ACUTE TOXICITY BIOASSAY AND BEHAVIOURAL RESPONSES INDUCED BY SODIUM FLUORIDE IN FRESHWATER FISH *PUNTIUS SOPHORE* (BLOCH)

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SUMMARY: Acute toxic effects and behavioural alterations induced by exposure of the freshwater fish *Puntius sophore* (Bloch) to sodium fluoride (NaF) are reported. Ten healthy specimens of the same size in each group were exposed to seven concentrations of NaF (120, 122, 124, 126, 128, 130, and 132 mg/L) along with a control group. After 96-hr exposure at a water temperature of 17.9–18.6ºC, their behavioural patterns including swimming and surfacing activity, opercular movements, and feeding habits were observed. Alterations in behavioral patterns were well marked during the period of the experiment. The 96-hr LC_{50} value of NaF to *Puntius sophore* was found to be 126.12 mg/L.

Key words: Acute toxicity; Behavioural alterations; Freshwater fish; NaF 96-hr LC_{50}; *Puntius sophore* (Bloch); Teleost fish.

INTRODUCTION

The toxic effects of elevated levels of fluoride (F) on various aquatic species are well documented.[1,2] For example, the toxicity of F on rainbow trout has been shown to depend not only on the concentration of F but also on the pH, hardness and temperature of the water, and the presence of ion exchange minerals.[3] As expected, F toxicity on fish depends very much on increasing F concentration in the aquatic medium, exposure time, as well as water temperature and uptake of F directly from water or indirectly via food.[4,5] F causes adverse biological effects such as changes in carbohydrate, lipid, and protein metabolism, reproduction, impairment, reduced embryonic and development of life stages, and alteration of size and growth in fish[6] and is therefore a potent hazardous pollutant to fish.[7]

In the present communication we report results of a study of acute toxicity and behavioural responses induced by F in freshwater teleost, *Puntius sophore* (Bloch), using NaF as the compound often used for investigating F toxicity in fish.[8]

MATERIALS AND METHODS

Young specimens of *Puntius sophore* (Bloch) weighing 10.9±0.67 g, and 6.82±0.22 cm in length were selected for the experiments. The fish were collected from the Gwalior Tighra Reservoir in Madhya Pradesh, India, and were transported to the laboratory in large plastic containers filled with reservoir water to minimize stress and mortality. The fish were checked for disease as well as injury, if any. They were treated with 0.1% $KMnO_4$ solution and acclimatized to standard laboratory condition for 20 days in de-chlorinated tube-well water contained in a large aquarium with proper aeration. During acclimation the fish were fed with Tokyu special fish food from M/S. Discus, Japan. No mortality was observed during the period of acclimatization. Analytical reagent grade NaF

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(99.0% minimum assay), manufactured by Qualigens Fine Chemicals Limited, Mumbai, India was used as the test compound.

Determination of median lethal concentration: A stock solution was prepared by dissolving 1.00 g of NaF in one L of distilled water. Test concentrations were prepared by diluting appropriate aliquots of the stock solution with tap water essentially free of F. Physico-chemical characteristics of the test solution were determined according to standard procedures.[9,10] The exploratory range of acute toxicity concentrations was determined by conducting a series of range finding experiments. Thereafter, a definitive acute toxicity bioassay was conducted by exposing the fish to seven different concentrations of NaF, i.e., 120, 122, 124, 126, 128, 130, and 132 mg/L in 20-L aquaria along with one control. The control group was kept in tap water without addition of NaF, keeping all other conditions the same. The bioassay was conducted in a semi-static system in three sets, with 10 specimens exposed per concentration per set, following standard procedures for determination of acute toxicity bioassay.[10] During the experiment, the water in the aquaria was changed every 24 hr to maintain the appropriate concentration of F in the test solutions. The experiments were conducted under the natural 12-hr photo period for 96 hr with no food being given to the fish during the period of study. Mortality was recorded at 24, 48, 72, and 96 hr of exposure. Fish were considered dead when there was no visible movement (e.g., no opercular motion), and touching the caudal peduncle produced no reaction. The dead fish were removed from the aquaria as and when noticed. After the 96-hr study period, food was supplied to observe the feeding response. Behavioral toxicity effects were recorded when they exceeded the normal range of variability.[11]

Data analysis: The acute toxic effect was determined as LC_{50} values using SPSS version-6.02, 2008[12] and the Trimmed Spearman-Karber Method version-1.5.[13]

RESULTS AND DISCUSSION

The physico-chemical properties of the aquaria test media during the experimentation were monitored and are given in Table 1. These parameters were fairly constant throughout the study.

Table 1. Physico-chemical properties of test water during exposure of freshwater fish *Puntius sophore* to NaF

Test	Parameter	Unit	Range	(±SEM)
1	Ambient temperature	°C	19.2–20.2	19.94±0.30
2	Water temperature	°C	17.9–18.6	18.33±0.07
3	pH	-	8.6–8.8	8.7±0.02
4	Conductivity	µS cm^{-1}	582.4–660.8	624.4±9.40
5	Dissolved oxygen	mg /L	8.10–8.92	8.72±0.11
6	Total alkalinity	mg /L as $CaCO_3$	100–120	113.75±3.09
7	Total hardness	mg /L as $CaCO_3$	284–302	291.25±1.89

Behavioural changes: Table 2 records the behavioural responses including body position, habit, opercular movement, food sensitivity and swimming movement.

Table 2. Behavioural changes in *Puntius sophore* after exposure to various concentrations of NaF.

Test	Parameter	Control group	F-exposed experimental groups
1	Body position	At bottom of aquaria during rest	Mostly swimming near the upper surface of water
2	Habit	Calm quiet	Active, mostly swimming
3	Operculum movement	30–35/ minute	45–48/ minute
4	Food sensitivity	Slow response	Full response to the food, fast capture food and settle down to the bottom
5	Swimming movements		
I	Normal condition	Horizontal and slow	Mostly vertical
II	At time of exposure	Slowly come to the surface of water	Actively come to the surface to catch the food by vertical swimming
III	At time of feeding	Slowly come to the surface of water	Actively come to the surface to catch the food by vertical swimming

*Experimental groups include exposure to various test concentrations of NaF.

During the 96-hr study period, the exposed fish were more active and restless compared to the control group. By contrast, the control fish were calm and quiet and preferred to confine themselves to the bottom of the aquarium, whereas the exposed fish were found hanging most of the time in a column of water. In regard to swimming movement, the control fish swam horizontally and slowly compared to the treated fish that swam in a slanting manner from a lower level to an upper level, and sometime they jumped above the water. At the end of the 96-hr F exposure, food intake was faster in the F group than in the control group. Opercular movements were slightly greater in the exposed fish than in the control fish. Thus, behavioural changes in feeding activity, jumpy and erratic swimming movements, orientation of body, opercular motion, and surfacing activity were observed when the fish were exposed to various F concentrations. In the F exposed fish, these swimming activities were found to increase in comparison to the control group (Table 2). These observations are well supported by earlier findings from other toxicity studies,[14,15] including a mostly vertical swimming pattern toward the surface compared to controls of fish.[16]

Mortality findings: Median lethal concentration values of Na F with 95% confidence limits at various time points to the *Puntius sophore* are given in Table 3. The LC_{50} values were estimated by SPSS and Trimmed Spearman-Karber methods. Both methods gave similar values at all the time points. Mortality at various time points after exposure to NaF shows a proportional relationship between percentage mortality of fish and NaF concentration (Figure 1 A–D).

Table 3. LC_{50} values at various time points with 95% confidence limits (in parentheses) for NaF, estimated by SPSS and Trimmed Spearman-Karber methods

Method	Estimated concentrations LC_{50} (mg /L) (95% confidence limits)			
	24 hr	48 hr	72 hr	96 hr
SPSS	133.85 (131.48-138.22)	129.60 (128.07-131.13)	128.47 (127.33-129.41)	126.12 (124.03-127.28)
Trimmed Spearman-Karber	Not calculable	Not calculable	128.07 (162.37-129.79)	126.76 (125.57- 127.96)

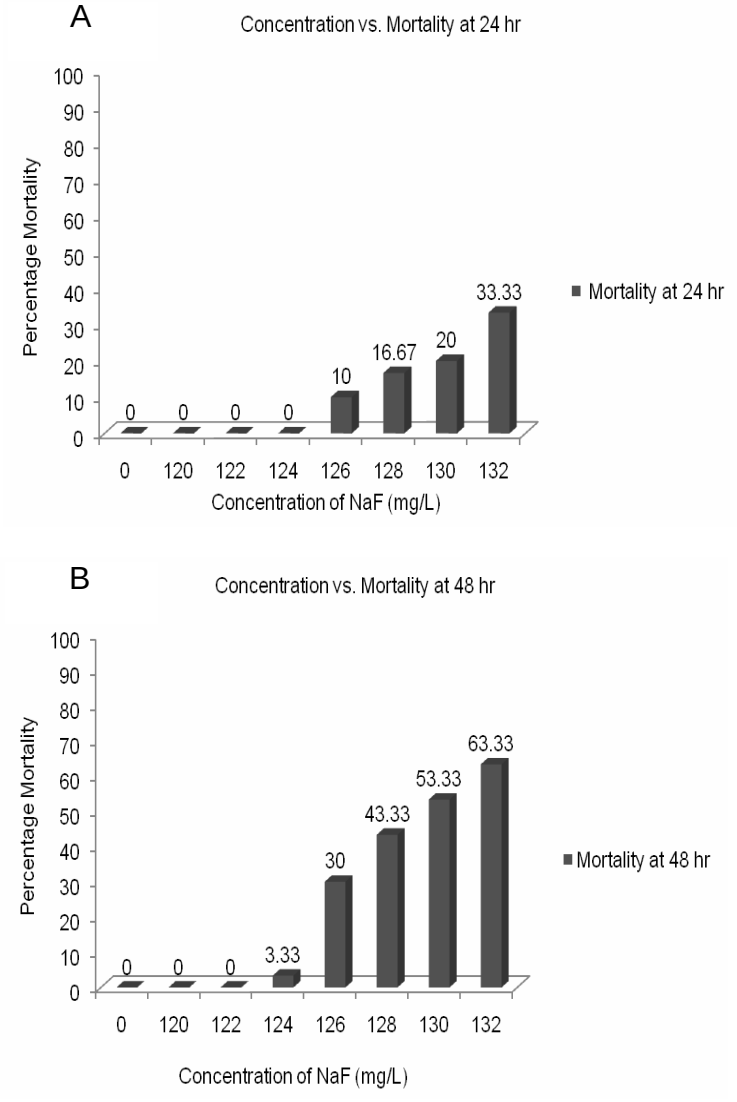

Figure 1 (A–B). Mortality of *Puntius sophore* at various time intervals after exposure to NaF. Figures show proportionate relationship between percentage mortality of fish (n=30) versus NaF concentration after 24, 48, 72, and 96 hr of exposure.

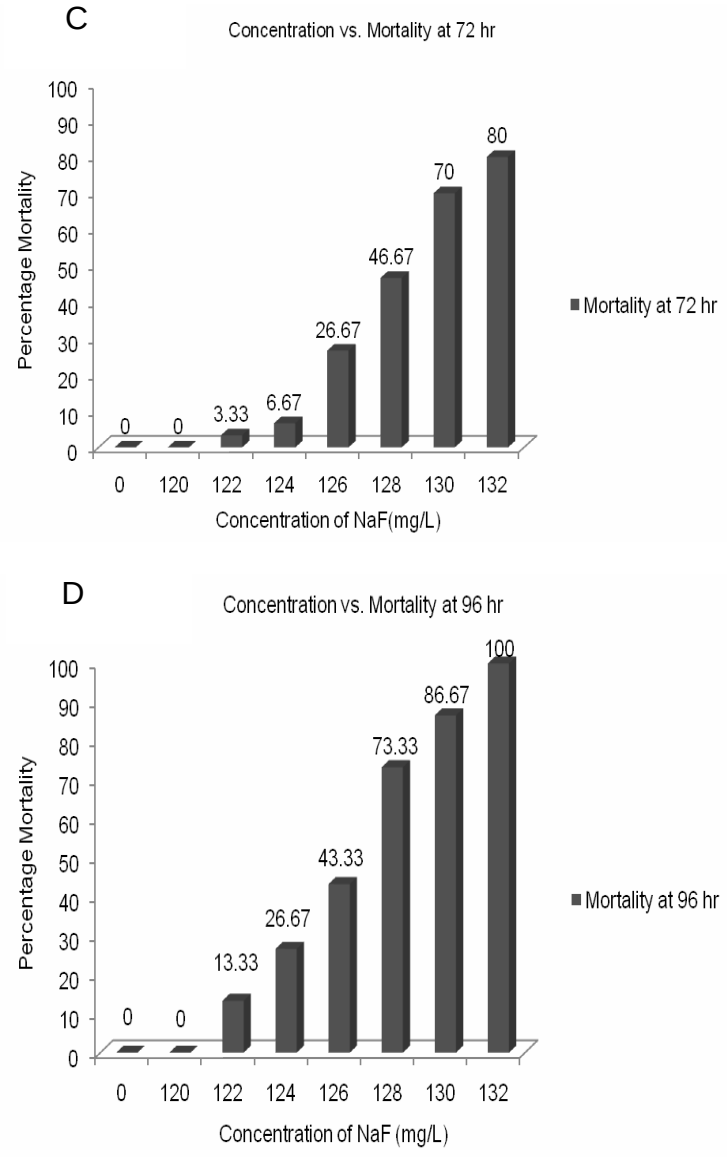

Figure 1 (C–D). Mortality of *Puntius sophore* at various time intervals after exposure to NaF. Figures show proportionate relationship between percentage mortality of fish (n=30) versus NaF concentration after 24, 48, 72, and 96 hr of exposure.

The variation in toxicity of a toxic substance to different kinds of fish is attributable to specific characteristics of fish such as variations in size and weight, sex, and maturity, species biological behaviour, and physico-chemical conditions of ambient water. In the literature, highly variable 96-hr LC_{50} values have been reported for F in different species of fish. In rainbow trout a 96-hr LC_{50} value of 107.5 mg/L at a water temperature of 15.3±0.22ºC was recorded for NaF, and in brown trout it was 160.5 mg/L at 16.1±0.13ºC.[8,17] In tilapia (*Oreochromis mossambicus*), the 96-hr LC_{50} of NaF was estimated to be 54.0 mg/L,[7] whereas, in *Labeo rohita*, this value was recorded as 935 mg/L at a water temperature of 18–30ºC.[18] Moreover, rainbow trout and other species of freshwater fish may withstand higher F concentrations in hard water than in soft water.[2,19] In rainbow trout a 96-hr LC_{50} figure of 51.0 mg NaF/L at 17 mg $CaCO_3$ mg/L was reported

for 58-mm long specimens. In this connection, it should be noted Ca tends to bind F ions in blood and bone.[2]

Here the toxic effects of NaF on the freshwater fish *Puntius sophore* appear to be the result of F entry into the soft-tissue parts of the fish body, but with greater accumulation occurring in osseous tissues.[3] Although different species of fish differ in their sensitivity to environmental pollution,[20] it is clear that F can exert adverse effects on fish.

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