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ASSESSMENT OF SODIUM CYANIDE TOXICITY ON FRESHWATER TELEOSTS

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Abstract

Short term definitive test by static renewal bioassay method were conducted to determine the toxicity of sodium cyanide on freshwater teleosts, *Catla catla*, *Labeo rohita*, *Cirrhinus mrigala, Cyprinus carpio* and *Oreochromis mossambicus*. Fishes were exposed to various concentrations of sodium cyanide for 96 h and the mortalities were recorded. The acute toxicity (LC_{50}) of sodium cyanide calculated over 96 h period for *C. catla, L. rohita, C. mrigala C. carpio* and *O. mossambicus* were found to be 0.11 mg/L, 0.19 mg/L, 0.33 mg/L, 1 mg/L and 0.420 mg/L, respectively. Behavioural patterns were observed in lethal (1, 2, 3 and 4 d) concentration. Some of the common behavioural changes exhibited by teleosts in toxic media include hyperexcitability, erratic and darting movements with imbalanced swimming activity, which might 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 and myocardium, which are the most sensitive critical sites for anoxia, resulting in respiratory arrest and death.

Key Words: Sodium cyanide; freshwater fishes; 96 h acute toxicity (LC_{50}); Behaviour.

Introduction

Cyanide is one of the most toxic chemical substances on Earth and is toxic to most aquatic life and humans, even at low concentrations. Cyanides are used widely and extensively in the manufacture of synthetic fabrics, plastics, electroplating baths, and metal mining operations, as pesticidal agents, intermediates in agricultural chemical production and for some therapeutic applications. Unlike toxic metals, cyanide is not an element but a compound composed of only carbon and nitrogen. The in-depth chemistry of cyanide and its chemical behaviour in streams and sediments is complex and its toxicity is influenced by several factors, including acidity or alkalinity (Moran, 2004). Free cyanide does not bioaccumulate up the food chain, or persists like toxic metals. Its toxicity is instant or acute and, once reacted, leaves little residual trace.

In Hungary, the spill was reported to have caused a five km carpet of dead fish and left a quarter of the population without drinking water (Cunningham, 2005). There were adverse impacts on local communities, economies and also tourism, all because of the lack of a fishing industry (UNEP/OCHA 2000a, b). The scale of the disaster resulted in calls for gold mining using cyanide to stop (Korte and Coulston, 2000).

In mining, cyanide is typically used to dissolve gold from crushed ore. The gold is then adsorbed onto charcoal and the excess cyanide stored for recycling in ponds. Problems occur when there are breaks in the ponds and a pulse of cyanide enters watercourses. Often, tailings and cyanide are released in these accidents causing multiple environmental effects. Mine accidents involving cyanide spills result in devastating ecological effects. Typically, the cyanide kills all life in the streams it enters for a considerable distance. Waste water containing cyanide from these industries must be treated so that it contains a low level (<1 mg/L) of cyanide before being released into the environment (Raybuck, 1992). The in-depth chemistry of cyanide and its chemical behaviour in streams and sediments is complex and its toxicity is influenced by several factors, including acidity or alkalinity (Moran, 2004).

Sodium cyanide (NaCN) is important in metallurgy. Highly toxic sodium cyanide is used increasingly by the international mining community to extract gold and other precious metals which require cycling of millions of liters 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.

However, data are scarce on background concentrations of cyanides in various non-biological materials and hence, there is a need to asses the relative environmental hazards of cyanide on aquatic ecosystem. Since, fish were the most cyanide sensitive aquatic organisms tested (Eisler, 1991), consequently; an attempt has been made in the present study to determine the acute toxicity (LC_{50}) of sodium cyanide and relative sensitivity of fish species followed by its impact on behaviour.

Materials and Methods

C. catla (2 ± 0.2 g, 4.0 ± 0.45 cm), *L. rohita* (2 ± 0.2 g, 4.5 ± 0.7 cm), *C. mrigala* (2 ± 0.2 g, 5 ± 0.8 cm) and *C. carpio* (2 ± 0.2 g, 4 ± 0.4 cm) fingerlings were procured from the State Fisheries Department, Dharwad, Karnataka, India and are acclimatized to laboratory condition for 15 days at 24 ºC and are held in 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, 22 ± 2 ºC; pH, 7.2 ± 0.1 at 24 ºC; dissolved oxygen, 8.2 ± 0.9 mg/L; total hardness, 23.4 ± 3.4 mg as $CaCO_3$/L; salinity-nil; specific gravity, 1.003 and conductivity less than 10 μScm^{-1}. Water was renewed thrice in a week. Fish were fed regularly with oil cake and rice bran and feeding was stopped two days prior exposure to test medium with 12-16 h of photoperiod daily during acclimation and test periods.

Sodium cyanide of 95% purity was procured from Loba Chemie Pvt. Ltd., Mumbai, India. The characteristics of sodium cyanide are given in Table 1.

Table 1. Some properties of sodium cyanide (EPA 1989).

Property	Sodium cyanide
CAS number	143-33-9
Chemical formula	NaCN
Molecular weight	49.01
Physical state	Solid
Boiling point (ºC)	1,496
Melting point (ºC)	563.7
Specific gravity	1.6
Solubility in water (g/L)	480 at 10 ºC

Test solutions of respective quantity of sodium cyanide were prepared by dilution of a stock solution. Stock solution was prepared by dissolving sodium cyanide in double distilled water in a standard volumetric flask.

Concentrations of the test compounds used in short term definitive tests were between the highest concentration at which there was 0% mortality and the lowest concentration at which there was 100% mortality in the range finding tests. Water was renewed every day over four days. The control and the sodium cyanide exposed fish were aerated frequently to prevent hypoxic condition of the medium and are kept under continuous observation during the experimental period 24, 48, 72 and 96 h for lethal concentration. Henceforth, the replacement of the water medium was followed by the addition of the desired concentration of the test compound. The fish were exposed in batches of ten to varying concentrations of sodium cyanide with 20 L of water in three replicates for each exposure. Exposure concentrations were confirmed in the test media by the method described in the APHA (2005). For LC_{50} calculation, mortality was recorded every 24 h and dead fish were removed when observed. Duncan's multiple range test (DMRT) was employed for comparing mean mortality values after estimating the residual variance by repeated measures ANOVA (Winner, 1971) for arc sine transformed mortality data (dead individuals/initial number of individuals). Time of exposure was the repeated measure factor while treatment (concentration and control) was the second factor. In addition, LC_{50} were compared by the method of APHA (2005). The LC_{50} with 95% confidence limit for sodium cyanide were determined/estimated for 96 h by probit analysis (Finney, 1971).

Results and Discussion

Cyanide acts rapidly in aquatic environments and does not persist for extended periods, and is highly species selective. Many chemical forms of cyanide are present in the environment, including free cyanide, metallocyanide complexes, and synthetic organocyanides, also known as nitriles. But only free cyanide (i.e., the sum of molecular hydrogen cyanide, HCN, and the cyanide anion, CN^-) is the primary toxic agent, regardless of origin. The observed toxicity to aquatic life of simple and complex cyanides were attributed almost entirely *too* molecular hydrogen cyanide (undissociated, HCN) derived from ionization, dissociation, and photodecomposition of cyanide containing compounds. All simple cyanides ionize in water to release cyanide ion which, depending on pH, will form hydrocyanic acid (HCN). Only free cyanide is considered to be a biologically meaningful expression of

cyanide toxicity. Adverse effects of cyanide on fish include delayed mortality, pathology, impaired swimming ability and relative performance, susceptibility to predation, disrupted respiration, osmoregulatory disturbances, and altered growth patterns (Leduc *et al.,* 1982; Leduc, 1984). At lethal levels, cyanide is primarily a respiratory poison and one of the most rapidly effective toxicants known (Leduc *et al.,* 1982). Cyanide's toxic effect is due to its affinity for the ferric heme form of cytochrome a_3, also known as cytochrome c oxidase, the terminal oxidase of the mitochondrial respiratory chain (Towill *et al.,* 1978; Egekeze and Oehme, 1980; Solomonson, 1981; Way, 1981 and 1984; Leduc *et al.,* 1982; Biehl, 1984; Ballantyne, 1987; Marrs and Ballantyne, 1987; Yamamoto, 1989).

The acute toxicity (LC_{50}) of sodium cyanide calculated over 96 h period for *C. catla, L. rohita, C. mrigala, C. carpio and O. mossambicus* were 0.11 mg/L, 0.19 mg/L, 0.33 mg/L, 1 mg/L and 0.42 mg/L, respectively. The Relative toxicity (RT) of sodium cyanide for *C. catla* was followed by *L. rohita*, *C. mrigala* and *O. mossambicus* i.e., 9.09, 5.26, 3.03 and 2.38-fold greater than that of *C. carpio,* respectively (Table 2).

Table 2. Acute toxicity (LC_{50}) of sodium cyanide calculated over 96 h periods for *C. catla, L. rohita, C. mrigala, C. carpio* and *T. moosambicus.*

Experimental time (h)	**LC_{50} (mg/L)**				
	C. catla	***L. rohita***	***C. mrigala***	***C. carpio***	***O. mossambicus***
96	0.11 (0.07-0.14)	0.19 (0.15-0.26)	0.33 (0.30-0.39)	1.0 (0.96-1.17)	0.42 (0.40-0. 43)
Relative toxicity	9.09	5.26	3.03	1.0	2.38

Copious biological and abiotic factors are known to modify the biocidal properties of free cyanide, including water pH, temperature, and oxygen content; life stage, condition, and species assayed; previous exposure to cyanide compounds; presence of other chemicals; and initial dose tested. There is general agreement that cyanide is more toxic to freshwater fish under conditions of low dissolved oxygen (Doudoroff, 1976; Towill *et al.,* 1978; Smith *et al.,* 1979; EPA, 1980; Leduc, 1984); that juveniles and adults were the most sensitive life stages tested and embryos and sac fry the most resistant (Smith *et al.,* 1978, 1979; EPA, 1980; Leduc, 1984); and that substantial interspecies variability exists in sensitivity to free cyanide (Smith *et al.,* 1979; EPA, 1980). Initial dose and water temperature both modify the biocidal properties of hydrocyanic acid (HCN) to freshwater teleosts. Sodium cyanide toxicity expressions in the tested teleosts exhibited substantial interspecies variability and relative sensitivity in the present investigation.

Some of the common behavioural abnormalities of *C. catla, L. rohita, C. mrigala, C. carpio* and *O. mossambicus* on exposure to sodium cyanide. Some of the common behavioural abnormalities of sodium cyanide on *C. catla, L. rohita, C. mrigala* and *C. carpio* include irregular, darting and erratic swimming movements, hyperexcitability, loss of equilibrium followed by muscular incoordination, convulsions, tremors and sinking to the bottom. Fish attempted to leap out of test solutions and body turned to light blue colour, which pronounced on dorsal surface and enhanced with exposure. Finally, surfacing phenomenon and death with widely extended gill covers. Similar observations that cyanide has a strong, immediate and long-lasting inhibitory effect on the swimming ability of fish were made (Leduc, 1984).

Animal behaviour is a neurotropically regulated phenomenon, which is mediated by neurotransmitters (Bullock *et al.,* 1977). Loss of equilibrium follows erratic and darting swimming movements with muscular incoordination and imbalanced body activity, which might be due to inhibition of brain cytochrome c oxidase activity causing cytotoxic hypoxia, which is a critical site for anoxia trailed by changes in electrical activity of brain thus causing damage to the region of the brain associated with the maintenance of equilibrium (Sambasiva and Chandrashekar, 1987). Changes in ventilation rate and surfacing frequencies are the general symptoms noticed in the fish after exposure to sodium cyanide which may probably due to cyanide induced tissue anoxia through inactivation of cytochrome c oxidase, causing cytotoxic hypoxia in the presence of normal haemoglobin oxygenation (Leduc, 1984) thus elicit fish to haul out more oxygen via surfacing, so as to ease respiratory stress and these activities help the fish to avoid contact with poison and fight against stress (Ray and Munshi, 1987).

Alteration in body colour to light blue colour is presumably due to the formation of ferrihaemoglobin-cyanide complex, in view of the fact that, Dimethylaminophenol (DMAP) forms methemoglobin by setting up a catalytic cycle inside the erythrocyte, in which oxygen oxidizes the DMAP to N-N-dimethylquinoneimine, the latter oxidizing the haemoglobin to methemoglobin (Marrs, 1987). The 4-dimethylamino-phenol induced ferrihaemoglobin

production, which combined with the cyanide in the red cells forms ferrihaemoglobin-cyanide complex (Christel *et al.,* 1977), may induce change in body colour to light blue.

Fish mortality could be due to rapid inhibition of cytochrome c oxidase, the terminal oxidase of the mitochondrial respiratory chain thus blocking electron transport chain of vital organs due to passage of free cyanide (HCN) through critical sites. Critical sites for cyanide toxicity in freshwater organisms include the gills, and other sites where gaseous exchange and osmoregulatory processes occur. On passing through these semipermeable membranes, the hydrocyanic acid molecules are usually distributed by way of the circulatory system to various receptor sites where toxic action or detoxification occurs. Once in the general circulation, cyanide forms stable cytochrome c oxidase-cyanide complex in the mitochondria, thus resulting impasse 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 induced by the inactivation of cytochrome c oxidase causes a shift from aerobic to anaerobic metabolism, resulting in the depletion of energy-rich compounds such as glycogen, phosphocreatine and adenosine triphosphate, and the accumulation of lactate with decreased blood pH. Death in the lethal concentration may be due to the combination of cytotoxic hypoxia with lactate acidosis depresses the central nervous system and myocardium, which are the most sensitive critical sites of anoxia, resulting in respiratory arrest and death. Among the tested species of fishes; *C. catla* was the most sensitive and *C. carpio* most hardy to cyanide concentrations.

The analysis of data from the present investigation evidenced that sodium cyanide is primarily toxic to fishes and induced high physiological stress on teleosts at lethal concentrations, resulting in profound impact on behaviour.

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