Bull. Environ. Contam. Toxicol. (1991) 46:479–484
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Environmental Contamination and Toxicology

Toxic Impact of Aldrin on Acid and Alkaline Phosphatase Activity of Penaeid Prawn, *Metapenaeus monoceros: In Vitro* Study

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The increasing contamination of the aquatic environment by the indiscriminate and widespread use of different kinds of pesticides is a serious problem for environmental biologists. Organochlorine insecticides are more hazardous since they are not only more toxic but also leave residues in nature. The deleterious effects of aldrin on several crustaceans have been studied (Ratnam 1989; Omkar et al. 1984). But studies concerning the impact of aldrin on biochemical aspects of crustaceans are very much limited. Though aldrin interacts with the physiological functions of hepatopancreas, muscle and gill tissues (Ratnam 1989), the exact mode of action is not yet clearly known. The present study is aimed to probe into the in vitro effects of aldrin on the acid and alkaline phosphatase activity levels in selected tissues of penaeid prawn, Metapenaeus monoceros (Fabricius). M. monoceros selected in the present investigation is considered to be a sensitive indicator of marine or estuarine pollution (Butler 1966).

MATERIALS AND METHODS

Penaeid prawns, Metapenaeus monoceros (Fabricius) 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 to laboratory conditions for 1 wk 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. The media in which prawns were placed was changed periodically at regular intervals and continuous aeration was provided to fulfil the oxygen requirement of the prawns. After acclimatization prawns

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were killed by keeping them in ice cold and hepatopancreas, muscle, gill, stomach and brain tissue were dissected out and weighed quantities of these tissues were homogenized in 0.25 M ice cold sucrose solution (3% w/v) and centrifuged at 1000 g for 15 min. The supernatants were used as the enzyme source. Sodium β-glycerophosphate of the pH 5 and 9 was used as substrate for acid and alkaline phosphatase, respectively. Aldrin (1,2,3,4,10,10-hexachloro 1,4-endo, 5-8-exodimethylene-1,4,4a,5,8,8a hexahydronaphthalene; 98.5%) was used as test chemical. Technical grade aldrin was dissolved in 2 mL of acetone and further dilutions were made as desired with glass distilled water. Three concentrations were selected (8,16,24 µg, LC_{50} value for 96 hr is 32 µg) for *in vitro* addition of insecticide in the reaction mixture. The activity levels of acid phosphatase and alkaline phosphatase in selected tissues were determined by the method of Bodansky (1933). The protein content in the enzyme source was estimated with the Folin phenol reagent (Lowry et al. 1951). Each experiment was replicated six times and the data were subjected to statistical analysis as per Bailey (1965).

RESULTS AND DISCUSSION

The activity levels of acid phosphatase (ACPase) and alkaline phosphatase (ALPase) were assayed in selected tissues of prawn, *M. monoceros* (Tables 1 & 2). Both ACPase and ALPase activities were significantly inhibited after the addition of aldrin, an organochlorine insecticide *in vitro*. The degree of inhibition is dose-dependent and considerable inhibition occurred even in the low concentrations. Maximum inhibition in ACPase activity was observed in 24 µg/L of aldrin *in vitro* addition in hepatopancreas (-66%; $P < 0.001$) as compared to control values, whereas minimum inhibition was observed in the muscle and brain tissues (-28%; $P < 0.001$) after treatment of 8 µg/L of aldrin. However, ALPase was inhibited maximum in stomach (-62%; $P < 0.001$) and minimum in the brain tissue (-8%; $P < 0.001$).

Generally, lysosomal hydrolases are thought to contribute to the degradation of damaged cells and hence to facilitate their replacement by normal tissue (De Duve 1963). ACPase is an enzyme of lysosomal origin, which hydrolyses the phosphorous esters in acidic medium and also helps in autolysis of the cell after its death. In the present study ACPase was inhibited significantly in all the *in vitro* aldrin concentrations. Bhagyalakshmi (1981) reported that acute exposure of sumithion enhanced the activity of ACPase while chronic exposure inhi-

bited the activity of the enzyme. On the contrary Kozik et al (1977) observed that acute exposure of mercuric chloride inhibited ACPase activity. In earlier *in vivo* studies authors Reddy and Rao (1988) and Reddy et al (1984) have found elevation in ACPase activity after phosphamidon and sumithion exposure, respectively, in penaeid prawn, *Penaeus indicus*, and crab, *Oziotelphusa senex senex*.

Table 1. Specific activity levels of acid phosphatase in selected tissues of *M. monoceros* after *in vitro* addition of aldrin. (Each value is mean ± SD of 6 observations).

Aldrin Concentration (µg/L)	Acid phosphatase, µmol Pi/mg/hr Tissues				
	Hepatopancreas	Muscle	Gill	Stomach	Brain
Control	3.45 ±0.43	1.02 ±0.12	3.34 ±0.44	3.14 ±0.29	0.75 ±0.06
8	1.89 ±0.28 (-45)	0.74 ±0.10 (-28)	2.05 ±0.29 (-39)	1.98 ±0.21 (-37)	0.54 ±0.05 (-28)
16	1.42 ±0.24 (-59)	0.62 ±1.10 (-39)	1.94 ±0.20 (-42)	1.81 ±0.18 (-42)	0.43 ±0.03 (-43)
24	1.18 ±0.21 (-66)	0.51 ±0.08 (-50)	1.73 ±0.18 (-48)	1.65 ±0.12 (-47)	0.38 ±0.03 (-49)

Values expressed in µ moles of Pi liberated/mg protein/hr.

All values for exposed enzyme are statistically significant from control at $P < 0.001$.

Values in parentheses are per cent change over their respective control.

ACPase is lysosomal enzyme and cellular damage is usually accompanied by increase in the activity of this enzyme. *In vitro* inhibition of ACPase activity

have shown that aldrin is an inhibitor of ACPase but the increase in the activity levels of ACPase during in vivo studies was most probably due to rapid destruction of lysosomal membranes and thereby excessive release of acid phosphatase. Similar kinds of observations were reported in M. monoceros during methylparathion exposure (Reddy & Rao 1989).

ALPase is a brush border enzyme which splits various phosphorous esters at alkaline pH and mediates membrane transport (Goldfisher et al. 1964) and also involved in transphosphorylation reactions. The decrease in the activity of ALPase in different tissues indicates that transphosphorylation was inhibited in these tissues. Sastry and Gupta (1978) reported the ALPase inhibition in liver and intestine of Heteropneustes fossilis during in vitro addition

Table 2. Specific activity levels of alkaline phosphatase in selected tissues of M. monoceros after in vitro addition of aldrin. (Each value is mean ± SD of 6 observations).

Aldrin Concentration (µg/L)	Alkaline phosphatase, µmol Pi/mg/hr Tissues				
	Hepato-pancreas	Muscle	Gill	Stomach	Brain
Control	5.15 ±0.42	1.14 ±0.12	5.45 ±0.35	3.74 ±0.29	0.95 ±0.06
8	3.84 ±0.28 (-25)	0.93 ±0.10 (-18)	4.03 ±0.28 (-26)	1.85 ±0.20 (-51)	0.68 ±0.03 (-8)
16	3.03 ±0.22 (-41)	0.82 ±0.08 (-28)	3.72 ±0.24 (-32)	1.63 ±0.15 (-56)	0.63 ±0.02 (-13)
24	2.81 ±0.21 (-45)	0.71 ±0.06 (-18)	3.09 ±0.21 (-43)	1.41 ±0.12 (-62)	0.79 ±0.03 (-17)

Values expressed in µ moles of Pi liberated/mg protein/hr. All values for exposed enzyme are statistically significant from controls at $P < 0.001$. Values in parentheses are per cent deviation over their respective control.

of mercuric chloride. In earlier in vivo studies, Bhagyalakshmi (1981) have also reported inhibition in the ALPase activity in most of the tissues of freshwater field crab, Oziotelphusa senex senex. However, ACPase and ALPases present in nucleolus are reported to be involved in the synthesis of nucleic acids (Cox & Griffin 1965) and thus any change in the activities of these enzymes also disturbs the protein synthesis.

The present investigation indicates that any alteration in the activities of ACPase and ALPase creates disturbances in the normal functioning of the various tissues of M. monoceros after in vitro addition of aldrin, an organochlorine insecticide.

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 October 22, 1989; accepted April 2, 1990.