

Cadmium Chloride Toxicity on Bighead Carp (*Hypophthalmichthys nobilis*)

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Abstract: The main objective of the present study was to investigate acute effects of cadmium chloride as potential dangerous additives to assess mortality effects of this heavy metal on one of freshwater cultured fish *Hypophthalmichthys nobilis*, to use it in the toxicity test programs. LC₅₀ is the ambient aqueous chemical activity causes 50% mortality in an exposed population. All fishes were exposed to the wide range of cadmium chloride (0, 0.2, 1, 2, 4, 6, 10 and 15 ppm) and mortality was recorded at 24, 48, 72 and 96 h. Toxicity tests were determined with probit analysis in SPSS software. The 96h LC₅₀ was 5.42 (4.78-6.28) and indicated that cadmium chloride had much toxic to cultured fish *Hypophthalmichthys nobilis*.

Key words: Freshwater Fish • Bighead Carp • Cadmium Chloride • LC₅₀ • Toxicity Test

INTRODUCTION

Toxic heavy metals are increasingly being released into the environment with the advent of agricultural and industrial revolution. Heavy metals can produce a range of hazard effects in aquatic organisms, ranging from alterations to a single cell, up to changes in whole populations [1]. Contaminants can be divided into three principle groups: bulk metals, essential (trace) metals and non-essential (heavy) metals. Most metals do not form stable alkylated forms, but some (e.g. Cu, Hg) have high affinity for organic material and may be found associated with organic macromolecules in aquatic ecosystems [2]. Essential metals, i.e. elements which all living organisms need to exist, include Fe, Cu, Zn, Mn, Mo and Ni. Whereas the lack of one or more of these elements is not uncommon in terrestrial animals, such deficiencies have not been reported for marine organisms. Non-essential metals, e.g. elements for which there is no known function, include Cd, Hg, Pb, Ag and Au [3]. Among the toxic heavy metals present in water bodies, cadmium chloride is the most abundant and is an emerging global concern due to their potential hazards on the public health. Heavy metals can accumulate in the tissues of aquatic organisms and as these tissue concentrations of heavy metals can be of public health concern to both

organisms and humans [4]. In determining the toxicity of a new chemical to fish, an acute toxicity test is first conducted to estimate the median lethal concentration (LC₅₀) of the chemical in water to which organisms are exposed [3]. LC₅₀ is the ambient aqueous chemical activity causes 50% mortality in an exposed population. These calculations are based on two important assumptions. The first assumption is that the exposure time associated with the specified LC₅₀ is sufficient to allow almost complete chemical equilibration between the fish and the water. The second assumption is that the specified LC₅₀, the minimum LC₅₀ that kills the fish during the associated exposure interval. Fortunately, most reliable LC₅₀ satisfy these two assumptions [5]. The 96-h LC₅₀ tests are conducted to measure the susceptibility and survival potential of organisms to particular toxic substances such as copper sulphates pollution. Higher LC₅₀ values are less toxic because greater concentrations are required to produce 50% mortality in organisms [6]. The heavy metals those are toxic to aquatic animals at very low concentrations and are never beneficial to living beings are mercury, cadmium and lead [7] so the aim of the present study was to investigate acute effects of Cadmium Chloride to assess mortality effects of this pollutant on valuable freshwater fish, Bighead Carp (*Hypophthalmichthys nobilis*).

MATERIALS AND METHODS

The toxicant used in this research, was cadmium chloride ($\text{CdCl}_2 \cdot \text{H}_2\text{O}$, Merck). Acute toxicity tests were conducted on Bighead carp (~50g). Groups of 21 fish were exposed for 96 h in fiberglass tanks. Samples transferred to a 400-L aerated tanks equipped with aeration with 200 L of test medium. Water quality parameters (temperature, dissolved oxygen (DO), salinity and pH) were also determined before and periodically during the tests. All samples were acclimated for one week in fiberglass tanks at 25°C under a constant 12:12 L:D photoperiod. Acclimatized fish were fed daily with a formulated feed. The concentrations of cadmium chloride solution were 0, 0.2, 1, 2, 4, 6, 10 and 15 ppm CdCl_2 . The control group was kept in experimental water without adding the cadmium chloride, keeping all other conditions same. The number of dead fish was counted every 12 h and removed immediately from the fiberglass tanks. Only healthy fish, as indicated by their activity and external appearance, were maintained alive on board in a fiberglass tanks. Dead fish was immediately removed with special plastic forceps to avoid possible deterioration of the water quality [8]. Test medium was not renewed during the assay and no food was provided to the animals. Acute toxicity tests were carried out in order to calculate the 96h- LC_{50} for cadmium, based on Hotos and Vlahos [9]. Mortality was recorded at 24, 48, 72 and 96h and LC_{50} values and its confidence limits (95%) were calculated by Boudou and Ribeyre [5]. Percentages of fish mortality were calculated for each cadmium concentration at 24, 48, 72 and 96 h of exposure. Also LC_{50} values were calculated from the obtained data in acute toxicity bioassays, by Finney's [10] method of "probit analysis" and with SPSS computer statistical software. In Finney's method, the LC_{50} value is derived by fitting a regression equation arithmetically and also by graphical interpolation by taking logarithms of the test chemical concentration on the X axis and the probit value of percentage mortality on the Y axis [10]. The 95% confidence limits of the LC_{50} values obtained by Finney's method were calculated with the formula of Mohapatra and Rengarajan [11]. Probit transformation adjusts mortality data to an assumed normal population distribution that results in a straight line. Probit transformation is derived from the normal equivalent deviate (NED) approach developed by Tort *et al.* [12], who proposed measuring the probability of responses (i.e., proportion dying) on a transformed scale based in terms of percentage of population or the standard deviations from the mean of the normal curve [3].

The $\text{LC}_{1,10,30,50,70,90,99}$ values were derived using simple substitution probit of 1, 10, 30, 50, 70, 90 and 99 respectively for probit of mortality in the regression equations of probit of mortality vs. cadmium. The 95% confidence limits for LC_{50} were estimated by using the formula $\text{LC}_{50} (95\% \text{ CL}) = \text{LC}_{50} \pm 1.96 [\text{SE}(\text{LC}_{50})]$. The SE of LC_{50} is calculated from the formula: $\text{SE}(\text{LC}_{50}) = 1/b\sqrt{pmw}$ Where: b=the slope of the cadmium/probit response (regression) line; p=the number of cadmium used, n= the number of animals in each group, w= the average weight of the observations [9]. At the end of acute test, the Lowest Observed Effect Concentration (LOEC) and No Observed Effect Concentration (NOEC) were determined for each endpoint measured. In addition, the maximum acceptable toxicant concentration (MATC) was estimated for the endpoint with the lowest NOEC and LOEC [13].

RESULTS AND DISCUSSION

Results showed that the relation between the cadmium chloride concentration and mortality of Bighead carp according to Finney's Probit Analysis. The mortality of Bighead carp in this study were examined during the exposure times at 24, 48, 72 and 96 h (Table 1).

Fishes exposed to cadmium chloride during the period 24-96h had Significant increase in number of dead individual with increasing concentration. There were no mortality until 2 ppm within the exposure times for Bighead carp fishes. Because mortality (or survival) data were collected for each exposure concentration in a toxicity test at various exposure durations (24, 48, 72, or 96 hours), data can be plotted in other ways; the straight line of best fit is then drawn through the points. These were time-mortality lines. Median lethal concentrations of 1%, 10%, 30%, 50%, 70%, 90% and 99% test have been showed in Table 2.

Table 1: Cumulative mortality of Bighead carp during acute exposure to cadmium chloride (n=21 for each concentration)

Concentration (ppm)	No. of mortality			
	24h	48h	72h	96h
Control	0	0	0	0
0.2	0	0	0	0
1	0	0	0	0
2	0	0	2	3
4	0	1	3	4
6	4	5	7	13
10	9	14	19	21
15	12	15	19	21

Table 2: Lethal Concentrations (LC₁₋₉₉) of cadmium chloride depending on time (24-96h) for Bighead carp

Point	Concentration (ppm) (95 % of confidence limits)			
	24h	48h	72h	96h
LC ₁	0.98 (0.54-3.24)	1.10 (0.37-2.86)	1.69 (0.43-2.92)	1.38 (0.59-2.41)
LC ₁₀	5.31 (3.02-6.94)	4.37 (2.6-5.67)	4.04 (2.76-4.90)	3.19 (2.07-3.89)
LC ₃₀	9.56 (7.98-11.41)	7.53 (6.28-8.76)	5.74 (4.86-6.56)	4.51 (3.80-5.17)
LC ₅₀	12.50 (10.75-15.17)	9.72 (8.50-11.25)	6.91 (6.11-7.93)	5.42 (4.78-6.28)
LC ₇₀	15.44 (13.23-19.21)	11.91 (10.49-13.95)	8.09 (7.20-9.44)	6.33 (5.61-7.53)
LC ₉₀	19.69 (16.63-25.23)	15.07 (13.18-18.04)	9.79 (8.63-11.76)	7.64 (6.68-9.47)
LC ₉₉	25.55 (21.20-33.67)	19.43 (16.76-23.82)	12.14 (10.51-15.07)	9.45 (8.08-12.22)

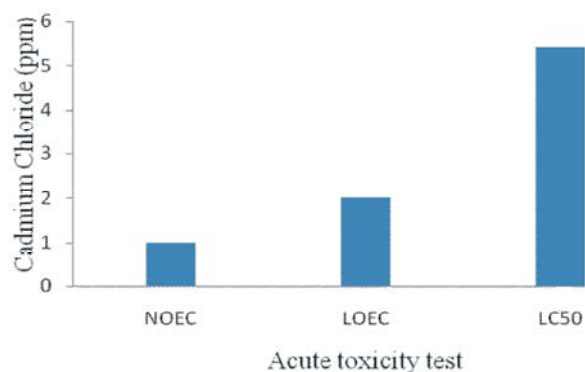


Fig. 1: Acute toxicity testing statistical endpoints of cadmium chloride

In this study LOEC (Lowest Observed Effect Concentration) and NOEC (No Observed Effect Concentration) were the same for all Bighead carp fishes. The Maximum Acceptable Toxicant Concentration (MATC) for Bighead carp was 0.54 ppm of cadmium chloride. Toxicity Testing Statistical Endpoints are in Fig 1.

The fish that is highly susceptible to the toxicity of one metal may be less or even non-susceptible to the toxicity of another metal at the same level of that metal in the ecosystem. Also, the metal which is highly toxic to a fish species at low concentration may be less or even non-toxic to other species at the same or even higher concentration. The concentration of heavy metals in aquatic animals is related to several parameters, such as the food habits and foraging behavior of fish [14], trophic status, source of a particular metal, distance of the animal from the pollutant source and the presence of other ions in the ecosystem [15], temperature, transport of metal across the membrane and the metabolic rate of the animal [16] and the seasonal variation in the taxonomic composition of different trophic levels affecting the level and accumulation of heavy metal in the fish tissue [14]. In current study the LC₅₀ values and the accumulation of heavy metals in the body of fish depends upon several factors, it is evident from the present study that

concentrations of cadmium and physiological response of fish species affect the LC₅₀ values of fish. It may be due to the increased resistance of some species to the cadmium through acclimatization. During acclimatization, some proteins, are released in the body of fish and detoxify the metal ions. This may cause higher levels of heavy metals being required to cause effects, resulting in higher LC₅₀ amounts [17]. Variability in acute toxicity even in a single species and single toxicant depending on the size, age and condition of the test species along with experimental factors [18]. Onorati *et al.* [19] reported 96h LC₅₀ range of 2.91 to 4.28 ppm of Cadmium for *C. orientalis*. Similarly, the 96-h LC₅₀ values of aquatic animals are different from metal to metal and from species to species. Johansson Sjobeck and Larsson [20], Gill and Pant [21] also found 96-h LC₅₀ levels of 20.0 and 12.65 ppm Cadmium for *Puntius conchoni* and *Pleuronectes flesus* respectively. It was observed 100% mortality in common carp, after 3-4 weeks of exposure to 2 ppm of Cadmium [22]. Das and Banerjee [23] found 300 ppm Cadmium for the 96-h LC₅₀ of *Heteropneustes fossilis*. The behavioral changes were also noted at high concentration of cadmium chloride. Higher percent of mortality occurred with increase in concentration of cadmium chloride agreement with result salmonids, *Oncorhynchus mykiss*, *Salvelinus confluentus* and *Oncorhynchus tshawytscha* [24]. Because of the lack of available data on the effects of cadmium on Bighead carp, so the results of the present study have been compared with other studies and discussed accordingly. In the present study, LC₅₀ values indicated that cadmium chloride as much toxic to cultured fish and in compare with other studies that have been published for other species of fish, show different LC₅₀ of cadmium chloride in different species and even different time, but lower value of LC₅₀ for some studied fishes was important and confirm sensitively of aquaculture species to low cadmium chloride doses. Although the LC₅₀ under a defined set of environmental conditions can provide useful information, the numeric value can't used in the field, so in continue we used some biomarkers for better understanding of cadmium chloride toxicity.

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