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TOXICITY OF METHYLMERCURY TO AN INDIGENOUS AIRBREATHING FISH, *ANABAS TESTUDINEUS* (BLOCH, 1792)

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Abstract: The objective of the present work was to deduce the toxicity of methylmercury, as median lethal concentration in freshwater climbing perch, *Anabas testudineus*. Range finding tests were conducted to determine the range of concentrations needed to be utilized in the final exposure experiments. In the final trials, mortality of fish was recorded at 24, 48, 72 and 96 hours of exposure. After 96 hours of exposure the LC_{50} value of MeHg was observed at 0.09 ppm.

INTRODUCTION

Among the heavy metals, mercury is one of the most dangerous due to its high toxicity, bioaccumulative properties, abilities to react with proteins and to pass through most of the tissue barriers in the living organisms and as the causative agent for genetic alteration or mutagenesis (WHO, 1990). The organic compound, methylmercury (MeHg) has been classified by the International Agency for Research on Cancer (IARC) as a group 2B substance, that has a tendency to be carcinogenic (Hallenbeck, 1993). Toxicity test is conducted to estimate the concentration at which a response is elicited by an animal to a foreign chemical under controlled conditions. These are routine tests conducted during toxicological studies and are also called bioassays. The endpoint for the test is death. The concentration of the toxicant at which 50% of the test population dies is defined as the Median Lethal Concentration (LC_{50}) (Eisler, 1987). The test organism used in this experiment is an air breathing freshwater fish, *Anabas testudineus*, common to the freshwater resources in Kerala. It is an indigenous teleost belonging to the Family Anabantidae in the Order Perciformes of the Subclass Actinopterygi. It is capable of surviving for about 6 hours out of water by the help of accessory organs called rosettes (Hughes and Singh, 1970).

There have been many studies on the effect of mercury on various fishes throughout the world. The studies have been concentrated on the effects of the inorganic salts of mercury (Guedenon *et al.*, 2012; Vutukuru and Basani, 2013) and on methylmercury chloride (Akter *et al.*,2008) with the test fish ranging from the temperate freshwater species as well as the highly prized commercial species. Very few work has been conducted in India and the ones done are on the Indian major carps (Shrivastava *et al.*, 1988) and catfishes (Rani *et al.*, 2011 and Dhara et al., 2014) commonly cultivated. This study tries to narrow the lacunae in the field regarding the effect of an organic salt of mercury on an Indian airbreathing fish having a good aquaculture potential.

MATERIAL AND METHODS

Laboratory Conditioning

The fishes were collected from Pulimugham Hatcheries, Alappuzha, Kerala and transported to the laboratory in oxygen filled polyethene bags. On arrival at the laboratory, they were acclimatised in FRP tanks (1000 litres) in tap water in which mercury was not detected (pH of 7.2 ± 0.2, temperature at 27 ± 1.27 ^{0}C, continuous aeration, natural photoperiod). Commercial fish feed was provided *ad libitum.* The water was exchanged every 3 days to prevent build-up of ammonia. Fishes were conditioned for 21 days

before being used for the experiment. Individuals measuring 7.066 ± 0.70 cm in total length and weighing 6.48 ± 2.112 g were randomly selected for the study.

Test Chemical

The chemical in the form of its iodide salt (MeHgI) of 98% purity was procured from Alfa Aeasar, England. Stock solution of 100 ppm was prepared from the salt which was diluted as needed for the experiment.

Definitive Test

Static renewal tests were undertaken to study the toxicity of methylmercury to the test organism (APHA, 2012). The acclimatised fish of uniform length and weight were selected to assess the median lethal concentration of the toxicant. Four concentrations (0.0375, 0.075, 0.15, 0.3 ppm) were used in duplicate with 6 fishes each in 10 litres of test solution. Control in duplicate were also run simultaneously. Fish were not fed during the 96 hours of experimental period and were left undisturbed. The responses along with the mortality was recorded every 6 hours, and the dead fishes were removed. Cessation of visible movements including gills and loss of reaction on application of external stimuli to the caudal peduncle were considered signs of mortality following OCED (1992). The median lethal concentration (LC_{50}) was calculated with Probit Analysis (Finney, 1978). The behaviour of the fish used in the experiment was analysed and the changes caused by the introduction of the toxicant used were recorded.

RESULTS

Table 1 shows the median lethal concentration of methylmercury on *A. testudineus*. From the probit analysis, the 96h LC_{50} was observed to be 0.09 ppm. In the range finding test, the higher concentrations showed increased mortality. Fish in the experimental tanks exhibited abnormal behaviour. At the beginning of the experiment, the fishes stopped swimming and remained motionless to the changes in the ambient environment which was followed by vigorous swimming and jumping. Opercular movement became rapid along with surfacing and gulping of air. In tanks with higher concentrations of test chemical, the fishes swam erratically. On exhaustion, they remained in a vertical position for a few minutes, with mouth up at the surface of water, gulping the air and the tail in a downward direction before settling to the bottom of the tank with their bellies turned upward.

From the graph (Fig. 1), it can be clearly seen that the higher concentration of methylmercury leads to increased mortality in the experimental fish.

DISCUSSION

The manner of death with the positioning of the body vertically as well as exhaustion, lethargy and finally settling at the bottom of tank with bellies upturned was recorded in *Esox lucius* (Rahimibashar and Alipoor, 2012). These are indications of body function impairments like enzyme inhibitions, delay and disruptions in neural transmission, blockage of nervous transmission between the nervous system and effector sites, along with disturbances in metabolic pathways (Rand, 1985). Similar behavioural changes with some degree of variations were reported by researchers in fishes like *A. testudineus, Danio danio, Clarias batrachus* and *C. gariepinus* on exposure to mercury (Akter *et al.*, 2008; Rani *et al.*, 2011; Guedenon *et al.*, 2012; Vutukuru and Basani, 2013). The control fishes remained active and calm throughout the experimental duration whereas the

Table 1. Determination of the 96h LC_{50} of methylmercury in *Anabas testudineus*

Concn. MeHg (ppm)	No. of fishes dead			No. of fish in each trial	Mean % mortality	Log conc (x)	Probit value (y)
	Trial 1	Trial 2	Control				
0	0	0	0	6	0	0	0
0.0375	0	1	0	6	8	-1.43	3.59
0.075	1	2	0	6	42	-1.13	4.8
0.15	3	2	0	6	67	-0.83	5.44
0.3	6	6	0	6	100	-0.53	8.09

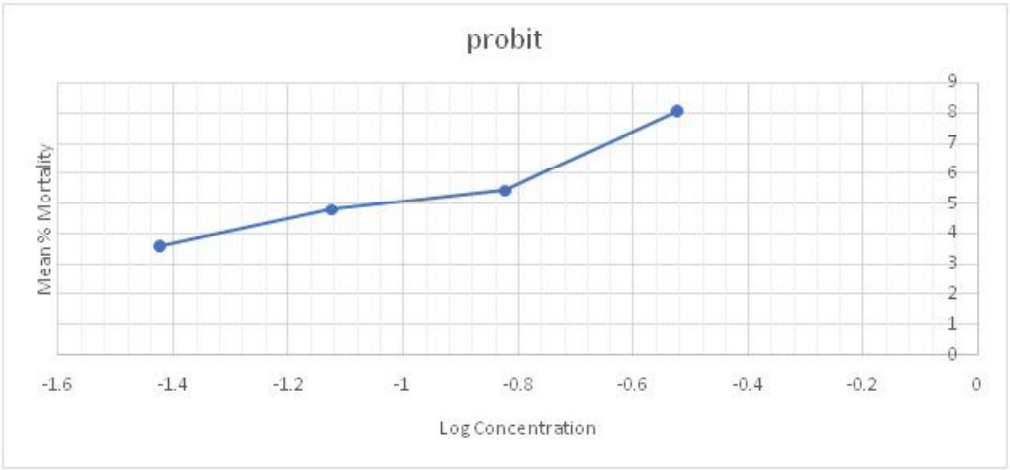

Fig. 1. Graph detailing the LC_{50} of methylmercury on *A. testudineus*

treated fishes were hyperactive initially and then showed restlessness. Erratic and fast swimming as well as tendency to jump out of the medium were observed when the concentrations were increased. The progress of time and increase in the concentration causes the fish to exhibit sluggish movement and to cease swimming. Fish also showed the signs of stress like difficulty in breathing by gulping of air at the surface at higher toxicant concentrations. These were in conformity with the observations of Rani *et al.* (2011) and Dhara (2014) on *C. batrachus*.

The toxicity of mercury varies widely among organisms including fish. Different sensitivities for different species can be attributed to physiological differences and species-specific effects or due to changes in the test chemical and methods applied, test species used, their habitat and diet, age, sex and size or even their metabolic rates or it could be due to the water quality of the test medium like pH, presence of sulphur or dissolved organic matter or carbon (Hodson *et al.*, 1982; McCahon and Pascoe, 1988; Kaviraj and Das, 1990; Boening, 2000; Gooley *et al*., 2006; Akter *et al.*, 2008; El-Moselhi *et al.*, 2011; Dhara *et al.*, 2013). These observations stand true for the present study as the LC_{50} value for *A. testudineus* (0.09 ppm) is found to be about 3 times lower than that reported by Rajan and Banerjee (1991) for *Heteropneustes fossilis* (96h LC_{50} of $HgCl_2$ was 0.300 ppm), Khangarot (1981) for *Channa marulius* (96h LC_{50} of $HgCl_2$ was 0.314 ppm) and almost 7 times lower than the same observed for *A. testudienus* by Akter *et al.* (2008). According to Vutukuru and Basani (2013) the 96h LC_{50} value of mercury in case of *Danio rerio* was 0.077 mgL^{-1}. Shrivastava *et al.* (1988) found an LC_{50} of 50 μgL^{-1} in *Cyprinus carpio* and *Cirrhinus mrigala* has an LC_{50} of 100 μgL^{-1}. In case of *Clarias batrachus*, the 96h median lethal concentration (LC_{50}) value of mercury is 0.698 mgL^{-1} suggesting that the metal was highly toxic to the fish (Dhara, 2014). This difference in the toxicity could be due to the differences in fish species, size as well as the difference in the chemistry of the toxicant. Moreover, fresh water perch is considered to be hardier than freshwater catfish, snakeheads and most of the fish species used in toxicological experiments (Akter *et al.*, 2008). In the present study higher mortality percent was observed in relation to increase in toxicant concentration and exposure duration. Similar observation was reported in *Varicorhinus barbatulus* and *Zacco barbata* (Shyong and Chen, 2000), *Anabas testudineus* (Akter *et al.*, 2008), *Oreochromis niloticus* (Kaoud and Mekawy, 2011), *Clarias batrachus* (Rani *et al.*, 2011) and *Mugil cephalus* (Rajkumar, 2013). Thus, it can be concluded that mercury effects fish mortality directly and methylmercury is more toxic than inorganic salts of mercury to *Anabas testudineus.* Only fish mortality as an endpoint was investigated in this study and this result may be used for further studies into the sublethal effects of mercury to *A. testudineus.*

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