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Chronic toxic effects of ekalux on some biochemical parameters in *Cyprinus carpio* (Lin.)

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Abstract
The effect of exposure to sublethal concentrations of the organophosphate pesticide, ekalux (1.27, 0.28mg/l) on biochemical parameters of muscle, gills and enzyme activities in liver and kidney of the Common carp, *Cyprinus carpio* was studied after 7, 14 and 21 days. The muscle, gill protein and acid phosphatase were elevated. Similarly, alkaline phosphatase was depleted. Lactate dehydrogenase levels in liver were elevated the maximum percentage of reduction was recorded at 21 days post-exposure in 1.27 mg/l.

Keywords: Hyperglycemia, *Cyprinus carpio,* Muscle, gill protein, Acid phosphatase, Alkaline phosphatase and Lactate dehydrogenase

1. Introduction
Due to their lower persistence in the environment, organophosphates are used judiciously to control a wide variety of agricultural pests as well as ectoparasites in fish in aquaculture. However, the uncontrolled use of these pesticides in agriculture and public health operations has increased the scope of ecological imbalance and thus many non-target organisms have become victims. Among the aqua fauna, fish form an important group due to their nutritive value. Therefore, it becomes a matter of great concern when aquatic pollution due to pesticides is discussed.

Fish accumulate xenobiotic compounds, especially those with poor water solubility, from water or food. The uptake from water occurs because of the very intimate contact with the medium that carries the chemicals in solution or suspension and also because fish have to extract oxygen from the medium by passing enormous volumes of water over their gills. A number of workers have investigated the toxicity, uptake and tissue distribution of pesticides in a number of fishes [1-3]. However, very little information is available on the alterations in enzyme activities due to pesticides in the common carp, which forms an important food item of most people in India. The effect of pesticides on muscle, gill protein has been studied by many workers [4-9]. Changes in acid phosphatase (ACP) and alkaline phosphatase (ALP) of brain induced by pesticides have been recorded by Sastry and Sharma (1981). Ghosh (1987) has reported that there was an increased activity of lactic dehydrogenase (LDH) in brain, liver and muscle and decreased activity in kidney and intestine of *Clarias batrachus* when exposed to sublethal concentrations of Tara 909, Suquin and Croton 36. Sastry and Siddiqui (1982) have reported increases in LDH of liver and brain of *Channa punctatus* exposed to sublethal concentrations of sevin. The present study was undertaken to examine the effect of quinalphos on certain biochemical parameters and enzyme activities of common carp, Cyprinus carpio

2. Materials and Methods
Fingerlings of *Cyprinus carpio* (Lin.) weighing 8.6±2.0 g and with mean body length of 6.1±1.9 cm were collected from the pond of the Thiruvallur Dist (Lat 13.2544°, North Long. 80.0088° East), Tamil Nadu. Fish were brought to the laboratory and acclimatized for 3 weeks prior to experimentation. Dechlorinated tap water was used throughout the course of the experiments. The physico-chemical characteristics of the test water were: temperature 27.4±1.0 °C; pH 7.1; hardness 78 ppm (as $CaCO_3$); alkalinity 86 ppm (as $CaCO_3$) and dissolved oxygen concentration 6.4 ppm. The LC_{50} value was determined in the laboratory as per the methods of Finney (1991) starting with range finding tests to acute toxicity trials. The 96-h LC_{50} was found to be 12.7 ppm. One-tenth and 1:50th of the 96-h LC50, i.e. 1.27 and 0.28ppm were selected for sublethal test trials.

Stock solution of Ekalux was prepared, using glass distilled water and desired degrees of concentrations were prepared. Based on the progressive bisection of intervals on a logarithmic scale, lethal concentrations were selected as experimental concentrations. These concentrations were fixed after conducting the range finding test (APHA, 1990).

Ninety common carp fingerlings were selected for the present study and were divided into 9 equal groups, each group containing10 fingerlings. The first three groups were exposed to 1.27 mg eklaux/1 (*O*, *O*-diethyl, *O*-quinoxalin-2-ylphosphorothiate, in acetone) and the second three groups were exposed to 0.28 mg ekalux/l for 7, 14 and 21 days, respectively. The remaining three groups were maintained as controls in ekalux-free tap water. The pesticide-exposed fish and the control group were fed with pelleted feed prepared in the laboratory (rice bran and oil cake in an equal ratio 1:1) twice daily, and the water in the glass tank (30 l) was changed every 24 h to maintain a constant concentration of ekalux during the period of exposure. Aeration was provided to each tank. No mortality could be seen in any group during the entire experimental period.

2.1 Sub lethal exposure

Anderson and Peterson (1969) reported that sublethal exposures to longer periods may be dangerous to the organisms. Even when the animal is exposed to low concentrations continuously, many behavioural abnormalities and physiological alterations would be observed. In the present study 1/5th, 1/10th and 1/15th of 96 hr LC_{50} value was selected as sublethal concentration to study the behavioral alterations and physiological alterations (As per the recommendations of committee on toxicity studies – Anon, 1975 and APHA *et al*., 1998)

One-third of fish from each group were killed on the 7th, 14th and 21st day. The liver, kidney and muscle, gill tissues were removed immediately and frozen until required (not more than 1 h). Total protein (Lowry *et al.,* 1951) contents of muscle, gills were quantitatively estimated.

For the estimation of enzyme activities, kidney and liver tissues were weighed, homogenized in a glass homogenizer at 4 °C and extracts were prepared in phosphate buffer saline (pH 7.2). The final concentrations of the homogenates were adjusted to 10% (w/v) with 0.25 M sucrose solution before estimation of enzyme activities (Sastry and Siddiqui, 1982). ACP and ALP activities were analyzed according to the methods of Jafee and Badansky (1943). Sodium b-glycerophosphate was used as a substrate and the enzymatic reaction was stopped using 30% cold trichloroacetic acid. Potassium dihydrogen orthophosphate was used as a standard. The inorganic phosphate liberated from the substrate was estimated at 660 nm. The quantitative estimation of the enzyme was made using the formula µmol/min per ml at 37 °C-14.71-Δ*A* where Δ*A* is the increased absorbance °C orrected for the blank and 14.71 is the common factor. LDH was measured according to the procedure of Raghuramulu *et al*. (1983). Sodium lactate (0.5 M) was used as a substrate and glycine buffer (pH 10.0, 0.1 M) was used to stop the reaction. The increase in extinction at 340 nm, followed at 30-s intervals for 2 min, was measured and LDH activity was expressed as change in optical density (OD/min) of tissue extract. Protein in the homogenates was determined by the method of Lowry *et al*. (1951). Analysis of variance (ANOVA) was employed followed by Duncan's new multirange test to calculate the significance difference between control and experimental means (SPSS Version, 20.0).

3. Results

The muscles, gills protein in the exposed animals although reduced at 0.28 and 1.27 ppm concentrations, were however, not significantly low in comparison to the unexposed control on the 7th day. But the reduction of muscle protein at the above two concentrations was significant (P<0.05) on the 14th and 21st days. Similarly, the gill tissue increased significantly at 1.27 ppm exposure on the 7th, 14th and 21st days without any significant change at 0.28 ppm. Simultaneously, the RNA of muscle tissue decreased significantly at 1.12 ppm exposure on all three occasions in comparison to control groups and fishes exposed to 0.22 ppm (Table 1).

Table 1: Alteration in the level of muscle, gill protein of *Cyprinus carpio* *

Protein(mg:100 mg tissue)	7 days			14 days			21 days		
	control	1.27 ppm	0.28 Ppm	control	1.27 ppm	0.28 ppm	control	1.27 ppm	0.28 ppm
Muscle	16.0±1.2^{a}	14.5±0.3^{a}	14.1±0.3^{a}	18.7±0.9^{a}	17.1±0.3^{b}	16.9±0.3^{b}	18.5±1.2^{a}	16.9±0.3^{b}	16.5±0.4^{b}
Gill	22.3±1.4^{a}	18.9±0.6^{a}	18.5±0.4^{a}	23.5±1.5^{a}	20.1±0.7^{a}	18.7±0.6^{b}	22.8±1.3^{a}	19.4±0.3^{b}	18.2±0.2^{b}

* Data are represented as mean ± S.D.; data with same superscript letter are not significant to each other and data with different superscript letters in a row are significant to each other at 5%.

Among the enzyme activities, ALP activity was significantly (P<0.05) inhibited (-4 to -30%) at both the concentrations whereas the ACP activity was significantly elevated except at 0.28 mg/l concentration after 14 days exposure (Table 2).

Table 2: Alteration in the ALP and ACP level of liver and Kidney of *Cyprinus carpio* *

Protein(mg:100 mg tissue)	7 days			14 days			21 days		
	control	1.27 ppm	0.28 ppm	control	1.27 ppm	0.28 ppm	control	1.27 ppm	0.28 ppm
ALP	0.76±0.03^{a}	0.89±0.06^{a}	0.90±0.08^{a}	0.91±0.03^{a}	0.61±0.04	0.77±0.04	0.89±0.02^{a}	0.44±0.05^{b}	0.35±0.04^{b}
ACP	0.35±0.02^{a}	0.47±0.03^{b}	0.43±0.02^{a}	0.43±0.02^{a}	0.47±0.02^{a}	0.49±0.02^{a}	0.39±0.02^{a}	0.57±0.03^{b}	0.59±0.05^{b}

* Values are in mg/mg protein per min as mean ± S.D.; data with same superscript letter are not significant to each other and data with different superscript letters in a row are significant to each other at 5%.

The maximum percentage of reduction was recorded at 21 days post-exposure in 1.27 mg/l. The elevation of LDH was the highest in liver. The activity was depleted in kidney in all three treatment periods (Table 3).

Table 3: Alteration in kidney and liver LDH (ΔOD/mg protein per min) of *Cyprinus carpio**

Protei(mg:100 mg tissue)	7 days			14 days			21 days		
	control	1.27 ppm	0.28 ppm	control	1.27 ppm	0.28 ppm	Control	1.27 Ppm	0.28 ppm
Liver	1.31±0.06[a]	1.94±0.11[b]	1.94±0.07[b]	1.58±0.03[a]	1.99±0.09[b]	1.99±0.1[b]	1.67±0.05[a]	2.11±0.03[b]	2.17±0.03[b]
Kidney	2.08±0.09[a]	1.22±0.03[b]	1.47±0.02[b]	1.63±0.08[a]	0.96±0.05[a]	1.37±0.01[b]	1.65±0.07[a]	0.55±004[b]	1.03±0.05[a]

* Data are represented as mean ± S.D.; data with same superscript letter are not significant to each other and data with different superscript letters in a row are significant to each other at 5%.

4. Discussion

Exposure of fingerlings of *C. carpio* to sublethal concentrations of ekalux produced changes in the protein levels of muscle, gill and the activities of ALP, ACP and LDH in different tissues. A fall in muscle protein is indicative of reduced protein synthesis and low assimilation of food uptake for protein synthesis. Organophosphates are known to methylate and phosphorylate cellular proteins directly (Wild, 1975). Murty and Devi (1982) recorded a decrease in the protein levels in the tissues of *C. punctatus* following acute exposure to technical grade malathion. Similarly, reduction in the protein content of brain, liver and ovary of *C. punctatus* was recorded due to cython (Narayan Ram and Satyanesan, 1986). Shanmugam (1977) suggested that tissue proteins are broken down to maintain plasma proteins in a condition of protein deficiency.

Organophosphates inhibit acid phosphatase and alkaline phosphatase activity in different tissues of fishes which may adversely affect nucleic acid synthesis (Sastry and Sharma, 1981). In the present study, we noticed an increase of ACP and decrease of ALP (Table 2). The decrease in protein content of muscle as already discussed earlier by other workers was also observed in the present study. As mRNA has a direct relationship with protein content, it may be concluded that the reduction of RNA was due to proteolysis as well as retardation of protein synthesis. Subsequently, the reduction of ALP and elevation of ACP were sequelae to alteration of DNA and RNA in muscle tissue. Similar observations were made by Sastry and Sharma (1981) in brain tissue of *C. punctatus* exposed to mercuric chloride, Das (1998) in brain tissue of *L. rohita* exposed to sublethal concentration of malathion, cypermethrin, and Mohapatra (1988) in *Liza parsia* exposed to Dichlorvos.

The unequivocal depression of elevation of LDH indicated anaerobic metabolism in eklaux-treated fish. Koundinya and Ramamurthi (1979) have also observed similar elevations in LDH activity in the muscle tissue of *T. mossambica* exposed to sumithion and suggested that aerobic oxidation through the Krebs cycle was adversely affected in pesticide exposed fish. From the findings of dehydrogenase activity, it appeared that the normal physiological functioning of different tissues is greatly disturbed by exposure to eklaux. Decreased activity of different enzymes may be attributed to a repressor effect in their synthesis or to the direct action of pesticides on the enzymes. The ATPase activity was depleted significantly at both concentrations over a period of 21 days which was an indication of tissue hypoxic conditions, attributed to mucous accumulation on the gill as reported by Das (1998) while working with malathion-induced stress on *L. rohita*.

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