Agricultural Science Digest, Volume 41 Special Issue: 242-246 (April 2021)

Acute Toxicity Assessment of Mercury Chloride to Freshwater Air Breathing Fish *Clarias batrachus* (*Linnaeus*, 1758): *In vivo* Study

Khushbu Kumari, Gyanendra Bahadur Chand

10.18805/ag.D-5195

ABSTRACT

Background: Mercury is the most noxious heavy metal. Because of its environmental persistence and ability to be accumulated in the fatty tissues of aquatic organism, it poses serious threat to the fish community.

Methods: In the present study, freshwater air breathing fish *Clarias batrachus* were exposed separately to different concentrations of Mercury chloride ($HgCl_2$) for 24 hrs, 48 hrs, 72 hrs and 96 hrs. Firstly, median lethal concentration (LC_{50}) of $HgCl_2$ to fish was determined by Probit analysis and was confirmed by pilot test. The behavioral and locomotary changes were monitored.

Result: Test group showed random irregular and rapid swimming, restless activity. Along with these activities the test group became hyperactive, started jumping and changing their direction and position in an irregular manner. Some of the noticeable physiological symptoms during the initial stage of toxicity assessment were rapid opercular movement and frequent gulping of air. Both jerky movement and rapid respiratory response were reported to be occasional and slower in the longer duration exposure. Acute toxicity assessment provides first hand information to keep a check on pollution and to observe and track rigours of aquatic ecosystems.

Key words: Acute toxicity, *Clarias batrachus,* LC_{50}, Median lethal concentration, Mercury chloride ($HgCl_2$).

INTRODUCTION

Due to rapid industrialization or advancement of technologies and non judicious anthropogenic interventions, environmental pollution has reached up to an alarming rate. Heavy metals are considered as the most noxious pollutant. Heavy metals have the ability to accumulate in the fatty tissues of the aquatic organism by the process of bioaccumulation as they possess environmental perseverance due to their bioavaibility (Veena and Radhakrishnan, 1997). Heavy metal contamination has severe impacts on ecosystem that can severely damage the ecological balance and the quality of the environment. There are many reports supporting the fact that mortality is directly proportional to the pollution (Barak, 1955). Introduction of anthropogenic activity like waste disposal leads to deterioration of environment as well as destruction of environment (Farombi *et al*., 2007). World Health Organization has declared 10 metals of major public concern among which mercury is considered to have extremely toxic effects along with cadmium, arsenic and lead. There are negative effects on physiological functions of the fish living in the waters polluted by the metals. It is also stated that the fish which are exposed to metal pollution have weakened immune system and so the risk of catching contagious diseases and death increases (Ahmed *et al*., 2016).

The health of an ecosystem can be predicted by assessing toxicity of a particular xenobiotic on a particular group of organism. It also influences the screening of strategies for the protection and conservation of an aquatic system. Toxicity may be defined as the ability of individual organism's response to a natural or artificial dosage or chemical at a particular concentration at specific duration of time. The toxicity test helps us to determine the maximum permissible limit of the toxicants in an ecosystem by showing sensitivity for a particular chemical in a given period of time. All the toxicants are capable of severely interfering with the biological system altering metabolic and physiological activities of the organisms and ultimately affecting their survival. Acute toxicity test is one of the most pertinent toxicological tests to determine the concentration of the test material on a group of a test organisms during short term exposure under controlled condition. It is inexpensive as well as provides basic information about toxic impacts of toxicants on test organisms and also provides reproducible limits of the toxicants in a short duration of time (Spacie and Hamelink, 1985).

Mercury is released in the environment by several resources like mining, fossil fuel combustion, thermal power projects and by using fungicides, bactericides and pharmaceuticals (Khangarot, 2003). Mercury is an element

Aquatic Toxicology Laboratory, P.G. Department of Zoology, Patna University, Patna-800 005, Bihar, India.

Corresponding Author: Khushbu Kumari, Aquatic Toxicology Laboratory, P.G. Department of Zoology, Patna University, Patna-800 005, Bihar, India. Email: khushburanjan@yahoo.com

How to cite this article: Kumari, K. and Chand, G.B. (2021). Acute Toxicity Assessment of Mercury Chloride to Freshwater Air Breathing Fish *Clarias batrachus* (*Linnaeus,* 1758): *In vivo* Study. Agricultural Science Digest. 41 (Special Issue): 242-246. DOI: 10.18805/ag.D-5195.

Submitted: 20-04-2020 **Accepted:** 03-10-2020 **Online:** 29-01-2021

and has an obstinate nature that's why it can't be broken down, it can only change its form (Maheswaran *et al*., 2008). Mercury exists in three forms in nature-elemental mercury, inorganic mercury and organic mercury. Mercury chloride ($HgCl_2$) has been listed as a violent poison in the Merck's index (1996). It is corrosive in nature as it has been reported that GI tract is lost, if ingested orally. Aneugenicity and clastogenicity of mercury chloride are a probable mechanism which leads to a chromosomal genotoxicity (Bonacker, 2004; Cavas, 2008). Mercury enters the fish body through gills, skin and mostly absorbed through body surfaces. Among several techniques available for short term toxicity assessment, the determination of median tolerance limit (TLM) or median lethal concentration (LC_{50}) by probit analysis (statistical bioassay) is so far most widely accepted techniques (APHA, 2005; Chaoua, 1994; Clarkson and Gatzy, 1961; Dean *et.al.,*1972; Miller and Tainter, 1944; Finney, 1971). The present study is planned to get the acute toxic impacts of mercury chloride on a freshwater air breathing fish *Clarias batrachus* (Linn.) based on probit analysis.

MATERIALS AND METHODS

Healthy groups of *Clarias batrachus* of 40±10 g and 16±2 cm in length were collected from NMCH pond, Patna during their spawning season in June 2019 and brought to the aquatic toxicology laboratory, Department of Zoology, Patna University, Patna, where the entire research work was done. They were disinfected with 0.01% $KMnO_4$ solution and the healthy fish that showed active movement were transferred into glass aquaria of 90 litre capacity @ 10 fish each containing well aerated, dechlorinated ground water. The fishes were acclimated in the laboratory with ideal physico-chemical condition for 15 days. Fishes were fed *ad libitum* @ 3-4% of body weight daily.

In the experimental protocol, commercial brand analytical grade mercuric chloride manufactured by Merck Pvt. Ltd., Cochin was used. The experiment required an aqueous mode. So, all the test media were prepared using deionized water. Mercuric chloride stock solution was prepared in the laboratory by dissolving amorphous $HgCl_2$ in double distilled water. Acute toxicity test was conducted as per standard method of APHA (2005). Since feeding tends to increase the metabolic activities such as respiration, excretion and production of other waste products which ultimately affects the toxicity of test solution. So the test group of fish @ 10 per aquarium were starved for 24 hrs, 48 hrs, 72 hrs and 96 hrs. The different concentrations of the test solution were added very carefully in each of the aquarium. Any changes in behaviour and other responses were carefully monitored. The specific doses were determined by trial and error method. Number of dead fishes were recorded properly and removed immediately from the rest of lot in the aquaria. The water was changed regularly after every 24 hrs in the morning and desired concentration of test solution was maintained continuously in the aquarium. At the end of the test duration, number of fish survived was counted and a proper record of behavioural and physiological responses was maintained. Determination of LC_{50} was carried out by probit regression analysis (Finney, 1971). For regression lines of probit logarithmic transformation of concentration were made (Doudoroff *et al*., 1951). Slope function (S), Upper and Lower confidential limit (UCL and LCL) of the regression line along with chi square test were calculated following the standard procedure of UNEP/FAO/IAEA (1987).

RESULTS AND DISCUSSION

In the present study results obtained clearly suggest that the fish mortality is directly proportional to concentration of mercuric chloride. The relationship between the logarithm of aqueous concentration of mercuric chloride (log_{10} of dose) and the logarithm of fish mortality (probit) appears to be linear for all the treatment (Table 1).

The LC_{50} value of $HgCl_2$ for *Clarias batrachus* at 24 hrs, 48 hrs, 72 hrs and 96 hrs of exposure were calculated as 1.85 ppm, 1.46 ppm, 1.10 ppm and 0.89 ppm respectively by probit regression method. From the above regression equation mentioned in Table 1, it is highly evident that mortality increased with the increase of exposure period (Fig 1-4). At the initial stages of the experiment, fishes started showing various locomotary responses like irregular and rapid swimming and restless activities due to the sub lethal impact of mercuric chloride. Along with these activities the test group became hyperactive, started jumping and changing their direction and position in an irregular manner. Some altered physiological responses like rapid opercular movement, frequent gulping of air were also seen during the initial stage of exposure but afterwards the changes became casual. The changes in the behaviour might be due

Table 1: Assessment of acute toxicity showing tolerance of *Clarias batrachus* to mercuric chloride.

Exposure period (Hrs.)	LC_{20} value (ppm)	LC_{50} value (ppm)	LC_{90} value (ppm)	Regression equation Y=ax+c	95% confidence limit		Slope function (S)	Chi square value	
					Lower limit	Upper limit		Calculated value	Table value
24	1.15	1.85	3.46	3.77 + 4.59 X	2.05	7.86	0.505	2.272	7.815
48	0.88	1.46	3.31	4.41 +3.58 X	0.23	6.94	0.838	3.284	7.815
72	0.75	1.10	1.97	4.47 + 5.11 X	2.41	7.80	0.697	4.460	7.815
96	0.61	0.89	1.57	5.26 + 5.15 X	3.18	7.11	0.518	3.232	7.815

N = 10 for each group; chi square table value taken at 3df and $p<0.05$.

to the hyper activity of the neural mechanism or secondary stress response in the fish due to the release of stress hormones from respective endocrine glands.

Metal ions and their complexes exhibit a wide range of toxicity from sub lethal to lethal to the organism depending

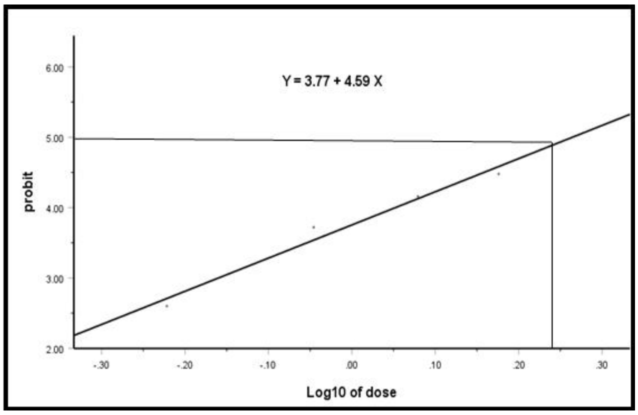

Fig 1: Regression line based on Probit analysis of log10 of concentration of mercury chloride Vs probit of percent mortality of *C. batrachus* at 24 hrs.

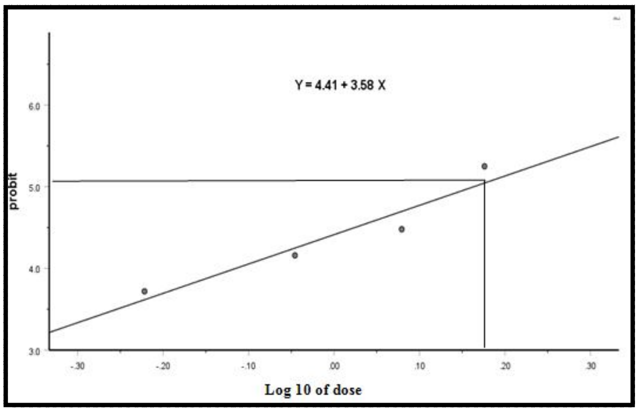

Fig 2: Regression line based on Probit analysis of log10 of concentration of mercury chloride Vs probit of percent mortality of *C. batrachus* at 48 hrs.

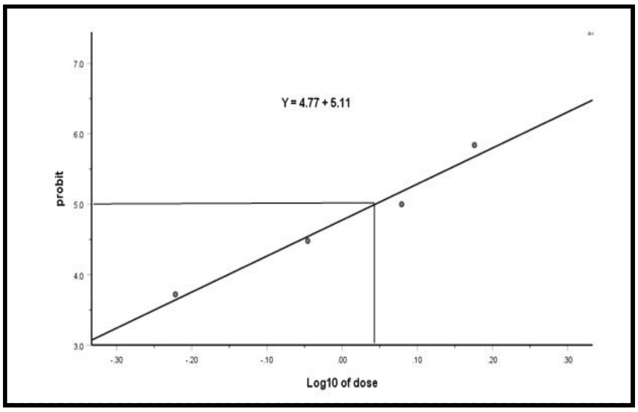

Fig 3: Regression line based on Probit analysis of log10 of concentration of mercury chloride Vs probit of percent mortality of *C.batrachus* at 72 hrs.

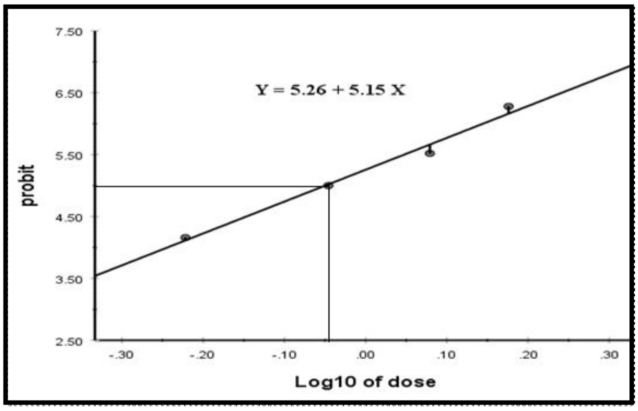

Fig 4: Regression line based on Probit analysis of log10 of concentration of mercury chloride Vs probit of percent mortality of *C. batrachus* at 96 hrs.

upon the time of exposure and prevailing conditions in the ambient water (Goel, 1997).

Mercuric chloride is a cumulative poison and is considered as a direct acting toxicant. It easily forms organomercury complexes with protein (Lorschieder *et al*., 1985). Once the mercury chloride is bound to GSH, it can leave the cell to circulate in serum or lymph and be deposited in other tissues. It is a powerful food oxidant and leads to generation of reactive oxygen species (ROS) which triggers many pathological changes in fish.

Heavy metal toxicity to the aquatic environment is primarily contributed by discharge of battery factories, drainage water from mine, effluents discharged from the tailing ponds and drainage water from soil heaps. Bioaccumulation of heavy metals in the fatty tissues of organisms signifies higher concentration of heavy metals of several magnitudes in the aquatic organism than that of ecosystem (Laws, 2000). There are some influencing factors that affect toxicity test are age, size (Sanders, 1993), sex (Victoriamma and Radhakrishnan, 1982) and supplied nutrition (Arunachalam, 1980; Eisler, 1970; Trivedy and Dubey, 1978). Singh *et al.* (2013) have also shown their concern about the negligence in implementing inland fisheries development programmes in the Indo- Gangetic Plain(IGP) apart from severe anthropogenic interventions.

These heavy metals show a characteristic pattern of their food chain magnification at subsequent higher trophic levels (Wyn *et al*., 2007). The calculated value of LC_{50} for the same toxicant may differ from species to species due to their mode of action (King, 1992).

Syed (2005) reported 96 hrs LC_{50} for mercury as 1 ppm in *Tench tinca*. Madhab and Kumar (1999) recorded 96 hrs TLM value for mercuric chloride as 1.21 ppm in *Anabas testidunius.* Ashwini and Ashok (2006) worked extensively on the acute toxicity of mercury for different fingerlings of Indian major carps. They reported that acute toxicity of mercury for fingerlings of catla, rohu and mrigal ranged in between 1.13 to 1.22 µg Hg/l, 1.05-1.06 µg Hg/l and 1.09-1.20 µg Hg/l respectively. Similar kind of acute toxicity assessment of dimethoate and chloropyrifos for juvenile common carp have been done earlier. The median lethal concentration (LC_{50}) of dimethoate and chlorpyrifos was reported as 1.1±0.053 ppm and 3.8±0.1 ppb respectively for juvenile common carps (Qayoom *et al*., 2018). They have shown a strong co-relation between dose of the toxicant given and response elicited by fishes. Besides the basic reason behind the small LC_{50} values recorded was attributed to small size of fishes, which have potentially weak immune system for biotransformation and elimination of toxicants from the body.

CONCLUSION

Acute toxicity test provides the data showing severe harmful biological effects of any chemical toxicants. The test is highly appreciated to predict the safe concentration in the environment. The present study furnishes a launching pad for the further study of various toxicological hazards of mercuric chloride on the fish at molecular level.

ACKNOWLEDGEMENT

Authors are thankful to the Head, P.G. Department of Zoology, Patna University, Patna for providing infrastructural facilities.

REFERENCES

Ahmed, Q., Benzer, S. and Yousuf, F. (2016). Distribution of heavy metals in different tissues of Indian mackerel from Karachi fish harbour, Karachi, Pakistan. Indian J. Anim. Res. 50(5): 759-763.

APHA (2005). Standard Methods for the Examination of Water and Wastewater (21st Ed.). A joint publication of the American Public Health Association (APHA), the American Water Works Association (AWWA) and the Water Environment Federation (WEF). 1368 pages.

Arunachalam, S. (1980). Toxic and sublethal effects of carbaryl in the freshwater catfish, *Mystus vittatus* (Bloch). Arch. Environ. Contam. Toxicol. 9: 307-316.

Ashwani, K. and Ashok, K.G. (2006). Acute toxicity of mercury to the fingerlings of Inidan major carps (Catla, Rohu and Mrigal) in relation to water hardness and temperature. J. Envir. Biol. 27(1): 89-92.

Barak, (1955). Concentration of Zinc and Copper in Some Fauna from Basrah. Iraq. Mar. Res. 4: 1-6.

Bonacker, D. and Stoiber, T. (2004). Genotoxicity of inorganic mercury salts and based on disturbed microtubule function. Arch. Toxicol. 78: 575-583.

Cavas, T. (2008). *In vivo* genotoxocity of mercury chloride and lead acetate: Micronucleus test on acridine orange stained fish cells. Food and Chemical Toxicology. 46(1): 352-358.

Chaoua, L. (1994). Studies on heavy metals in Commercial fishes from the northern part South China Sea. J. Fish. Sci. China Zhongguoshuichan-Kexue. 1: 68-74.

Clarkson, T.W., Gatzy, J., Dalton C. (1961). Studies of the equilibrations of mercury vapour with blood, Univ. of Rochester. UR RepNCBI (PMID: 24545514). 582: 1-64.

Dean, J.G., Bosqui, F.L. and Lannovette, V.H. (1972). Removing heavy metals from waste water. Environ. Sci. Technol. 6: 518-522.

Doudoroff, P., Cherman, B.G. anderson, G.E., Burdice, P.S. and Galtsoff, (1951). Bio assay methods for the evaluation of acute toxicity of industrial wastes to fish. Swe. Ind. Wastes. 23: 1380-1397.

Eisler, R. (1970). Factors affecting pesticide induced toxicity in estuarine fish. Technical papers of the Bureau of sports for fish and wildlife.

Farombi, E.O., Adelowo, O.A. and Ajimoko Y.R. (2007). Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African Cat fish *Clarias gariepinus* from Nigeria Orgun River. Int. J. Environ Res. Public Health. 4(2): 158-165.

Finney, D.J. (1971). Probit analysis, 3rd Edn. Cambridge University Press. UK, p. 25-26.

Goel, P.K. (1997). Water pollution causes effects and control. New Age International (P.) Ltd. Publishers, New Delhi.

Khangarot, B.S. (2003). Mercury induced morphological changes in the respiratory surface of Asian freshwater catfish *Sacobranchus fossilis.* Bull. Environ. Contam. Toxicol. 70: 705-712.

King, S.G. (1992). Some effects of DDT on the guppy and brown trout. Spec. Scient Res. U.S. Fish Wild. Surv. 399: 1-22.

Lorschieder, F.L., Vimy M.J. and Summers A.Q. (1995). Mercury exposure form silver tooth filling: emerging evidence questions a traditional dental paradigm. FASEBJ. 9: 504-508 (Pub Med).

Laws, E.A. (2000). Aquatic Pollution - An introductory text. John Wiley and Sons, New York, USA. 309-430.

Madhab, P.B. and Kumar Ajit A. (1999). Determination of 96h median tolerance limit (TLM) of *Anabas testudineus* for cadmium chloride, mercuric chloride and metacid-50. J. Ecotoxicol. Enviro. Nonit. 9(4): 237-242.

Maheswaran, R., Devpaul, A., Muralidharan, S., Velmurugan. B., Ignacimuthu. S. (2008). Hematological studies of fresh water fish, *Clarias batrachus* (L.) exposed to mercuric chloride. International Journal of Integrative Biology. 2(1): 49-54.

Miller L.C. and Tainter (1944). Estimation of ED-50 its error by means of logarithmic probit graph paper. Proc. Soc. Exp. Biol. Med. 57: 261-264.

Qayoom, I., Balkhi, M.H., Shah, F.A. and Bhat, B.A. (2018). Toxicological evaluation and effects of oganophosphate compounds on hematological profile of juvenile common carps (*Cyprinus carpio var. Communis*). Indian J. Anim. Res. 52(10): 1469-1475.

Rani, K.J., John Milton, M.C., Uthiralingam, M. and Azhaguraj, R. (2011). Acute toxicity of mercury and chromium to *Clarias batrachus* (Linn.). Bioresearch Bulletin. 1: 104-108.

Sanders, B.M. (1993). Stress proteins in aquatic organisms: an environmental perspective. Crit. Rev. Toxicol. 23: 49-52.

Singh, R., Sharma, R., Pandey, P.K. and Chaturvedi, C.S. (2013). Geospatial mapping of fisheries profiles of Indo-Gangetic Plains of India through GIS. Indian J. Anim. Res. 47(2): 113-119.

Spacie, A. and Hamelink, J.L. (1985). Bioaccumulation, In: Fundamentals and Aquatic Toxicology Methods and Applications [(Eds) Rand, G.M. and Petrocelli, S.R.] Hemisphere Publishing Corporation, New York. 495-525.

Syed, L.S. (2005). Effects of heavy metal accumulation on the 96h LC_{50} values in *Tench tinca* L., 1758. Turk J. Vet. Anim. Sci. 9: 139-144.

Trivedy, R.C. and Dubey P.S. (1978). Evaluation of toxicity of some industrial waste to fish by bioassay. Environ. Pollut. 17: 75-80.

UNEP/FAO/IAEA (1987). Test of the acute lethal toxicity of pollutants to marine fish and invertebrates. Reference methods for marine pollution studies, 43.

Veena, K.B. and Radhakrishnan, C.K. (1997). Heavy metal induced biochemical effects in an estuarine teleost. Indian J. Mar. Sci. 26: 74-78.

Victoriamma, D.C., Radhakrishnan (1982). Mercury tolerance of fresh water field crab, *Ozinokeiphces senex. Symp*. Physiol. Rep. Anim. Poiluf. 67: 82-89.

Wyn, B., Sweetman, J.N., Leavitt, P.R. and Donald, D.B. (2007). Historical metal concentrations in lacustrine food webs revealed using fossil ephippia from Daphnia. Ecolog Applic. 17: 754-764.