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In vivo Recovery of Acetylcholinesterase Activity from Phosphamidon and Methylparathion Induced Inhibition in the Nervous Tissue of Penaeid Prawn (*Metapenaeus monoceros*)

M. Srinivasulu Reddy and K. V. Ramana Rao

Division of Toxicology, Department of Marine Zoology, S.V. University P.G. Centre, Kavali 524 202, India

Increased production and utilization of organophosphorous (OP) pesticides due to suspended or cancelled registration of persistant and highly toxic chlorinated pesticides, has lead to the pollution of freshwater, marine, estuarine environments (Mrak 1969), thereby proving highly toxic to several aquatic biota, including some important members of the food chain. Phosphamidon and methylparathion, an organophosphorous insecticides, are being extensively used as broad spectrum insecticides in agricultural purposes in Andhra Pradesh. The insecticidal mode of action of phosphamidon and methylparathion are similar to that for the organophosphate group in general, in that acetylcholinesterase (AChE) appears to be the primary target. Inhibition of AChE is regarded as a significant parameter to asses the complex effects of various toxicants (Coppage 1972; Coppage & Matthews 1974; Coppage et al. 1975). The present investigation is oriented to evaluate the in vivo inhibitory potentiality of lethal and sublethal concentrations of phosphamidon and methylparathion on the AChE activity in the nervous tissue of penaeid prawn, Metapenaeus monoceros and to assess the recovery of AChE activity after transfer of these prawns to pesticide free water. 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 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. The media in which prawns were placed was changed periodically

at regular intervals and continuous aeration was provided.

Technical grade phosphamidon (92% w/v; 0,0-dimethyl-0-(1-methyl-2-chloro-2-diethyl-carbomoyl-vinyl) phosphate) and methylparathion (80% w/w; 0-0-dimethyl, 0-4 nitrophenyl thiophosphate) were used as test chemicals. A stock solution of 1000 ppm (1 mg/1 ml) and appropriate working concentrations were prepared by dilution with seawater. Toxicity evaluation studies were conducted in static bioassay system (Doudoroff et al. 1951) and the results were tabulated for computization of LC_{50} values as per Finney (1964). LC_{50} values were found to be 1.2 ppm for phosphamidon, 0.12 ppm for methylparathion to the intermolt prawn for 48 h period. After 48 h exposure in the respective pesticide media the prawns were transferred to toxicant-free media, to study the rate of elucidation of recovery of pesticide induced AChE inhibition and AChE was again measured after 2, 4 and 7 days.

The entire nervous tissue was isolated in cold ($4^{o}C$) from the prawns of the control and different experimental groups after a specified time interval of treatment and used for enzyme assay. The activity of AChE was assayed according to the method of Metcalf (1957) after initial standardization (Srinivasulu Reddy 1986). The reaction mixture of 2 ml contained 100 μ moles of sodium phosphate buffer (pH 7.4), 8 μ moles of acetylcholine chloride and 1.0 ml of the homogenate (1% w/v in 0.25 M sucrose solution).

The protein content in the enzyme source was estimated with the Folin phenol reagent (Lowry et al. 1951) using bovine serum albumin as standard. The data were subjected to statistical analysis as per Bailey (1965).

RESULTS AND DISCUSSION

The activity levels of acetylcholinesterase was assayed in the control and experimental prawn, M. monoceros nervous tissue. The AChE activity of nervous tissue was significantly inhibited in M. monoceros, when exposed to both lethal and sublethal concentrations of phosphamidon and methylparathion up to 48 h period (Tables 1 & 2). The degree of inhibition is dose-dependent and considerable inhibition occurred even in the low concentrations. Higher inhibition could be observed at lethal concentrations compared to sublethal concentrations. Maximum inhibition was observed in prawns exposed to methylparathion (-63.60%) than to phosphamidon (-53.61%). Most of the

Table 1 : Activity levels of acetylcholinesterase in the nervous tissue of prawn, M. monoceros during and after exposure to lethal and sublethal concentrations of phosphamidon. (Each value is mean ± SD of 6 observations).

Phosphamidon concentration (ppm)	Enzyme activity (μ moles of acetylcholine hydrolysed/mg protein/h)				
	Control	48 h after exposure	Reclamation period (in days)		
			2	4	7
1.2	7.35 ±0.48	3.41 ±0.25	4.45 ±0.32	5.72 ±0.29	6.93 ±0.39
	% Change	-53.61	-39.46	-22.18	-5.72*
		PDE	(+30.50)	(+67.74)	(+103.23)
0.4	7.42 ±0.51	5.34 ±0.31	6.03 ±0.28	6.72 ±0.29	7.24 ±0.44
	% Change	-28.03	-18.73	-9.43**	-2.43***
		PDE	(+12.92)	(+25.84)	(+35.58)

PDE = Percent deviation over experimental (48 h). All values are significant at $P < 0.001$ except $^{*}P < 0.1$; $^{**}P < 0.01$; ***NS Not significant.

Table 2 : Activity levels of acetylcholinesterase in the nervous tissue of prawn, M. monoceros during and after exposure to lethal and sublethal concentrations of methylparathion. (Each value is mean ± SD of 6 observations).

Methylparathion concentration (ppm)	Enzyme activity (μ moles of acetylcholine hydrolysed/mg protein/h)				
	Control	48 h after exposure	Reclamation period (in days)		
			2	4	7
0.12	7.72 ±0.45	2.81 ±0.22	4.45 ±0.38	5.22 ±0.39	6.60 ±0.43
	% Change	-63.60	-42.36	-32.38	-14.51
		PDE	(+58.36)	(+85.77)	(+134.88)
0.04	7.54 ±0.50	4.91 ±0.29	5.68 ±0.34	6.05 ±0.39	6.98 ±0.45
	% Change	-34.88	-24.67	-19.76	-7.43*
		PDE	(+15.68)	(+23.22)	(+42.16)

PDE : Percent deviation over experimental (48 h). All values are significant at $P < 0.001$ except $^{*}P < 0.05$.

phosphorothionate insecticides are considered as latent inhibitors, wherein they are converted to active AChE inhibitors by the microsomal oxidative systems in the presence of NADPH or NADH (O'Brien 1967). There is a strong evidence to show that methylparathion is metabolically altered to a more active AChE inhibitor by the oxidation of the thiono-sulphur atom (P=S) to an oxygen atom (P=O). The resulting oxygen analogue (methylparaoxon) is several times a more potent inhibitor of AChE (Benke et al. 1975). The high level of AChE inhibition under 48 h methylparathion exposure suggests its convertion to methylparaoxon. The degree of inhibition is dose dependent i.e., at higher (lethal) concentrations of both pesticides inhibition of AChE is more, compared to lower (sublethal) concentrations. Coppage & Matthews (1974) observed 72% inhibition of AChE in the ventral nerve cord of shrimp, Penaeus monodon exposed to lethal concentration (LC_{50}/48 h) of malathion.

After transfer of pesticide exposed prawns to toxicant free water, the nervous tissue AChE activity was shown a progressive recovery. Almost near normalcy was obtained on 7 days of recovery period or reclamation period in pesticide free water of prawns exposed to sublethal concentrations of phosphamidon and methylparathion (Tables 1 & 2). Prawns exposed to lethal concentrations failed to reach control values indicating inhibitory action of the pesticides to still persist. This trend is greater with methylparathion than with phosphamidon. However, at lethal exposures both pesticides were causing serious damage to the nervous tissue as observed through AChE inhibition. This must be due to higher concentrations of insecticides used or that the recovery period is rather short or both. The retention of these insecticides in tissues of lethally (LC_{50}/48 h) exposed prawns is suspected. Spontaneous recovery of organophosphate inhibited esterases was observed in vertebrates such as fish during malathion exposure (Coppage et al. 1975), and invertebrates such as the house fly, Musca domestica (Ahmad 1970), and the freshwater field crab, Oziotelphusa senex senex during sumithion exposure (Bhagyalakshmi & Ramamurthi 1980). Wilson (1951) reported an interesting observation, that eel cholinesterases inhibited by TEPP showed 45% recovery in 28 days. Coppage et al (1975) reported almost absolute recovery of brain AChE activity of fish poisoned by malathion in 40 days only. Complete (97%) recovery of AChE activity was noticed in Musca domestica exposed to malathion (Ahmad 1970). This process of recovery attributed to dephosphorylation of the OP compound and resynthesis of the

fresh enzyme. A similar kind of situation might also be operating in sublethally exposed prawns when subjected to reclamation period in pesticide free water. However, lethally exposed prawns might be requiring still longer periods of reclamation in pesticide free media to overcome the insecticide toxic stress. In addition to hydrolysis, biodegradation and rapid excretion of toxic chemicals on transfer of pesticide exposed prawns to pesticide free water may facilitate quick recovery. In view of these above reasons it is suggested that an interruption in the application of pesticides and/or reexposure of the animal to medium free of toxic chemicals may be used as a step to protect the animals of economic importance from deleterious effects.

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