The Scientific Temper
Vol. XI, No 1-2, January-July, 2020
ISSN 0976 8653, E-ISSN 2231 6396
A Web of Science Journal
Doc ID: https://connectjournals.com/03960.2020.11.67

Chlorpyrifos Induced Disruption in Serum Ca^{2+}, Mg^{2+} and Pi Electrolytes Level in Freshwater Catfish *Heteropneustes fossilis* (Bloch)

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ABSTRACT

Chlorpyrifos is widely used organophosphate (OP) insecticide in agricultural and domestic applications and possess anti-cholinesterase activity. Fishes are inadvertently exposed to pesticides and their metabolic byproducts through runoff water entering the fish habitat. The present study was carried out to observe short-term (24h, 48h, 72h and 96h) and long-term (7d, 14d, 21d and 28d) effect of chlorpyrifos on serum electrolytes (Ca^{2+}, Mg^{2+} and Pi) in freshwater air-breathing catfish *Heteropneustes fossilis*. The concentration of chlorpyrifos for short-term exposure was kept at 1.56 mg/l (75% of 96 h LC_{50}) and 0.52 mg/l (25% of 96 h LC_{50}) for long-term. Fishes exposed to sublethal concentrations for short-term (24h, 48h, 72h and 96h) and long-term (7d, 14d, 21d and 28d), exhibited hypocalcaemia, hypophosphatemia and hypermagnesaemia. The present study may be used as valuable biochemical parameters to monitor the health of fish, affected by insecticides.

Keywords: Chlorpyrifos, serum electrolytes, Toxicity, *Heteropneustes fossilis*

INTRODUCTION

Pesticides used for controlling pests of agricultural and other products, are major cause of concern for aquatic life due to their persistence in the environment, biotransformation and toxicity (Joseph and Raj, 2011; Pandey *et al*. 2009 a; Pandey *et al*. 2015). Chlorpyrifos is highly toxic to freshwater fish, aquatic invertebrates and estuarine and marine organisms (US EPA 1989). It is a widely used organophosphate pesticide and second largest selling in India. Fishes, especially carnivorous and omnivorous such as *H. fossilis*, are the most affected organisms in aquatic environment contaminated with pesticides and other chemicals. In India as much as 70% of the chemical formulations used for agriculture practices are believed to affect non-target organism and to find their way to freshwater fish (Bhatnagar *et al*. 1992).

Chlorpyrifos has a wide range of persistence. The soil persistence of this pesticide has been reported to occur from few days to several years depending on rate of application, type of ecosystem and other environmental conditions (Gebremariam *et al*. 2012). Under normal environmental condition chlorpyrifos is mainly degraded ingenerally persistent compound 3,5,6-trichloro-2-pyridinol (TCP). Due to its persistence in environment, chlorpyrifos undergoes bioaccumulation after entering the food chain. A bio-concentration factor (BCF) as high as 5,100 in gulf toadfish (*Opsanus beta*) has been reported by Hanson *et al*. (1986). In another report Log BCF values 3.84 was reported in Zebra fish (El-Amraniet al. 2012). Chlorpyrifos accumulates in the tissues of aquatic organisms and studies involving continuous exposure of fish during the embryonic through fry stages have shown bio-concentration values ranging from 58 to 5100 (Racke 1992).

Chlorpyrifos is highly toxic to aquatic organisms. Many studies have reported its mutagenic and genotoxic effects in *Channa punctatus* (Ali *et al*., 2008; Ismail *et al*. 2014). Exposure of pesticides causes short term and

long term damage to nervous system, lung damage, reproductive dysfunction, and possible disruption of the endocrine gland (hormone) and immune system (Pandey, 2017). A number of toxicants have been reported to affect the physiological functions of aquatic organisms specially the fish and disturb their water and ion homeostasis (Bakshi and Panigrahi 2018; Rashmi *et al*. 2019). Effects of pesticides on electrolytes and various biochemical parameters have been reported by many workers (Kavitha and Rao 2007; Begum 2008; Logaswamy *et al*. 2007; Srivastav *et al*. 2009; Pandey *et al*. 2009 b) Das *et al*.2013; Pandey *et al*. 2015; Pandey and Das 2015 a, b). Ghayyur *et al*.(2019) have reported alterations in hematological and biochemical components in *Oreochromis mossambicus* after short term exposure. In Nile tilapia chlorpyrifos exposure caused lowered serum cortisol, estradiol and testosterone levels (Oruc, 2010).

The present work aims to study short-term (96h) and long term (36d) effect of chlorpyrifos exposure in *Heteropneustes fossilis.* The electrolytes, $Ca^{2+,}$ Mg^{2+} and Pi are vital for many biochemical and physiological functions. Most of the biological functions like cell signaling, neural transmission, muscle contraction, blood coagulation, enzymatic cofactor, membrane and cytoskeleton functions are controlled by the calcium. Phosphorus is required for the maintenance of bones and teeth, and normal functioning of nerves and muscles. Phosphate is a major cytoplasmic buffer and forms essential component of membranes and nucleic acids. Phosphate in plasma may occur in the form of an esters, phosphate ions (ortho-phosphoric acid), or in form of protein calcium-phosphate complex (Loken *et al*; 1960). Magnesium is essential for proper functioning of the cells and is involved in a variety of enzymatic reactions and affects neuronal activities (Sato and Fukuda, 2004). In fish magnesium occurs in ionized (Mg^{2+}) form or complexed with proteins or mineralized in bony tissues. The total magnesium concentration of blood plasma in normally does not exceed 2 mmol L^{-1}, and the ionic concentration generally remains less than 1 mmol L^{-1} (Bijvelds *et al*. 1998). Stress of pesticides exposure alters electrolyte levels in body. These alterations are useful indicators to assess the health of fish and suitability of aquatic environment to its inhabitants.

MATERIALS AND METHODS

Fresh and adult *Heteropneustes fossilis* (both sexes, size 17±1.5 cm, weight 23±2 g) were procured locally and disinfected with 0.05% $KMnO_4$ solution for 2 minutes. Fish were kept under favorable environmental conditions and all precautions were taken to minimize stress and mortality. Fish were acclimatized to the laboratory conditions (under natural photoperiod 11 h light and 13 h dark) for 10 days. Dead fish were removed immediately from the tank. Handling and sacrifice of fish were carried out following the guidelines provided by Animal Ethics Committee of the institute. Fish were daily fed (about 0.1gm/ fish) with a mixture of wheat flour, mustard cake, dried prawn powder and soybean in a ratio of 2:1:1:1. Fish were not given any food 24 hours before and during experiment. Sub-lethal concentrations of chlorpyrifos for short-term and long-term experiment were kept at 1.56 mgL^{-1} (75% of 96 h LC_{50}) for short-term and 0.52 mgL^{-1} (25% of 96h LC_{50}) respectively. The experiment was carried out to study the effect of chlorpyrifos on serum calcium, magnesium and inorganic phosphate levels after short-term (96h) and long-term (28d) sub-lethal exposure. In short-term experiment acclimatized fish were separated in two equal groups: control and test. Similarly for long-term fish were separated in two equal groups - control and chlorpyrifos exposed test group. Six fish were sacrificed on each time intervals from control and experimental groups after 24h, 48h, 72h, and 96h in short-term and 7d, 14d, 21d and 28d of long-term exposure. Blood was collected and analyzed for calcium and phosphate levels. Serum Ca^{2+} Mg^{2+} and Pi levels were measured using Erba Mannheim diagnostic kit (manufactured by Erba diagnostics Mannheim GmbH, Germany). The serum Ca^{2+} Mg^{2+} and Pi levels were statistically evaluated by Student's t-test (two tailed) for significance ($P < 0.05$ benign accepted as significant).

RESULTS

Physicochemical properties of test water were- temperature (23 2.5) pH (7.2) dissolve oxygen (7.50.42 mg/l) and hardness as $CaCO_3$ (121.45±2.24 mg/l. The LC_{50} of chlorpyrifos in fish was determined (2.08 mgL^{-1} for 96h) earlier using SPSS-20 computer program.

In short-term experiment, serum Ca^{2+} levels of *H. fossilis* were recorded insignificant ($P>0.05$) at sub-lethal concentration of chlorpyrifos (75% of 96 h LC_{50}= 1.56 mgL^{-1}) following 24h of exposure (Fig. 1a). Significant hypocalcaemia was recorded after 48h and continued up to 96h in exposed fishes. No appreciable change in serum Ca^{2+} level ($P>0.05$) in control fish was observed. Similar trends were observed in long-term chlorpyrifos exposure (0.52 mgL^{-1}= 25% of 96h LC_{50}), and an increase in serum Ca^{2+} level ($P<0.05$) after 7 days was observed but there after fishes exhibited hypocalcaemia (significant ($P<0.0001$).

The serum Mg^{2+} level in chlorpyrifos exposed fish show hyper magnesaemia at 24 h (significant $p < 0.05$) and the level of Mg^{2+} increased consistently up to 96 h (Fig. 2a) in short-term experiment. In long-term, hypermagnesaemia was also observed after 7d exposure and continued to increase up to 28d (Fig. 2b).

However, the serum Pi level exhibited a transient increase at 24h of dimethoate exposure but gradually declined thereafter up to 96h (Fig. 3a). The Pi level in exposed fish was always higher than the Pi (control) and showed significant ($p < 0.001$) increase. In long-term, increase in Pi level was observed after 7d, and exhibited significant ($p<0.05$) increase up to 28d (Fig.3b).

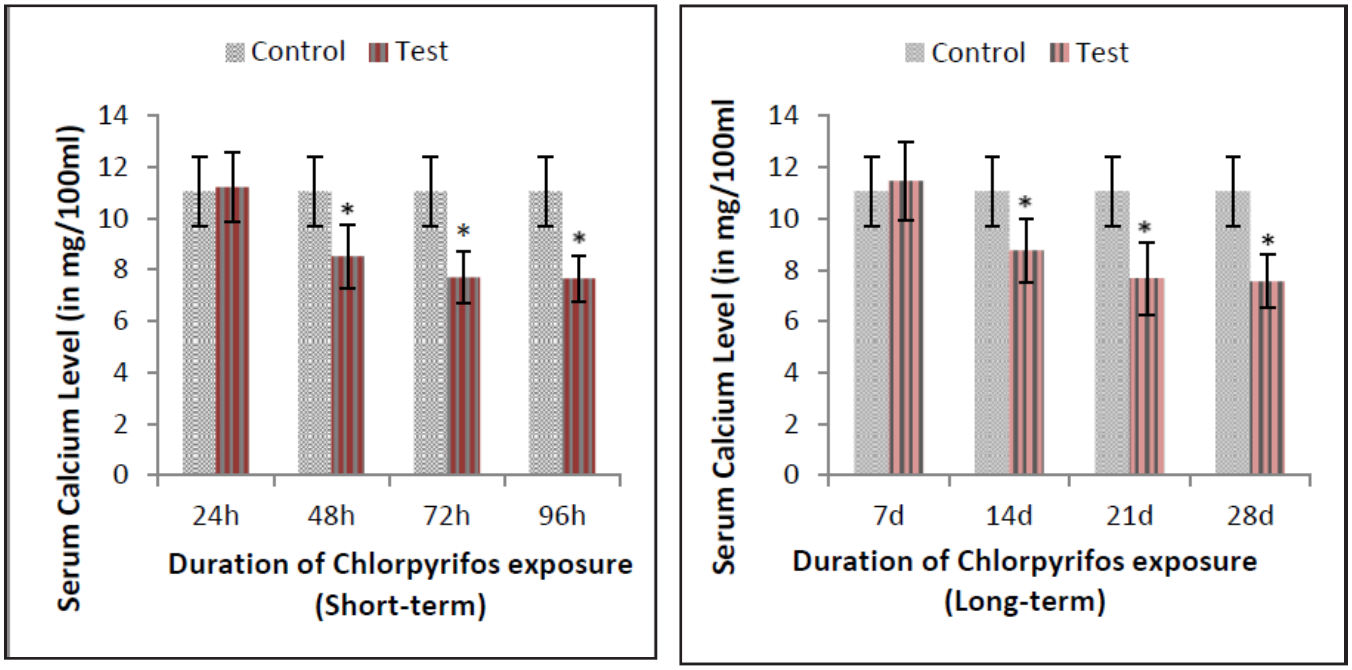

Fig. 1a (left) and Fig 1b (right): Serum Ca^{2+} level after short term (75% of 96h LC_{50}=1.56 mg/L) and Long-term (25% of 96h LC_{50}= 0.56 mg/L) Chlorpyrifos exposure in *Heteropneustes fossilis*. Values are mean ± S.E. of six specimens. Asterisk indicates significant differences (P< 0.05) from control.

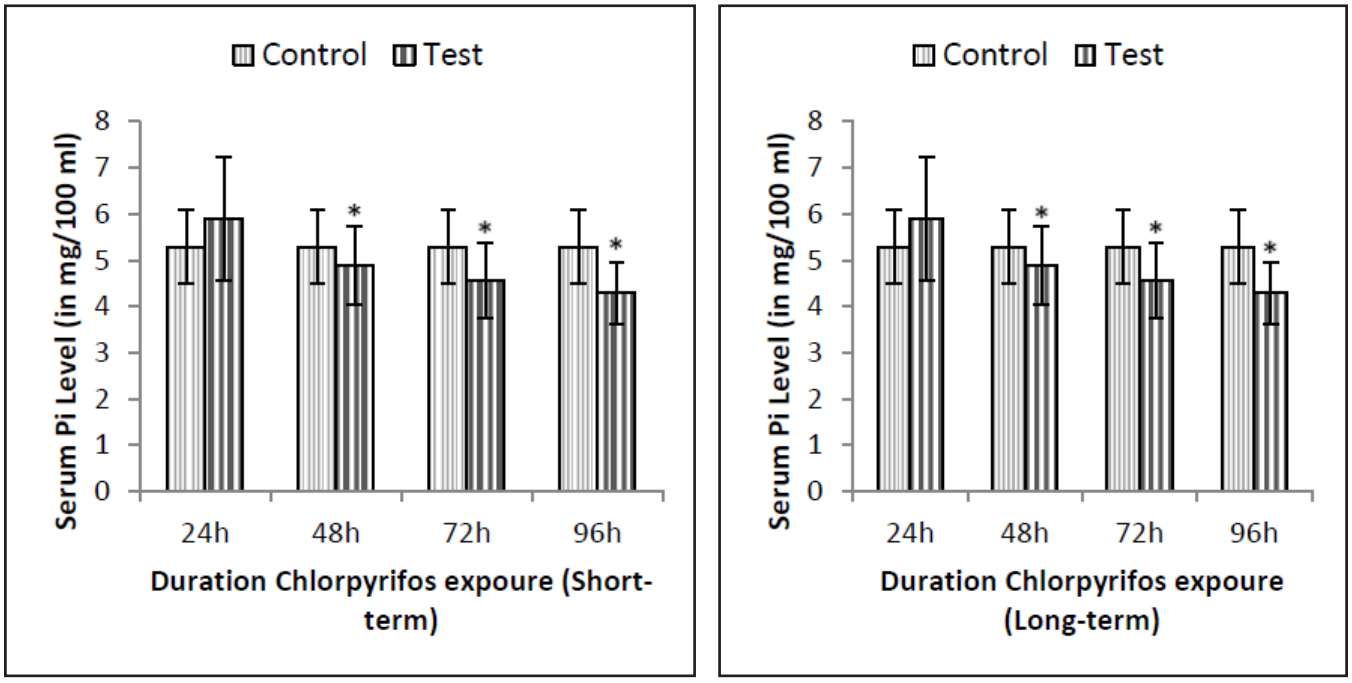

Fig. 2a (left) and Fig 2b (right): Serum Pi level after short term (75% of 96h LC_{50}=1.56 mgL^{-1}) and Long-term (25% of 96h LC_{50}= 0.56 mgL^{-1}) Chlorpyrifos exposure in *Heteropneustes fossilis*. Asterisk indicates significant differences (P< 0.05) from control.

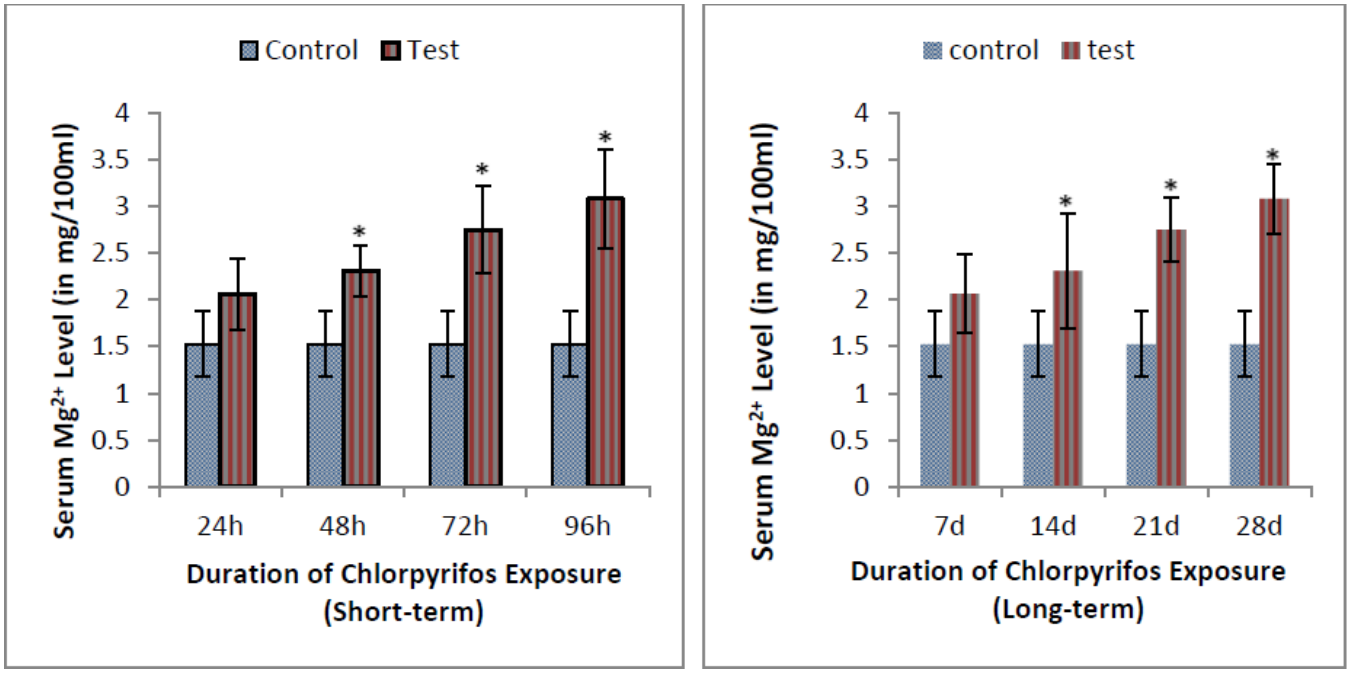

Fig. 3a (left) and Fig 3b (right): Serum Mg^{2+} level after short term (75% of 96h LC_{50}=1.56 mgL^{-1}) and Long-term (25% of 96h LC_{50}= 0.56 mgL^{-1}) Chlorpyrifos exposure in *Heteropneustes fossilis*. Asterisk indicates significant differences (P< 0.05) from control.

DISCUSSION

Fish exhibited increase in serum electrolytes (Ca^{2+}, Pi and Mg^{2+})levels in short-term as well as in long-term experiment during early duration. The initial increase in blood parameters in this study may be due to an attempt of exposed fish to cope with toxic effects of chlorpyrifos. Hypocalcaemia was observed after short-term and long-term chlorpyrifos exposure in *Heteropneustes fossilis*. Chlorpyrifos exposure leading to hypocalcaemia (Fig. 1a and 1b) as observed in this study may be due tubular necrosis in kidney resulting decreased Ca^{2+} ion reabsorption and enhanced urinary excretion in exposed catfish. Similar observations have been reported byYildirimet al.(2006), Velmurugan *et al*.(2007), Pandey *et al*.(2009 b), Das *et al*.(2013); Pandey and Das (2015a, b),Pandey *et al*.(2015). Fish exhibiting hypocalcaemia during short-term and long-term exposure of chlorpyrifos in this study is similar to the observations made by other workers (Srivastavet al. 2009; Pandey *et al*. 2009 b; Srivastav *et al.* 2010). However, Thangavel *et al*. 2005 have reported a decline in serum Ca^{2+} levels in a freshwater teleost, *Sarotherodon mossambicus* when exposed to dimecron. Decline in serum calcium level was observed in this study after 48 h in short-term and 7 d in long-term exposure to *Euphorbia royleana* latex (Prasad *et al*. 2011). Das *et al*. (2013) have also reported hypocalcaemia in *Heteropneustes fossilis* after dimethoate exposure.

In fishes exposed to chlorpyrifos and exhibiting acute and chronic hypocalcaemia may be due to depletion of Ca^{2+} from calcium reservoirs and reduced uptake of calcium from damaged gills and kidney tissues owing to pesticide exposure (Velmurugan *et al*. 2007; Peebua *et al*. 2008; Parikh *et al*. 2010, Pandey, 2017; Srivastav *et al*. 2021). Hypocalcaemia and hypophosphatemia have been observed in teleosts exposed to cypermethrin, deltamethrin and metacid-50 by Mishra *et al*. (2010).

The observed hypophosphatemia in chlorpyrifos exposed fish is supported from the studies of earlier investigators who have also recorded a decrease in blood phosphate content after exposure of the fish (Srivastav *et al*. 1997; Pandey *et al*. 2009 b; Kumar *et al*. 2011a; Prasad *et al*. 2011; Srivastav *et al*. 2021). However, contrast to the findings of this study, hypophosphatemia has been reported after exposure of fish to various toxicants (Gill *et al*. 1991; Pandey *et al*. 2009 b).

Increase in serum Mg^{2+} level observed in this study is in agreement with the reports of many authors in a number of fishes exposed to variety of insecticides (Singh *et al*. 1996; Singh *et al*., 1997; Pandey *et al*. 2009b). Dabrowska *et al*. (1991) suggested an inverse correlation between $Mg^{2+}$and Ca^{2+}levels in common carps. Thangavel *et al*. (2005) have reported no change in serum Mg^{2+} level. However, more evidence point to the fact that fishes having hypocalcaemia during insecticide exposure exhibit increased Mg^{2+} level, which could be due to kidney damage (Gill *et al*. 1989). Giles (1984) have earlier noted hypermagnesaemia with decrease in serum calcium and urinary Mg^{2+} level in cadmium exposed rainbow trout.

Biochemical alterations in toxicant exposed fishes are a manifestation of toxic substances interfering with biochemical process and inducing apparent physiological stress. It may be concluded from the this study that chlorpyrifos is toxic to fish *H. fossilis* and damages the homeostatic balance of body electrolytes, especially Ca^{2+}, Mg^{2+}, and Pi. The study shows that serum electrolyte indices can be used as bio-indicator for early warning to the possible pesticide contamination and exposure of aquatic organism.

ACKNOWLEDGEMENT

The authors are grateful to the Principal, Kamla Nehru Institute of Physical and Social Sciences, Sultanpur and to the head, department of zoology of the institute for facilitating the research work. The author Rakesh Kumar Pandey, gratefully acknowledges and thankful to the Department of Higher Education government of U.P. for expending research grant under "Research and Development Plan (Number-46/2021/603/ Sattar-4-2021-4(56)/2020).

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