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ACUTE TOXICITY OF METAL CYANIDES TO INDIAN MAJOR CARP *Labeo rohita* (HAMILTON)

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Abstract: Static renewal bioassay tests were carried out to determine the acute toxicity (LC_{50}) of metal cyanides to the Indian major carp *Labeo rohita*. The 96 hour LC_{50} value for the sodium cyanide, zinc cyanide and copper cyanide to the fish *L. rohita* were 0.32 mg/L 0.35 mg/L, and 1.1 mg/L respectively. Among the metal cyanide tested, sodium cyanide is found to be more toxic than the other cyanide complexes. In general behavioral responses of the fishes exposed to cyanide included uncontrolled swimming, erratic movements, loss of balance, moving spiral fashion with sudden jerky movements, vertical movements lying on the sides of the test chamber and rapid flapping of the opercular movements with opened mouth finally settles to the bottom

Key words: LC_{50}, Behaviour, metal cyanides, *Labeo rohita*.

Introduction

The pollution of rivers and streams with chemical contaminants has become one of the most critical environmental problems of the century. As a result of the pollutants transported from industrial areas into the environment and their chemical persistence, many freshwater ecosystems are faced with high levels of xenobiotic chemicals *(Brack, et al., 2002; Chebbi and David, 2010)*. Among the different sources which cause environmental deterioration cyanide is the most important one (Mathangi and Namashivayam 2000). A cyanide is any chemical compound that contains the cyano group (C≡N), which consists of a carbon atom triple-bonded to a nitrogen atom. Inorganic cyanides are generally salts of the anion CN^- *(Greenwood and Earnshaw 1997)*. The cyanide anion is a potent ligand for many transition metals *(Sharpe 1976)*. The very high affinities of metals for this anion can be attributed to its negative charge, compactness, and ability to engage in π-bonding.

Cyanides may be released into the aquatic environment through waste effluents from the organic chemical and gold mining and milling industries, as well as from industrial processes such as gas works, coke ovens, gas scrubbing in steel plants, metal cleaning and electroplating. Cyanide in the aquatic environment may also be associated with non-point sources, including runoff from the application on land and water of salt containing cyanide compounds as anti-caking agents *(ASTDR 1998)*. Consequently, the waste waters generated by these industries often contain cyanide complexed with heavy metals (viz., copper, nickel, zinc, cadmium, iron, silver, gold, etc.) of variable stability and toxicity *(Patil and Paknikar 1999)*.

Many cyanide-containing compounds are highly toxic, but some are not. Nitriles (which do not release cyanide ions) and hexacyanoferrates (ferrocyanide and ferricyanide, where the cyanide is already tightly bound to an iron ion) have low toxicities, while most other cyanides are deadly poisonous. The most dangerous cyanides are hydrogen cyanide (HCN) and salts derived from it, such as potassium cyanide (KCN) and sodium cyanide (NaCN). These cyanides when dissolved in water they get dissociated and highly toxic free cyanide ion gets released, which get binds to the transition elements viz, copper and zinc forming the metal cyanide complexes. These metal cyanide complexes exists in solution as the anionic cyanometallates and are highly stable *(Aguilar et al. 1996)*.

Fish are able to take up and retain chemicals dissolved in water via active or passive processes. They can be used to detect and document pollutants released into their environment. Knowledge of acute toxicity of a xenobiotic often can be very helpful in predicting and preventing acute damage to aquatic life in receiving waters as well as in regulating toxic waste discharges (APHA 2005). The Indian major carp *L. rohita* together with *Catla catla* and *Cirrihinus mrigala*, contribute 81.53% of total aquaculture production in India (2.04 million metric tons) (FAO 2001). Information on the acute toxic effects of metal cyanide complexes to fishes is limited in Indian context and its effects on the widely consumed carp *L. rohita*, which forms an important link in the aquatic food chain are not known. In view of this, short-term acute toxicity tests were performed over a period of 96h to determine the LC_{50} value so as to elucidate the acute effects of metal cyanides on the survival of these fishes. The corresponding results are discussed in this paper.

Materials and Methods

Sample collection, maintenance and acute toxicity test. Healthy and active fingerlings of *L. rohita* (2±0.5 g, 5±0.75 cm), were procured from the State Fisheries Department, BRP, and Shimoga, India. Fish were brought to the laboratory in large aerated crates. Later they were acclimatized for 30 days in large cement tanks (25 x 15 x 5 feet) and fed with commercial dry feed pellets (Nova, Aquatic P. Feed). The carp (2±0.5 g, 5±0.75 cm) were acclimatized to laboratory conditions for 15 days at 25 ± 1 ºC and are held in 100 L glass aquaria (100 x 35 x 50 cm) containing dechlorinated tap water of the quality used in the test, whose physico-chemical characteristics were analyzed following the methods mentioned in APHA (2005) and found as follows, temperature 25±1 ºC, pH 7.2±0.2 at 25ºC, dissolved oxygen 6.3±0.8 mg/L, carbon dioxide 6.3±0.4 mg/L, total hardness 23.4±3.4 mg as CaCO3/L, phosphate 0.39±0.002 µg/L, salinity 0.01ppm, specific gravity 1.001 and conductivity less than 10 µS/cm.

Water was renewed every day and a 12-12 h photoperiod was maintained during acclimatization and test periods. The fish were fed regularly with commercial fish food pellets during acclimatization and test periods, but feeding was stopped two days prior to exposure to the test medium for acute toxicity test only. Care was taken in order to keep the mortality rate of fish not more than 5% in the last 4 days before the experiment was started. Also, the experimental medium was aerated in order to keep the amount of oxygen not less than 4 mg/l.

Sodium cyanide (99.9 %), copper cyanide (99%) and zinc cyanide (97%) was procured from the Loba chemicals Pvt. Ltd, Mumbai, India. Stock solution was prepared by dissolving the sodium cyanide in double distilled water in a standard volumetric flask. Copper cyanide and zinc cyanides were prepared by dissolving dilute alkalis, and then make up to the mark with double distilled water. Required quantity of cyanide was drawn directly from these stock solutions using micropipette. The concentrations of test compounds used in short term definitive tests were between the highest concentrations at which there was no mortality and the lowest concentration at which complete was mortality observed in the range finding tests.

Each aquarium was stocked with fish with a ratio of 2 g fish per litre water. The amount of cyanide solution to be added in each aquarium was calculated after the volume of each aquarium was accurately determined. The fish, in batches of 10, were exposed to varying concentrations of sodium, zinc and copper cyanide with 20 litres of water using three replicates for each concentration. There was a simultaneous control group together with the actual experiments. The control group was kept in experimental water without adding the toxicant keeping all other conditions constant. The experiment was repeated thrice for accuracy. The mortality rate in the control group did not exceed 10% and 90% of the fish looked healthy throughout the experiment.

Acute toxicity tests were carried out for a period of 96 h. The mortality rate was determined at the end of 24, 48, 72 and 96 hours and dead fish were removed as and when observed. During the experimental period the control and cyanide exposed fishes were kept under constant observation to study the behavioural changes. Acute toxic effect of metal cyanides to the fishes were determined by the use of Finney Probit Analysis *(Finney 1971)*, Dragstedt- Behrens equation *(Carpenter 1975)*, as mentioned by *Chebbi and David (2010).*

Results and Discussion

In the present investigation no mortality was observed in the control groups of all the experiments. The highest concentration at which there was zero percent mortality for sodium cyanide exposed fish was 0.29 mg/L and hundred percent mortality 0.34 mg/L (Table. 1).The estimated 96 h LC_{50} value (95% confidence limits) was found to be 0.32 mg/L .

The highest concentration at which there was zero percent mortality for zinc cyanide was 0.33 mg/L and hundred percent mortality 0.372 mg/L (Table. 2). The estimated 96 h LC_{50} value (95% confidence limits) was found to be 0.35 mg/L. Where as for copper cyanide zero percent mortality was observed at 0.95 mg/L and hundred percent mortality 1.2 mg/L (Table. 3).The estimated 96 h LC_{50} value (95% confidence limits) was found to be 1.1 mg/L (Table. 4).

Table 1. Mortality of *Labeo rohita* fingerlings in different concentrations of sodium cyanide at 96 h exposure periods

Conc. of Sodium Cyanide (mg/l)	Log Conc.	No. of fish alive out of ten	% Corrected mortality	Probit Kill
0.290	-0.5376	10	0	---
0.292	-0.5346	9	10	3.72
0.295	-0.5301	8	20	4.16
0.300	-0.5228	7	30	4.48
0.315	-0.5016	6	40	4.75
0.320	-0.4948	**5**	**50**	**5.00**
0.325	-0.4881	3	70	5.52
0.330	-0.4814	2	80	5.84
0.335	-0.4749	1	90	6.28
0.340	-0.4685	0	100	---

Table 2. Mortality of *Labeo rohita* fingerlings in different concentrations of zinc cyanide at 96 h exposure periods

Conc. of Sodium Cyanide (mg/l)	Log Conc.	No. of fish alive out of ten	% Corrected mortality	Probit Kill
0.330	-0.4814	10	0	---
0.338	-0.4710	9	10	3.72
0.341	-0.4672	8	20	4.16
0.342	-0.4659	7	30	4.48
0.343	-0.4647	6	40	4.75
0.350	**-0.4559**	**5**	**50**	**5.00**
0.367	-0.4353	3	70	5.52
0.370	-0.4317	2	80	5.84
0.372	-0.4294	0	100	---

Table 3. Mortality of *Labeo rohita* fingerlings in different concentrations of copper cyanide at 96 h exposure periods

Conc. of Sodium Cyanide (mg/l)	Log Conc.	No. of fish alive out of ten	% Corrected mortality	Probit Kill
0.950	-0.0222	10	0	---
0.970	-0.0132	9	10	3.72
0.980	-0.0087	8	20	4.16
1.020	0.0086	7	30	4.48
1.070	0.0294	6	40	4.75
1.100	**0.0414**	**5**	**50**	**5.00**
1.120	0.0492	3	70	5.52
1.150	0.0607	2	80	5.84
1.170	0.0682	1	90	6.28
1.200	0.0792	0	100	---

Table 4. 96 h LC_{50}, slope and 95% confidence limits of sodium, zinc and Copper cyanide to the fish, *L. rohita*

Toxicant	96 h LC_{50} (mg/L)	Slope	95% Confidence limits	
			Upper	Lower
Sodium cyanide	0.32	1.698	0.322	0.321
Zinc cyanide	0.35	1.702	0.33	0.372
Copper cyanide	1.1	1.742	1.02	1.224

In the present study, the control fish were active and alert to slightest of the disturbance with their well-synchronized movements. The behavior did not significantly vary between the control groups; therefore, these results were taken as standards for the entire experimentation. Fish exposed to sodium cyanide and zinc cyanide exhibited disrupted shoaling behavior, localization to the bottom of test chamber and independency (spread out) in swimming. This followed loss of co-ordination and occupancy of twice the area to that of control group were the early responses of the fish following exposure to both sodium and zinc cyanide concentrations. Subsequently, fish moved to the corners of the test chambers, which can be viewed as an avoidance behavior of the fish to sodium cyanide. Further, fish exhibited irregular, erratic and darting swimming movements and loss of equilibrium followed by hanging vertically in water. Fish slowly became lethargic, restless, and secreted excess mucus all over the body. Intermittently some of the fish were hyper excited resulting in erratic movements. An excess secretion of mucus in fish forms a non-specific response against toxicants, thereby probably reducing the toxicant contact. Mucous also forms a barrier between the body and the toxic medium, to minimize its irritating effect, or to scavenge it through epidermal mucus.Nno significant behavioural changes were observed in the fishes exposed to copper cyanide except for the secretion of mucous all over the body.

Thus, we can infer from our studies that toxicity of sodium cyanide (0.32 mg/L), zinc cyanide (0.35 mg/L) and copper cyanide (1.1 mg/L). Thus the study shows that sodium cyanide can be rated as highly toxic to fish followed by zinc cyanide and copper cyanide.

Mine accidents involving cyanide spills results in devastating ecological effects particularly when there are breaks in the ponds and a pulse of cyanide entire the watercourses (UNEP/ OCHA 2000). Along with water containing cyanides often the spill involves solid waste products mainly the muds containing the heavy metals such as copper, lead, zinc and cadmium. These can cause long term, effects on ecosystems as they persist for many years (Macklin et al. 2003). Metal cyanides also found in the waste waters in various forms depending upon the concentration of metal and cyanide. The complex cyanides of copper and nickel are commonly encountered in waste waters emanating from the plating industry waste waters. In most countries the statutory limits for the discharge of cyanide, copper and nickel in the water bodies are 0.2, 3.0 and 3.0 mg/ L, respectively *(Patil and Paknikar 1999).*

Metal cyanide complexes and breakdown products of cyanide degrading, although less toxic than the free cyanide, persist in the environment for long time because of their stable nature. These break down products could cause long term effects in addition to the immediate effects from the free cyanide. In the present study sodium cyanide is shown to be relatively toxic to fish exposed and copper cyanide is least toxic among the cyanides tested because of its stable nature and will not readily dissociate to give free. LC_{50} values of sodium cyanide to *Catla catla*, *Labeo rohita*, *Cirrhinus mrigala*, *Cyprinus carpio* and Oreo*chromis mossambicus* was found to be 0.11 mg/L, 0.19 mg/L, 0.33 mg/L, 1 mg/L and 0.420 mg/L (*David et al., 2010*) respectively. Whereas there is no data available on the acute toxicity of metal cyanide complexes particularly to Indian major carps. On comparing the LC_{50} values of different metal cyanides to the Indian major caprs, it is obvious that the sodium cyanide is more toxic as the fishes are less resistant to it followed by zinc and copper cyanide. However, the results of the present study are comparable with that of the other workers who have reported more or less similar values for different fish species *(David et al., 2010)*. Difference in the 96h LC_{50} value between the metal cyanides may be attributed the fact that cyanide induced changes in physiology and survival of aquatic organisms under stress is complicated because such changes differ from compound to compound, species to species and from one experimental condition to another. The exact causes of death due to cyanide poisoning are multiple and depend mainly on time-concentration combinations. However, there is no clear-cut explanation on the exact mode of action of different metals causing the mortality in aquatic animals.

For the sake of comparison of acute toxicities of different toxicants on the Indian major carps have showed that, carboryl toxicity to the fish *Catla catla* 64 ppm *(Tilak et al.1981)*, *Labeo rohita* exposed to hexavalent chromium 111.45 mg/L (Vutukuru 2005), *Cirrhinus mrigala* exposed to nitrite 10.4 mg/L (*Das et al. 2004*), *Labeo rohita* exposed to cypermethrin 0.139 ppm *(Das and Mukharjee 2003)*. These results show that the cyanides are more toxic to the carps. Carps are much sensitive to the copper *(De Boeck et al. 2004)*, even though copper is essential metal for metabolic processes, can be acutely toxic to the fishes as low as 10µg/L *(Lyndersen et al. 2002; Schjolden et al. 2007)*. Exposure to copper causes a number of effects in fish and lethal copper concentrations for carp can vary depending on fish size and water composition *(Sorensen 1991)*. *Alam and Maughan (1992)* found 96-h LC_{50} values varying from 4.7 to 15.8 µmol/L, depending on fish size whereas *Peres and Pihan (1991)* found 48-h LC50 values for carp juveniles (3.555 g) between 1.9 and 11.8 pmol/L depending on water hardness.

Different behavioural responses of metal cyanide complexes to *Labeo rohita* were also observed throughout the experimental periods. The control group showed the normal behaviour during the whole experimental periods. When exposed to lowest concentration of the metal cyanide complexes, fishes showed normal responses as the control along with staying motionless in group at the bottom. When the concentrations of metal cyanides increased gradually the neurotoxin effects resulting in the loss of balance, rapid opercular movements, followed by vertical movements. At the lethal concentration the fish started to swim spirally with sudden jerks. Finally the fish started to settle at the bottom of the test chamber. The appearance of jerks in this case is in accordance with findings of *Fukuto (1990)*.

Beauvais et al. (2000) and *Scholz et al. (2000)* reported that behavioral study is one of the important parameter in the assessment of toxicity of pesticides in fish. In the present study fishes showed normal behavior in control group but severity in different responses was observed with the increase in cyanide concentrations and passage of time. The abnormal behavior includes loss of balance, staying motionless in a group at bottom, lying laterally at bottom, swimming in spiral fashion with jerks, revolving in water, opened mouth and rapid opercular movements. Our results are in agreement with other studies *(Sarikaya and Yilmaz 2003; Selvi et al. 2005)* applying different pesticides on other fish species. Current study shows that metal cyanide complexes are highly toxic to the fingerlings of *L. rohita* particularly the sodium and zinc cyanide. Owing to high toxicity of metal cyanides, it is strongly recommended to handle the toxic compounds carefully using all the precautionary measures so that its harmful effects on aquatic life can be minimized.

Conclusion

Cyanides are one of the most rapidly acting poisons. Toxicity of the cyanide is due to its ability to inhibit the cytochrome oxidase, terminal respiratory chain enzymes. Cyanide has a special affinity for the heme ion and there by forming cyanide-heme cytochrome oxidase complex, which renders the enzyme incapable of utilizing the oxygen. Resultant anaerobic metabolism with severely decreased ATP generation and concomitant increase in lactic acid production eventually leads to tissue hypoxia and metabolic acidosis. Cyanide is also potent stimulator of neurotransmitter release both in the CNS and peripheral nervous system. All of these events contribute to the acute toxic syndrome. Subacute or chronic cyanide poisoning is characterized by prolonged energy deficit, loss of ionic homeostasis and oxidative stress leading to CNS pathology. Current study shows that metal cyanide complexes are highly toxic to the fingerlings of *L. rohita* particularly the sodium cyanide as compared to zinc and copper cyanide.

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Ispoljavanje aktune toksičnosti metal cijanida kod *Labeo rohita* – indijskog velikog šarana (Hamilton)

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Rezime

Statički, biološki testovi su urađeni kako bi se odredilo ispoljavanje akutne toksičnosti (LC_{50}) metal cijanida kod indijskog velikog šarana *Labeo rohita.* 96-časovna vrednosti LC_{50} za natrijum-cijanid, cink-cijanid i bakar-cijanid utvrđena kod *L. rohita* su bile 0.32 mg/L 0.35 mg/L, i 1.1 mg/L respektivno. Od svih ispitanih metal cijanida, natrijum cijanid je bio više toksičan u odnosu na ostale cijanid komplekse. Opšta reakcija riba izloženih cijanidu je uključivala

nekontrolisano plivanje, čudni pokreti, gubitak ravnoteže, kretanje u spiralnim pokretima sa iznenadnim pokretima, vertikalni pokreti, ležanje na strani u testnim komorama i brzi operkularni pokreti sa otvorenim ustima i konačno smirivanje na dnu.

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