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# Diazinon Effect on the Activities of Brain Enzymes from *Ophiocephalus (Channa) punctatus*

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Organophosphate insecticides reduce the quality of fresh waters and are hazards to the fish population. Organophosphorus insecticides because of their rapid biodegradability are replacing the more persistent organochlorines (ANEES 1975). They are now produced in greater quantities than the chlorinated hydrocarbon insecticides (FOWLER & MAHAN 1971; LAWLESS et al. 1972). These pesticides act as nerve poisons by blocking synaptic transmission in the cholinergic parts of the nervous system (HEALTH 1961, KARCZMAR 1970, KOELLE 1963, METCALF 1971, O'BRIEN 1967). The effects of guthion, malathion phorate, parathion and other organophosphates on the brain of fish has been studied by COPPAGE (1972) and COPPAGE & METTHEWS (1974). POST & LEASURE (1974) observed the sublethal effect of malathion on three salmonid species. WEISS (1961) investigated the physiological effect of organophosphorus insecticides on several species of fish.

Diazinon (0,0-diethyl, O-(2-isopropyl, 6-methyl, 4-pyrimidyl phosphorothioate) has wide spread usage as an insecticide and acaricide all over India. In the present study a fresh water teleost, Ophiocephalus (Channa) punctatus, was exposed to LC(50) and a sublethal concenstration of diazinon. The alterations in the activities of alkaline phosphatase, acid phosphatase, $Na^+/K^+$ activated adenosine triphosphatase, glucose-6-phosphatase, lactic dehydrogenase, pyruvic dehydrogenase, succinic dehydrogenase and cholinesterase were observed after 96 hr, and 15 and 30 days, respectively.

Living specimens of Ophiocephalus (Channa) punctatus were collected from local fresh water sources and were allowed to acclimatize to the laboratory conditions for 4 days. Water used in the tests had a temperature of 20 $\pm$ 3°C and pH 7.4, hardness of 160 ppm (as $CaCO_3$) alkalinity of 87 ppm (as $CaCO_3$) and dissolved oxygen concentration of 7.5 ppm. Fish were fed with commercial fish food twice a day during the tenure of the experiments. Bioassays were conducted to determine the LC(50) and sublethal concentrations of diazinon. 3.1 mg/l of diazinon produced 50% mortality by 96 hr while 10 to 20% mortality was obtained after exposure to 0.31 mg/l of diazinon for 30 days.

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100 fish, 14-16 cm long and 50-60 gm in weight were selected. They were divided into four groups, three groups of 20 fish and the fourth consisting of 40 fish. The first group of fish was exposed to 0.31 mg/l of diazinon for 30 days. After 15 days, the second group was also exposed to the same concentration of diazinon. After 26 days, the fourth group of fish was exposed to 3.1 mg/l of diazinon for 96 hr. The third group of fish served as non-treated controls for first, second and fourth groups, respectively. In the first group of fish, one fish each died on 6th, 17th and 25th days and in the second group only one fish died on second day. In the fourth group 19 fish died within 96 hr. No mortality was recorded in control fish. All the surviving fish were sacrificed after completing the experiments and the brains were immediately removed and weighed to the nearest milligram. For preparing enzyme extracts, the brains were homogenized in cold 0.25 M sucrose solution using a chilled Potter-Elvehjem homogenizer. 0.016 M sodium B-glycerophosphate was used as the substrate at pH 9.3 and 5.0 for alkaline phosphatase and acid phosphatase assays, respectively. The enzyme activities were determined by the method of MORTON (1955). Glucose-6-phosphatase activity was determined by the method of SWANSON (1955). $Na^+/K^+$ activated adenosine triphosphatase activity was estimated according to the method of KOCH (1969). The activities of lactic dehydrogenase, pyruvic dehydrogenase and succinic dehydrogenase were estimated by modifying the method of SRIKANTAN & KRISHNAMOORTI (1955). Cholinesterase activity was determined according to the method of BOCKENDAHL & AMMON (1955) using $4.5 \times 10^{-2}$ M acetylcholine as substrate. Protein in the homogenates was determined by the method of LOWRY et al. (1951) using bovine serum albumin as standard. For each enzyme assay triplicate samples of the enzyme extracts were incubated and the enzyme assays were repeated three times as 37°C. The 't' test described by FISHER (1950) as employed to calculate the significance of differences between control and experimental values and 'P' values of 0.05 or less were considered significant. Ditaf® (Diazinon) was obtained from Rallis India Ltd., Bombay.

## RESULTS

Alkaline phosphatase activity was reduced in the brain of fish exposed for 96 hr to diazinon. However, exposure to diazinon for 15 days produced no alteration in the activity of this enzyme, while after 30 days of exposure there was increase in enzyme activity (Table 1). Acid phosphatase showed significant decrease and the activity was lowest after 30 days. There was slight elevation in the activity of sodium-potassium activated adenosine triphosphatase after 96 hr and 15 days which was followed by an insignificant inhibition

TABLE I

Effect of diazinon on the activities of brain enzymes of Ophiocephalus punctatus.

| Enzyme | Control | Experimental activity/mg protein/hr | | | | | |
|---|---|---|---|---|---|---|---|
| | | 3.1 mg/l for 96 hr | % Alter. | 0.31 mg/l for 15 days | % Alter. | 0.31 mg/l for 30 days | % Alter. |
| Alkaline phosphatase[b] | 0.55±0.01 | 0.43±0.01 | 22(+)[a] | 0.54±0.01 | 2(-) | 0.61±0.01 | 11(+) |
| Acid phosphatase | 0.30±0.04 | 0.27±0.002 | 10(+) | 0.28±0.003 | 7(+) | 0.19+0.02 | 37(+) |
| Adenosine triphosphatase | 1.01±0.01 | 1.13±0.01 | 12(+) | 1.05±0.01 | 4(+) | 0.99±0.02 | 2(-) |
| Glucose-6-phosphatase | 0.36±0.01 | 0.25±0.02 | 31(+) | 0.30±0.02 | 17(+) | 0.19±0.00 | 47(+) |
| Lactic dehydrogenase[c] | 0.07±0.002 | 0.05±0.006 | 29(+) | 0.06±0.001 | 14(+) | 0.06±0.003 | 14(+) |
| Pyruvic dehydrogenase | 0.07±0.001 | 0.05±0.008 | 29(+) | 0.05±0.005 | 14(+) | 0.04±0.007 | 43(+) |
| Succinic dehydrogenase | 0.09±0.001 | 0.02±0.014 | 78(+) | 0.07±0.005 | 22(+) | 0.05±0.007 | 44(+) |
| Cholinesterase[d] | 133±4 | 38±6 | 71(+) | 66±4 | 50(+) | 88±5 | 34(+) |

Values are mean ± S.E.
a (+) indicates significant differences at P = 0.05 from control value.
b μ mole of phosphate.
c mg of formazan.
d μ mole of acetylcholine.

by 30 days of exposure. Glucose-6-phosphatase, and all the three dehydrogenases showed significant reduction in activities after 96 hr, 15 and 30 days of exposures. Lactic dehydrogenase and succinic dehydrogenase activities were lowest after 96 hr of exposure to LC(50), while pyruvic dehydrogenase showed marked decrease in activity after 30 days of exposure to the sublethal concentration. Cholinesterase activity was decreased in all the experimental groups of fish exposed to the insecticide. However, the decrease in activity was greater after 96 hr of exposure to LC(50) of diazinon.

## DISCUSSION

The mode of action of organophosphate insecticides in vertebrates is generally regarded as disruption of nerve impulse transmission in the central and peripheral nervous system (COPPAGE & METTHEWS 1975). Acute and chronic exposures to difference organophosphates resulted in marked decrease in the acetylcholinesterase activity in different fish (COPPAGE & METTHEWS 1975, COPPAGE et al. 1975). The present study demonstrates that the organophosphates affect the activities of acetylcholinesterase, alkaline and acid phosphatases, and lactic, pyruvic and succinic dehydrogenases. The decreases in the activities of enzymes may be either due to enzyme inhibition or decreased synthesis of the enzymes.

Alkaline phosphatase activity in the nervous tissue is believed to be involved in the permeability processes (SETHI et al. 1969, TEWARI & BOURNE 1963a,b) and acid phosphatase is associated with nucleic acid synthesis (COX & GRIFFIN 1965, TEWARI & SOOD 1969). The reduction in the activities of the two enzymes indicates that the permeability processes and nucleic acid synthesis in brain may be adversely affected by diazinon, and this in turn may effect the synaptic transmission. DALELA et al. (1978) working on the chronic effects of Rogor®, another organophosphate insecticide, have reported inhibition in acid phosphatase activity of liver, muscle and kidney of Channa gachua.

Adenosine triphosphatase accumulates at the neuronal cell membrane and may supply energy for ion pumping and the transport of substances across the cell membrane (HYDEN 1962). Thus, the alteration of $Na^+/K^+$ activated adenosine triphosphatase activity may also interfere with transmission processes. Adensoine triphosphatase activity was found to be elevated in the liver and kidney after intraperitoneal administration of diazinon in rats, while a decrease in enzyme activity was noted in testis (DIKSHITH et al. 1975).

Glucose-6-phosphatase is a specific enzyme which catalyses the breakdown of glucose-6-phosphate into glucose and phosphate. Glucose is by far the most important substrate for brain respiration. The glycogen stores of brain are low, but because of the dependence of functional activity of neurons on glucose and oxygen, brain exhibits a particular requirement for a continuous and adequate blood supply. Thus the transmission processes are dependent on glucose availability, and the decrease in glucose-6-phosphatase activity may interfere with the transmission processes.

Organophosphate insecticides are considered to be specific inhibitors of cholinesterase activity. In the present study 71% inhibition was recorded by exposure to LC(50) for 96 hr and chronic exposure produced 38% and 50% inhibition of the enzyme activity after 15 and 30 days, respectively. COPPAGE & METTHEWS (1974) observed 70% inhibition in brain acetylcholinesterase of spots exposed to 1250 ppb of malathion for 24 hr. For the same period of exposure, 75 ppb of naled produced 85% inhibition while 20 ppb of guthion produced 96% inhibition in acetylcholinesterase activity. Exposure to 0.1 ppm of chlorothion for six days resulted in 60% reduction in cholinesterase levels of fat head minnows (WEISS 1961). In the present study the inhibition of enzyme activity increased with exposure time to sublethal concentration. Similar results were obtained by COPPAGE & METTHEWS (1974) and COPPAGE et al. (1975).

It has been shown with labelled organophosphates in vitro, that the actual toxic agents are deacylated metabolites which irreversibly phosphorylate O'-serine in the esterolytic site of "purified cholinesterase" and that further alteration of the organophosphate may occur by dealkylation. The covalent phosphorus serine bond is maintained long after parent compound has disappeared. Additional exposure would increase the number of these bonds and enzyme inhibition (KARSCZMAR 1970, KOELLE 1963, O'BRIEN 1967, ALDRIDGE 1971, FUKUTO 1971, O'BRIEN 1960, O'BRIEN 1969, SCHAFFER 1954).

O'BRIEN (1960) lists the mechanisms involved in death as bronchoconstriction, lowered blood pressure, neuromuscular block of the respiratory muscles, and failure of the respiratory centre. The sequence of events is (i) inhibition of cholinesterase; (ii) acetylcholine accumulation; (iii) disruption of nerve function either centrally or peripherally; (iv) respiratory failure; and (v) death or asphyxia.

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