a short time at low cost, marking lasts for several months to years, the dye can be used on fish of all ages (from larvae to adult), the labeling process is quite simple and there is no excessive stressing of fish. Dyes Alizarin Red S and Alizarin Complexone demonstrated good efficiency of fluorescent pigment marking in bone structures with minimal negative effect on the survival of marked fish (Lü et al. 2015). The best results were obtained during an experiment on the embryo whitefish (Coregonus lavaretus) 28 days after fertilization with a concentration of Alizarin Red S 1000 mg/L, and on rainbow trout (Oncorhynchus mykiss) with a concentration of Alizarin Complexone 10 mg/L (Eckman 2003, Walt and Faragher 2003).
MATERIAL AND METHODS

Characteristics of the tested substances

Alizarin Red S (C₁⁴H₇NaO₇S) and Alizarin Complexone (C₁⁹H₁₅NO₈) are fluorescent dyes which are used for marking of fish. Alizarin Red S was previously used for textile dyeing. Alizarin Red S forms a complex with calcium that is stored in the bones (Puchtler et al. 1968, Eckmann, 2003). Adding sodium chloride will increase the osmotic pressure, which improves the dye transport to the bone structures (Baer and Rösch 2009).

Alizarin Red S is a dark red powder. Alizarin Complexone is dark yellow powder with a boiling point of 190 °C. They have poor water solubility, better solubility in ethanol (Safety Data Sheet, Alizarin Red S Alizarin Complexone 2013).

Acute toxicity test on fish

Acute toxicity tests were performed on aquarium fish zebra fish (at the age of 4 months, the total body length of 20±5 mm). 7 days before testing fish were acclimatized to the medium, in which the test was performed. Dilution water was prepared according to methodology ČSN EN ISO 7346 1 (ÚNMZ, 1999). Fish were exposed to various concentrations of Alizarin Red S and Alizarin Complexone for 96 hours. Toxic effect of dye was evaluated even in the variant with 10 g/L of sodium chloride. Temperature during laboratory testing was constant (25°C) with controlled lighting mode – 13 hours light, 11 hours dark. For all the tested substances were selected concentrations of 150; 300 and 600 mg/L. Tested concentrations were chosen on the basis of other authors, who tested the same concentration of dyes on other fish species. Tests were carried out in glass tanks with 3000 ml of water without aeration with 10 fish per concentration and control group in triplicate. Fish were not fed during the test. Mortality of the fish was determined every 24 hours during the test. Temperature, pH and dissolved oxygen content in water were measured using device HACH HQ40d and conductivity using device Hanna combo.

Data from acute toxicity test on fish were processed using probit analysis to calculate 72h LC₅₀.

RESULTS AND DISCUSSION

Values of hydrochemical parameters are presented in the Table I. During the acute toxicity test with Alizarin Red S and Alizarin Complexone the temperature and conductivity throughout were steady without significant fluctuations. The pH in all tested tanks ranged from 6.00 to 8.22. The value of the percentage saturation of oxygen in the test solutions during the whole test did not fall below 60% of saturation. Decrease of oxygen saturation under 60% was monitored only in the concentration of 600 mg/L during the test with Alizarin Complexone + 10 g/L NaCl. The decrease in oxygen saturation occurred at 48 h. The high value of the percentage saturation of oxygen (129.8 and 126.4%) was at the beginning of testing, which was caused by aeration of dilution water 24 hours before testing. Differences in pH among the aquaria were probably caused by different concentrations of fluorescent colors. There were no pH differences in control group (pH 7.86 to 8.09). Oxygen saturation in control group did not fell under 60% (84.6 to 99.3 %) for whole duration of the test. Temperature and conductivity did not fluctuated too.

Table I Values of physical and chemical parameters during the acute toxicity tests (min.–max.).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>Control</th>
<th>Alizarin Red S</th>
<th>Alizarin Red S 10 g/L NaCl</th>
<th>Alizarin Complexone</th>
<th>Alizarin Complexone 10 g/L NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen saturation</td>
<td>%</td>
<td>84.6–99.3</td>
<td>73.1–99.6</td>
<td>71.3–129.8</td>
<td>80.9–100.3</td>
<td>59.4–126.4</td>
</tr>
<tr>
<td>Temperature of water</td>
<td>°C</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Conductivity</td>
<td>mS/m</td>
<td>49.2–54.9</td>
<td>49.8–56.6</td>
<td>&gt;400.0</td>
<td>49.9–57.6</td>
<td>&gt;400.0</td>
</tr>
</tbody>
</table>
For Alizarin Red S mortality did not occur at any tested concentration. The process of mortality in different concentrations Alizarin Red S in combination with 10g/L is shown in Figure 1. Median lethal concentration at 72 hours (72hLC50) was calculated by probit analysis. Toxicity value (72hLC50) for zebra fish with a combination of Alizarin Red S + 10 g/L of sodium chloride is 546.42 mg/L. The highest mortality at a concentration of 150 mg/L and 10g/L was caused most likely by NaCl. The NaCl is probably fixed into the complex by fluorescent color which is the cause of lower mortality at higher concentrations of color. More tests will be conducted to support this hypothesis in a future. LC50 is calculated only from three values so it is just approximate. More tests with larger scale of concentrations will be conducted to specify the LC50 value more precisely.

Mortality was 100% at concentrations of 300 and 600 mg/L for Alizarin Complexone in 24 hours. At the concentration of 150 mg/L, there was no mortality. Mortality in all tested concentrations was 100% for Alizarin Complexone + 10g/L NaCl in 72 hours (see Figure 2). Mortality was monitored at the concentrations of 150 and 600 mg/L in a few minutes after test begun. This is the reason why the curves start at 30 and 40% in Figure 2. There was no mortality in the control group during the test.

Baer and Rösch (2008) states, that the best dyeing was obtained using a concentration of 300 mg/L of Alizarin Red S. However, the concentration caused the death of 95% of the tested brown trout (Salmo trutta). Fluorescent marking with good quality and low mortality of brown trout, which did not differ from the control group (0 mg/L), was observed at concentrations of 150 mg/L of Alizarin Red S with the addition of 10 g/L of sodium chloride. Dying of brown trout with Alizarin Red S was carried out at a water temperature of 12 °C for 3 hours. The initial pH value was 7.6. In concentration of 50 and 150 mg/L of Alizarin Red S the pH was relatively stable (7.6 and 7.7). At a concentration of 300 mg/L of Alizarin Red S pH decreased rapidly. For this reason 1 mg/L of NaOH was added to the highest concentration to maintain the pH constant. Throughout the test the fish were fed by commercial feed (57% protein, 17% fat). During the test with Alizarin Complexone supplemented with 10 g/L of sodium
chloride Baer and Rösch (2008) indicates, that the environment temperature was 8 °C. After addition of the dye there was an immediate drop in pH to 5.8. By adding 20 mg/L of NaOH they were able to increase the pH to 6.8. After stabilization of the pH they added brown trout to the tested tank. During the test the tanks were aerated. Liu et al (2009) tested the Alizarin Red S concentrations from 200 to 400 mg/L and Alizarin Complexone concentration from 50 to 300 mg/L on juvenile (20–30 mm) of Japanese flounder (Paralichthys olivaceus). Deaths were avoid except for one fish at a concentration of 300 mg/L of Alizarin Red S, and for one piece with the concentration of 300 mg/L of Alizarin Complexone.

Environmental conditions were the same in both tests. Testing was carried out in seawater at a temperature of 20±5 °C, water was heavily aerated to increase pH, which was maintained in the range of 7.23 to 7.86. Eckman (2008) carried out testing for 15 minutes in a solution of a fluorescent dye on the fertilized roe of whitefish (Coregonus lavaretus). The experiment was performed in the recirculation water, where 5% of the total of 250 l of water was exchanged every other day. The water temperature was 4.5 °C and increased to 12 °C during 3 days. In the initial experiment with dye Alizarin Red S in a concentration of 400 mg/L were immersed embryos 5 days after fertilization. In this test dying of otoliths was avoided. Marking quality was increased on older embryos (38 days after fertilization) at the time of immersion. During immersion of embryos (28 days from fertilization) into a concentration of 1000 mg/L of Alizarin Red S the mortality was 35%, which was considered tolerated dose for multiple marking of whitefish. Marking was visible for 8 months. In our experiments with Alizarin Red S there was observed no mortality in 96 hours in any concentrations tested. Mortality was recorded in combination Alizarin Red S with 10 g/L of sodium chloride. When testing Alizarin Complexone there were no mortalities only at concentration of 150 mg/L, from concentration of 300 mg/L mortality was 100% within 24 hours. When testing Alizarin Complexone supplemented with 10 g/L of NaCl the mortality at 72 hours was 100%. Causes of different results of toxicity dyes effect on different fish species are likely to be caused by the different susceptibility of each species, different life stage of the experimental fish and different environmental conditions during testing.

CONCLUSION

Based on our results, marking of zebra fish by immersion in Alizarin Red S can be recommended as a simple and secure method. Dyeing using dye Alizarin Complexone is safe only to a concentration of 150 mg/L. Using fluorescent dyes in combination with the addition of 10 g/L of sodium chloride increased mortality on zebra fish. In comparison with the results of other authors who tested fluorescent dyes on other fish species (Salmo trutta, Paralichthys olivaceus, Coregonus lavaretus) the results are different in experiments with zebra fish, both when tested dye alone and in combination of dye and sodium chloride. Based on our results and the results of other authors is obvious different susceptibility of different species to the used dyes. In case of use of tested dyes for marking other species is therefore necessary to test susceptibility (toxicity) of given species.

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REFERENCES


