



## Determination of Median Tolerance Limit(LC50) Of sodium fluoride For *Clarias batrachus* (Linn.).

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### Abstract:

The aim of present study was to determine the LC50 value of sodium fluoride in *Clarias batrachus*. Experimental fishes were procured from fish markets/fish ponds, which were measured an average length  $16 \pm 4$  cm and weight  $28 \pm 5$  g. Thereafter they were kept in sterile aquaria and fed with fish meal @ of 4% body weight of fish. A similar static test was conducted by taking a series of 6 concentrations of sodium fluoride, ranging from 1.0 to 10.0 mg/l in order to make fairly precise estimation of the LC50 values for *C. batrachus*. The test was performed for 96 hours at  $26 \pm 1^\circ\text{C}$  with a batch of 10 acclimated fishes in glass aquaria containing 20 liters of water with artificial aeration. Parallel controls were run under identical conditions. The test solutions were kept unchanged throughout the duration of experiment. Mortality of fishes was recorded every 24 hours (24h, 48h, 72h and 96h) for each test concentration. During experimentation, dead fishes were removed as quickly as possible. Finally, percentage mortality was calculated. The 96h LC50 value was then calculated using regression analysis as well as by straight line graphical interpolation.

**Keywords:** LC50; sodium fluoride; Catfish; *Clarias batrachus*, Toxicity, Graphical interpolation.

### Introduction

Fluorine (F) is the most reactive nonmetal and the most electronegative element, and therefore almost never occurs in nature in its elemental state; it combines with all elements, except oxygen and the noble gases to form fluorides. Sodium fluoride appears as an odourless white powder or colourless crystals, with a water solubility of 4% at 150C and a pH (saturated solution) of 7.4. Hydrogen fluoride or hydrofluoric acid is a colorless gas or fuming liquid with strong irritating odor, highly corrosive, very soluble in water and with a vapor pressure of more than 1 atmosphere. Odor detectable limits are 0.033–0.1333 mg/m<sup>3</sup>, while the irritating concentration is 4.17 mg/m<sup>3</sup>.

Fluoride is found in insecticides, rodenticides, floor polishes, in the petroleum and aluminium industries, glass etching and timber preservation and in dietary supplementation and toothpastes (up to 1 mg/g of toothpaste), and it is added to water supplies.

To determine the relative toxicity of a new chemical to organisms, an acute toxicity test is first conducted to estimate the median lethal concentration of the chemical in the medium to which replicates of test organisms are exposed. The median lethal concentration (LC50) is the concentration of a harmful chemical, generally in aqueous solution, which can be expected to cause the death of 50% of a specified population of organisms under a defined set of experimental conditions.

The purpose of this test is to determine the acute lethal toxicity of a substance to fish in soft water. It is desirable to have information on the water solubility, chemical stability and biodegradability of the substance to help in the selection of the most appropriate test method (Static, semi-static or flow-through) for ensuring statistically constant concentrations

of the test substance over the period of the test. In the present test, acute lethal toxicity is expressed as the median lethal concentration (LC50) in water which kills 50% of a test batch of fish within a continuous period of exposure. All concentrations of the test substance are given in weight by volume (milligrams per liter).

The median lethal concentration (LC50) which is very significant in toxicological research is usually determined by the probit analysis.117,118. Hamilton119have critically analyzed this method and described the limitations. Acute toxicity test is another method used to estimate safe concentration of toxicants in the environment, although they provide little information on the mode of action of the chemical. In general, research in this direction has received very little attention by toxicologists. By combining physiological, biochemical and histological studies with more acute and chronic toxicity test, it is possible to evaluate the mode and site of action of the toxic chemical as well as the determination of environmentally safe concentrations of toxicants.

From LC50 value test one can understand the survivance, mortality and behaviour of fish exposed to the toxicants for a definite period of time at a particular temperature. The LC50 values are dependent on the size and weight of the animal. In order to select sublethal doses of sodium fluoride for demonstration of histological, biochemical and physiological effects under acute and chronic toxic conditions, determination of LC50 value is absolutely necessary.

### EXPERIMENTAL PROTOCOL:

Materials and Method:

Chemicals: Sodium fluoride (Merck India Ltd)

Maintenance of fish:

Healthy fish *C. batrachus* of size (15-20 cm) and weight (50-60 gm) were collected from

local market. They were brought to the laboratory and disinfected by the treatment with 0.01% potassium permanganate solution for 1-2 min. The fish were then acclimated in the laboratory conditions ( $26 \pm 2$  °C) for 15 days in glass aquaria (18" X 12" X 12"). The fish were exposed to natural photoperiodism i.e., 12L:12D photoperiod. The pH and dissolved oxygen content of the water were  $7.0 \pm 1$  and  $7.5 \pm 0.5$  ppm respectively. The water used was tap water filtered by Dee Bee Filters (India) to remove excess of iron. The entire experiment was conducted at a controlled room temperature of  $25 \pm 2$  °C. During acclimation, water was changed every day to discard the metabolic waste products. Dry foods are available as granules which were given as food and were starved for one day prior to transfer into the toxic environment for test.

#### **Principle of the test:**

In determining LC<sub>50</sub> values, three types of procedure can be used: (a) static test, (b) semi-static test, (c) flow-through test. In static test, no flow of test solution occurs. Solutions remain unchanged throughout the duration of the test. Semi-static test is without flow of test solution, but with regular batch wise renewal of test solution after prolonged periods (e.g. 24 hr). In flow through test, water is renewed constantly in the test chambers. The chemical under test being transported with the water is used to renew the test medium.

At first a preliminary test is performed by taking wide range of test concentrations i.e. doubling the amount. The fish are exposed to the test substance added to water at a range of concentrations for a period of 96 hours. Mortalities are recorded at least at 24 hr. intervals. Then to get a fairly precise estimation of LC<sub>50</sub> value, at least five test concentrations are taken differing by a constant factor and as far as possible spanning the range of 0 to 100% mortality. Dead fish are removed when observed and mortalities are recorded. Records are kept of visible abnormalities (e.g. loss of equilibrium, changes in swimming behaviour, altered respiratory function, pigmentation, etc.). Measurements of pH, dissolved oxygen and temperature are carried out daily.

#### **Static Bioassay Method:**

The method of Doudoroff was followed for the determination of 96 hr LC<sub>50</sub> values. The chosen test concentrations were prepared by diluting the test substance, sodium fluoride, in distilled water, in which, the test fish were exposed to observe their reactions for a definite time period.

#### **Preliminary exploratory test:**

Small scale preliminary exploratory tests were made with 5 fishes in each glass aquaria containing 10 liters of water. Test solutions were made over wide range of concentrations, i.e. 0.5, 1.0, 2.0, 5.0 and 10.0

mg/l. The experiments were conducted by supplying oxygen artificially through air pump. The test concentration 10.0 mg/l caused 100% mortality of the test fish within 48 hours. There was no significant change or mortality in 0.5 mg/l. Even upto 60% mortality was observed at 5.0 mg/l after 96 h exposure. These observations over a short period indicated the test concentrations necessary for full scale experiment.

#### **Full scale experiment:**

A similar static test was conducted by taking a series of 6 concentrations of sodium fluoride, ranging from 1.0 to 10.0 mg/l in order to make fairly precise estimation of the LC<sub>50</sub> values for *C. batrachus*. The test was performed for 96 hours at  $26 \pm 1$ °C with a batch of 10 acclimated fishes in glass aquaria containing 20 liters of water with artificial aeration. Parallel controls were run under identical conditions. The test solutions were kept unchanged throughout the duration of experiment. Mortality of fishes was recorded every 24 hours (24h, 48h, 72h and 96h) for each test concentration. During experimentation, dead fishes were removed as quickly as possible. Finally, percentage mortality was calculated. The 96h LC<sub>50</sub> value was then calculated using regression analysis as well as by straight line graphical interpolation.<sup>121</sup>

#### **RESULT:**

##### **Dosage-mortality studies:**

The dosage-mortality studies were conducted for 96 hours. The percent mortality for *C. batrachus* at different doses of sodium fluoride was recorded. No mortality seen in 1.0 mg/l, while 100% mortality was found at 10.0 mg/l within 96h exposure period. 20% fish mortality was observed upto 2.0 mg/l. In 4.0 mg/l, 40% mortality was seen. Whereas, 50% mortality was recorded at 5.0 mg/l and in 8.0 mg/l, 80% mortality was observed (Table 1.1). The concentration causing 50% mortality was determined by regressing percentage mortality (y-axis) against concentration of sodium fluoride in mg/l (x-axis) and obtaining a linear regression curve. The median lethal concentration (LC<sub>50</sub> value) of sodium fluoride for 96 h was found to be 5.0 mg/l for the Indian catfish, *Clarias batrachus*. (Fig.1.2)

##### **Behavioural responses:**

Fishes after being exposed to different concentrations of sodium fluoride showed abnormal behavioural responses followed by mortality. On transferring fishes to respective aquaria, initial restlessness was observed for few minutes both in control and treated groups. The control fish, after settlement for few minutes, underwent its normal swimming activity, while the exposed fish of variable concentrations showed increased rate of opercular movement for few hours of exposure. As the exposure time increased, the rate of opercular movement decreased than normal, probably due to reduced metabolic activities.

Meanwhile, swimming activity along with surfacing activity (air gulping) were reduced in the first 24 hr and gradually declined till the end of experiment. Sometimes they showed erratic movements and loss of buoyancy with occasional jerking of their bodies. Finally the fish subsided to bottom of the aquaria showing lack of sensitivity to the external stimulus. Apart from this, few assumed a diagonal to vertical position in the water with the head directed towards the surface, indicating loss of equilibrium.

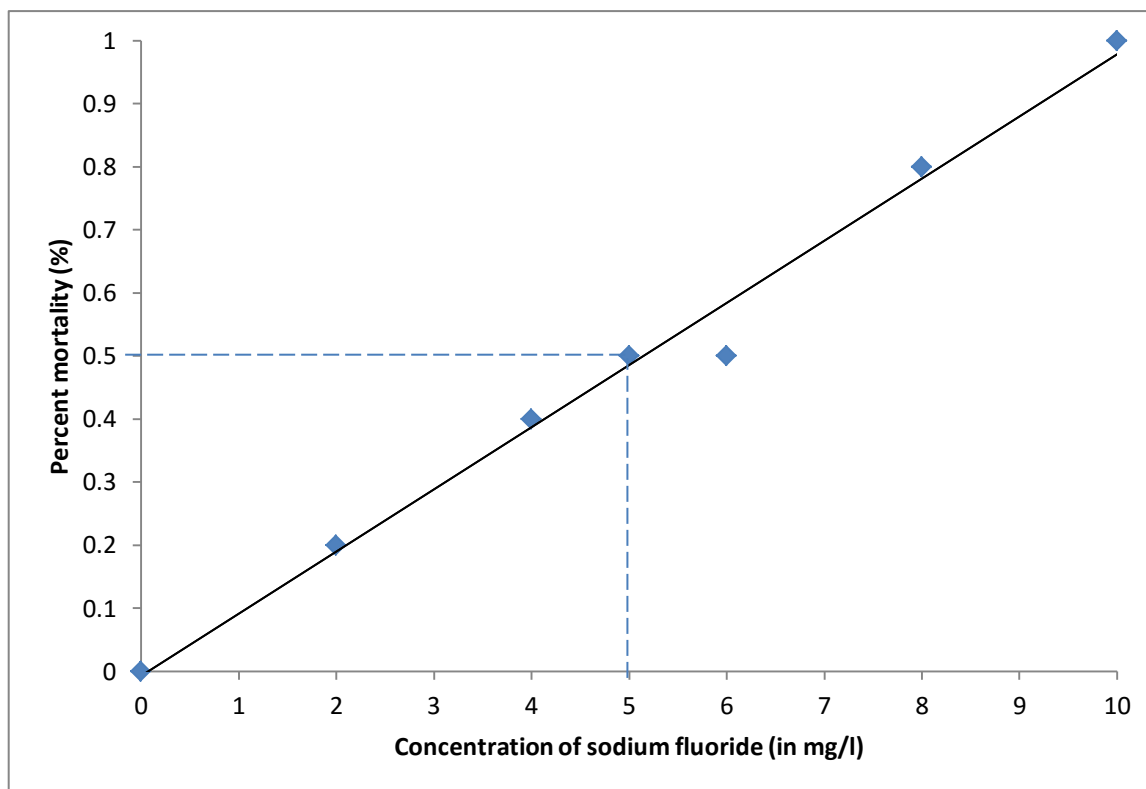
The behavioural response like feeding activity was totally depressed in the higher doses of sodium fluoride exposure, while this

activity declined in other sub lethal doses. Also there was excessive mucus secretion in the fishes, when exposed even to a lower concentration of toxicants and the mucus increased with the increased dose of sodium fluoride. Furthermore, fish of treated groups exhibited altered body pigmentation, being darker. The above behavioural changes were more pronounced with the increased concentration of sodium fluoride.

Percentage mortality of *Clarias batrachus* (Linn.) exposed to various concentrations of sodium fluoride for a period of 96 hours.

Concentration of sodium fluoride (in mg/l)	No. of fishes exposed	No. of fishes dead	Percent mortality (%)
0	10	0	0.0
2	10	2	0.2
4	10	4	0.4
5	10	5	0.5
6	10	5	0.5
8	10	8	0.8
10	10	10	1.0

**Fig1.2.** Estimation of median lethal concentration (LC<sub>50</sub>) of 96h exposure under Sodium Fluoride toxicity in *clarias batrachus* (Linn.)



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