Modulation in Behavior and Respiratory Dynamics of the Freshwater Fish, *Cyprinus carpio* under Sublethal Chlorpyrifos Exposure

by

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INTRODUCTION

Recent evidence indicates that fish, an extremely valuable resource, are quickly becoming scarce. One consequence of this scarcity is the increasing concern for fish survival and a growing interest in identifying the levels of various chemical pollutants, which are safe for fish and other aquatic life.

Organophosphorus pesticides (OPs) are one of the most important classes of insecticides used for protecting crops, livestock, and human health during the past 60 years (Eto, 1974; Ware, 2000; Tomlin, 2003). It is estimated that approximately 200 different OPs are currently used commercially worldwide (Klaassen, 1996).
The main advantage of the OPs is their low cumulative ability and short term persistence in the environment (Svoboda et al., 2001).

Many organophosphates are potent neurotoxins, functioning by inhibiting the action of AChE in nerve cells. Neurotransmitters such as acetylcholine (which is affected by organophosphate pesticides) are profoundly important in the brain’s development, and many OPs have neurotoxic effects on developing organisms even at low levels of exposure.
The primary effect of chlorpyrifos and other OPs on vertebrate and invertebrate organisms is the inhibition of AChE activity, the enzyme that degrades the neurotransmitter acetylcholine in cholinergic synapses (Pan and Dutta, 1998). Duration of exposure, type of OP, as well as species of fish has an effect on the extent of AChE expression.
• The problem of pollutant bioaccumulation and subsequent physiological transma is often reflected in the rate of oxygen uptake, either through disrupted metabolism or in the mobilization of compensatory homeostatic mechanism.

• Consequently index of environmental suitability and the cost of survival of aquatic organisms. Any change in the behaviour and physiology of fishes indicates the deterioration of water quality, as fishes are the biological indicators.
Objectives of the Study

- The objective of the present investigation is to determine the sublethal effects of chlorpyrifos (organophosphate) on respiratory rate and AChE activity of brain, gill and muscle in C. carpio (Linnaeus, 1758).

- And to elucidate relationship of AChE activity and respiratory rate from this exposure as a way to evaluate the toxicity risk of chlorpyrifos to the test species.
MATERIALS AND METHODS

Carp Collection and Maintenance

Healthy and active *C. carpio* (2 ± 0.2 g, 4 ± 0.25 cm) fingerlings were procured from the State Fisheries Department, Dharwad, India.

Fish were brought to the laboratory in large aerated crates. Later they were acclimatized for 30 days in large cement tanks (22 x 12 x 5 feet) and fed with commercial dry feed pellets (Nova, Aquatic P. Feed).
Toxicity expression (LC50) of chlorpyrifos

• By Semi-static test by the method of APHA (2005).

• The LC50 with 95% confidence limit for chlorpyrifos were determined/estimated for 96 h by probit analysis (Finney, 1971).
• **Experimental Design and Toxicant Concentrations**

• Sublethal concentrations of 14% (0.0224 mg/L) and 7% (0.0112 mg/L) of the acute toxicity value (LC50) were selected for subacute studies. Fish were exposed to both the sublethal concentrations for 1, 7 and 14 days and allowed to recover in toxicant free medium for seven days (designated as -7) along with the control sets.

• Concentrations of chlorpyrifikos in the test medium were confirmed by GC-MS analysis.
Assay of Respiratory Rate

- Respiratory rate (oxygen consumption) of chlorpyrifos exposed fish was measured besides control by following the method of Welsh and Smith (1953) as described by Shivakumar (2008). The values are expressed as ml of oxygen consumed/g wet wt. of fish/h.

Estimation of AChE (E.C. 3.1.1.7) Activity

- Tissues were excised in physiological saline (0.9% NaCl). Homogenates (4%) of brain, gill, liver and muscle were prepared in cold 50 mM Tris-HCl (pH 6.8) extraction buffer using a glass-teflon homogenizer (Remi Motors Ltd., Mumbai, India) and then centrifuged at 3000 rpm for 15 min. Supernatants were used to determine enzyme activity. AChE activity was determined by the method of Ellman et al. (1961).

- Protein contents were measured according to the method of Lowery et al. (1951) using bovine serum albumin as standard.

Statistical Analysis of Data

- Data correspond to the average of six replicates. The data obtained were analyzed statistically by following Duncan’s multiple range test.
RESULTS AND DISCUSSION

Chlorpyrifos Toxicity

• Acute toxicity of chlorpyrifos for the freshwater fish, *C. carpio* was found to be 0.16 mg/L. The upper and lower 95% confidence limits were found to be 0.168 mg/L and 0.151 mg/L respectively. It is evident from the results that the chlorpyrifos can be rated has highly toxic to fish.

• No significant mortality was observed during the sublethal experimental tenures, but the fish were under stress and showed symptoms of dullness, loss of equilibrium, loss of feeding, and erratic swimming.
AChE Inhibition and Respiratory Toxicity of Chlorpyrifos

• The AChE activity and respiratory rate of chlorpyrifos and their relation in fish exposed to 14% and 7% of lethal concentration of chlorpyrifos is presented in Figure 1, 2 and 3.
Figure 1: Percent variation of AChE activity in the brain and gill and respiratory rate of the fish, *C. carpio* following exposure to 14% of the lethal concentration of chlorpyrifos.
Figure 2: Percent variation of AChE activity in the brain and gill and respiratory rate of the fish, *C. carpio* following exposure to 7% of lethal concentration of chlorpyrifos.
Impact on morphological features by chlorpyrifos

- Caudal bending (left side) was noticed in both the sublethal concentrations with time and persisted even under recovery periods. The extent of caudal bending was pronounced in higher toxicant concentration. This greatly retarded the normal swimming pattern. Caudal bending may be a sort of paralysis, which is due to the inhibition of muscular AChE activity resulting in blockage of neural transmissions.

- Bending of caudal region is owing to the fact that caudal portion is the thinnest structure and hence can be conferred any sort of orientation due to paralysis of caudal musculature by inhibition of AChE activity as evidenced in the present study. Further inhibition of AChE activity results in a progressive accumulation of ACh, especially during periods of repetitive stimulation, leading to desensitization of nAChRs (nicotinic acetylcholine receptors) and consequent muscular weakness (Giniatullin et al., 1998). Thus chlorpyrifos reduced instinctive behavioural responses and affected morphological features by depression of AChE activity.
Figure 3: Percent inhibition of AChE activity in the muscle of the fish, *C. carpio* following exposure 14% and 7% sublethal concentrations of chlorpyrifos.
CONCLUSION

- The present study evidenced neurotoxic potential of chlorpyrifos by inhibition of the AChE activity in the tissues of the fish, *C. carpio* at sublethal concentrations. Inhibition of AChE activity in the brain appears to be an early process in response to sublethal exposures, and could be a more sensitive biomarker than inhibition of AChE activity in the muscle and gill to characterize toxicological impacts.

- Depression in AChE activity had a critical impact on fish neurophysiology which ultimately leads to impaired behavioral responses and morphological deformities. Deviation in the respiratory rate is due to impaired oxidative metabolism and altered respiratory physiological response to chlorpyrifos stress. Respiratory rate in the fish, *C. carpio* is independent of AChE activities of brain and gill.

- Decline in AChE activity and altered respiratory distress even under recovery periods can be viewed as greater half-life of chlorpyrifos *in vivo* and biotransformation of sequestered chlorpyrifos in the storage organs. Further inhibition of AChE activity by sequestered chlorpyrifos may be competing with the process of *denovo* synthesis of AChE.
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