



Research Paper

Determination of LC₅₀ value of mercury chloride in common carp (*Cyprinus carpio*)

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Abstract: Common carp (*Cyprinus carpio*) is of great importance in toxicological work together with economic aspects. The aim of present work was to determine LC₅₀ value of mercury chloride on *Cyprinus carpio* at different time intervals. The fish were treated with different concentration of mercury chloride with increasing concentration for 96 hours. The mortality was recorded after a period of 24h, 48h, 72h and 96h. A dose response curve between % mortality and concentrations of toxicant was plotted. To calculate LC₅₀ by probit analysis the concentrations were converted into log concentration. LC₅₀ values for different time interval were obtained from the curves by drawing a perpendicular line at 5 probit corresponding to the 50% mortality and taking the inverse log of the concentrations associated with it. We calculated LC₅₀ value of 1.15Mg/L, 1.10Mg/L, 1.07 Mg/L and 1.03 Mg/L for the periods 24h, 48h, 72h and 96 h respectively.

Keywords: Common carp, *Cyprinus carpio*, Lc50, Mercury chloride, Probit analysis, Toxicity.

Introduction:

Fishes are source of livelihood for millions of marginalized peoples. Fishes are also ideal model for toxicological research and largely used as bioindicator for aquatic ecosystems. Fishes are most important aquatic food and frequently consumed by humans. Fishes have expressed large economic importance and highly sensitive to heavy metals and toxicants discharged in the aquatic ecosystems. Fish consumes heavy metal by contaminated water or indirectly through eating live things that have bio accumulated the metals (Javed, 2005; Singh and Zahra 2017). Pollution of water bodies with heavy metals is now well documented and its effect of aquatic organisms such as fishes is an active area of research now a day (Sadauki *et. al.*, 2024). Even though some heavy metals are important for aquatic animals in balance amount for their physiological function, however, if present at higher concentration they concentrated in different organs depends on the type of heavy metal and their reaction with target organs, damage tissues, disturbs biochemical and hematological parameters, slow growth and proliferation (Majeed 2023; Alkarkhi, *et. al.*, 2009). Mercury chloride has been considered as most dangerous aquatic pollutant, because

of its toxicity, bioaccumulation, and bio-magnification properties at higher trophic levels and also due to its non-biodegradable nature. In fishes, mercury chloride targets mainly the body's vital organ like kidney, liver and gastrointestinal tract, organic mercury and its vapor also affects the central nervous system Vahter *et. al.*, 2000; Ghosh and Sil, 2008). Biochemical, histopathological, physiological and behavioral changes in response to $HgCl_2$ exposure is well described in fishes (Jasim *et. al.*, 2016; Saswan *et. al.*, 2012; Kousar *et. al.*, 2018; Abdullah *et. al.*, 2018; Latif and Javed, 2018). In long term heavy metals can accumulate in human body that is able to start hematological and histopathological alterations (Clarkson, 1990).

Different indices are adopted to estimate the influence of a given pollutant on the mortality of a given species, e.g., the TL (Tolerance Limit), the LD (Lethal dose), the LC (Lethal concentration) and the ST (Survival time). Several of these criteria are routinely used to establish maximum acceptable values of pollutants (Rajan, 2002). The most important is the LC_{50} (median lethal concentration), which is the theoretical value, causing 50% mortality in the test population. The LC_{50} value is determined after 24hours, 48hours and in some cases after 96 hours Parray *et. al.*, 2017; Vutukuru, 2005). The basic protocol for the determination of LC_{50} is well established and consists of treating groups of animals with a mathematically related series of doses in order to determine the dose that kills 50% of the group and the dose response function (Rengarajulu, 2002). The aim of the present study is to determine LC_{50} value of mercury chloride in *Cyprinus carpio* for 24 hrs, 48 hrs, 72 hrs, 96 hrs. This fish is very common animal model for toxicological research and have great economic value. Result will definitely aid future research and development work in Bundelkhand region.

Materials and Methods:

Present work was conducted at department of Zoology, Bipin Bihari degree college, Jhansi affiliated to Bundelkhand University, Jhansi, U.P., India. Alive, healthy/ disease free common carp (*Cyprinus carpio*) of either sex from a single population having mean length and weight of 17.0 ± 2.0 cm and 100.0 ± 10.0 gm respectively were purchased from the nearby fish farm during November to march, 2023. Only healthy fish were employed for the experiment after being properly inspected for damage and disease conditions. Prior to reaching the fish laboratory, the specimens were carried in plastic containers filled with chilled, oxygenated water to reduce their activity and stress. The fish were kept in clear glass tanks so that any abnormal signs could be seen. The fishes were checked against any dermal injury or infection by keeping in 0.2% potassium permanganate solution for 2-4 min. The fishes were acclimatized in laboratory conditions for 5-10 days under natural photoperiod. During acclimatization the fishes were fed with commercial diet. The aquarium used was washed with soap, rinsed with clean water then disinfected with chlorine for 24 hours and sun dried. Each aquarium was filled with 05 carps. Mean temperature of alkaline water ($pH 7.5 \pm 0.2$) with total hardness of about 220 ppm was $25 \pm 2.5^\circ C$. Dissolved O_2 and CO_2 ranged between 5-6 ppm and 3-4 ppm respectively. Total dissolved solid (TDS) was around 10 ppm in all the experimental tanks. Alkalinity was 450 ppm for MO (Methyl Orange) and nil for PHP (Phenolphthalein). All the tanks tested negative for Lead nitrate. The optimal values for temperature, pH, and dissolved oxygen were established through regular measurements taken during the acclimation and experimentation phases. Fishes were not given feed 24h before experiment. Mercury chloride (mercury (II) chloride) supplied by manufacturer Alpha Chemika pvt. Mumbai

was used in the toxicity test. Two range finding tests for determination of LC₅₀ of mercuric chloride (HgCl₂) were performed. Wide concentration range was based on previous studies. First test was performed with 3 concentrations i.e. 0.1, 1.0 and 2.0 mg/L respectively and a control group in duplicate taking 5 fish in each aquarium to determine mortality between 0% to 100%. Second test was also performed in duplicate having concentration of 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 1.5, 2.0, mg/L respectively and a control group with same number of fish. Based on the range finding test results, definitive test was performed with concentrations 0.5, 0.75, 1.0, 1.10, 1.20, 1.25 and 1.50 mg/L respectively (3 replicates with 5 fish per aquaria). The mortality was recorded after a period of 24h, 48h, 72h and 96h and dead fish were removed when observed. The concentrations from the definitive test were employed to determine the LC₅₀ values by plotting a dose response curve between %

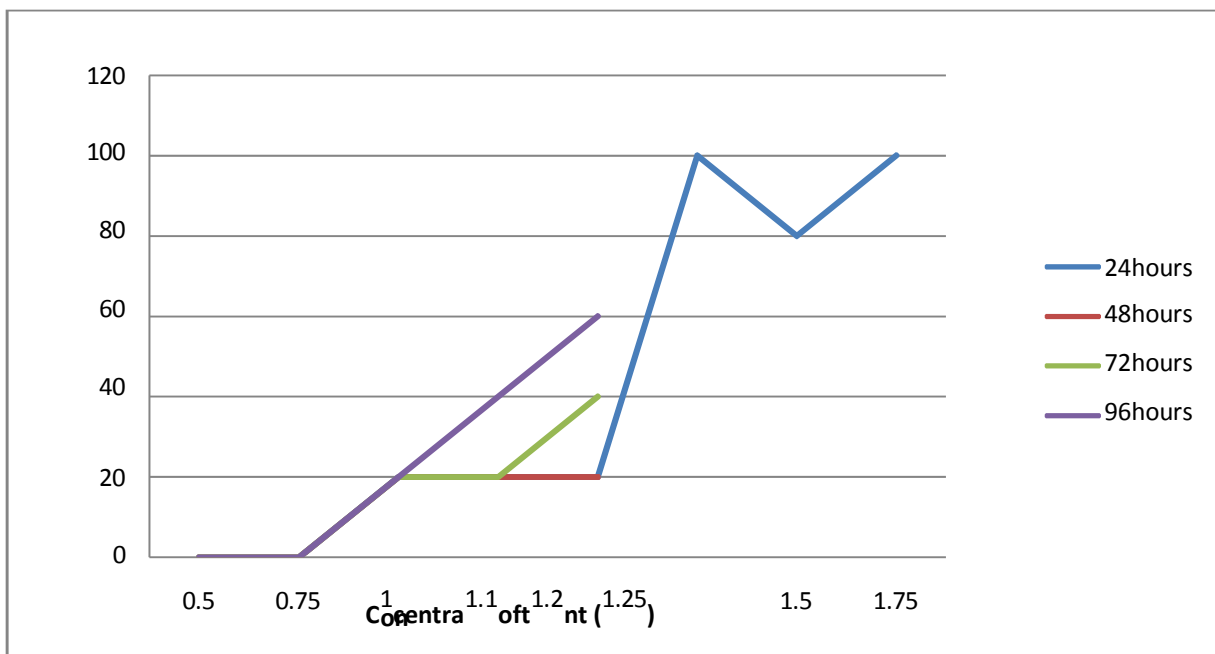
mortality and concentrations of toxicant. To calculate LC₅₀ by probit analysis the concentrations obtained from definitive test were converted into log concentration. LC₅₀ values for different time interval were obtained from the curves by drawing a perpendicular line at 5 probit corresponding to the 50% mortality. The actual LC₅₀ was determined by taking the inverse log of the concentrations associated with it.

Results:

First range finding tests observed 100% mortality after 24h at 2.0 mg/L concentration. Second range finding test observed 80% mortality at 1.5 mg/L concentration after 24 h and 100% mortality was observed after 48h respectively. On the basis of second range finding test, we have taken concentration viz. 0.5, 0.75, 1.0, 1.10, 1.20, 1.25 and 1.50 mg/L for definitive test and observed mortality at different time intervals as mentioned in table/graph 1.

Table: 1. Definitive test for direct interpolation method

Conc. of toxicant (Mg/L)	No. of fishes	24 hours		48 hours		72 hours		96 hours	
		M	M%	M	M%	M	M%	M	M%
0.50	5	0	0	0	0	0	0	0	0
0.75	5	0	0	0	0	0	0	0	0
1.00	5	1	20	0	20	0	20	0	20
1.10	5	1	20	0	20	0	20	1	40
1.20	5	1	20	0	20	1	40	1	60
1.25	5	5	100	-	-	-	-	-	-
1.50	5	4	80	1	100	-	-	-	-
1.75	5	5	100	-	-	-	-	-	-



Graph: 1. Percentage mortality after applying different concentration of toxicant at different time intervals

The concentrations taken in definitive test were converted into log concentrations. The correct % and their corresponding probit values are shown in table 2 & 3. After plotting

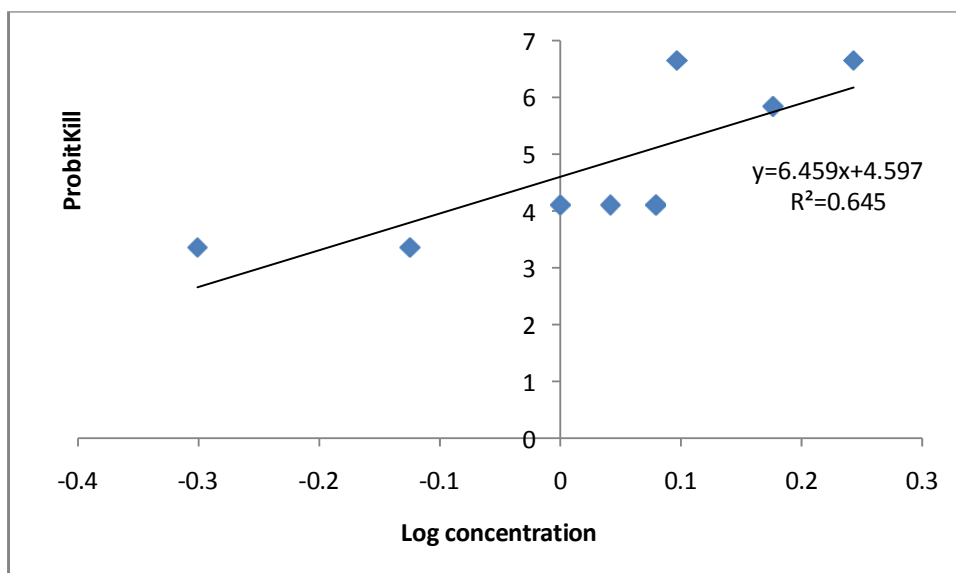
a graph between the log concentration and probit, we observed LC_{50} at 1.15Mg/L, 1.10Mg/L, 1.07Mg/L and 1.03Mg/L after 24h, 48h, 72h and 96hr respectively (**Graph 2-5**).

Table: 2. Log concentrations and probit values when exposed to $HgCl_2$ after 24 and 48h

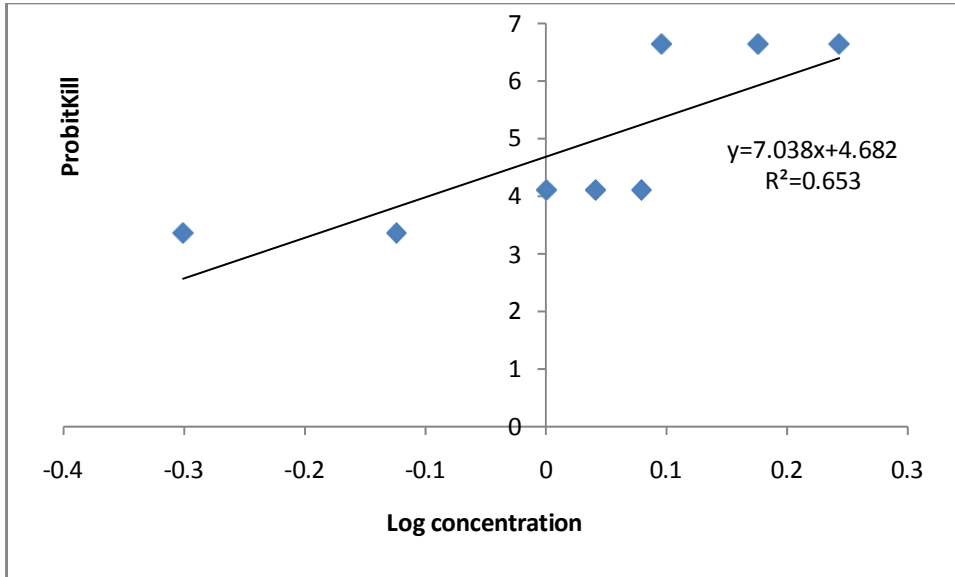
Conc. (Mg/L)	Log Conc.	No. of fishes	24 hours			48 hours		
			% dead	Correct %	Probit	% dead	Correct %	Probit
0.50	-0.301	5	0	5	3.36	0	5	3.36
0.75	-0.124	5	0	5	3.36	0	5	3.36
1.00	0	5	20	20	4.10	20	20	4.10
1.10	0.041	5	20	20	4.10	20	20	4.10
1.20	0.079	5	20	20	4.10	20	20	4.10
1.25	0.096	5	100	95	6.64	100	95	6.64
1.50	0.176	5	80	80	5.84	100	95	6.64
1.75	0.243	5	100	95	6.64	100	95	6.64

Table: 3. Log concentrations and probit values when exposed to HgCl₂ after 72 and 96h

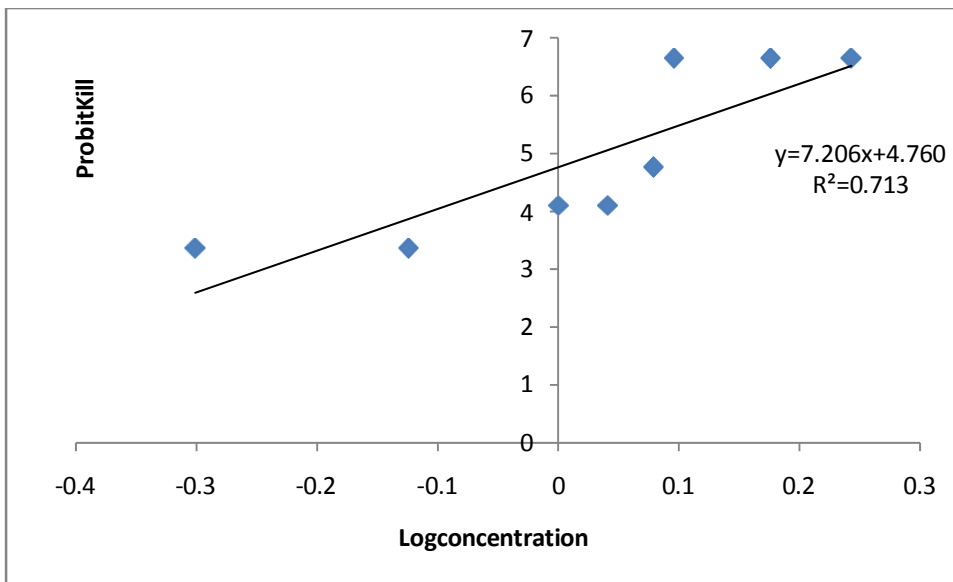
Conc. (Mg/L)	Log Conc.	No.of fishes	72 hours			96 hours		
			% dead	Correct %	Probit	% dead	Correct %	Probit
0.50	-0.301	5	0	5	3.36	0	5	3.36
0.75	-0.124	5	0	5	3.36	0	5	3.36
1.00	0	5	20	20	4.10	20	20	4.10
1.10	0.041	5	20	20	4.10	40	40	4.76
1.20	0.079	5	40	40	4.76	60	60	5.25
1.25	0.096	5	100	95	6.64	100	95	6.64
1.50	0.176	5	100	95	6.64	100	95	6.64
1.75	0.243	5	100	95	6.64	100	95	6.64



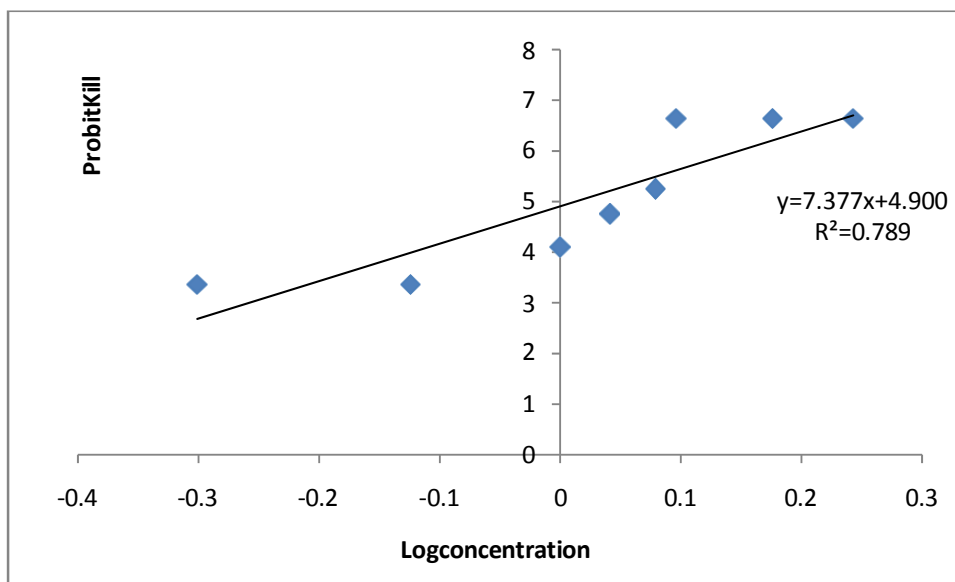
Graph: 2. Determination of LC₅₀ at 24h



Graph: 3. Determination of LC_{50} at 48h



Graph: 4. Determination of LC_{50} at 72h



Graph: 5. Determination of LC₅₀ at 96h

Discussion:

Various workers carried out determination of LC₅₀ value of HgCl₂ on different fish models. It is important to mention that workers denoted concentration of HgCl₂ by different units of measurement. The LC₅₀ values of HgCl₂ were 1.339, 0.649, 0.411 and 0.310 (Pg/L) at 24, 48, 72 and 96 hours respectively in marine fish *Theraponjarbua* and observed significant increase in MN (micro nucleus) frequency in erythrocytes (Nagrani, *et. al.*, 2009). The 96 h LC₅₀ value of mercuric chloride was 0.6ppm in *C. batrachus* using the probit analysis method (Maheswaran *et. al.*, 2008). The acute toxicity for 96 h LC₅₀ was found to be 0.25Mg/L for freshwater fish *Labeo rohita* (Naik *et. al.*, 2017). Median lethal concentration (LC₅₀) for mercury chloride to fish *Cyprinus carpio* for 96 hours was found to be 1.3ppm (Lone *et. al.*, 2021). The median lethal concentration of HgCl₂ for 24hours was 0.3 ppm and for 96 hours it was 0.1 ppm (Rajan, 2002). The LC₅₀

values of Hg within 24, 48, 72 and 96 h recorded for *Oreochromis sp.* with 95% confidence limits were 1.09 (0.92-1.40mg/L), 0.75 (0.47-1.32mg/L), 0.54 (0.12- 0.96 mg/L) and 0.30 (0.17- 0.44 mg/L). Furthermore, it was added that tolerance to mercury decreases with the increased in time of exposure (Jasim *et. al.*, 2016). The LC₅₀-96h of mercury was 0.24 Mg/L for Nile tilapia *Oreochromis niloticus* (Kaoud and Mekawy, 2011). LC₅₀-96h of mercury had higher value (1.15 mg/L) in air-breathing fish *Channa punctatus* (Pandey *et al.*, 2008; 2005). Histopathology of liver, kidney, spleen, gills and muscles of fresh water fish exposed to ½ LC₅₀ (96 hours) for 7 days and for 1/10 LC₅₀ (96 hours) found Hg residues 0.22 ppm/gm at 2nd day, 0.411 ppm/gm in the 5th day ended with 0.96 ppm/gm in the 7th day in acute toxicity. In chronic toxicity it was 1.1320, 1.7140, 2.3620 and 3.5640 ppm/gm respectively from the 2nd to the 8th week of exposure (Saswan *et. al.*,

2012). Common carp (*Cyprinus carpio*) exposed to water borne HgCl₂ at levels of 0.01, 0.05 and 0.1 Mg/L respectively for 30 days showed significantly decreased in lymphocyte transformation index, phagocytic/lysozyme activities, albumin and globulin content and increased blood glucose, urea particularly at higher dose of HgCl₂ (Mustafa and Al-Rudainy, 2021). There was decrease of erythrocytes by 1,678,000 cells/mm³, leukocyte increase by 16,360 cells/mm³, hemoglobin decrease by 6.7g/dl, hematocrit decrease by 10.24% and an increase in blood sugar by 99 mg/dl in treatment with 0.015 ppm HgCl₂ (Setiyowati *et. al.*, 2019). Restlessness, erratic swimming, jerky body movement, rolling body, convulsion, mucous secretion, loss of equilibrium, rapid opercular movement, difficulty in breathing, lethargic are some observed behavioral responses (Majeed, 2023). The concentration of electrolytes such as Na⁺, K⁺ and Cl⁻ is also get affected by HgCl₂ (Clarkson, 1990). It is clear that mercury chloride affect organisms at every biological parameter even at very low concentrations. So far LC₅₀ value is concerned, we observed comparatively higher concentration that may be due to variation in species local population and/or experimental conditions.

Conclusion:

The present study was an attempt to find the toxicity mercury chloride on common carp (*Cyprinus carpio*) and the results conclusively showed that this is highly toxic to fishes even at very low concentration. Mercury chloride is now leading toxicant present in aquatic ecosystem affecting aquatic environment and organisms from cellular to behavioral level. Common carp is

a model organism for ecotoxicological research together with its economic importance. Determination of LC₅₀ of mercury chloride for this fish will help researchers in ecotoxicological research and economic wellbeing of peoples living in socioeconomically backward region of Bundelkhand of this country.

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