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Determination of Median Tolerance Limit (LC50) of *Clarias batrachus* for Cadmium Chloride and Mercuric Chloride

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Abstract

Industries are the major sources of heavy metal pollution and it is released into water and soil. Heavy metals cause several ill effects to aquatic living organisms and environment. The acute toxicity of chosen metals (Cadmium and Mercury) against fresh water *Clarias batrachus* (Cat fish) and the chronic toxicity of sublethal concentrations of heavy metals on the different organs such as liver, gill, muscle, kidney and brain of the chosen fresh water fish. Adult cat fish treated separately with Cadmium Chloride ($CdCl_2$) and Mercury Chloride ($HgCl_2$) for 24, 48, 72, and 96hr at different dose levels showed significant behavioral changes in the Lethal concentration were determined by probit analysis method. The LC 50 values for $CdCl_2$, $HgCl_2$ were found as 8.21ppm, 1.85ppm; respectively. Among the toxicants selected, $HgCl_2$ is more toxic than $CdCl_2$ in cat fish.

Key Words: : Lethal concentration, *Clarias batrachus*, Cadmium chloride, Mercuric chloride.

Introduction

In India, there has been a tremendous pace in the development and agricultural practices since independence with the progress in industrial development due to advancement of technology; environmental pollution has grown in alarming proportion. The problem of pollution of the water where the wastes are usually discharged has increased to a great extent in recent years. Aquatic life is strongly influenced by physical properties of a water body. It is known that heavy metals as well as agro-pollutants are potentially harmful to the aquatic lives. All pesticides applied for the pest control eventually pollute the water resources either in their original chemical form or in some degraded variety. On the other hand, all industries discharge their effluents indiscriminately in the adjoining water areas and frequently cause serious hazards to aquatic life. Among the aqua fauna, fishes are affected to a significant extent. There are ample reports dealing with mortality and pollution [1].

Short term toxicity tests had been designed for the estimation of lethality of the xenobiotics to the test specimens. The most widely adapted technique for short term bio assay is the evaluation of a Medium Tolerance Limit (TLM). Determination of 24, 48, 72 and 96h TLM value by probit analysis (static bio assay) procedure is generally applied to determine the toxicity levels of the xenophobic materials to various aquatic organisms under laboratory conditions [2]. These toxicity tests are necessary to predict the safe concentration of the chemicals in the environment [3].

TLM or Lethal Concentration$_{50}$ (LC_{50}) is the particular concentration of a chemical in the experimental water at which just 50% of the text organisms are able to survive for a specific period of exposure [4],[5]. Many investigators had accepted this method for the determination of acute toxicity[6], [7], [8] even in recent years for scientific, economic and ethical reasons the TLM test have been prescribed as one of the toxicity tests. APHA [4] suggested that the preferable duration for a toxicity test should be at least 24, 48, 72 and 96h. For the present study, the selected exposure period was 96 in the fish *Clarias batrachus* [9].

Materials and Methods

C. batrachus were obtained from the fish hatchery at (Tamil Nadu Fish Seed Farm) Poondi near by Thiruvallore. The fishes were transported from the Farm in oxygenated bags to the laboratory and immediately transferred into glass aquaria of 50L capacity containing well aerated, unchlorinated ground water. The water was discharged every day. The healthy fish that showed active movement were only used for the experimentation. Fishes weighing about 10-15g and measuring about 6-7 cm were acclimatized in the laboratory condition for one month. Fishes were feed with fish Food.

Two heavy metals Cadmium Chloride ($CdCl_2$), Mercuric chloride ($HgCl_2$) were chosen as xenobiotics for estimation of their lethality on *C. batrachus.* A batch of 10 adults, healthy disease - free laboratory acclimated individual of some age irrespective of sex, were released in plastic aquaria measuring 5L diluted water. The text fish were starved for 24, 48, 72, and 96h; Test period. Feeding tends to increase the metabolic activities such as rate of respiration, excretion and production of other waste products altogether affect the toxicity of the test solution, so food supply to the text specimen was avoided.

For the determination of 96h TLM for $CdCl_2$, $HgCl_2$ a set of 4 plastic aquaria were arranged for each chemical, containing 5L of water. After releasing the text individuals, different constructions of each chemical were added to respective aquaria very carefully and mixed thoroughly. Any change in behavior and other responses were carefully noted. The specific doses had been determined by trial and error method. The individuals were considered dead when they failed to respond to touch stimulus. After the death of each fish, its body was removed immediately from the containers along with water allotted for each fish. The aquaria water containing the compounds was renewed after each 24h keeping the concentration constant throughout the experiment. After 96h (the end of the test), survived individuals in each container were counted. Determination of 96h TLM values of $CdCl_2$, $HgCl_2$, in adult *C. batrachus* had been done by the Finney [8] method of probity regression analysis. Percentage of mortality in staticbio assay was converted into probit mortality following the method of Doudoroff *et al* [10].

Fish exposed to different doses of pollutants displayed marked behavioral changes. Fish exposed to sub lethal doses of the test chemicals exhibit cessation of all movements and became very sluggish. Toxicity is a relative property of a chemical which refers to its potency to induce harmful effects on an organism. It is a function of concentration of the toxicant and the length of exposure of the animal [11]. Fish exposed to lethal doses of the xenobiotics showed drastic changes in the behavior. Respiratory upset, insensitivity to external stimuli, erratic and jerky movements along with a tendency to jump outside the convulsion were the general responses in all the treatments.

The toxic effects may include both lethal and sublethal concentration which may change the growth rate, development, reproduction, histopathology, biochemistry, physiology and behaviour [12].The determined 96h TLM values for $CdCl_2$, $HgCl_2$ were 8.51 ppm and 1.81 ppm, respectively (Table 1). The point of Intersection with the calculated 50% mortality was determined as the TLM value Fig. 1.

Result and Discussion

Table: 1 Acute Toxicity Test showing tolerance of *Clarias batrachus* to Cadmium

Exposure Period (h)	LC20 (ppm)	LC50 (ppm)	LC 90 (ppm)	Regression equation	Slope `S' function	95% confidence Limit		Chi-square Value	
						Lower Limit	Upper Limit	Calculated value	Table Value
24 h	8.31	8.56	8.95	Y=3.330+ (-28.53) x	5.130	8.25	8.35	0.345	5.99
48 h	7.97	8.29	8.79	Y = 2.85+ (-21.45) x	4.485	8.22	8.34	0.006	5.99
72 h	7.90	8.20	8.66	Y =2.784 +(-22.84) x	4.673	8.09	8.26	0.060	5.99
96 h	7.88	8.13	8.51	Y = 3.330+(-27.88) x	5.130	8.45	8.64	0.345	5.99

Table: 2 Acute Toxicity Test showing tolerance of *Clarias batrachus* to Mercury

Exposure Period (h)	LC20 (ppm)	LC50 (ppm)	LC 90 (ppm)	Regression equation	Slope `S'	95% confidence Limit		Chi-square Value	
						Lower Limit	Upper Limit	Calculated value	Table Value
24	1.61	1.86	2.25	Y = 3.330+ (-6.21) x	5.130	1.802	1.997	0.345	5.99
48	1.37	1.70	2.19	Y = 2.585+ (-4.39) x	4.480	1.652	1.771	0.006	5.99
72	1.27	1.59	2.09	Y = 2.585 + (-4.13) x	4.480	1.647	1.647	0.006	5.99
96	1.18	1.43	1.81	Y = 3.330+ (-4.77) x	5.130	1.497	1.497	0.345	5.99

In present investigation, it has been endeavored to evaluate the environmental risk of man made chemicals mostly focusing on Fish due to their infusion of trial and error methods applying acute and sub lethal exposures of $CdCl_2$ and $HgCl_2$ may be explained unsuitable stress. Further, metal pollution may make mercury shift in the community structure to metal tolerant organisms, which are often capable of accumulating large amounts of metals [13]. The changes in behavior might be due to hyperactivity of the neural mechanism, as well as, for hypersecretion of stress hormones secretary endocrine glands.

Studies on the median tolerance limits usually provide measures of relative toxicity of different chemicals and also the sensitivity of fish species, but not throw right on the maximum concentration to toxic materials which would not affect the metabolism of the fish. Tolerance of an organism is often measured in terms and of the level of lethal factor and length of time the animal survives.

The relationship between the logarithm of the water concentrations of the test toxicants ($CdCl_2$ and $HgCl_2$) and the logarithm of fish mortality [8] appear to be linear for all the treatments and accordingly the regression lines have been drawn detecting the TLM values (Fig. 1-2) by probit analysis method (Table 1 and 2). The toxicity tolerance values may vary in different size, weight and age groups of the organism in a stable environmental condition. The 96h TLM values for $CdCl_2$ (TLM 8.51ppm) than that of $HgCl_2$ (TLM: 1.81ppm). Metal ions and their complex exibit widening toxicity to the organism that ranges from sublethal to lethal depending upon the time of exposure and the prevailing conditions in the ambient water [14]. LC_{50} values differ from species to species for the same toxicant due to the mode of action and responses of the animals [15]. The toxicity tests have also been influenced by the size, age [16], sex [17] and the nutrient supply [18],[19],[20].The present study reveals that of the two toxicants under investigation the $HgCl_2$ has caused great harm to the fish. This is evidenced by the facet that a slight higher concentration of $HgCl_2$ in the medium causes death of a much higher percentage of fish.

The lack of proportionality associated with the xenobiotics is more pronounced in mercury. Possibly, Synthesis of the detoxifying proteins (Metallothionein etc) due to cadmium induction to combat its poisoning is seemed to be much more prominent than $HgCl_2$ (because $HgCl_2$ also has a specific detoxification system guided by the proteins like metallothionein or glutathione). On the other hand, the inhibitory effect of $HgCl_2$ and $CdCl_2$ prevailing on the enzyme system (controller or bioaccumulation and tissue metabolism of the xenobiotics),probably gets much more prominent than that of cadmium induction.

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