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RESPIRATORY DISTRESS AND BEHAVIOURAL ANOMALIES OF INDIAN MAJOR CARP, *LABEO ROHITA* (HAMILTON) EXPOSED TO SODIUM CYANIDE

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Abstract
Sodium cyanide, is highly contaminating aquatic ecosystems as a toxic pollutant, was investigated in the present study for acute toxicity on freshwater fish *Labeo rohita.* The toxicity tests were conducted by static renewal bioassay method on the juveniles of fish was evaluated. The LC_{50} value of sodium cyanide to *Labeo rohita* was found out to be 320 µg/L. One third (106 µg/L) and one fifth (64 µg/L) of the LC_{50} value was selected for sublethal studies. Behavioural patterns and oxygen consumption were observed in both (1/3rd and 1/5th) sublethal concentrations (1, 5, 10 and 15 days). Sodium cyanide is highly toxic to the animal tested. Fish behaved irregular, erratic, and dartic movements, by followed hyperexcitability, loss of balance, finally settled to the bottom of the test chamber. A decrease in oxygen consumption was observed in 1/3rd (11.62% and -4.52%) and 1/5th (9.11% and -2.82%) sublethal concentrations. Fish under sublethal concentration were found to be under stress but not fatal.

Key Words: Behavior, *Labeo rohita*, Sodium cyanide, Oxygen consumption.

Introduction
The use of cyanide compounds by the mining industry, coupled with limitations in current analysis and monitoring of these compounds, raises serious concerns regarding public safety and environmental protection at mine sites using cyanide processing. Cyanide and its salts are used extensively in electroplating, extraction of ores (Sliver and gold), metal processing, photographic processes, production of synthetic rubber, chemical synthesis, manufacture of plastic, pesticides, dehairing of hides, laboratory processes, manufacture of dyes and pigments (ATSDR, 1988, UPA, 1985 and WHO, 1984) and are found in water and food consumed by animals and humans. Chronic exposure to low levels of cyanide is suspected to be responsible for various neuropathic and thyrotoxic conditions in humans. However, cyanide also tends to react readily with many other chemical elements, and is known to form, at a minimum, hundreds of different compounds (Flynn and Haslem, 1995). Many of these breakdown compounds, while generally less toxic than the original cyanide, are known to be toxic to aquatic organisms. In addition, they may persist in the environment for long periods of time, and there is evidence that some forms of these compounds can be accumulated in plants (Eisler, 1991) and fish tissues (Heming, 1989).

The decision whether a certain xenobiotic is dangerous for the aquatic system and the food cycle, can only be made after the (a) mammalian acute toxicity (b) bacteria acute toxicity (c) fish acute toxicity and (d) biological dissociation tests have been carried out in detail (Ardali, 1990). The fact that increasing use of contaminating chemicals in many industrialised parts of the world makes the development of ecotoxicity measurement techniques an absolute necessity (Brando et al., 1992). The first step is the acute toxicity test on fish in order to show the potential risks of these chemicals (OECD, 1993). What is important is the toxicity in fish which is the last chain in the food cycle (Castano et al., 1996). *Labeo rohita* is a prime cultured and important staple freshwater fish generally found in rivers, ponds, and reservoirs.

The most affective indications of toxic pollution are the behavioural changes (Richmonds and Dutta, 1992). Hence, fish are ideal sentinels for behavioural assays of various stressors and toxic chemical exposure due to their 1) constant, direct contact with the aquatic environment where chemical exposure occurs over the entire body surface, 2) ecological relevance in any natural systems (Little et al., 1993), 3) ease of culture, 4) ability to come into reproductive readiness (Henry and Atchison, 1986), and 5) long history of use in behavioural toxicology. Behaviour provides a unique perspective linking the physiology and ecology of an organism and its environment (Little and Brewer, 2001). The metabolic response to changes in oxygen availability may vary, depending on the physiological state of the animal, level of activity and temperature

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(Burggren and Roberts, 1991). Like most fish, carps are oxygen regulators, meaning that they maintain their oxygen consumption at a constant level along a gradient of environmental oxygen concentrations, until a critical oxygen concentration, is reached, below which oxygen consumption begins to fall. Under conditions of stress, this critical oxygen is likely to increase, reflecting the decreased capacity of the fish to cope with environmental perturbations. The present study aimed to determine the acute toxicity of sodium cyanide, with reference to its effects on behaviour and dysfunction in the oxygen consumption to the freshwater fish *Labeo rohita*.

Materials and methods

A total of 320 healthy and active *L. rohita* fingerlings were procured from the State Fisheries Department, BRP, Shimoga, Karnataka, 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 (3.5±0.5 g, 6±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.

Sodium cyanide (99.9 %) 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. Required quantity of sodium cyanide was drawn directly from this stock solution using micropipette. The concentrations of test compounds used in short term definitive tests were between the highest concentrations at which mortality was 0%, and the lowest concentration at which mortality was 100% in the range finding tests. Each aquarium was stocked with fish with a ratio of 2 g fish per litre water. The amount of sodium cyanide to be added in each aquarium was calculated after the volume of each aquarium was accurately determined. The fish, in bathes of 10, were exposed to varying concentrations of sodium cyanide with 20 litres of water using three replicates for each concentration. There was a simultaneous control group together with the actual experiments. 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 24th, 48th, 72nd and 96th hours and dead fish were removed as and when observed. During the experimental period the control and sodium cyanide exposed fish were kept under constant observation for both sublethal concentrations to study behaviour and whole animal oxygen consumption. One third (106 µg/L) and one fifth (64 µg/L) of the LC_{50} values were selected as sublethal concentrations for chronic study (1, 5, 10 and 15 days).

The behavioural changes of the healthy fish and the fish subjected to various doses of sodium cyanide and evaluated as regard to behaviour anomalies. The experiments were carried out with static acute experimental method. Acute toxic effect of sodium cyanide on *L. rohita* was determined by the use of Finney Probit Analysis (Finney, 1971), Dragstedt-Behrens equation (Carpenter, 1975), as mentioned by Bhargava and Rawat, (1999). Whole animal oxygen consumption was measured for both sublethal concentrations beside the control by following the methods Welsh and Smith (1953), as described by Saroja (1959). Difference in the dissolved oxygen content of the water before and after experiment yielded the amount of oxygen consumed by the fish during the period of experimentation. The oxygen consumed by normal and treated fish was determined. After experimentation, the fishes were individually weighed and their unit metabolism was calculate and expressed as millilitres of oxygen consumed per gram wet weight of fish per hour. The data were subjected to analysis of variance (ANOVA), and the means compared using Duncan's new multiple ranges to test at a 0.05 % confidence level (Duncan, 1955) to draw the mean comparison among the results.

Results and Discussion

Acute toxicity (96 h LC_{50}) of sodium cyanide for the freshwater fish, *L. rohita* was found to be 320 µg/L (Table 1). The upper and lower 95% confidence limits are presented in Table 2. No significant mortality was observed during the experimental periods in both the sublethal concentrations. We can infer from our results that sodium cyanide is highly toxic to freshwater fish, *L. rohita*.

In the present study, the control fish were active for feeding 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 exhibited disrupted shoaling behavior, localization to the bottom of test chamber and independency (spread out) in swimming. This is 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 sodium cyanide in both the sublethal 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. Mucus also forms a barrier between the body and the toxic medium, to minimize its irritating effect, or to scavenge it through epidermal mucus. Disrupted shoaling behavior, easy predation, gulping air, and swimming at the water surface (surfacing phenomenon) were observed on the day of exposure to sublethal concentrations of sodium cyanide.

A change in respiration rate is one of the common physiological responses to toxicants and is easily detectable through changes in oxygen consumption rate, which is frequently used to evaluate the changes in metabolism under environmental deterioration. It is clearly evident from the studies (Table 3) that sodium cyanide affected oxygen consumption of *L. rohita* under sublethal concentrations. Fish exposed to one third sublethal concentration depicted increased oxygen consumption on day 1 to day 5 and decreased on day 15. In one fifth sublethal concentration exposure, oxygen consumption decreased on day 1 and increased on day 5 and but day10 to 15 witnessed decrement as compared to control . However, it is interesting to note that on both sublethal day 5 depicted extremely high oxygen consumption (21.30% and 15.13%) (Fig1).

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

Conc. of Sodium Cyanide (µg/l)	Log Conc.	No. of fish alive out of ten	% Corrected mortality	Probit Kill
290	2.4623	9	10	3.72
295	2.4698	8	20	4.16
300	2.4771	7	30	4.48
315	2.4983	6	40	4.75
320	2.5051	5	50	5.00
325	2.5118	3	70	5.52
330	2.5185	2	80	5.84
335	2.5250	1	90	6.28
340	2.5314	0	100	---

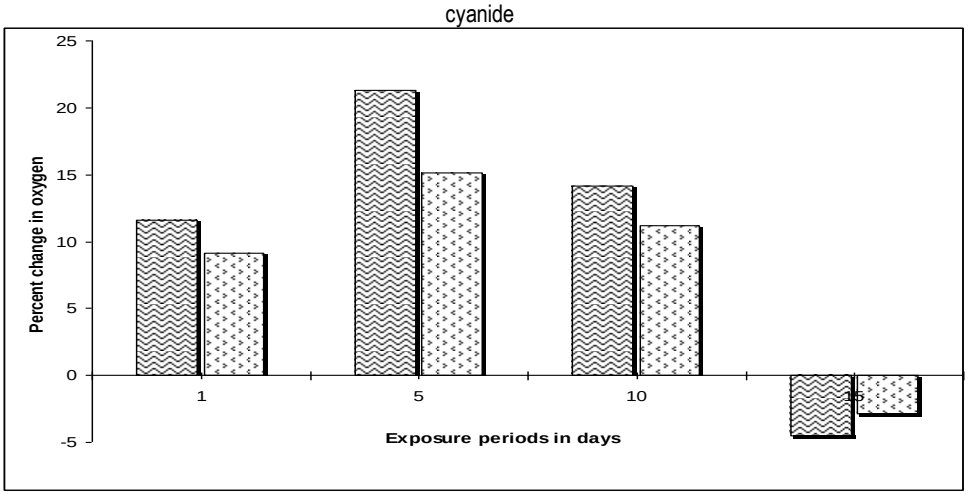
Table 2. 96 h LC50, slope and 95% confidence limits of sodium cyanide to the freshwater fish, *Labeo rohita*

Toxicant	96 h LC_{50} (µg/L)	Slope	95% Confidence limits	
			Upper limit	Lower limit
Sodium cyanide	320	1.6980	322.34	321.78

Table 3. Oxygen consumption (ml of oxygen consumed/g wet wt. of fish/h) of the fish, *L. rohita* following exposure to sublethal 1/3rd (106 µg/L) and 1/5th (64 µg/L) concentrations of sodium cyanide.

Estimations	Control	Exposure periods in days							
		One third				One fifth			
		1	5	10	15	1	5	10	15
Oxygen consumption	0.1662	0.4699	0.5279	0.4847	0.4857	0.4558	0.4903	0.4676	0.3938
±SD	0.0002	0.0003	0.0001	0.0002	0.0001	0.0002	0.0004	0.0003	0.0002
% Change	------	11.62	21.31	14.18	-4.52	9.12	15.13	11.22	-2.82

Fig 1: Oxygen consumption of the fish, Labeo rohita following exposure to sublethal concentrations of Sodium cyanide



With the increasing industrialisation human beings are continuously disturbing the delicate ecological balance in aquatic ecosystems. Among the various chemicals which cause environmental deterioration, cyanide is the most important one concerned to public and environmental health (Mathangi and Namashivayam, 2000). Acute cyanide poisoning in man and other animals have resulted in incordinated movements, convolutions, coma and respiratory distress (Burrows et al., 1973). Cyanide is regarded as notorious chemical dating back to antiquity (Bhattacharya, 2000). In aquatic ecosystem cyanide may affect the fish populations by direct lethal toxicity or through sublethal toxic effects imparted by pollutants of industrial wastes. The extent of cyanide toxicity to fish depends upon the rate of its intoxification in vivo (Cheng et al., 2001).

Behavioural responses meet the criteria as rapid tool for bioassay testing and could be easily standardised using sodium cyanide as reference toxicant. The development of behavioral methods in fish as important tools in aquatic toxicology has now reached the stage where they have been standardized. Behavioral responses represent an integrated response of fish species to toxicant stress (Kane et al., 2005). Changes in spontaneous locomotor activity and respiratory responses are sensitive behavioral indicators of sublethal exposure in fish (Scherer, 1992). A guide covering some general information on methods for qualitative and quantitative assessment of the behavioral responses of fish (locomotory activity, feeding, and social responses) during standard laboratory toxicity tests to measure the sublethal effects of exposure to chemical substances (ASTM, 2008) and a guide covering information on methods to measure and interpret ventilatory behavioral responses of freshwater fish to pollutants are available.

Behavior provides a unique perspective linking the physiology and ecology of an organism and its environment (Little and Brewer, 2001). Behavior is both a sequence of quantifiable actions, operating through the central and peripheral nervous systems and the cumulative manifestation of genetic, biochemical and physiologic processes essential to life, such as feeding, reproduction and predator avoidance. Behavioral endpoints have been slow to be integrated in aquatic toxicology because, until recently, there was a poor understanding of how alterations in behavior may be related to ecologically-relevant issues such as predation avoidance, prey capture, growth, stress resistance, reproduction and longevity (Kane et al., 2005).

The avoidance response by fishes is one form of phenotypic adaptation allowing fishes to survive in altered environment (Svecevieius, 1999). This situation further continued intensely throughout the test periods, which is in accordance with the observations made by Ural and Simsek (2006). Gulping of air may help to avoid contact of toxic medium and to ease respiratory stress. Surfacing phenomenon i.e., significant preference of upper layers in exposed groups may be due to elevated demand for oxygen during the exposure periods. Of all, phenomenon easy predation is one of the most serious damage caused by a pollutant on sensitive species like fish, which ultimately decide the survival of a species in a given ecosystem. Hyper extension of fins, dullness in body colour, and fish body became lean towards abdomen, and fish under stress were observed with time and dose in experimental periods. Intermittently, some of the fish sank to the bottom with their least opercular movements, failing to fight sodium cyanide stress in both the sublethal exposures. Leaning of fish indicates reduced feeding behavior, and diversion of fish metabolism towards adaptability to the toxic media.

Feeding preferences were affected and consumption of food in fish was impaired and reduced drastically. This was more pronounced in one-third of sublethal exposure periods. For these animals, it might be profitable to decrease their food uptake under toxic

environmental conditions to lower the energetic costs of digestion. Depression in appetite is a common response of fish to stress and intermittence of feeding for longer periods can have a clear impact on growth and reproduction. A substantial growth reduction caused by toxicant stress has important implications for survival in the natural situations. Dembele et al., (2000) indicated that the abnormalities in fish behavior observed in exposure with OP insecticides could be related to failure of energy production or the release of stored metabolic energy, which may cause severe stress, leading to the death of the fish. Fish in the lowest that is in one fifth ($1/5^{th}$ of 96 h of LC_{50}) sublethal concentration of sodium cyanide were alert, and fed actively.

Respiratory activity of a fish is often the first physiological response to be affected by the presence of contaminantes in the aquatic environment. Although many biological early warning systems monitor abnormal opercular movement as an indicator of respiratory stress, a more direct measurement of stress in this sense necessitates the quantification of oxygen consumed by the fish. The oxygen consumption is not often used as a bioindicator of pollution associated stress in biological early warning systems. Respiratory responses were found to be less sensitive, but also could be successfully used in bioassay testing of treated industrial and municipal effluents before they are discharged into receiving waters. Gill ventilation frequency and coughing rate are intimately associated with respiratory demands and gill irritation or blockage (Scherer et al., 1986). Oxygen consumption measurements provide a robust indicator of whole animal stress and concomitant water quality. Cyanide mode of toxic action towards mitochondria is well known. It acts on cytochrome oxidase competing with oxygen for the heme site. This effectively inhibits the final transfer of electrons to oxygen, destroys the mitochondrial membrane potential and stalls the production of ATP. Cyanide acts through the inhibition of cytochrome c oxidase in the respiratory electron transport chain of the mitochondria, impairing both oxidative metabolism and the associated process of oxidative phosphorylation. The high value recorded on 5^{th} day of both sublethal exposures, may be attributed to the initiation of specific protein synthesis or increased to detoxify the toxicant (Connell et al., 1999). Since most fish breathe in water in which they live, changes in the chemical properties thereof may be reflected in the animals ventilatory activity, particularly if the environment factors affect respiratory gas exchanges (Mushigeri, 2003). The fluctuated response in respiration may be attributed to respiratory distress as a consequence of the impairment of oxidative metabolism. Disturbance in oxidative metabolism was reported earlier under cypermethrin toxicity in *Tilapia mossambica* (David et al., 2003). Gills are the major respiratory organs and all metabolic pathways depend upon the efficiency of the gills for their energy supply and damage to these vital organs causes a chain of destructive events, which ultimately lead to respiratory distress (Magare and Patil, 2000). Pronounced secretion of mucus layer over the gill lamellae has been observed during sodium cyanide stress. Secretion of mucus over the gill curtails the diffusion of oxygen, which may ultimately reduce the oxygen uptake by the animal. If gills would be destroyed due to xenobiotic chemicals (Grinwis et al., 1998) or the membrane functions are disturbed by a changed permeability (Hartl et al., 2001), oxygen uptake rate would even rapidly decreased. On the other hand, the metabolic rate (in relation to respiration) of fish could be increased under chemical stress. Kalavathy et al., (2001) reported that the dimethoate is efficiently absorbed across the gill and diffuse into the blood stream resulting toxic to fish.

Conclusion

The analysis of data from the present investigation evidenced that sodium cyanide is toxic and had profound impact on behaviour and respiration in *L. rohita* in both sublethal concentrations. Thus it has led to the altered fish respiratory physiology. Variation in the oxygen consumption in sodium cyanide treated fish is probably due to impaired oxidative metabolism and sodium cyanide induced respiratory stress. Hence, dysfunction of behaviour and respiration can serve as index sodium cyanide toxicity in the fish L. rohita under laboratory condition.

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