Acute Effects of Zinc Cyanide on the Behaviour and Oxygen Consumption of the Indian Major Carp Cirrhinus mrigala (Hamilton)

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Abstract: An attempt has been made in the present study to evaluate the acute toxicity of Zinc cyanide and its toxicological effects on behaviour and oxygen consumption parameters of widely consumed Indian major carp Cirrhinus mrigala. Short-term acute toxicity tests were conducted by static renewal bioassay test over a period of 96 h, using different concentrations of zinc cyanide to the fish and LC₅₀ value was found to be 343 µg/L. Results revealed that the normal respiratory activity (O₂ consumption) of the fish was significantly affected and there is a depression in the metabolic rate at the end of exposure periods (24, 48, 72 and 96 h). Fish in toxic media exhibited passive drift, active upstream movement, erratic movements, loss of balance, hyper excitability, moving in spiral fashion with sudden jerky movements, vertical movements and rapid flapping of the opercular movements with opened mouth finally settles to the bottom. This study reflects the extent of the toxic effects of zinc cyanide and induced cumulative deleterious effect in the widely consumed freshwater fish, Cirrhinus mrigala.

Key words: Behaviour • Cirrhinus mrigala • Oxygen consumption • Zinc cyanide

INTRODUCTION

With the industrialisation the water of the streams, lakes and rivers are receiving an increasing load of industrial wastes. Besides polluting waters, in many cases these waters kills the fish and other aquatic organisms. Freshwaters are highly vulnerable to pollution since they act as immediate sinks for the consequences of human activity always associated with the danger of accidental discharges or criminal negligence [1]. Primary effects such as fish kills can be detected but secondary effects may go unnoticed [2]. The ability to detect, identify and properly respond to natural chemical stimuli is an important component of the environmental physiology of fishes. The classic ecotoxicology approach to testing aquatic toxicity is to measure the direct effect in simple experiments using death, more often than not, as the endpoint [3]. In situations where no toxicity are available, bioassays become extremely important. Development of acute toxicity bioassay data must be viewed as a necessary step for providing comparative toxicity information on different toxicants and species of organisms [4].

Cyanide is a fast acting poison because it binds to key iron-containing enzymes required for cells to use oxygen and as a result tissues are unable to take up oxygen from the blood [5]. In the absence of first aid, poisoning from gas inhalation, ingestion or absorption through the skin, can kill within minutes [6]. Some of the cyanide is changed to thiocyanate, which is less harmful and leaves the body in the urine. Some can also combine with hydroxocobalamin to form vitamin B12. A small amount of cyanide is converted in the body to carbon dioxide, which leaves the body in the breath. Most of the cyanide and its products leave the body within the first 24 hours after exposure [7].

Cyanide is considered as a potent suicidal, homicidal, genocidal and chemical warfare agent. 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 [8].

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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. 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 exist in solution as the anionic cyanometallates and are highly stable [9]. The degree of dissociation of various metallo cyanide complexes at equilibrium increases with decreased concentration and decreased pH and is inversely related to their highly variable stability. The zinc- and cadmium- cyanide complexes dissociate almost completely in very dilute alkaline solutions; thus these complexes can result in acute toxicity to fish at pH 8 or below. However, dilute solutions of these complexes are subject to extensive and rapid photolysis on exposure to direct sunlight yielding toxic HCN [10,11].

Zinc cyanide is an inorganic chemical compound with the formula Zn(CN)₂. It adopts a polymeric structure consisting of tetrahedral zinc centres linked by bridging cyanide ligands. It is employed as a catalyst for the cyanoisilylation of aldehydes and ketones [12]. It is also used to introduce the formyl group in organic synthesis. 2-Hydroxy-1-naphthaldehyde has been prepared from 2-naphthol, zinc cyanide and anhydrous hydrogen chloride [13,14]. Zinc cyanide is also a byproduct of gold extraction, where zinc metal is added to the gold cyanide solution to precipitate the metallic gold.

Fish have become an indispensable model system for the evaluation of the extent of aquatic pollution. Fish are used as biomarkers of not only acute toxic effects but also of the consequences of long-term exposure to low concentrations of pollutants [15,16]. Indian Major Carps form an important commercially exploited species and are an ideal animal for studying the impairment caused by the effects of toxic chemicals that are often detected in the aquatic environment. Information on the acute toxic effects of metal cyanide complexes to fishes is limited and its effects on the widely consumed carp Cirrhinus mrigala, which forms an important link in the aquatic food chain, are not known. The objective of the present study was to determine the acute toxicity of Zinc cyanide to Cirrhinus mrigala and its effects on behavioral and oxygen consumption. The reported results would be a useful contribution in the ecotoxicity risk assessment studies of Zinc cyanide on these fish species.

**MATERIALS AND METHODS**

**Procurement of Fish:** Fingerlings of *C. mrigala* (6±0.5cms, 3±0.2gms) were procured from the State Fisheries Department, Bhadra Reservoir Project, Shimoga, Karnataka, India and healthy fishes were acclimatized to laboratory conditions for 15 days at 27±1°C and were held in 100 L glass aquaria (100 x 35 x 50 cm) containing dechlorinated tap water of the quality used in the test. 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.

**Water Quality Analysis:** The physico-chemical characteristics were analyzed following the methods mentioned in APHA [10] and found as follows, temperature 27±1°C, pH 7.4±0.2 at 27°C, dissolved oxygen 6.8±0.5 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. All the water quality parameters (Dissolved oxygen, temperature, pH, salinity, alkalinity, conductivity) were monitored throughout the experimental period except for minimal variation which is tolerated by the fish in the wild.

**Preparation of Stock Solution:** Zinc cyanide (97%) was procured from the Loba chemicals Pvt. Ltd, Mumbai, India. Stock solution was prepared by dissolving the Zinc cyanide in known amount of alkali (1% NaOH) and diluted with double distilled water in one litre standard volumetric flask. The working concentrations were prepared from this standard stock and required quantity of zinc cyanide was drawn directly from this working solution using micropipette. The concentrations of test compounds used in short term definitive tests were between the lowest concentrations at which mortality was 100% and highest the concentration at which mortality was 0% in the range finding tests. There was a simultaneous control group together with the actual experiments.

**Acute Toxicity Tests:** The acute toxicity of Zinc cyanide to fish *C. mrigala* exposed to different concentrations for a period of 96 h was evaluated, taking into consideration the ecological importance of this
species and the problems related to pollution of the aquatic environment. The mortality rate was determined at the end of 24, 48, 72 and 96 h and dead fish were removed as and when observed. After every 12 h the opercular beat and tail beat frequencies were counted.

In this study the acute toxic effect of zinc cyanide on *C. mrigala* was determined by the use of Finney Probit Analysis [17] and the data were also evaluated using Dragstedt- Behrens equation [18], as mentioned by Bhargava and Rawat, [19]. The behavioral changes of the healthy fish and the fish subjected to various doses of zinc cyanide were photographed and evaluated as regard to behaviour anomalies. Observations were taken for every 12 h to record the opercular beat and tail beat frequencies of the fish exposed to zinc cyanide and the experiment ended for 96 h.

**Oxygen Consumption:** Since respiratory distress is one of the important manifestations of acute cyanide toxicity and is known to produce physiological imbalance. In the present study, the respiration rate of the fish was measured from 24 h to 96 h with a 24 h interval. To minimize the effect of low oxygen level and metabolite accumulation on the metabolism, the experiment duration was regulated so that the oxygen concentration by the end of experiments was above 70% of its initial concentration. The dissolved oxygen was determined by Winkler's iodometric method [20]. No deaths occurred during the oxygen consumption tests. The oxygen consumed by the fish is expressed as mgO₂/g/h. The respiratory measurements were made in diffused daylight and the time of the experiment was kept constant to avoid the effect of time of day on the respiration of the fish. The temperature and pH during the course of the experiments were 27°C±1°C and 7.4±0.2, respectively.

**RESULTS**

**Mortality:** No mortality was observed in the control, however, mortality increased with the increase in the concentration and the exposure duration. The concentration at which there was zero percent mortality was 335 µg/L and hundred percent mortality 350 µg/L (Table 1). The estimated 96 h LC₅₀ value (95% confidence limits) was found to be 343 µg/L.

**Behavioural Manifestation:** The behaviour and condition of the fishes in both the control and test solution was noted every 24 h up to 96 h (Fig 1, 2, 3 and 4). The fishes showed marked changes in their behaviour when exposed to the test solution of different concentrations. In lower concentrations of zinc cyanide (335 µg/L) the fishes showed rapid swimming than in control. Behavioural manifestations of acute toxicity like hyperactivity, loss of balance, rapid swimming, increased surfacing activity,
Table 1: The mortality rate of Indian Major Carp (Cirrhinus mrigala) individuals in 96-h at different zinc cyanide concentrations and empirical probit values

<table>
<thead>
<tr>
<th>Conc. (µg/l)</th>
<th>Log Conc.</th>
<th>Number fish exposed</th>
<th>Number of dead fish</th>
<th>Mortality %</th>
<th>Empirical Probit</th>
</tr>
</thead>
<tbody>
<tr>
<td>335</td>
<td>2.5250</td>
<td>10</td>
<td>1</td>
<td>10</td>
<td>3.72</td>
</tr>
<tr>
<td>340</td>
<td>2.5314</td>
<td>10</td>
<td>2</td>
<td>20</td>
<td>4.16</td>
</tr>
<tr>
<td>341</td>
<td>2.5327</td>
<td>10</td>
<td>3</td>
<td>30</td>
<td>4.48</td>
</tr>
<tr>
<td>342</td>
<td>2.5340</td>
<td>10</td>
<td>4</td>
<td>40</td>
<td>4.75</td>
</tr>
<tr>
<td>343</td>
<td>2.5352</td>
<td>10</td>
<td>5</td>
<td>50</td>
<td>5.00</td>
</tr>
<tr>
<td>345</td>
<td>2.5378</td>
<td>10</td>
<td>7</td>
<td>70</td>
<td>5.84</td>
</tr>
<tr>
<td>347</td>
<td>2.5403</td>
<td>10</td>
<td>8</td>
<td>80</td>
<td>5.93</td>
</tr>
<tr>
<td>348</td>
<td>2.5415</td>
<td>10</td>
<td>9</td>
<td>90</td>
<td>6.28</td>
</tr>
<tr>
<td>350</td>
<td>2.5440</td>
<td>10</td>
<td>10</td>
<td>100</td>
<td>8.09</td>
</tr>
</tbody>
</table>

Table 2: Impact of Zinc cyanide (343 µg/L) on the behavioural patterns of C. mrigala at different time intervals

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>24h</th>
<th>48h</th>
<th>72h</th>
<th>96h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperactivity</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Loss of balance</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Rate of Swimming</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Surfacing activity</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Rate of opercular activity</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Convolutions</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

The increase or decrease in the level of behavioral parameters is shown by numbers of (+) sign. The (-) sign indicate normal behavioral conditions.

Opercular Beat Frequency (OBF): The effect of Zinc cyanide on OBF of C. mrigala is shown in Table 3. In all cases except in control (89.4 counts/ minute). Progressive decline in the OBF was observed with the exposure time.

<table>
<thead>
<tr>
<th>Exposure period (hr)</th>
<th>Control</th>
<th>96h LC50 (343 µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>8.2±0.8</td>
<td>16.4±0.5</td>
</tr>
<tr>
<td>24</td>
<td>8.2±0.1</td>
<td>15± 3.5</td>
</tr>
<tr>
<td>36</td>
<td>8.7±0.6</td>
<td>14± 2.7</td>
</tr>
<tr>
<td>48</td>
<td>8.6±0.2</td>
<td>11± 3.8</td>
</tr>
<tr>
<td>60</td>
<td>8.3±0.5</td>
<td>8.5±2.1</td>
</tr>
<tr>
<td>72</td>
<td>8.5±0.3</td>
<td>5.1±2.8</td>
</tr>
<tr>
<td>84</td>
<td>8.1±0.9</td>
<td>4.3±0.6</td>
</tr>
<tr>
<td>96</td>
<td>8.0±0.2</td>
<td>1.9±3.5</td>
</tr>
</tbody>
</table>

Tail Beat Frequency (TBF): The fishes in control experiment showed limited variation in the TBF, whereas in the treated animals the TBF showed wide variation with the initial increase and decrease in the TBF (Table 4).

Fig. 4: Secretion of mucous at 96 h of exposure
Oxygen Consumption: The mean metabolic rates of the control and exposed fishes at the end of 24, 48, 72 and 96 h along with the percent decrease from control are given (Table 5). For fish acclimatized to the 27°C temperature, the specific oxygen consumption decreased 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 oxygen/g/L/h, respectively. Oxygen consumption increased in the initial 24 h of exposure to zinc cyanide concentrations. However, after 24 h of exposure to zinc cyanide showed decrease in the oxygen consumption (-25.99% and -61.82%) respectively, relative to the control (Fig 5.). Average oxygen consumption at different time intervals of zinc cyanide exposure (24, 48, 72 and 96 h) was significantly different from the control (0.4812 mg/L). The decrease in the oxygen consumption of C. mrigala exposed to zinc cyanide indicates the onset of acute hypoxia under cyanide stress. Further, the fact that the drop in metabolic rate of the fish as a protective measure to ensure that there is a low intake of toxic substance also cannot be ruled out.

DISCUSSION

In the course of 96 h toxicity test of zinc cyanide to C. mrigala fingerlings, there was no mortality observed in control fish. Oxygen saturation of water did neither drop below 60% in any concentration tested, nor in the control group. Presence of the substance tested (above 80% of the nominal concentration) was provided by means of daily exchange of the testing bath. Fulfilling these conditions, the test may be considered valid. Acute toxicity (96 h LC50) of sodium cyanide to *Cyprinus carpio* was 1 mg/L [21]; whereas in the present study the acute toxicity of zinc cyanide to C. mrigala was found to be 343 µg/L. This difference in the 96 h LC50 value between the C. mrigala and *Cyprinus carpio* may be attributed to 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.

Behavioral changes are the most sensitive indication of potential toxic effects studied. In the control group the behavioral and the swimming patterns of the fishes were normal and there was no mortality. In the initial period of exposure to zinc cyanide, the fish stayed motionless and settled to the bottom. This may 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 behavioral response started appearing only after 3 h 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 [22], increased swimming activity and entails high expenditure of energy [23]. 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 [24]. 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 atmosphere [25]. Loss of equilibrium follows erratic and darting swimming movements, which might be due to the inhibition of brain cytochrome C oxidase activity, causing cytotoxic hypoxia, thus causing the brain damage to the region of the brain associated with the maintenance of equilibrium [21].

Ferrando et al., [26] in their study on the effects of eight selected organochlorine pesticides such as endosulphan, diazinone, phenylthiran and methylparathion on eels, determined their 96 h LC_{50} values and reported behavioral changes in 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, behavioral changes observed are similar to our study. Bradbury and Coast [27] 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 [28].

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 cyanide (0.3561 mg/L) and there after decrease in the oxygen consumption (0.1837 mg/L) was noticed at the end of 96 h. Similar results have also been observed in different fish species for different chemical substances [29-32]. The decrease in the consumption of oxygen is probably the result of alterations of energy metabolism [33]. 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 [34-37]. The oxygen consumption endpoint also provides an index for sublethal stress and for biomonitoring the potentially toxic effects of chemicals [32,38]. Downing [39], 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 of C. mrigala exposed to zinc cyanide indicates the onset of acute hypoxia under stress. Further, the fact that the drop in metabolic rate of the fish as a protective measure to ensure that there is a low intake of toxic substance also cannot be ruled out. Reduced oxygen consumption at higher concentrations of cyanide could also arise as a result of respiratory inhibiting factors that come into play. The primary site of action of cyanide is presumed to be the central nervous system (CNS). In acute cyanide poisoning a rapid inhibition of cytochrome oxidase results in an energy deficit within the target tissue [40,41]. 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 [42,43]. Additionally a number of other enzymatic processes are inhibited which exacerbate the toxicity [44]. 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 [45].

The observed increase in the OBF and TBF in the initial 24 h of exposure to zinc cyanide and decline thereafter has been reported by early workers [46]. The initial increase in the OBF as a primary response to sudden stress was also reported by Rajasekaran et al., [25]. In the present investigation the initial increase may be attributed to the sudden shock caused by the toxicant. Grillitsch et al., [47] reported that organisms exhibit behavioural responses to chemical stress both at acute and sublethal toxicity. This elicits the potency and sensitivity of the fish C. mrigala to the test chemical. The ecological importance of this is that the damage to non-target species in the environment and such attribute of the organism could be effectively used as toxicity biosensor of chemical stress. Other studies on different toxicants especially petroleum related hydrocarbon compounds indicated damage to epithelial cells of the gill chamber, destruction of liver, possible nervous breakdown, failing organs and retarded physiological processes in fish body functions [46,48]. Chindah et al., [49] noted that aquatic organisms (shell and fin fishes) which is indirect continuous contact with the medium in addition to breathing and feeding will be vulnerable to respiratory tract damage and other organs of the body.
CONCLUSION

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. mirigala*. Variation in the oxygen consumption in zinc cyanide treated fish is probably due to impaired oxidative metabolism and cyanide induced respiratory stress. In the present study, copious mucous secretion and bulging of gills were also observed. The drop in the oxygen consumption rate of *C. mirigala* exposed to zinc cyanide can also be attributed to clogging of gills by mucous. Gills are vital respiratory and osmoregulatory organs and cellular damage induced by the metal might impair the respiratory function of the fish by reducing the respiratory surface area. These findings clearly suggest decreased respiratory surface area, which can also account for the drop in the metabolic rate of the fish. Hence, dysfunction of behaviour and respiration can serve as index zinc cyanide toxicity.

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