Bull. Environ. Contam. Toxicol. (1988) 40:389–395

Environmental Contamination and Toxicology

Effects of Technical and Commercial Grade Phosphamidon on the Carbohydrate Metabolism in Selected Tissues of Penaeid Prawn, *Metapenaeus monoceros* (Fabricius)

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

Extensive and indiscriminate use of insecticides have contributed much to environmental pollution, leading to disturbances in the ecological balance (Holden 1972). Organophosphorous (OP) insecticides are widely used since they are biodegradable and seldom leave residues but for a short time (Bookhout & Monroe 1977). The organophosphates are neurotoxic and they inhibit acetylcholinesterase activity with subsequent disruption of nervous functions (Fest & Schmidt 1973; Coppage & Matthews 1974), thereby interfering with some of the vital physiological functions (Reddy et al. 1986). Among them, energy metabolism has a key role as the animal is forced to expend more energy to mitigate toxic stress. In the present investigation, the changes in carbohydrate metabolism in selected tissues of prawn, Metapenaeus monoceros were studied during induced toxicity of technical and commercial grade phosphamidon, an organophosphorous insecticide. Phosphamidon is toxic to several aquatic biota, including crustaceans (Reddy & Rao 1986). M. monoceros is considered to be a sensitive indicator of marine and estuarine pollution (Butler 1966) and also forms one of the commercially important fishery of India.

MATERIALS AND METHODS

Penaeid prawn, Metapenaeus monoceros (Fabricius) were collected from the Buckingham canal, near Kavali seacoast, Andhra Pradesh, India. Only intermolt uninjured prawns of the size 75 ± 5 mm and weight 2.5 ± 0.5 g were selected and acclimatized to the laboratory for 1 week at constant salinity of 15 ± 1 ppt, pH 7.1 ± 0.2 and temperature of 23 ± 2°C. They were fed ad lib diet of oil cake powder. The media in which they were placed was changed for every 24 h.

Technical grade (92% w/v) and commercial grade (85% w/v) phosphamidon (0,0-dimethyl-0-(1-methyl-2-chloro-2-diethyl-carbomoyl-vinyl) phosphate) obtained from CIBA-GIEGY, Bombay was used as the test chemical. A stock solution of 1000 ppm phosphamidon (1 mg/1 ml) was prepared in glass distilled water and appropriate working concentrations were prepared by dilution with seawater. The concentrations selected ranged from 0.1 to 2.4 ppm with a difference of 0.2 ppm. LC_{50} values were found to be 1.2 ppm for technical (TgP) and 0.9 ppm for commercial grade phosphamidon (CgP) for 48 h. Laboratory acclimatized prawns were exposed to a sublethal concentrations 0.4 ppm (technical grade) and 0.3 ppm (commercial grade) phosphamidon as described earlier (Reddy & Rao 1987) for 96 h in the present study.

Determination of total carbohydrates, glycogen and lactate levels :

10% Tissue homogenates were prepared in 10% trichloroacetic acid (TCA) and centrifuged at 750 g for 10 min. The supernatants were collected, and the total carbohydrates and glycogen contents were determined by the method of Carrol et al (1956). The lactate levels were determined by the method described by Barker and Summerson (1941).

Assay of aldolase activity :

5% Tissue homogenates were prepared in ice cold distilled water and centrifuged at 1000 g for 15 min. The supernatants were used for the assay of aldolase activity by the method described by Bruns and Bergmeyer (1965).

Assay of lactate (LDH), succinate (SDH) and malate (MDH) dehydrogenases :

5% Tissue homogenates were prepared in ice cold 0.25 M sucrose solution and centrifuged at 1000 g for 15 min to remove cell debris. A clear cell free extract was used for the assay of the dehydrogenases by the method described by Nachlas et al (1960).

The protein content in the enzyme source was estimated following 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 total carbohydrates, and glycogen levels decreased in the tissues of prawn on exposure to both technical and commercial grade phosphamidon. The decrease was more in the midgut gland compared to

gill and muscle tissues. Whereas, the lactate levels increased and the increase is more in the muscle followed by midgut gland and gill (Table 1). An increase in the aldolase activity and a decrease in LDH, SDH and MDH activity levels were observed in the tissues of prawn exposed to sublethal concentrations of technical and commercial grade phosphamidon (Table 2). The above trends are more pronounced in the midgut gland followed by gill and muscle, more so in commercial grade phosphamidon exposed prawns.

The potency of toxic stress induced by CgP is found to be more than that of TgP, suggesting greater effectiveness of CgP over TgP with respect to the certain parameters investigated. The observed greater potency of CgP is most probably due to the presence of emulsifier system in CgP, since the emulsifier may lead to a better penetration of phosphamidon. Whether with TgP or CgP, changes in the carbohydrate metabolism of prawn tissues were significant. The decrease in total carbohydrates and glycogen contents in the tissues of prawns exposed to CgP and TgP suggest its mobilization to meet the energy demand warranted by the toxic environment. This indicates that phosphamidon toxicity to induce glycogenolysis in tissues and also suggests that the tissue carbohydrate and glycogen reserves may first be broken down into glycosyl units via the glycogenolysis pathway (Reddy et al. 1985). Reddy (1986) reported synthesis and utilization of glycogen are altered during phosphamidon toxic stress. 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). Increase in the glycolytic enzymes like aldolase and the decrease in krebs cycle enzymes particularly of mitochondrial origin demonstrates shift in tissue respiratory metabolism towards anaerobic glycolysis. The occurrance of anaerobic glycolysis under stress conditions has been reported in invertebrates (Hochachka 1973). The organophosphorous and organochlorine insecticides are found to effect mitochondrial structure, function and integrity (Mivoglaw 1973) and also inhibits respiration (O'Brien 1960; Keister & Buck 1974). The prevalence of anaerobic glycolysis generally results in the decreased oxidative metabolism (Reddy 1986). The decreased activity levels of tricarboxylic acid (TCA) cycle enzymes, SDH and MDH could be attributed to the decrease in respiratory rate during organophosphate pesticide stress (O'Brien 1967). Most of

Table 1 : Levels of total carbohydrates, glycogen and lactate (mg/g wet wt. tissue) in tissues of control (C), technical grade phosphamidon (TgP) and commercial grade phosphamidon (CgP) exposed prawns (Each value is mean ± SD of 6 observations).

Midgut gland			Muscle			Gill		
C	TgP	CgP	C	TgP	CgP	C	TgP	CgP
			Total carbohydrates					
21.0 ±1.5	6.3 ±0.9 (-70)	5.4 ±0.8 (-74)	5.6 ±0.4	2.8 ±0.9 (-50)	2.2 ±0.7 (-60)	3.8 ±0.5	2.4 ±0.1 (-36)	2.1 ±0.2 (-44)
			Glycogen					
11.4 ±1.3	6.6 ±0.8 (-42)	5.7 ±0.8 (-50)	2.5 ±0.2	1.6 ±0.4 (-36)	1.4 ±0.4 (-44)	2.1 ±0.4	1.5 ±0.3 (-29)	1.3 ±0.3 (-38)
			Lactate					
0.20 ±0.02	0.36 ±0.03 (+80)	0.37 ±0.04 (+85)	0.99 ±0.08	1.91 ±0.11 (+92)	2.21 ±0.13 (+123)	0.89 ±0.08	1.44 ±0.18 (+61)	1.51 ±0.18 (+70)

Values in parantheses are per cent deviation over control. All values are statistically significant over control at $P < 0.001$.

Table 2 : Activity levels of aldolase (μ moles of fructose 1,6 diphosphate cleaved/mg protein/h) and selected dehydrogenases (μ moles of formazan formed/mg protein/h) in tissues of control (C) technical grade phosphamidon (TgP) and commercial grade phosphamidon (CgP) exposed prawns. (Each value is mean ± SD of 6 observations).

Midgut gland			Muscle			Gill		
C	TgP	CgP	C	TgP	CgP	C	TgP	CgP
				Aldolase				
5.44 ±0.39	10.15 ±0.48 (+87)	11.42 ±0.75 (+110)	2.42 ±0.17	3.14 ±0.21 (+29)	3.60 ±0.25 (+50)	4.88 ±0.31	7.34 ±0.32 (+50)	7.65 ±0.49 (+57)
				LDH				
0.291 ±0.011	0.192 ±0.015 (-56)	0.117 ±0.013 (-60)	0.057 ±0.003	0.021 ±0.003 (-63)	0.018 ±0.004 (-69)	0.129 ±0.021	0.076 ±0.004 (-41)	0.064 ±0.003 (-50)
				SDH				
0.831 ±0.022	0.344 ±0.028 (-59)	0.304 ±0.022 (-63)	0.064 ±0.009	0.041 ±0.005 (-36)	0.037 ±0.004 (-42)	0.409 ±0.021	0.212 ±0.024 (-48)	0.194 ±0.019 (-53)
				MDH				
0.293 ±0.028	0.154 ±0.018 (-47)	0.141 ±0.015 (-52)	0.072 ±0.012	0.051 ±0.004 (-30)	0.046 ±0.004 (-36)	0.205 ±0.022	0.130 ±0.014 (-37)	0.117 ±0.011 (-43)

Values in parantheses are per cent deviation over control. All values are statistically significant over control at $P < 0.001$.

the TCA cycle enzymes are mitochondrial in their origin, and any change in the mitochondrial integrity and structure by pesticide poisoning might influence the activity levels of these enzymes (Mivoglaw 1973). The present study concludes that either commercial or technical grade, phosphamidon decreases oxidative metabolism in the tissues of the prawn, M. monoceros, consequently switch over to anaerobic pathways as an adaptive measure to combat the induced pesticide toxicity and also to survive through the phosphamidon polluted habitats.

Acknowledgments : The authors thank CSIR, New Delhi for financial assistance and CIBA-GEIGY for providing technical grade phosphamidon and to Prof. K. Sasira Babu for his encouragement.

REFERENCES

Bailey NTJ (1965) Statistical methods in biology. The language book society and the English University Press Ltd. Great Britain.

Barker SB, Summerson WH (1941) The colorimetric determination of lactic acid in biological material. J Biol Chem 138 : 535-554.

Bookhout CC, Monroe RJ (1977) Physiological responses of marine biota to pollutants (eds) FJ Vernberg, A Calabrese, FP Thunberg and WB Vernberg. Academic Press New York. p 3-19.

Bruns FH, Bergmeyer HU (1965) Methods of enzymatic analysis (ed) HU Bergmeyer Academic Press New York. p 724.

Butler PA (1966) The problems of pesticides in estuaries. In a symposium on estuarine fisheries. Pub No.3. Am Fish Soc p 110-115.

Carrol NV, Longley RW, Row JH (1956) Glycogen determination in liver and muscle by use of anthrone reagent. J Biol Chem 220 : 583-593.

Coppage DL, Matthews E (1974) Short-term effects of organophosphate pesticides on cholinesterases of estuarine fishes and pink shrimp. Bull Environ Contam Toxicol 11 : 483-488.

Dezwaan A, Zandee DI (1972) The utilization of glycogen and accumulation of some intermediates during anaerobiosis in Mytilus edulis L. Comp Biochem Physiol 43 : 47-54.

Fest C, Schmidt KJ (1973) The chemistry of organophosphorous insecticides. Springer Verlag New York.

Hochachka PW (1973) Comparative animal physiology (ed) CL Prosser Saunders, Philadelphia.

Holden AV (1972) The effect of pesticides on life in fresh waters. Proc R Soc London B 180 : 383.

Keister M, Buck J (1974) The physiology of insecta (ed) M Rockstein Academic Press New York.
Lowry OH, Rosenbrough NT, Farr AL, Randall RJ (1951) Protein measurement with Folin phenol reagent. J Biol Chem 193 : 265-275.
Mivoglaw GR (1973) Rocz Panstam Zakl Hig 6 : 741.
Nachlas MM, Margulius SP, Seligmen AM (1960) A colorimetric method for the estimation of succinic dehydrogenase activity. J Biol Chem 235 : 499-503.
O'Brien RD (1960) Toxic phosphorous esters : Chemistry, metabolism and biological effects Academic Press, New York.
O'Brien RD (1967) Insecticides Academic Press New York.
Reddy MS (1986) Subacute toxic impact of phosphamidon on the carbohydrate metabolism of a penaeid prawn, Metapenaeus monoceros (Fabricius) - a tissue metabolic profile. Ph.D. thesis. S.V.University, Tirupati, India.
Reddy MS, Murthy BN, Venkateswarlu Y, Rao KVR (1985) Toxicity of insecticide phosphamidon on tissue carbohydrate catabolism of penaeid prawn, Penaeus indicus (H.Milne Edwards). Indian J Mar Sci 14 : 224-225.
Reddy MS, Rao KVR (1986) Phosphamidon induced alterations in the nitrogen metabolic profiles of penaeid prawn, Penaeus indicus during acute and chronic exposure. Proc Indian Acad Sci (Anim Sci) 95 : 533-537.
Reddy MS, Rao KVR (1987) Changes in carbohydrate metabolism in selected tissues of marine prawn, Penaeus indicus (H.Milne Edwards) exposed to phosphamidon. Nat Acad Sci Letters 10 : 39-43.
Reddy MS, Venkateswarlu Y, Murthy BN, Rao KVR (1986) Effect of phosphamidon on respiratory potentials in the penaeid prawn, Penaeus indicus (H.Milne Edwards) Nat Acad Sci Letters 9 : 93-95.

Received August 31, 1987; accepted November 19,1987.