ORIGINAL ARTICLE

Comparison of the sensitivity of different fish species to medical substances

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Abstract

OBJECTIVES: Formaldehyde, sodium chloride and potassium permanganate belong to the commonly used substances for fish treatment. The aim is to define and compare their acute toxicity and therapeutic index between two fish species – *Danio rerio* and *Poecilia reticulata*.

DESIGN: To determine acute toxicity of these compounds, the semistatic method was implemented in compliance with the OECD No. 203 (Fish acute toxicity test). In each test series, 4 acute toxicity tests were performed. The results were subjected to the probit analysis to determine the 96hLC50 values. Therapeutic index (TI) was calculated with respect to short-term LC50 and effective therapeutic concentrations commonly used for fish treatment.

RESULTS: The mean 96hLC50 values for sodium chloride were 21.69 ± 0.92 g l^{-1} (TI = 1.4) for *P. reticulata* and 10.39 ± 0.12 g l^{-1} (TI = 1.0) for *D. rerio*. The acute toxicity of formaldehyde for *D. rerio* expressed as 96LC50 was 0.12 ± 0.003 ml l^{-1} (TI = 5.24) and for *P. reticulata* 0.1 ± 0.003 ml l^{-1} (TI = 4.9). The acute toxicity of potassium permanganate for *D. rerio* expressed as 96LC50 was 1.25 ± 0.15 mg l^{-1}(TI = 1.5) and for *P. reticulata* 1.43 ± 0.05 mg l^{-1} (TI = 2).

CONCLUSION: *P. reticulata* showed significantly higher ($p < 0.05$) tolerance to sodium chloride than *D. rerio,* whereas *D. rerio* showed significantly higher ($p <$ 0.05) tolerance to formaldehyde than *P. reticulata.* The acute toxicity of potassium permanganate was comparable for both fish species. Calculated therapeutic indexes of all tested substances were low; therefore it is important to conduct preliminary tolerance tests before aplication of treatment baths.

INTRODUCTION

Fish in the aquatic environment are threatened with contaminants of anthropogenic origin, e.g. pesticides (Modra *et al.* 2008) and polycyclic aromatic hydrocarbons (Blahova *et al.* 2008) as well as with infectious agents.

In aquaculture we have limited options in using pharmaceuticals. Basically, they are restricted to anaesthetic agents and disinfectants or anti-infective agents for parasitic, fungal and microbial diseases. Anaesthetics are commonly used in aquaculture to reduce handling stress and mechanical damage that fish may suffer during manipulation, breeding or veterinary interventions (Macova *et al.* 2008). The anti-infective agents are used to control disease, and the choice of drugs depends on many factors (efficacy, ease of application, human safety, target animal safety including stress to the fish, environmental impact, costs, etc.) (Burka *et al.* 1997). The disinfectants are commonly used in both intensive fish farming and aquarium fish breeding as a preventive disinfection. The tolerance of chemicals can vary depending on the species and the treatment conditions; hence the toxicity of a chemical to fish should be determined before the application of chemical therapies. This is particularly important as the recommended concentrations and treatment times for some therapeutics are near the lethal level for certain cultured species (Fajer-Avila *et al.* 2003; Hohreiter & Rigg, 2001). Formaldehyde, potassium permanganate and sodium chloride are among the most important chemicals used in bath treatment of fish.

Formalin is saturated (37%) aqueous solution of formaldehyde gas widely used as fish egg fungicide and for the treatment of ectoparasitic infections (Bills *et al.* 1977; Schreier *et al.* 1996). Svobodova *et al.* (2007) recommend preparing the formaldehyde bath treatment with respect to the water temperature. At temperatures up to 10 °C, the formaldehyde recommended concentration is 0.25 ml l^{-1}, at temperatures between 10–15 °C it is 0.20 ml l^{-1}, and at temperatures above 15 °C the concentration is 0.17 ml l^{-1}. The treatment length is 30–60 minutes. Formalin solutions will degrade with precipitation of paraformaldehyde, which is toxic and must be removed prior to the use. An extensive comparative review about formaldehyde toxicity in different fish species was presented by Hohreiter & Rigg (2001).

Potassium permanganate ($KMnO_4$) is a crystalline violet compound with oxidizing properties. Potassium permanganate has been used to treat external pathogens including fungus, bacteria, and some parasites (Lay, 1971; Francis-Floyd & Klinger, 1997; Straus & Griffin, 2002). For most fish, potassium permanganate can be administered at a concentration of 0.3–0.6 mg l^{-1} as a long-term bath (12 hours). Potassium permanganate can also be used as a short-term bath at concentrations of 0.01 g l^{-1} for 60–90 minutes or 0.1 g l^{-1} for 5–10 minutes. The treatment concentrations are close to lethal concentrations for fish (Svobodova *et al.* 2007); therefore, several studies were performed on different fish species to determine potassium permanganate toxicity (Marking & Bills, 1975; Tucker, 1987; Bills *et al.* 1993; Da Silva *et al.* 2006). Moreover, several factors, including pH, total alkalinity, total hardness, and chemical oxygen demand (COD), have been found to affect the toxicity of this chemical to fish (Marking & Bills, 1975; Tucker, 1987; Da Silva *et al.* 2006).

Sodium chloride is a common and easily available antiparasitic compound, which can be used in fishery to prevent nitrite toxicity in fish (Svobodova *et al.* 2005). Sodium chloride is characterised as a low regulatory compound, but the large quantities required limit its applicability (Marking *et al.* 1994). In fishery it is used for antiparasitic treatment from early fish stages up to the market size (Marking *et al.* 1994; Mischke *et al.* 2001). The bath treatment is to be prepared by dissolving 10–30 g NaCl in one litre of water for 15–30 minutes. The effect of sodium chloride bath is significantly decreased when water temperature drops below 5 °C (Svobodova *et al.* 2007).

The toxicity tests were performed on two fish species – *Danio rerio* and *Poecilia reticulata*; both species are recommended by OECD as model organisms in toxicity tests. However, their sensitivity to different compounds can vary. Zebrafish (*D. rerio*), belonging to family *Cyprinidae*, are small tropical freshwater fish that originate from India. Guppies (*P. reticulata*), family *Poeciliidae*, are small tropical fish, native to the coastal streams of northeast South America.

We defined two different aims in our work. The first was to determine and compare 96hLC50 of the above mentioned compound in two fish species to recognize the species specific sensitivity. Secondly, we performed short-term acute toxicity tests (in compliance with the recommended treatment times) and determined the therapeutic index.

MATERIAL AND METHODS

Experimental fish. Both adult zebrafish (aged 2–3 months, length 30 ± 5 mm, weight 0.3 ± 0.1 g) and adult guppies (aged 2 months, length 25 ± 5 mm, weight 0.3 ± 0.1 g) were procured from a permanent local supplier. All fish were held for a minimum of 7 days for the acclimatization period in the aquariums. During this period they were fed with commercial fish pellets. Food was withheld 72 h prior to the test commencement. Experimental procedures were in compliance with the national legislation (Act No. 246/1992 Coll., on the protection of animals against cruelty, as amended, and Decree No. 207/2004 Coll., on the protection, breeding and use of experimental animals).

Acute toxicity test. To determine the acute toxicity of sodium chloride, formaldehyde (36–38% solution) and potassium permanganate, the semistatic method according to OECD No. 203 (Fish acute toxicity test) was pursued. Both zebrafish and guppies were exposed to progressive concentrations series of each substance. In each test series, 4 acute toxicity tests were performed, with 10 fish used for each concentration and for the control group. The tests were carried out using the semi-static method with solution replacement after 48 hours. The total duration of acute toxicity tests was 96 hours.

Therapeutic index. Therapeutic index represents the range between therapeutic concentration and lethal concentration, often expressed as ratio of lethal concentration (LC50) and effective therapeutic concentration (EC50). Common treatment concentrations of each

Table 1. Lethal concentrations (LC50) of formaldehyde, sodium chloride and potassium permanganate in *Poecilia reticulata* and *Danio rerio* at different treatment times (mean ± SD).

	Poecilia reticulata	*Danio rerio*
Formaldehyde		
96h	0.1 ± 0.003 (ml l^{-1})	0.12 ± 0.003 (ml l^{-1})
60 min	0,86 ± 0,068 (ml l^{-1})	0,9 ± 0,089 (ml l^{-1})
Sodium chloride		
96h	21.69 ± 0.92 (g l^{-1})	10.39 ± 0.12 (g l^{-1})
30 min	42.03 ± 1.345 (g l^{-1})	29.55 ± 1.52 (g l^{-1})
Potassium permanganate		
96h	1.43 ± 0.05 (mg l^{-1})	1.25 ± 0.15 (mg l^{-1})
90 min	20.0 (mg l^{-1})	15.0 ± 5.77 (mg l^{-1})

Table 2. Median lethal (LC50) and effective therapeutic concentrations in *Danio rerio* used for the calculation of therapeutic index

	Median lethal concentration	Effective concentration
Formaldehyde	0.89 ml l^{-1}	0.17 ml l^{-1}
Sodium chloride	29.5 g l^{-1}	30 g l^{-1}
Potassium permanganate	15 mg l^{-1}	10 mg l^{-1}

Table 3. Median lethal (LC50) and effective therapeutic concentrations in *Poecilia reticulata* used for the calculation of therapeutic index

	Median lethal concentration	Effective concentration
Formaldehyde	0.84 ml l^{-1}	0.17 ml l^{-1}
Sodium chloride	41.75 g l^{-1}	30 g l^{-1}
Potassium permanganate	20 mg l^{-1}	10 mg l^{-1}

Table 4. Therapeutic indexes

	Poecilia reticulata	Danio rerio
Formaldehyde	4.9	5.24
Sodium chloride	1.4	1
Potassium permanganate	2	1.5

substance were used as effective concentrations. Lethal concentrations were established in short-term toxicity tests using common treatment times for each substance, and the median was calculated from the results (Fajer-Avila *et al.* 2003). The tests were performed in 5 litres jars; each compound was tested in four different concentrations and four test series. Ten fish were randomly placed in each jar from the storage tank. The treatment times were as follows: in formaldehyde 60 minutes, in sodium chloride 30 minutes and in potassium permanganate 90 minutes.

Water quality parameters. The basic physical and chemical parameters of diluted water used in toxicity tests were: $ANC_{4.5}$ 3.6–3.7 mmol l^{-1}; COD_{Mn} 1.4–1.9 mg l^{-1}; total ammonia below the limit of determination; NO_3^- 24.5–31.4 mg l^{-1}; NO_2^- below the limit of determination; Cl^- 18.9–19.1 mg l^{-1}; Σ Ca ± Mg 14 mg l^{-1}.

The temperature of the experimental bath was 24 ± 1 °C, the dissolved oxygen concentrations did not fall below 60% (80–94%), and the pH was between 7.89 and 8.62. No fish died in the control tanks during the experiments.

Data analysis. The probit analysis (EKO-TOX 5.2 software) was applied to the results obtained (the number of fish dying at individual test concentrations) and the LC50 values for the tested substances were calculated. The statistical significance of the difference between LC50 values for zebrafish and guppies was calculated using the non-parametric Mann-Whitney test and the Unistat 5.1 software.

RESULTS

Acute toxicity. Values of the 96hLC50 and short-term lethal concentrations (30 min for sodium chloride, 60 min for formaldehyde and 90 min for potassium permanganate) used for *P. reticulata* and *D. rerio* are given in ***Table 1***.

In potassium permanganate, there were no significant differences in sensitivity of these two fish species. However, in sodium chloride and formaldehyde we recognized significant difference in sensitivity ($p < 0.05$). *P. reticulata* showed higher tolerance to sodium chloride toxicity, whereas *D. rerio* was more tolerant to formaldehyde toxicity.

Therapeutic index. Lethal and effective therapeutic concentrations which were applied to calculate therapeutic indexes are shown in ***Table 2*** and ***Table 3***. In ***Table 4***, there are presented calculated therapeutic indexes. Common treatment concentrations of each substance were used as effective concentrations. Lethal concentrations were established as a median from LC50's of short-term toxicity tests using common treatment times of each substance. The therapeutic index should be at least 4 or higher. A chemical with a high index can presumably be administered with greater safety than the one with a low index (Fajer-Avila *et al.* 2003).

DISCUSSION

Due to the fact that formaldehyde, sodium chloride and potassium permanganate are among the routinely used treatment substances in fishery, the acute toxicity and accompanying safety of these drugs need to be determined.

We conducted toxicity tests on two fish species (*P. reticulata* and *D. rerio*), which are recommended as standard organisms for toxicity testing to establish both 96hLC50 and therapeutic index of each substance. After that we compared LC50's for each substance in *D. rerio* and *P. reticulata* in order to recognize possible differences in their sensitivity.

Formaldehyde. In case of formaldehyde, the acute toxicity for *D. rerio* expressed as 96LC50 was calculated to be 0.12 ± 0.003 ml l^{-1} (mean ± SD). In *P. reticulata,* the 96hLC50 was 0.1 ± 0.003 ml l^{-1} (mean ± SD). In this case we found significant difference ($p < 0.05$) in sensitivity between *P. reticulata* and *D. rerio*.

The most sensitive freshwater fish were fingerlings of striped bass (*Morone saxatilis*) as Reardon & Harrell (1990) reported in their work, which was dealing with the tolerance to formalin in varying water salinities. The 96hLC50 value for saltless water was 4.96 mg l^{-1}. Wellborn (1969) reported a similar 96hLC50 value of 7.26 mg l^{-1} for striped bass under static conditions. Other fish species, for which the formaldehyde toxicity data are available, are salmonids. In rainbow trout formaldehyde 96hLC50 value ranged from 47.59 to 98.82 mg l^{-1} (Bills *et al.* 1977). Acceptable data for other salmonids include formaldehyde 96hLC50 of 40.3 mg l^{-1} for lake trout (*Salvelinus namaycush*), and formaldehyde 96hLC50 of 69.8 mg l^{-1} for Atlantic salmon (*Salmo salar*) (Bills *et al.* 1977). The differences in acute toxicity values might be influenced by water parameters such as pH, salinity and water hardness. However, the knowledge about influencing the formaldehyde toxicity is inconsistent (Meinelt *et al.* 2005; Piper & Smith, 1973). On the other hand, the increasing temperature decreased the viability of formaldehyde-exposed fish according to concordant reports by Kouril *et al.* (1984) and Bills *et al.* (1993).

Sodium chloride. The second tested compound was sodium chloride with the 96hLC50 mean values 21.69 ± 0.92 g l^{-1} for *P. reticulata* and 10.39 ± 0.12 g l^{-1} for *D. rerio*. There was a significant difference ($p < 0.05$) in sensitivity between these two fish species.

Information regarding the sodium chloride acute toxicity in freshwater fish species is scarce. Nevertheless, there are several studies that evaluated the effects of salinity on corporal growth and food intake, behavioural patterns and gill alterations (Velasco-Santamaria & Cruz-Casallas, 2008) as well as on the nitrite toxicity (Svobodova *et al.* 2005). Toxicity data published in the technical report of the Pest Management Regulatory Agency (PMRA, 2006) (96hLC50 in *Lepomis macrochirus* 9.62 g l^{-1}, in *Pimephales promelas* 10.83 g l^{-1}, in *Oncorhynchus mykiss* 11.11 g l^{-1}), 96hLC50 reported by King & Farrell (2002) (*Acipenser oxyrinchus* 9.73 g l^{-1}) and Velasco-Santamaria & Cruz-Casallas (2008) (*Metynnis orinocensis* 10.5 g l^{-1} for juvenile and 10.8 g l^{-1} for adult moneda) were similar to our data observed in *D. rerio*. According to Velasco-Santamaria & Cruz-Casallas (2008) *Gambusia affinis* (96hLC50 17.5 g l^{-1}) counts among more tolerant fish species; close to *P. reticulata* sensitivity.

Potassium permanganate. According to the conclusions reported, the susceptibility of aquatic organisms to potassium permanganate depends on water quality, exposure time and aquatic species. For example, Marking & Bills (1975) observed in *Onchorhynchus mykiss* higher potassium permanganate toxicity at lower water temperature, higher water hardness or at higher pH. Another water parameter that can have an impact on potassium permanganate toxicity is salinity. The increased water salinity caused the rise of $KMnO_4$ toxicity in *Poecilia latipinna* (Marecaux, 2006).

The acute toxicity of potassium permanganate for *D. rerio* expressed as 96LC50 was calculated to be 1.25 ± 0.15 mg l^{-1}. In *P. reticulata* the 96hLC50 was 1.43 ± 0.05 mg l^{-1}. In this case we didn't find significant difference in sensitivity between *P. reticulata* and *D. rerio*.

In general, the acute data on potassium permanganate toxicity to fish are similar and do not vary to any great extent. Comparable 96hLC50 values, which were obtained during our experiment, were reported by Bills *et al.* (1993), who conducted static toxicity tests in juvenile striped bass, *Morone saxatilis,* (1.58 mg l^{-1}). Hobbs *et al.* (2006) performed acute toxicity tests of potassium permanganate in order to evaluate its toxicity to nontarget aquatic organisms. In synthetic moderately hard water, the 96hLC50 value in *Pimephales promelas* was 2.13 mg l^{-1}. Kori-Siakpere (2008) conducted static bioassays to determine the 96hLC50 of potassium permanganate to fingerlings of African catfish, *Clarias gariepinus* (3.02 mg l^{-1}). Similar 96hLC50s of $KMnO_4$ were also calculated by Marking & Bills (1975) in *Carassius auratus* (3.6 mg l^{-1}). Tambaqui (*Colossoma macropomum*), American eel (*Anguilla rostrata*) and channel catfish (*Ictalurus punctatus)* appeared to be more tolerant to $KMnO_4$. The 96hLC50s were established as follows: 8.6 mg l^{-1} (Da Silva *et al.* 2006), 21.6 mg l^{-1} (Hinton & Eversole, 1980) and 4.5 mg l^{-1} (Tucker, 1987).

Therapeutic index. The therapeutic index was qualified as a safety factor for an applied drug. It is often calculated using the quotient between the lethal concentration (LC50) and effective therapeutic concentration (EC50). It is advisable for the therapeutic index to be 4 or more. A chemical with a high index can presumably be administered with greater safety than the one with a low index, which may allow prolonging of the exposure time to the chemical without affecting the fish (Fajer-Avila *et al.* 2003). In our study, we calculated therapeutic index for commonly used treatment substances in fishery, i.e. for formaldehyde (in *P. reticulata* it was 4.9 and in *D. rerio* 5.24), for sodium chloride (in *P. reticulata* it was 1.4 and in *D. rerio* 1) and for potassium permanganate (in *P. reticulata* it was 2 and in *D. rerio* 1.5). It could seem that according to our therapeutic index the safest drug is formaldehyde. Nevertheless, the formaldehyde baths could have negative effect after longer periods of time (Kouril *et al.* 1984). Both in potassium permanganate and sodium chloride it is known that the lethal concentrations are close to the effective ones. Hence it is important to conduct the preliminary tolerance test on a small sample of fish every time before the preparation of treatment baths.

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