Biomarkers of monocrotophos in a freshwater fish *Channa punctatus* (Bloch)

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Abstract: A few biomarkers have been investigated on freshwater fish *Channa punctatus* treated with monocrotophos for acute exposure to 18.56 ppm at 96 hr and subacute exposure viz. 0.46 ppm, 0.96 ppm and 1.86 ppm for 30 days. Biomarkers such as total protein, lipid peroxidation and acetylcholinesterase have been measured in different tissues of fish viz. gills, liver, brain and muscles. The protein levels were found to be depleted in all the tissues after pesticide exposure to lethal and sublethal concentration over the control, where as the lipid levels showed an increase under the stress of pesticide monocrotophos. The increased lipid level may be due to inhibition of lipase activity and other biomarkers of lipid metabolism. A significant inhibition of brain acetylcholinesterase (AChE) indicating its effects on nervous system have also been observed. These parameters can be used as biomarkers to predict the early toxicity of monocrotophos added to aquatic ecosystem.

Key words: Monocrotophos, Biomarkers, Channa punctatus.

Introduction

Biomarkers are now used routinely in environmental monitoring to examine the toxicity of chemicals to non-target species and have been successfully applied in a wide range of species including fish, birds and invertebrates (Adam et al., 1990, Lagadic et al., 1994 and Johnston, 1995). Acetylcholinesterase activities have appeared as biomarkers of exposure for some organophosphate pesticides and other pollutants on fish (Boquene et al., 1990; Najimi et al., 1997). Acetylcholinesterase (AChE) has been used to measure the extent of pollution in the aquatic environment by the indiscriminate application of pesticides (Holland et al., 1967; Gibson et al., 1969). The inhibition of AChE, as a result of phosphorylation, leads to accumulation of acetylcholine (ACh) and triggers the release of catcholamine as primary response and results in number of biochemical changes (Corbett, 1974). The biochemical changes induced by stress are described as secondary responses of the fish. Since, toxic pollutants often affect the activity of enzymes and changes in enzyme levels such as acetylcholinesterase and lipid peroxidation are logical candidates to use as biomarkers (Mayer et al., 1992).

Among organophosphate pesticides, monocrotophos is one of the important organophosphate pesticide, indiscriminately used in India. It reaches to the aquatic environment by direct application, spray drift, aerial spraying, washing from the atmospheric precipitation and runoff from agricultural lands where they ravage the biotic life (Thangnipon et al., 1995). Fest and Schmidt (1973) stated that monocrotophos act as a cholinesterase inhibitor and may cause disorders in the physiological state of nervous system. It can cause biochemical disorders by disrupting the passages of impulses across the neuromuscular junction and neural junction by inhibiting the enzyme acetylcholinesterase (AChE), which modulates the amount of neurotransmitter, acetylcholine. Joshi and Desai (1988) reported biochemical changes in the liver of *Tilapia mossambica* due to the exposure of monocrotophos. The measurement of brain AChE activity in animal indicates that AChE inhibition is directly related to the concentration of pesticide and the length of exposure (Macek et al., 1972). In the present study biomarkers viz: total protein, lipid peroxidation and acetylcholinesterase have been evaluated, in the freshwater fish *Channa punctatus* exposed to monocrotophos.

Materials and Methods

Disease-free fish, *Channa punctatus* (weighing 20 ± 2.0 g) collected from a local resource were washed in 0.1% KMnO₄ solution and acclimatized in the laboratory conditions for 15 days. They were kept in large holding tanks of 1000 liters capacity. During the acclimatization period, water was changed daily and fish were fed dry prawn twice a day.

Stock solution of the pesticide (36% EC) was prepared in acetone and prior to exposure the fish were examined carefully for pathological signs. After acclimatization, fish were transferred to 150 l glass aquaria containing chlorine free water. Physical and chemical indices of test water used in this acute toxicity test were as follows: temperature 25 ± 0.4°C, pH 6.9 ± 0.2, and DO 8.5 ± 2.0 mg/l, hardness (as CaCO₃) 120 ± 1.9 mg/l, alkalinity (as CaCO₃) 360 ± 1.4 mg/l were studied (APHA, 1998).

Median tolerance limit (LC₅₀) of monocrotophos to fish was estimated by exposing five groups of fish (6 fish per group) to different concentrations of the pesticide for the period of 96 hr. The recorded data was analyzed for LC₅₀ and upper/lower confidence limit using the Trimmed Spearman- Karber method (Hamilton et al., 1977).

Feeding was stopped 24 hr before the exposure. The fish were exposed to three different sublethal concentrations
**Fig 1:** Alteration in protein level (mg protein/1 gm body weight) in different tissues of *Channa punctatus* after exposure with 18.56 ppm for 96 hr of monocrotophos.

**Fig 2:** Alteration in protein level (mg protein/1 gm body weight) in different tissues of *Channa punctatus* after 30 days exposure of monocrotophos.

**Fig 3:** Alteration in lipid peroxidation activity (Malondialdehyde nmol) in different tissues of *Channa punctatus* after exposure with 18.56 ppm for 96 hr of monocrotophos.
Tissues viz. liver, muscle, brain and gills were dissected out from the control and treated fish from each concentration for the analysis of protein, lipid peroxidation and acetylcholinesterase (AChE). Total protein was estimated by the method of Lowry et al. (1951), using bovine serum albumin as standard. Lipid peroxidation activities were estimated by Okhawa et al. (1979) and acetylcholinesterase (AChE) were analyzed by the method of Ellman et al. (1961). Statistical significance of the data was assessed through Student “t” test.

**Results and Discussion**

Exposure of *Channa punctatus* to sublethal concentrations (0.46, 0.96 and 1.86 ppm) of monocrotophos for 30 days and 18.56 ppm for 96 hr decreased the total protein content of liver, muscle, brain and gill. Protein showed significant decreasing (p<0.001) trend in liver, brain, muscle and gill tissues of fish at the end of 96 hr (Fig. 1). In subacute exposure, sublethal concentrations (0.46, 0.96 and 1.86 ppm) of monocrotophos produced marked changes in the selected tissues (Fig. 2), showing maximum effect in the brain. Rao and Ramaneshwari (2000) also observed decrease of protein content in gill, liver, muscle and brain of *Labio rohita*, *Mystus vittatus* and *Channa punctatus* exposed to monocrotophos.
Rajyashree (1996) also observed decline in protein level in liver, muscle, gill and brain of carbamide exposed Labio rohita. Cythion exposures have been reported to reduce the protein level in brain and liver of Channa punctatus (Ram and Sathyanesan, 1985). Sastry and Dasgupta (1991) reported that decrease in total protein level in liver and muscle of Channa punctatus exposed to monocrotophos for 15, 30 and 60 days. Monocrotophos reduced the protein content of fish brain, Tilapia mossambica (Joshi and Desai, 1983; Richardson, 1981). A significant decrease was reported in the protein content in almost all tissues in exposed to sublethal and lethal concentration of fenvalerate (Joshi et al., 2003).

The total lipid content of all the selected tissues increased significantly after 96 hr and to the sublethal concentrations for 30 days (Fig. 3 and 4). Sastry and Dasgupta (1991) reported the increase of the lipid peroxidation level in the liver of Channa punctatus after the exposure of monocrotophos. Pandey et al. (2001) showed the significant induction of lipid peroxidation in liver, muscle and gill of Channa punctatus exposed to endosulfan for 24 hr. Pant and Gill (1987) reported an increase in lipid content of liver and muscle of Barbus chonchonius exposed to aldicarb for 15 and 30 days. Lal and Singh (1987) and Bhaskar et al. (1984) found increased lipid content in muscle, liver and gill of Clarias batrachus and Tilapia mossambica after pesticide exposure.

Exposure to 18.56 ppm for 96 hr and sublethal concentrations (0.46, 0.96 and 1.86 ppm) of monocrotophos for 30 days significantly inhibited the activity of acetylcholinesterase (AChE) in fish brain. However, the significant inhibition in brain (p<0.001) was noticed at end of 96 hr. It was interesting to note that all the three sublethal concentrations showed significant (p<0.1, <0.05 and <0.005) decrease in brain tissue of Channa punctatus (Fig. 5). The inhibition of brain AChE in the range of 70-80% is critical to fishes (Coppage et al., 1975). Maximum inhibition was observed in brain (95%) tissues of monocrotophos exposed fish, Oreochromis mossambicus (Venkateswar Rao, 2004). AChE inhibition in brain was observed in fish exposed to organophosphate insecticides such as chlorpyrifos and profenofos (Jeyarathi and Jebanesan, 2001; Kumar and Chapman, 1998, 2001 and Venkateswar Rao et al., 2003 a, b). Inhibition of acetylcholinesterase (AChE) after the exposure of monocrotophos in the fish brain has also been reported by Anderson et al. (1977), Wang and Murphy (1982 a, b) and Johnson and Wallace (1987). This study indicates that protein content, lipid peroxidation and cholinesterase activity can be used as biomarkers under stress of monocrotophos.

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