Bull. Environ. Contam. Toxicol. (1990) 45:350–357

Environmental Contamination and Toxicology

Methylparathion-Induced Alterations in the Acetylcholinesterase and Phosphatases in a Penaeid Prawn, *Metapenaeus monoceros*

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The indiscriminate use of pesticides can be considered as one of the factors which alters the environment, causing several imbalances in the ecosystem, especially to the denizens of the aquatic environment. It was already established that the organophosphorous insecticides induce toxic effects by the disruption of nerve impulse transmission in the nervous system by causing inhibition in the activity of acetylcholinesterase (AChE), the enzyme which modulates the neurotransmitter, acetylcholine (ACh) (O'Brien 1967). But the studies concerning the lethal action of these chemicals in relation to AChE inhibition could not establish a relationship between the degree of AChE inhibition and the death of the organism. Van Asperen (1958) pointed out that some times aliphatic esterase activity was more strongly inhibited than AChE. Later Stegwee (1959) proved that aliesterases are of minor importance in producing the toxic effects of organophosphorous insecticides. Further, the biochemical lesions in the carbohydrate metabolism have been reported to induce symptoms similar to anti-ChE insecticides (Reddy 1986). With this perspective backdrop, in the present investigation an attempt has been made to probe into study the changes in acetylcholinesterase, alkaline and acid phosphatase activities and also with the alterations in the carbohydrate metabolism of penaeid prawn, Metapenaeus monoceros during exposure to methylparathion to understand the nature of its impact.

MATERIALS AND METHODS

M. monoceros were collected from the Buckingham Canal, near Kavali seacoast, Andhra Pradesh, India. Only intermolt prawns (75 ± 5 mm in length and 2.5 ± 0.5 g weight) were selected and acclimatized

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to laboratory conditions for a week at constant salinity of 15 ± 1 ppt, pH 7.1 ± 0.2 and temperature of 23 ± 2°C. They were fed ad libitum diet of oil cake powder, which was stopped before 24 hr of experiments. The media in which prawns were placed was changed periodically at regular intervals and continuous aeration was provided. Technical grade methylparathion (80% w/w; 0-0-dimethyl, 0-4 nitrophenyl thiophosphate) was used as test chemical. 300 specimens were exposed to 40 µg/L (1/3 of 48 hr LC_{50}) of methylparathion in plastic containers of fifteen litres capacity. Suitable controls were maintained without pesticide. The whole set was aerated continuously. The treatments were continued up to 5 days and the desired tissues were taken out from control and experimental animals after every 24 hr and measured quantities of these tissues were used for the biochemical analysis. The activity of acetylcholinesterase (AChE) was recorded in the thorasic ganglionic mass of M. monoceros by adopting the method of Metcalf (1957). The reaction mixture of 2 ml contained 100 µmoles of sodium phosphate buffer (pH 7.4), 8 µmoles of acetylcholine chloride and 1.0 ml of the homogenate (1% w/v in 0.25 M sucrose solution). The activity levels of acid phosphatase (ACPase) and alkaline phosphatase (ALPase) of hepatopancreas were determined by the method of Bergmeyer (1967). The glycogen content was estimated in the hepatopancreas by the method of Carrol et al (1956) and the blood glucose level was determined by the method of Nelson-Somogyi as given in Oser (1979). Each experiment was replicated six times and the data were subjected to statistical analysis as per Bailey (1965). The protein content in the enzyme source was estimated with the Folin phenol reagent (Lowry et al. 1951).

RESULTS AND DISCUSSION

Prawns are active animals and are very sensitive to any change in their environment. During exposure to sublethal concentration of methylparathion, the prawns became restless and showed fast erratic swimming (hyperexcitability) and which was followed by tremors in the appendages, restriction of appendage movement, loss of coordination and subsequently equilibrium, violent action of chelate legs and ultimately death.

The activity levels of AChE, ACPase and ALPase were assayed in the control and experimental prawn, M. monoceros. The AChE activity or thoracic ganglionic mass was significantly inhibited in all the

Table 1: Activity levels of thoracic ganglionic mass acetylcholinesterase (AChE), hepatopancreas acid (ACPase) and alkaline (ALPase) phosphatase of M. monoceros after exposure to methylparathion (Each value is mean ± SD of 6 individual observations)

Enzyme	Group	Exposure period (in days)			
		2	3	4	5
AChE*	Control	7.35 ±0.48	7.51 ±0.43	7.24 ±0.39	7.42 ±0.35
	Experimental	3.03 ±0.35	3.68 ±0.32	4.41 ±0.43	5.29 ±0.41
	PDC	(-59)	(-51)	(-39)	(-29)
ALPase**	Control	5.15 ±0.42	5.18 ±0.38	5.23 ±0.43	5.21 ±0.33
	Experimental	2.54 ±0.28	2.88 ±0.25	3.14 ±0.29	3.29 ±0.32
	PDC	(-51)	(-44)	(-40)	(-37)
ACPase**	Control	3.45 ±0.43	3.49 ±0.42	3.54 ±0.38	3.63 ±0.34
	Experimental	7.49 ±0.49	8.65 ±0.48	8.94 ±0.45	9.48 ±0.52
	PDC	(+117)	(+148)	(+153)	(+161)

* µmoles of ACh hydrolysed/mg protein/hr. ** µmoles of Pi liberated/mg protein/hr. PDC: Percent diviation over respective control. All values are significant at $P < 0.001$.

Table 2: Changes in hepatic glycogen and hemolymph glucose of M. monoceros after exposure to methylparathion (Each value is mean ± SD of 6 observations)

Exposure period (in days)	Hepatic glycogen (mg/g wt tissue)		Hemolymph glucose (mg/100 ml)	
	Control	Experimental	Control	Experimental
2	11.35 ±1.28	5.18 ±0.74 (-54)	50.44 ±3.37	74.18 ±3.87 (+47)
3	11.20 ±1.15	5.73 ±0.78 (-49)	51.12 ±3.15	81.41 ±3.94 (+59)
4	10.84 ±1.32	6.24 ±0.82 (-42)	50.14 ±2.85	89.12 ±3.45 (+77)
5	11.19 ±1.25	7.08 ±0.81 (-36)	50.19 ±2.94	33.83 ±1.88 (-32)

Figures in parentheses indicate percent change over respective control. All values are significant at $p < 0.001$.

periods of exposure. The ACPase activity was enhanced in the hepatopancreas of M. monoceros significantly after exposure to methylparathion sublethal concentration.

Methylparathion exposure to M. monoceros inhibited the ALPase activity in the hepatopancreas (Table 1). The hepatic glycogen content of M. monoceros decreased after exposure to methylparathion, significantly. The hemolymph glucose level was increased upto 4 days of methylparathion exposure, leading to hyperglycemia (Table 2). After 5 days of methylparathion exposure hemolymph glucose was decreased significantly.

Most of the organophosphorous insecticides have structural complementarity with the target enzyme AChE (Matsumura 1976) hence the reaction between organophosphorous insecticides and AChE is analogous to the early stage of acetylcholine hydrolysis.

Methylparathion belongs to phosphorothionate group of organophosphorous insecticides, and were potent inhibitors of cholinesterase both *in vivo* and *in vitro*. There is a strong evidence to show that methylparathion is metabolically altered to a more active AChE inhibitor by the oxidation of the thionosulphur atom (p=S) to an oxygen atom (P=0). Further it was established that conversion of phosphorothionate and phosphorodithioate insecticides to their corresponding oxygen analogs is a necessary prerequisite for their action as cholinesterase inhibitors. The enzyme system in hepatopancreas that catalyses this reaction belongs to the group of NADPH-dependent mixed-function oxidases of the microsomes. The resulting oxygen analogue (methylparaoxon) is several times a more potent inhibitor of AChE (Schoor & Brausch 1980). Thus the strong electrophilic group (P=0), binds with the active site of the enzyme AChE, and thereby blocks the ACh hydrolysis, thus inhibiting AChE activity. Coppage & Matthews (1974) observed 72% inhibition of AChE in the ventral nerve cord of shrimp, *Penaeus monodon* exposed to lethal concentration (LC_{50}/48 hr) of malathion.

Alkaline phosphatase is a brush border enzyme which splits various phosphorous esters at alkaline pH and mediates membrane transport (Goldfisher et al. 1964). ALPase has also been shown to be involved in active transport (Danielli 1972), glycogen metabolism (Reddy & Rao 1988), protein synthesis (Pilo et al. 1972), secretary activity (Ibrahim et al. 1974) and in the synthesis of certain enzymes (Summer 1965). Thus any change in the ALPase activity will affect the physiological and biochemical pathways of animals. ALPase, like AChE, contains a serine residue at its active site (Mahendru & Agarwal 1983) and organophosphorous insecticides are reported to be the potent inhibitors of serine containing enzymes (Bell et al. 1970). Hence it is possible that methylparathion and its converted product methylparaoxon, having a strong electrophilic group, attacks the active site of the enzyme and thereby blocks its hydrolytic action.

ACPase is an enzyme of lysosomal origin, which hydrolyses the phosphorous esters in acidic medium and also helps in the autolysis of the cell after its death. The increase in the lysosomal activity occurs as a part of prenecrotic changes (Novikoff 1961). The increase in ACPase activity after methylparathion exposure to *M. monoceros* may be attributed to the rupture of cellular and lysosomal

membranes and effusion of their contents, resulting in the rapid autolysis of the cells. Similar kind of results are also reported in Metapenaeus monoceros after exposure to sublethal concentrations of phosphamidon (Reddy 1986).

The hepatic glycogen content was decreased considerably with an apparent increase in hemolymph glucose level may be due to decreased glycogen synthesis, possibly due to decreased activity of glycogen synthetase, a serine containing enzyme and or due to increased glycogen utilization. Reddy (1986) reported synthesis and utilization of glycogen are altered during phosphamidon toxic stress in the tissues of M. monoceros. The decrease in glycogen content observed in the present study might be due to the prevalence of hypoxic or anoxic conditions which normally increases carbohydrate and glycogen utilization (Dezwaan & Zande 1972). The increased glycogen phosphorylase activity levels in tissues of M. monoceros during pesticide exposure reveals that the glycogen utilization through the glycolytic pathway. This has been further evidenced by increased activity levels of aldolase and glycogen phosphorylase during pesticide exposure in tissues of M. monoceros and Penaeus indicus (Reddy 1986; Reddy & Rao 1988). In crustacean tissues, glycogen phosphorylase is responsible for the degradation of glycogen (Cowgill 1956). The phosphorylase activity is regulated by the neuroendocrine principle of the eyestalk (sinus gland complex) in the crustaceans (Ramamurthi & Venkataramanaiah 1982). Methylparathion exposure causes accumulation of acetylcholine at the synaptic junctions by inhibiting AChE activity in M. monoceros, which inturn stimulates the secretion of the sinus gland of the eyestalk and thus activates the phosphorylase activity. The changes in the carbohydrate metabolism in the hepatopancreas of M. monoceros after exposure to sublethal concentration of methylparathion may be attributed to the direct action of methylparathion on carbohydrate metabolism by its action on serine residue, the active site of an enzyme related to a rate-limiting step. The hyperglycemia may be attributed to the physiological response to meet the critical need of the brain for increased energy in the form of glucose. The hypoglycemia with an apparent decrease in the hepatic glycogen content may be ascribed to the rapid breakdown and utilization of glycogen and its allied carbohydrate precursors for energy requirement of M. monoceros due to the augmented toxic stress condition.

The present investigation concludes that methylparathion exposure causes significant inhibition of AChE activity of thoracic ganglionic mass and the accumulation of ACh at the synaptic junctions in M. monoceros. This may lead to the behavioural changes and create a derangement in the physiological and biochemical activities ultimately lead to the death of the organisms. The physiological, biochemical and ecological significance of methylparathion induced alterations in AChE activity and in tissue carbohydrate metabolism of penaeid prawn may be used valuable indices for determining the environmental pollution by methylparathion and the organisms adaptability to polluted habitats.

Acknowledgments. Dr.MSR is grateful to the Council of Scientific and Industrial Research, New Delhi, for providing financial support in the form of Research Associateship. We thank Prof.K.Sasira Babu for his encouragement.

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Received September 27, 1989; accepted February 22,1990