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Assessment of Perchlorate Induced Acute Toxicity Study in *Rasbora dandia*

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Abstract: ***Perchlorate (ClO_4^-) is an emerging pollutant affecting aquatic ecosystem as well as human health and widely reporting from ground as well as surface waters near military sites and manufacturing units. As a pollutant, perchlorate requires utmost concern and its contamination report seeks global attention in the current world. Previous studies showed that in animals and plants perchlorate caused various toxic effects. The effect of perchlorate in an aquatic environment needs great concern due to the solubility, stability and persistent nature of perchlorate salts. The high water solubility and mobility in aquatic environment, perchlorate present in the soil can be transported into ground water and might remain for decades. Due to these properties aquatic organisms are most susceptible to perchlorate. In the present study we observed acute toxic effect of potassium perchlorate on a fresh water fish Rasbora dandia. Static bioassays were performed on Rasbora dandia to evaluate the median lethal concentrations of perchlorate for 24, 48, 72 and 96 hrs. The LC50 values of perchlorate to Rasbora dandia were 2011, 2007, 1987 and 1971 ppm after 24, 48, 72 and 96 hours respectively. The results showed that the LC50 values decreased with increase in concentration with exposure period.***

Keywords: Acute toxicity, Bioassay, LC 50, Perchlorate, *Rasbora dandia*

1. Introduction

The fate and effect of perchlorate in an aquatic environment is of immense concern, because perchlorate salts are soluble, stable and persistent in water [25]. Due to high stability and low reactivity, ClO_4^- transport readily in surface waters and through soils to groundwaters [22]. In an aqueous medium perchlorate anion readily dissociate from its various cation and remains stable for a long period under normal environmental conditions [24]. Perchlorate present in ground water can be transported and might reside for decades [11] [18]. Due to these properties aquatic organisms are most susceptible to perchlorate toxicity. In an aquatic ecosystem fishes are more susceptible to stress than other animals due to their intimate dependence upon their surrounding environment. Perchlorate contamination in an aquatic environment may affect the fishes present in the contaminated water. The important health effect of perchlorate in humans and other ecological receptors are the inhibition of iodide uptake and subsequent thyroid hormone synthesis [26][5]. In teleost fishes, thyroid hormone promotes growth, reproduction, embryonic development and initiate metamorphosis [7][15][21]. For the assessment of pollution one of the widely accepted methods is bioassay study. Bioassay test are defined as the estimation of the amount of biologically active substance by the level of their effect on the test organisms. The present study mainly focused to evaluate acute toxic effect of perchlorate in a fish to assess perchlorate induced toxicity response under laboratory conditions.

2. Materials and Methods

Test organism

The fish selected for toxicity study was *Rasbora dandia* (Valenciennes, 1844). It is a fresh water fish occurs in different habitat such as ditches, ponds, canals, streams and rivers and in inundated fields. *Rasbora dandia was* selected for the toxicity study since it is commonly found in the freshwater ecosystem, easy availability and dominant species present in the perchlorate contaminated pond (study site).

Collection and maintenance of test organism

Healthy and adult *Rasbora dandia* were collected from a local fish collector for toxicity studies. The fish were transported to the lab in live condition with care to avoid any mechanical injury. They were kept in a aquarium tank having 2000 L capacity filled with de chlorinated tap water and provided continues aeration. Prior to experiment, fishes were acclimatized in laboratory conditions for a period of two weeks. During the acclimatization period fishes were fed with commercial fish food (Aqua mix pellet) twice per day. The physio -chemical parameters of the water were daily monitored using standard procedure [1] and maintained constant condition during the acclimatization period. The acclimatization was done at room temperature. Fishes free of any deformities, disease or lesions and showed good health conditions was selected for the experiments.

Preparation of Stock solution

Pottassium perchlorate ($KCLO_4^-$) purchased from Sigma Aldrich was used for the prepration of stock solution (Plate 4.2). It is a white colored crystal with molecular weight 138.55 g/mol. A stock solution (10000mg/L) was prepared with potassium perchlorate as per the standard procedure [1] prior to the experiment. Test solutions were made by diluting the stock solution to produce desired perchlorate concentration for each treatment.

Experimental protocol

Preliminary test were conducted to determine the range of the concentration of the toxicant used for the acute toxicity test. The test fishes were starved for 24 hrs prior to and during the 96 hrs acute toxicity test period. The fishes were exposed to different concentration of perchlorate to

determine 96 hrs LC50 values. For the calculation of LC50, acute toxicity test was done according to the standard procedure. After the exploratory test, acute toxicity test was conducted by exposing the fish to different concentration of perchlorate for 96 hrs. Fishes were divided into five groups, T1 to T5 and each group contain 10 healthy fishes having about uniform size (6.1 ± 2.4 cm of total length, 4.2 ±1.6 gm of weight) were kept separately in aquaria (100L capacity) filled with test medium with different concentration (1950, 1960, 1970, 1980 and 1990 mg/L in T1, T2, T3, T4 and T5 respectively). A control tank (100L capacity) was also maintained without perchlorate. Half of the perchlorate solution was replaced at every 24 hrs interval and replaced with fresh solution to maintain nominal concentration. The experiment was conducted in triplicate and static renewal method was applied for the experiment. The toxicity response of the fish was studied by observed its behavior and mortality at every 12 hrs interval up to 96 hrs was recorded. Dead fishes were removed immediately from the experiment tanks. The lethal concentration of water for 50% of fish death.

Statistical Analysis

Per cent mortality was calculated and the values were transferred into probit scale. Probit analysis was carried out as suggested by Finney (1971) [10]. LC50 for 96 hrs experiment was calculated by using SPSS, ver.10 software followed by probit analysis method [10].

Morphological assay

Morphological changes of the experimental fish (both test and control) was monitoring on daily basis throughout the exposure period. Behaviour changes such as fish general behaviour, feeding activity, swimming activity and opercular movement were monitored daily. For morphological analysis total length and wet body weight of the fish were measured and observed any deformities in scales, fins, gills and body also.

3. Results and Discussion

Acute toxicity

The present study is concerned the acute toxic effects of perchlorate on a freshwater fish, *Rasbora dandia.* The percentage of mortality was found to be increased with the increase in the concentration of perchlorate. The minimum mortality was recorded at lowest concentration (1950 ppm) of perchlorate exposure, and maximum mortality was recored at highest concentration (1990 ppm) of perchlorate. The LC50 values of perchlorate to *Rasbora dandia* was found to be from 2011 (24 hrs) to 1971 ppm (96 hrs) at different concentration.

The result of acute toxicity study showed that 96 hr LC50 of potassium perchlorate on *Rasbora dandia* was calculated as 1971 mg/L (fig: 4) and 72 hr LC50 was 1987 mg/L (fig: 3). Similarly the 48 hr LC50 of potassium perchlorate on *Rasbora dandia* was calculated as 2004 mg/L (fig: 2) and 24 hr LC50 was 2011 mg/L (fig: 1). The value obtained in the present study were compared with the previous report. Park *et al.* [19] reported that in mosquito fish (*Gambusia holbrooki*) the LC50 of sodium perchlorate was 404 mg/L. Goleman *et al.* [12] reported that in *Xenopus laevis* the 5 day medial lethal concentration (LC50) was 510 ± 36 mg/L. Liu *et al.* [16] reported that 96 h LC50 of perchlorate in Zebra fish (*Danio rerio*) was 2532 mg/L. While in another study the LC50 value of perchlorate in rainbow trout and mosquito fish (*Gambusia holbrooki*) was 2100 mg/L and 404 mg/L respectively [9][19]. The LC50 value of perchlorate in different stages of Zebra fish was obtained as 529mg/L (embryo), 1204 mg/L (larval stage), 2532mg/L (Juveniles) and 3352.8 mg/L (adult) [20] [17] [27]. The result of the present study revealed that environmentally relevant concentration of perchlorate does not induce acute toxic effect on *Rasbora dandia*. The 96 hr LC50 value obtained in the present study was lower than the LC50 reported from Zebra fish (2532 mg/L) and rainbow trout (2100 mg/L) [16] [9]. This may be due to species difference or due to size of the species. The LC50 value differs from species to species for the same toxicant due to the mode of action or response of the animals [14]. Similarly LC50 has been changed by age, size, sex and nutrient supply [23].

Morphological changes observed on *Rasbora dandia* during acute toxicity

Behavioural and morphological changes are considered as a good biomarker to access the health status of fish [13]. In the present study morphological changes were observed in fish (*Rasbora dandia)* exposed to ClO_4^- compared to control. The perchlorate exposed fishes showed faster opercular activity (75-78/ m) than that of the control fish (62-65/m). Similarly exposed fishes showed reduced body coloration, invisible lateral line pigmentation and erratic swimming activity. In exposed group the prominent blue black stripes from the eyes to the base of the caudal fin of the fish was completely invisible during the exposure period. However such changes were not observed in control fish. These findings are similar to the previous reports of perchlorate induced toxicity study in fathead minnows *(Pimephales promelas*) and three spine stickle back (*Gasterosteus aculeatus*). Previous studies reported that perchlorate (10mg/L and 100mg/L) causes morphological abnormalities such as reduction of body pigmentation, poor scale formation, low wet weight, standard length and retarded growth in fathead minnows *(Pimephales promelas*) and in three spine stickle back (*Gasterosteus aculeatus*) [6]. Similarly chronic perchlorate exposure in three spine stickle back caused abnormal lateral plate development, slower growth rate, reduced body pigmentation and decreased swimming performance [2][3][4]. These reports were also in consistent with the present observation.

4. Conclusion

This study elucidates acute toxic effect and morphological changes observed in a fresh water fish (*Rasbora dandia)* exposed to high concentration of perchlorate. The LC 50 determined in the present study was significantly higher than the ClO_4^- concentration reported from the contaminated sites. There for this study concluded that environmentally relevant concentration of perchlorate does not cause any acute toxic effect on *Rasbora dandia.*

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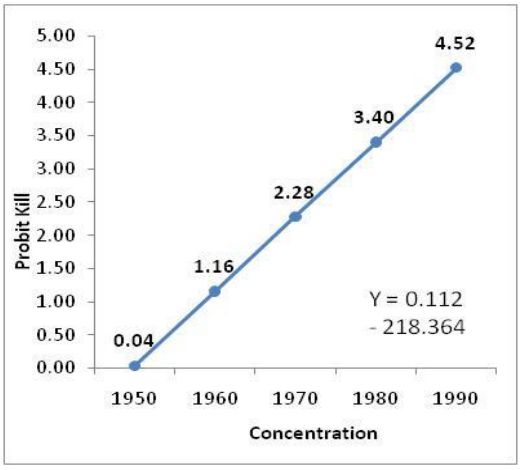

Figure 1: LC50 value of perchlorate to *Rasbora dandia* at 24 hours

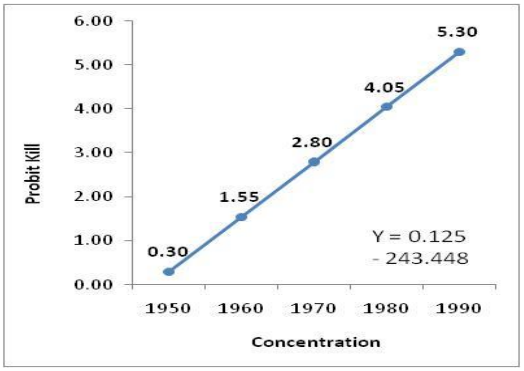

Figure 2: LC50 value of perchlorate to *Rasbora dandia* at 48 hours

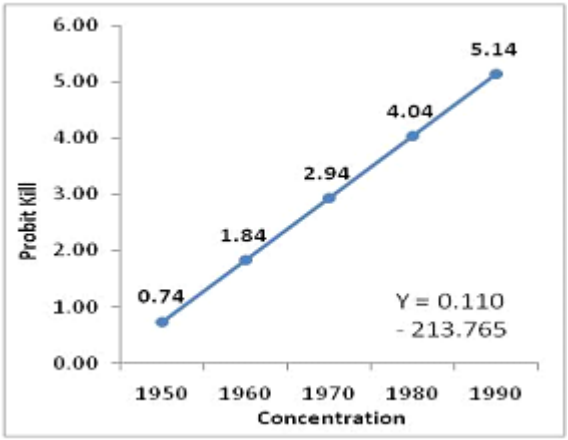

Figure 3: LC50 value of perchlorate to *Rasbora dandia* at 72 hours

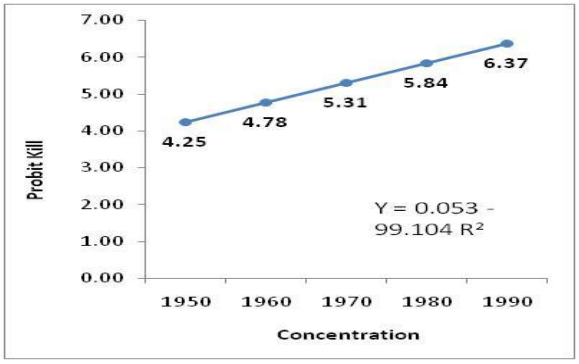

Figure 4 LC50 value of perchlorate to *Rasbora dandia* at 96 hours