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Effect of Zinc Cyanide on the Behavior and Oxygen Consumption in Air Breathing Fish *Channa gachua*

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ABSTRACT

Gills are vital respiratory and osmoregulatory organs in fishes. Cyanide is a fast acting poison because it binds to key iron containing enzymes required for cells to use oxygen as a result the tissue are unable to take oxygen from the blood. In the present investigation an attempt has been made to study the impact of zinc cyanide on the behaviour and oxygen consumption in air breathing fish, *Channa gachua*. Short term acute toxicity test was performed by static renewal bio-assay test over a period of 96 hours, using different concentrations of zinc cyanide and LC50 value was found to be 343 ug / liter. It was observed that the normal respiratory activity (oxygen consumption) was significantly affected due to the depression in the metabolic rate at the end of the exposure periods i.e. 24, 48, 72 and 96 hours respectively. The fish *Channa gachua* in different toxic media shows passive drift, active upstream movement, loss of balance, hyper excitability, moving in spiral fashion with sudden jerky movement and rapid flapping of the opercular movement was recorded. The variation in the oxygen consumption in zinc cyanide treated fish is probably due to impaired oxidative metabolism and cyanide induced respiratory stress. Hence, dysfunction of behaviour and respiration can serve as index of toxicity in *Channa gachua*. The details will be dealt in this paper.

Keywords: Zinc cyanide, behaviour, oxygen consumption, *Channa gachua*

INTRODUCTION

The rapid industrialization of streams, lakes and rivers are receiving an increasing load of industrial wastes. Beside water pollution in many cases these waters kills the fish and other aquatic organisms. Fresh water are highly vulnerable to pollution since they act as immediate sinks for the consequences of human activity and always associated with the danger of accidental discharges. The ability to detect, identify and properly respond to natural chemical stimuli is an important component of the environmental physiology of fishes. Cyanide is fast acting poison because it binds to key iron containing enzymes required for cells to use oxygen and as results tissues are unable to take up oxygen from the blood. In the absence of first aid poisoning from gas inhalation ingestion or absorption through the skin can kill within minutes Gosselin *et al.,* (1976). Some of the cyanide is changed to thiocyanate, which is less harmful and leaves the body urine. Some can also combine with hydroxo cobalamine to from B12. A small amount of cyanide is converted in the body to carbon dioxide, which leaves the body within the first 24 hours after exposure (WHO, 1996). Cyanide is considered as a potent suicidal, homicidal, genocidal and chemical warfare agent. Cyanides may be released into the aquatic environment through waste effluents 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 plant, metal cleaning and electroplating. Cyanide in the aquatic environment may also be associated with non-point sources including runoff from application on land and water of salt containing cyanide compounds as anti-caking agent. Many cyanide containing compounds are highly toxic, but some are not. Nitriles [which do not release cyanide ions] and Hexa cynoferrates [Ferrocyanide and Ferricyanide where the cynide is already tightly bound to an iron] have low toxicities, while most other cyanides are deadly poisonous. These cyanides when dissolved in water they get dissociated and highly toxic free cyanide ion gets released, which get binds to the transition viz. copper and zinc forming the metal cyanide complex. Cyanide complex exits in

solution as an ionic cyano metallates and is highly stable. Zinc cynide is an inorganic chemical compounds with the formula Zn(CN)2 . It adopts a polymeric structure consisting of tetrahedral zinc centers linked by bridging cyanide ligands. It is employed as a catalyst for the cyanosilylation of aldehydes and ketones. It is also used to introduce the formy1 groups in organic synthesis 2-Hydroxy-1Naphthaldehyde has been prepared from 2-Naphthol, zinc cyanide and anhydrous hydrogen chloride. Fish have become an indispintate model system for the evaluation of the extent of aquatic pollution. Fishes is used as biomarker of not only acute toxic effect but also of the consequence of long term exposure to low concentration of pollutants Whitfield and Elliot (2002). Information on the acute toxic effects of metal cyanide on complexes in fishes is limited and its effects forms an important links in the aquatic food chain, are not known. The objective of the present study was to determine the acute toxicity of zinc cyanide in *Channa gachua* and its effect on behavioral and oxygen consumption. The reported result would be useful contribution in the ecotoxicity risk assessment studies of zinc cyanide on this fish species.

MATERIALS AND METHODS

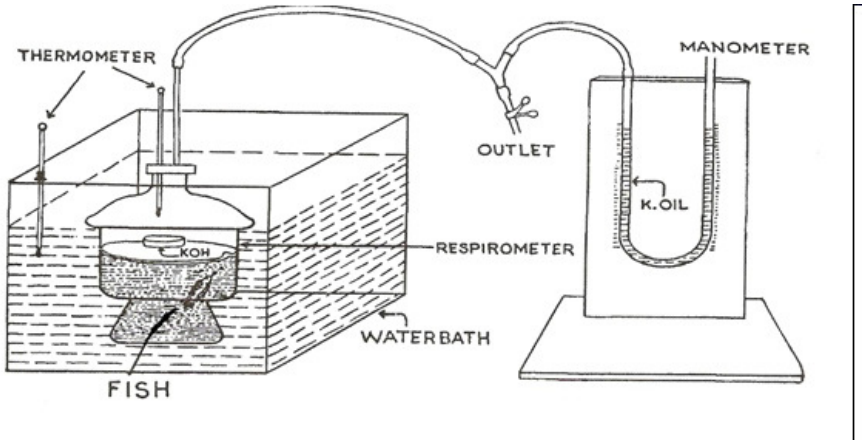

Fig. 1: Experimental set up for the measurements of dual mode of oxygen uptake in *Channa gachua*

Live specimens of *Channa gachua* were procured from local fish dealers at Hazaribag (Latitude 25º 59'N and Longitude 85º 22'E) and maintained in large glass aquaria size (90x60x60cm) with continuous flow of water. The specimens were fed on chopped goat liver daily during a minimum acclimation period of 15 days in the laboratory. Routine oxygen consumption from air and still water was measured in a closed glass respirometer containing 3 litres of water (initial O2 content = 6.5 mg O2/litre; pH = 7.2) and 0.51 ML of air (Fig.1). The fish had free access to air through a small semi circular hole (10 cm diameter) in a disc float. Carbosorb (B.D.H) or KOH in a petridish placed on the float absorbed CO2. Thus the fish could exchange gases with water by way of its gills as well as with the air using the suprabranchial chamber. The air phase of respirometer was connected to a differential manometer. Movement of the manometer fluid follow uptake of oxygen when the CO2 is absorbed by "Carbosorb" (KOH). The fish were acclimatized to the respirometers for at least 12 hours before the readings were taken. The concentration of dissolved oxygen in the water was estimated by Winklers volumetric method (Welch, 1948). The oxygen uptake through gills was calculated from the difference between the oxygen levels of the ambient water in the respirometer before and after the experiment and the reading of volume of water in the respirometer. Oxygen uptake from air was measured and calculated from the reading of volume change in the manometer and by the use of the combined gas law equations and vapour pressure (Dejours, 1975). Mean values of VO2 of a series of observations, on each fish at standard temperature pressure dry and standard errors were calculated. The experiments were conducted at 29.0 ± 1.5°C. The desired degree of concentrations was prepared by adopting the dilution techniques of APHA *et.al.,*(1971). The 96 hours bio assay tests were performed employing the technique of static bioassay tests (Doudoroff *et. al*., 1951). Five fish were used for each set of

experiment and mean values of oxygen uptake of all the fish of each set of experiment were taken and compared. The experimental fishes including controls were divided into different groups each containing ten fishes. The animals of control group got the treatment of normal saline. The difference of significance, if any between the control and experimental groups of fish was calculated by students 't' test at the level of 5%.

RESULTS AND DISCUSSION

No mortality was observed in the control ones however, mortality increased with an increase in the concentrations and the exposure of duration. The concentration at which there was zero percent mortality was (335ug/L) in Table 1. The analysis of data from the present investigation evidenced that zinc cyanide is highly toxic and had profound impact on behaviour and respiration in *C. gachua.* Variation in the oxygen consumption in treated fish is probably due to impaired oxidative metabolism and cyanide induced respiratory stress, copious mucus secretion and bulging of gills were also observed. The drop in the oxygen consumption rate in *C. gachua* exposed to zinc cyanide can also be attributed to clogging of gills by mucous. These findings clearly suggest decreased respiratory surface dysfunction of behaviour and respiration can serve as index zinc cyanide toxicity.

The behaviour and condition of fishes in both the control and treated solution was noted in every 24 h to 96 h. The fishes showed marked changes in their behaviour when exposed to the test solution of different concentrations. In lower concentrations of zinc cyanide [335ug/L] the fishes showed rapid swimming than in control ones. Behavioural manifestations of acute toxicity like hyperactivity, loss of balance and rapid swimming increased surfacing activity was seen. For fish acclimatized to the 27°C temperature, the specific oxygen consumptions decreases with increase in zinc cyanide concentration. The oxygen consumption of fish exposed to zinc cyanide for 24, 48, 72

and 96 h of median lethal concentration was 0.3561, 0.3102, 0.2836 and 0.1837 (mg O2/ L/h) respectively. Oxygen consumption increased in the initial 24 h of exposure to zinc cyanide concentrations. The oxygen uptake in *C. gachua* for 24, 48, 72 and 96 h was 0.3561, 0.3102, 0.2836 and 0.1837 (ml/kg/hr) the O2 up take increased in the initial 24 h of exposure to zinc cyanide concentration. However, an average O2 consumption in different time intervals of zinc cyanide exposure was significantly different from the control 0.4812 (mg/L). The decrease in O2 consumption in *C. gachua* exposed to zinc cyanide indicates the onset of acute hypoxia under cyanide stress because of the drop in metabolic rate in fishes.

In the course of 96 h toxicity test in zinc cyanide to *C. gachua*, there was no mortality observed in control fish. The oxygen saturation of water neither drop below 60 percent in any concentration test, nor in the control groups. Presence of the substance tested [above 80 percent of the nominal concentration] was provided by the means of daily exchange of the testing bath. Where as in the present study the acute toxicity of zinc cyanide to *C. gachua* may be attributed the fact that cyanide induced changes in the physiological 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 explanation on the exact mode of action of different metals causing the mortality in aquatic animals. Behavioral changes are most sensitive indication of potential toxic effects when studied. In the control groups the behavioural and swimming patterns of the fishes were normal and there was no mortality. The initial periods of exposure to zinc cyanide, the fishes stay in motion less and settled to the bottom. This can be attributed to the fact that, the sudden shock caused by the toxicant. The fishes began to swim naturally after an hour of exposure and the behavioural response

startedappearing only after 3 hors of treatment.

The shoaling behaviour was disrupted in the first day itself and they were spread out and appeared to be swimming independent of one another. The disturbance in the shoaling behaviour of the fish in the treated media indicates the loss of group hydrodynamic effect of fish Zuyev and Bolyayen (1970) increased swimming activity and entails high expenditure of energy. Erratic swimming of the treated fish indicates the loss of equilibrium. Cyanide has profound effect on the central nervous system. This is strongly supported by the changes in the neurotransmitter levels in the corpus striatum and cerebellum. It is likely that the region of the brain which is associated with the maintenance of equilibrium might have been affected by the cyanide intoxication. Surfacing phenomena as observed in the fish treated with lethal concentration of zinc cyanide indicates hypoxic condition. Such surfacing might be to procure definite proportion of its oxygen requirement from the atmosphe. Loss of equilibrium follows erratic and darting swimming movements, which might be due to the inhibition of brain cytochrome C-oxidase activity, causing the brain damage to the region of the associated with the maintenance of equilibrium David *et al*., (2007). In the study on the effects of eight selected organochlorine pesticides such as endosulphan, diazinone, phenyltrithian and methylparathion on eels, determined their 96 h LC50 values and reported behavioral changes in the fish. They observed anxiety, disorders in swimming pattern, loss of balance, excessive mucus secretion and lightening in colour. Although the modes of function of these insecticides are markedly different than zinc cyanide, behavioural changes observed are similar to our study. Bardbury and Coast (1989) reported signs of fenvelerate poisoning in fish, which included loss of schooling behaviour, swimming near the water surface, hyperactivity, erratic swimming, seizures, loss of buoyancy, elevated cough rate,

increased gill mucus secretions, flaring of the gill arches, head shaking and restlessness before death. Such effects may be due to osmotic stress which affects the nervous system of the animal.

The present investigation demonstrated that despite the regulatory capability of the fish exposed to the toxicant, the oxygen consumption rate was indeed increased in the initial 24 h of exposure to lethal concentration of zinc [0.3561 mg/L] and after decrease in the oxygen consumption [0.1837mg/L] was noticed at the end of 96 h. Similar results have also been observed in different fish species for different chemical substances (Chinni *et. al.,* 2002 and Wu and Chen 2004). The decrease in the oxygen consumption is probably the result of alterations of energy metabolism. Some studies of the pathological effects caused by chronic exposure to chemical substances evidenced the gradual destruction of gills filaments, killing the fish by asphyxia (Zaccone *et. al.,* 1985). The oxygen consumption endpoint also provides an index for sublethal stress and for bio monitoring the potentially toxic effects of chemicals. Downing (1953) studied the effect of oxygen concentration on the toxicity of potassium cyanide to rainbow trout and revealed that, as the oxygen concentration increases the toxicity of potassium cyanide decreased. The decrease in the oxygen consumption in *C. gachua* exposed to zinc cyanide indicates the onset of acute hypoxia under stress. Futher, the fact that the drop in metabolic rate of the fish as a protective measure to ensure a low intake of toxic substance that cannot be ruled out. Reduced oxygen consumption at higher concentrations of cyanide could also arise as result of respiratory inhibiting factors that come into play.

The primary site of action of cyanide is presumed to be the central nervous system. Cyanide acts through the inhibition of cytochrome C-oxidase in the respiratory electron transport chain of the mitochondria, impairing both oxidative phosphorylation Holland (1983) and Dreisenbach and Robertson (1987). Hence, a

number of other enzymatic processes are inhibited which exacerbate the toxicity and cyanides is also potent stimulators of events contribute to the acute toxic syndrome. Gills are vital respiratory organs and cellular damage induced by the metal might impair the respiratory function of the fish by reducing the respiratory surface area. The observed increase in the OBF and TBF in the initial 24 h of zinc cyanide and decline after has been reported by Qaisur (2012). The initial increase in the OBF as a primary response to sudden stress was also reported by Rajasekaran *et. al* (2009). In the present investigation the initial increase may be attributed to the sudden shock caused by the toxicant. This elicits the potency and sensitivity in the fish *C. gachua* in the test chemical. The ecological importance is that the damage to non target species in the environment and such attribute of the organisms could be effectively used as toxicity, biosensor of chemical stress.

Table1: The mortality of *Channa gachua* in 96 hours at different concentration of zinc cyanide.

Conc(ug/l)	Log Conc.	No.fish exposed	Death	Mortality	Emp.probit
280	2.5250	10	1	10	3.72
285	2.5314	10	2	20	4.16
290	2.5327	10	3	30	4.48
291	2.5340	10	4	40	4.75
292	2.5352	10	5	50	5.00
293	2.5378	10	7	70	5.84
294	2.5403	10	8	80	5.93
295	2.5415	10	9	90	6.28
296	2.5440	10	10	100	8.09

Table 2: The opercular beat of *Channa gachua* in 96 hours at different zinc cyanide concentrations and time interval

Exposue time	Control	96 h LC50 (343µg/L)
(Hr)		
12	89.1±2.1	115±3.5
24	68.85±0.6	113±3.5
36	89.34±0.8	91.86±2.7
48	89.00±0.4	79.5±3.8
60	89.70±0.0	70.13±0.8
72	89.79±1.5	53.67±1.8
84	89.58±0.2	40±2.5
96	89.15±1.9	24.75±0.0

Table 3: The tail beat frequency of *Channa gachua* at different zinc cyanide conc. and time interval.

Exposue time (Hr)	Control	96 h LC50 (343µg/L)
12	8.2±0.8	16.4±0.5
24	8.2±0.1	15±3.5
36	8.7±0.6	14±2.7
48	8.6±0.2	11±3.8
60	8.3±0.5	8.5±2.1
72	8.5±0.3	5.1±2.8
84	8.1±0.9	4.3±0.6
96	8.0±0.2	1.9±3.5

Table 4: Oxygen consumption of *Channa gachua* at different conc. of zinc cyanide and time interval

Exposue time (Hr)					
Oxygen consumption	control	24	48	72	96
O2/ml/kg/hr	0.4812	0.3561	0.3102	0.2836	0.1837
SD±	0.0001	0.0003	0.0003	0.0004	0.0003
% change	---------	-25.99	-35.53	-41.06	-61.82

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