



Sodium cyanide induced alteration in the whole animal oxygen consumption and behavioural pattern of freshwater fish *Labeo rohita*

Muniswamy David*, Jeyabalan Sangeetha and Etigemane R. Harish

Department of Studies in Zoology, Karnatak University, Dharwad – 580 003, India

*Corresponding Author E-mail: davidkcd@gmail.com

Publication Info

Paper received:
10 October 2013

Revised received:
26 March 2014

Accepted:
03 September 2014

Abstract

Sodium cyanide is a common environmental pollutant which is mainly used in many industries such as mining, electroplating, steel manufacturing, pharmaceutical production and other specialized applications including dyes and agricultural products. It enters aquatic environment through effluents from these industries. Static renewal bioassay test has been conducted to determine LC_{50} of sodium cyanide on indigenous freshwater carp, *Labeo rohita*. The behavioural pattern and oxygen consumption were observed in fish at both lethal and sub lethal concentrations. *Labeo rohita* in toxic media exhibited irregular and erratic swimming movements, hyper excitability, loss of equilibrium and shrinking to the bottom, which may be due to inhibition of cytochrome C oxidase activity and decreased blood pH. The combination of cytotoxic hypoxia with lactate acidosis depresses the central nervous system resulting in respiratory arrest and death. Decrease in oxygen consumption was observed at both lethal and sub lethal concentrations of sodium cyanide. Mortality was insignificant at sub lethal concentration test when fishes were found under stress. Consequence of impaired oxidative metabolism and elevated physiological response by fish against sodium cyanide stress showed alteration in respiratory rate.

Key words

Acute toxicity, Behaviour, *Labeo rohita*, Oxygen consumption, Sodium cyanide

Introduction

Cyanide is triple bonded carbon and nitrogen radical, which may be found in a wide variety of organic and inorganic compounds. It is very powerful and fast acting toxin, (Banerjee *et al.*, 2002; Maniyam *et al.*, 2011). The toxicity of cyanide is quite high due to its ability to poison the respiratory system by inhibiting the final transport of electrons from cytochrome C oxidase to oxygen and preventing production of ATP (Nůsková *et al.*, 2010). Cyanide comes from both natural and anthropogenic sources (O'Reilly *et al.*, 2003), such as mining, electroplating, steel manufacturing, polymer synthesis, pharmaceutical production, and other specialized applications including dyes, and agricultural products (Baxter and Cummings, 2006). Cyanide compound such as sodium cyanide (NaCN) and potassium cyanide (KCN) has been used for extraction of gold and silver from ore and also used in manufacturing industries for almost a

century. It forms tight complexes with heavy metals such as iron, gold and silver (Gonen *et al.*, 2004; Akcil, 2003). They also cause hazard to aquatic ecosystems in waste-receiving water ecosystem and to livestock.

Highly toxic sodium cyanide (NaCN) is increasingly used by the international mining community to extract gold and other precious metals through milling high grade-ores and heap leaching of low grade-ores, which requires cycling of millions of litres of alkaline water containing high concentrations of potentially toxic sodium cyanide, free cyanide and metal cyanide complexes that are frequently accessible to aquatic ecosystems and wildlife (Eisler *et al.*, 1999). Many species of fish and migratory birds were found dead in the immediate vicinity of gold-mining leach extraction facilities and tailing ponds (acute $LC_{50} < 1\text{mg l}^{-1}$), presumably as a result of contamination of cyanide in contaminated water (Eisler, 2003).

Fish are a biological indicator of water quality as they are more sensitive towards toxicant. Any changes in behaviour and physiology of fishes such as swimming, reproduction, susceptibility to predation, disrupted respiration, osmoregulatory disturbances and altered growth indicates decline of water quality (Eisler *et al.*, 1999). Behavioural changes and physiological response have been used as sensitive measures of stress syndrome in the organism (Rajamanickam and Karpaganapathi, 1988; David *et al.*, 2005). Determining oxygen consumption of aquatic animal undoubtedly provides information on the effect of toxicants on the physiology of aquatic life (Sarkar, 1999). Hence, the present study was carried out to evaluate the toxicity of sodium cyanide on the whole animal oxygen consumption and behavioural pattern of freshwater fish *Labeo rohita* exposed to lethal and sub-lethal concentration.

Materials and Methods

Labeo rohita, weighing 8 ± 2 g and measuring an average length of 10 cm were procured from the State Fisheries Department, Dharwad, Karnataka, India and acclimatized for 20 days under laboratory condition at 27°C. The fish were kept in large cement tank, previously washed with potassium permanganate solution to free walls of any microbial contamination.

Sodium cyanide (95%) (Batch No. V-1058/1; Cas No. 143-33-9) was procured from Loba Cheme Pvt Ltd. Mumbai, Maharashtra, India. A stock solution was prepared by dissolving sodium cyanide in double distilled water in standard volumetric flask. The fish, in batches of 10 were exposed to varying concentration of sodium cyanide with 10 l of water and maintaining three replicate for each concentration. Mortality was recorded every 24 hr and dead fish were removed and observed immediately. The concentration at which 50% mortality (LC_{50}) occurred were obtained graphically by Probit analysis, plotting concentration against fish mortality (Finney, 1971). During the experimental period, control and sodium cyanide exposed fish were kept under constant observation for lethal and sub lethal concentration to study the whole animal oxygen consumption and behavioural pattern. For further studies, 96 hr LC_{50} ($300 \mu\text{g l}^{-1}$

sodium cyanide) was selected for lethal concentration (1, 2, 3, 4 ds), whereas $1/5^{\text{th}}$ of LC_{50} ($60 \mu\text{g l}^{-1}$ Sodium cyanide) was selected for sub lethal concentration (1^{st} , 5^{th} , 10^{th} , 15^{th} day). The responses of fish to toxic medium was assessed in the form of change in the swimming pattern, group dynamics, avoidance to test medium and altered opercular movements as mentioned by Kumari *et al.* (1997).

Whole animal oxygen consumption was measured for both lethal and sub lethal concentrations besides control by following the method of Welsh and Smith (1953) as described by Saroja (1959). The difference in dissolved oxygen content of water and oxygen consumption of control and sodium cyanide treated fish were determined before and after the experiment. After the experiment the fishes were individually weighed and their unit metabolism was calculated and expressed as millilitre of oxygen consumed per gram wet weight of fish per hour (ml of oxygen consumed/g wet weight of fish/h). The data were subjected to analysis of variance and the means were compared using Duncan's Multiple Range Test (Duncan, 1955).

Results and Discussion

Cyanide compounds containing highly reactive cyanide anion (CN⁻), most commonly found in the environment as hydrogen cyanide and two cyanide salts such as sodium cyanide and potassium cyanide. The half-life of cyanide compound in aquatic ecosystems is about 5-15 days (Atkinson, 1994); and it acts rapidly in aquatic environment (ATSDR, 2006). 96 hr LC_{50} of sodium cyanide for the indigenous freshwater fish, *Labeo rohita* was found to be $300 \mu\text{g l}^{-1}$ (Table 1). Adverse effect of cyanide on fish include mortality, pathology, impaired swimming ability and relative performance, susceptibility to predation, disturbed respiration osmoregulatory disturbances and altered growth pattern (David *et al.*, 2010).

In the present study, the control group fish maintained a compact schooling and moved in coordinated manner, covering about $1/3^{\text{rd}}$ of the bottom during the initial 10 days of 15^{th} day experiment and on the 11^{th} day the school become less compact covering up to $2/3^{\text{rd}}$ of tank area. The fish were observed to scrub the

Table 1 : 96 hr LC_{50} of sodium cyanide for the indigenous freshwater fish, *Labeo rohita*

Concentration of sodium cyanide ($\mu\text{g l}^{-1}$)	Log Conc.	No of alive fish out of ten	% Mortality	Probit kill
280	2.4472	9	10	3.72
285	2.4558	8	20	4.16
290	2.4624	7	30	4.48
295	2.4698	6	40	4.75
300	2.4771	5	50	5.00
310	2.4983	3	70	5.52
315	2.5051	2	80	5.84
320	2.5119	1	90	6.28
330	2.5185	0	100	—

Table 2: The whole animal oxygen consumption of *Labeo rohita* on exposure to lethal and sub lethal concentrations of sodium cyanide

Estimation	Control	Lethal concentration exposure periods (Days)				Sub lethal concentration exposure periods (Days)			
		1	2	3	4	1	5	10	15
Mean	0.5722 ^A	0.4009 ^D	0.3827 ^F	0.2299 ^H	0.1586 ^I	0.4908 ^B	0.4249 ^C	0.4249 ^E	0.3018 ^G
±SD	0.0003	0.0014	0.0023	0.0004	0.0004	0.0003	0.0003	0.0003	0.0004
% Change	—	-28.36	-33.11	-59.82	-72.28	-14.22	-25.74	-29.67	-47.25

Values are Mean ± SD (n=6) for Oxygen consumption in a column followed by the same letters are not significantly different (Pd=0.005) from each other according to Duncan's Multiple range Test (DMRT).

bottom surface, when startled they instantly formed a tight school which was maintained briefly. They were sensitive to light and moved to the bottom of the tank when light was passed into the tank except for a lower response to form a dense school towards the end of the study, no other extraordinary behaviour was observed, the same had been reported by Shwetha and Hosetti (2009).

The lethal concentration of sodium cyanide induced irregular, erratic and sometimes jerky movements, hyperexcitability and loss of equilibrium, followed by muscular coordination, convulsion, tremors and sinking to the bottom. The schooling behaviour was disrupted on the 1st day itself and the fish occupied twice the area as that occupied by control group. The fish exposed to sodium cyanide were spread out and appeared to be swimming independent of each another. The fish attempted to leap out of test solution and the body turned slight by pale blue in color, which was pronounced on the dorsal surface and enhanced with exposure. A similar report was given by Hosetti and Praveen (2011). The fish were found to be in a state of excitement, and an increase in the pectoral, pelvic and caudal fin "Flicker" was observed; the fish progressively showed signs of tiredness and lost positive geotaxis. Finally they showed surface phenomenon and death with widely extended gills (Magare and Patil, 2000). Alteration in body color to light pale blue was presumably due to the formation of ferrihaemoglobin-cyanide complex as dimethyl amino phenol (DMAP) forms methaemoglobin by seating of a catalytic cycle inside the erythrocyte, in which DMAP is oxidized to N-N dimethylquinoneimine, which later oxidizes haemoglobin to methaemoglobin, 4-DMAP induced ferrihaemoglobin complexes may induce change in the body color to light blue (Govind, 2013).

During sub lethal treatment, the schooling behaviour of fish was slowly disrupted on 1st day. On exposure to sub lethal concentration of sodium cyanide on 10th and 15th day, the fish became alert at the slightest disturbance. The body leaned towards the bottom position when compared with control fish, which indicated reduced amount of dietary protein consumed by the fish at sodium cyanide stress which was immediately utilized and was not stored in the body. Similar observations were made in the fish *Sarathierodon mossambica* after exposure to dimethioate (Kalavathi *et al.*, 2001). The fish in sub lethal concentration were found under stress, but the stress was not fatal. Most of the absorbed sub lethal concentration of cyanide reacts with thiosulphate in the presence of rhodanase to produce the

comparatively non toxic thiocyanate, most of which is excreted in the urine. Similar observation revealed that rapid detoxification enables animals to ingest high sub lethal doses of cyanide over extended period without harm (Dube and Hosetti, 2010) Hence, the fish seem to have adapted to sub lethal concentration of sodium cyanide. Acclimatization of fish to low sub lethal levels of cyanide through continuous exposure might enhance their resistance to potentially lethal concentration (Eisler and Wiemeyer, 2004) which is in accordance with the current observation.

The rate of oxygen consumption in control and treated fish are presented in Table 2. The data indicates that oxygen consumption of fish exposed to lethal and sub lethal concentration of sodium cyanide decreased steadily with increasing time. The observed decrease in oxygen consumption by may be due to respiratory distress resulting due to impairment of oxidative metabolism. The fluctuated response in respiration may be attributed to respiratory distress as a consequence of impairment of oxidative metabolism. Disturbance in oxidative metabolism was reported earlier under cypermethrin toxicity in *Tilapia mossambica* (David *et al.*, 2003). Cyanide rapidly inhibits the electron transport chain of vital organ to the lethal level, cyanide is primarily a respiratory poison and one of the most rapidly effective toxicants. The toxic effect of cyanide is due to its affinity for ferric heme form of cytochrome A3, also known as cytochrome C oxidase, the terminal oxidase of mitochondrial respiratory channel (David *et al.*, 2009). The formation of stable cytochrome C oxidase CN complex in mitochondria produces a blockage of electron transfer from cytochrome C oxidase to molecular oxygen and cessation of cellular respiration, causing cytotoxic hypoxia in the presence of normal haemoglobin oxygenation. Tissue anoxia induce due to inactivation of cytochrome C oxidase causes a shift from aerobic to anaerobic metabolism, resulting in depletion of energy rich compound such as glycogen, phasopocreatinin and adenosine tri phosphate and accumulation of lactate with the decreased blood pH as impaired oxidative metabolism. Gills are the major respiratory organ and all metabolic pathway depend upon efficiency of gills energy supply damage to these vital organ causes a chain of destructive events, which ultimately lead to respiratory distress (Shwetha and Hosetti, 2009). In agreement with this, the present study also reported that depletion of oxygen consumption was due to disorganization of respiratory function caused by rupture of the respiratory epithelium of the gills.

Hosetti and Praveen (2011) reported that depletion in oxygen consumption was due to disorganization of the epithelium of gill and to the disturbance in mitochondrial integrity and decreased activity of some mitochondrial enzyme. Decrease can also be attributed to induction of hypoxic condition within the animal due to intimate contact of the respiratory surface with toxicant in water, resulting in the alteration of respiratory area of the animal. Secretion of mucus layer over gill lamella has been observed during sodium cyanide stress. Coagulation of mucus on gill causes demolition of various important processes such as gas exchange, nitrogen excretion balance and circulation of blood. Decrease in oxygen consumption at sub lethal concentration of sodium cyanide appears to be mainly due to decrease in the energy requirements which is considered to be adaptive and even strategic. Generalization of data from the present investigation provides evidence that sodium cyanide primarily induces high physiological stress in *Labeo rohita* at both lethal and sub lethal concentration, resulting in profound impact on whole animal oxygen consumption and behavioural pattern.

Acknowledgment

Authors are thankful to the University Grant Commission (UGC) -New Delhi (F. No. 41-103/2012(SR) Dated. 13/07/2012) for providing financial support.

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