Bull. Environ. Contam. Toxicol. (1989) 43:603-610

Bulletin of Environmental Contamination and Toxicology

In vivo Modification of Lipid Metabolism in Response to Phosphamidon, Methylparathion and Lindane Exposure in the Penaeid Prawn, *Metapenaeus monoceros*

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Chemical pollution by pesticides has been increasing in a large scale due to their vast usage for eradication of various pests and insects and to protect agricultural crops (Matsumura et al. 1972). Pesticides, even in very low concentrations have been reported to interfere with basal metabolism (Lal & Singh 1986; Reddy & Rao 1987). The consensus is that intoxication deranges intermediary metabolism (Reddy 1986). Most of the information regarding the effects of pesticides on crustaceans restricted to the survival test, growth rate assessment, fecundity and reproductive activity (Khorram & Knight 1977; Conklin & Ranga Rao 1978). Organochlorines and organophosphates are known to alter the lipid metabolism in non-target organisms. Except for the reports of Murthy and Devi (1982) in Channa punctatus and Madhu (1983) in Tilapia mossambica, apparently no study on lipid metabolism in response to pesticides is available. Practically little attention has been made towards the understanding of comprehensive account of lipid metabolism in response to pesticides. Since lipids undergo rapid breakdown, resynthesis and interconversion in response to different stimuli, it is essential that various lipid fractions in different tissues be considered simultaneously to provide a clear picture of lipid metabolism in response to pesticides. Therefore, in the present investigation an attempt has been made to determine the impact of an organophosphorous-phosphamidon and methylparathion and an organochlorine-lindane, on the lipids and its derivatives in the selected tissues of the penaeid prawn, Metapenaeus monoceros. This prawn is considered to be a sensitive indicator of marine and

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estuarine pollution (Butler 1966) and also forms one of the commercially important fisheries of India.

MATERIALS AND METHODS

Penaeid prawn, *Metapenaeus monoceros* (Fabricius) with an average weight of 2.5 ± 0.5 g and length of 75 ± 5 mm were collected from the Buckingham Canal near Kavali seacoast, Andhra Pradesh, India. Prawns were acclimatized to laboratory conditions for one week prior to experimentation under 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 (Fish and Prawn feed suppliers, Madras) and fresh prawn muscle pieces. The media in which they were placed changed for every 24 h.

Technical grade phosphamidon (92% w/v; 0,0-dimethyl-0-(1-methyl-2-chloro-2-diethyl-carbomoyl-vinyl) phosphate; CIBA-GEIGY, Bombay), methylparathion (80% w/w; 0-0-dimethyl, 0-4-nitrophenyl thiophosphate; Bharat Pulverising Mills Pvt. Ltd., Bombay) and lindane (99.8% w/v; gamma-Hexachlorocyclohexane; Pesticides India, Udaipur) 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. LC_{50} values were found to be 1.2 ppm for phosphamidon, 0.12 ppm for methylparathion and 0.005 ppm for lindane to the intermolt prawn for 48 h exposure. Laboratory acclimatized prawns were exposed to sublethal concentrations of phosphamidon (0.4 ppm), methylparathion (0.04 ppm) and lindane (0.0015 ppm) for 72 h in the present study and aerated twice a day. The tissues like midgut gland, muscle and gill were isolated from control, phosphamidon exposed (PE), methylparathion exposed (ME) and lindane exposed (LE), prawns and used for biochemical analysis.

The lipids were isolated by repeated extraction with a mixture of chloroform and methanol (2:1 v/v). Their amounts were determined gravimetrically according to the procedure of Folch et al (1957). The free fatty acids and total cholesterol were estimated by the method of Natelson (1971). Lipase activity levels was estimated as per the method outlined by Colowick and Kaplan (1965). Glycerol by the method of Lambert and Neish (1950). The protein content was estimated by Lowry et al (1951) using bovine serum albumin as standard. The statistical correlations between control and experimental values were conducted using student 't' test (Bailey 1965).

RESULTS AND DISCUSSION

In all the three control tissues studied, the levels of total lipids remained higher in midgut gland, when compared to muscle and gill tissues. Midgut gland of crustaceans is the principle site of lipid accumulation (Chang & O'Connor 1983). There is a general decrease in the total lipids and glycerol levels in prawns exposed to phosphamidon, methyl-parathion and lindane (Table 1), while the free fatty acids and total cholesterol showed a significant increase in all the PE, ME and LE prawn tissues (Table 2). This suggests the mobilization of energy-rich lipids for production of energy during toxic stress caused by pesticides. Generally under any type of stress condition the animals are bound to spend extra energy to overcome the stress through the oxidation of either carbohydrates or proteins or lipid constituents (Hochachka & Somero 1973). The decrease in total lipids correlates with the increased activity levels of lipase, the enzyme responsible for the breakdown of lipids into free fatty acids and glycerol. The elevated levels of lipase activity agrees with the enhanced tissue free fatty acid levels, since the later forms one of the products of lipid hydrolysis (West et al. 1967). The mobilization of lipid reserves testifies the imposition of high energy demand under pesticide toxicity. Since lipids form the rich energy reserves whose caloric value was reported to be twice than that of an equivalent weight of carbohydrates or proteins (Oser 1979), the mobilization of lipid reserves testifies to the imposition of high energy demands.

Sterols are necessary for membrane synthesis and for the synthesis of many other metabolic compounds. In the present study, the increase in quantitative levels of cholesterol might be due to the increased diversion of acetyl-CoA to acetoacetate formation for cholesterol biosynthesis. Increased levels of acetoacetate and β-hydroxybutyrate have been shown in the insecticide administered animals (Domschke et al. 1971). Since TCA cycle enzymes are inhibited during insecticide stress (Reddy & Rao 1987), the accumulation of acetyl-CoA is likely to cause that diversion. The acetyl-CoA, produced through augmented β oxidation and glycolysis (Reddy & Rao 1988) will produce more ketone bodies, particularly acetoacetate, which then act as precursors for the synthesis of cholesterol. The metabolic orientation of lipids and its derivatives can be represented in figure 1. All these pathways are well established in crustacean species including prawns (Chang &

Table 1. Levels of total lipids, lipase and glycerol in the selected tissues of control (C), phosphamidon exposed (PE), methylparathion exposed (ME) and lindane exposed (LE) prawns.

Midgut gland				Muscle				Gill			
C	PE	ME	LE	C	PE	ME	LE	C	PE	ME	LE
Total lipids (mg/g wet weight of tissue)											
20.18 ±1.15	13.43 ±0.84 (-33)	11.49 ±0.72 (-41)	10.24 ±0.62 (-49)	14.94 ±1.65	11.08 ±0.72 (-26)	9.72 ±0.64 (-35)	8.45 ±0.49 (-43)	4.15 ±0.28	3.41 ±0.21 (-18)	2.84 ±0.13 (-32)	2.59 ±0.15 (-38)
Lipase (units/mg protein/h)											
2.14 ±0.19	2.93 ±0.19 (+37)	3.27 ±0.21 (+53)	3.85 ±0.24 (+80)	1.03 ±0.15	1.38 ±0.17 (+34)	1.54 ±0.17 (+50)	1.79 ±0.18 (+74)	0.32 ±0.05	0.41 ±0.06 (+28)	0.45 ±0.07 (+41)	0.53 ±0.08 (+66)
Glycerol (μ moles/g wet weight of tissue)											
6.43 ±0.65	3.84 ±0.43 (-40)	3.39 ±0.40 (-47)	3.04 ±0.35 (-53)	3.85 ±0.34	2.80 ±0.28 (-27)	2.14 ±0.20 (-44)	1.94 ±0.15 (-50)	2.01 ±0.20	1.31 ±0.17 (-35)	1.24 ±0.15 (-38)	1.05 ±0.12 (-48)

Values are mean ± S.D of 6 individuals. Values in parentheses are % change over their respective controls. All experimental values are statistically significant at $P < 0.001$ when compared to their respective controls.

Table 2. Levels of free fatty acids and total cholesterol in the selected tissues of control (C), phosphamidon exposed (PE), methylparathion exposed (ME) and lindane exposed (LE) prawns.

Midgut gland				Muscle				Gill			
C	PE	ME	LE	C	PE	ME	LE	C	PE	ME	LE
Free fatty acids (mg/g wet weight of tissue)											
6.74 ±0.65	8.49 ±0.71 (+26)	10.15 ±0.74 (+51)	11.12 ±0.81 (+65)	2.58 ±0.21	3.28 ±0.24 (+27)	3.98 ±0.29 (+54)	4.15 ±0.28 (+61)	2.85 ±0.29	3.58 ±0.32 (+26)	4.08 ±0.35 (+43)	4.43 ±0.38 (+55)
Total cholesterol (mg/g wet weight of tissue)											
1.72 ±0.12	2.08 ±0.15 (+21)	2.45 ±0.24 (+42)	2.70 ±0.25 (+57)	0.74 ±0.05	0.89 ±0.07 (+20)	0.98 ±0.08 (+32)	1.09 ±0.08 (+47)	0.35 ±0.08	0.46 ±0.07 (+31)	0.48 ±0.08 (+37)	0.59 ±0.06 (+69)

All values are mean ± S.D of 6 individual observations. Values in parentheses are % change over their respective controls. All experimental values are statistically significant at $P < 0.001$ when compared to their respective controls.

O'Connor 1983).

From the results, it is evident that the effect produced by lindane was always greater than that by phosphamidon and methylparathion, indicating the higher toxicity of organochlorine insecticides than organophosphorous compounds, which might be attributed to the possibility of more specific action and quicker penetration of organochlorines than organophosphates. Similar type of findings are also available on organochlorine compound penetration through biological membrane and causes derangement in the lipid metabolism (Madhu 1983; Lal & Singh 1986; Singh & Singh 1980). Among the tissues selected the changes were more pronounced in the midgut gland, when compared to muscle and gill tissues. Since the midgut gland which simulates in function with the liver of vertebrates, is the center of lipid metabolism, the higher levels of free fatty acids can be expected in this tissue than in other tissues as evidenced in the present study. Since metabolically active tissues have been selected, it can be suggested that the free fatty acids and glycerol formed by the breakdown of lipids might be diverted to yield energy to mitigate the toxic stress due to sublethal concentrations of phosphamidon, methylparathion and lindane.

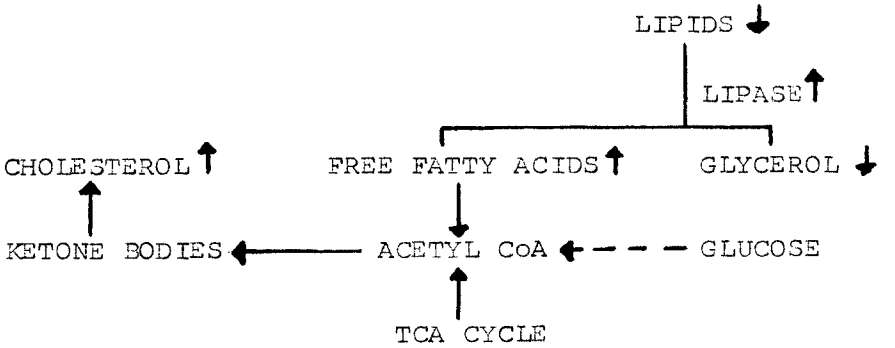

Figure 1. Metabolic orientation of lipids and its derivatives during pesticide treatment. ↑ indicates increased and ↓ indicates decreased levels during pesticide treatment.

The target action of the organophosphorous (phosphamidon and methylparathion) and organochlorine (lindane) insecticides are the enzymes acetylcholinesterase and adenosinetriphosphatase, respectively. Thus, the changes in the lipid metabolism observed in the present study represents a secondary manifestation.

Acknowledgments. Dr.MSR is grateful to the Council of Scientific and Industrial Research, New Delhi, for providing financial support in the form of Research Associate. We also thank 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.

Butler PA (1966) The problems of pesticides in estuaries. In a symposium on estuarine fisheries. Pub No.3. Am Fish Soc p110-115.

Chang ES, O'Connor JD (1983) Metabolism and transport of carbohydrates and lipids. In:The Biology of Crustacea. Vol.5. Internal anatomy and physiological regulation. (ed) LH Mantel. Academic Press New York. p263-287.

Colowick SP, Kaplan NO (1965) Lipase titrimetric method using water soluble substrates. In: Methods in Enzymatic Analysis. Vol.1 Preparation and assay of enzymes (eds) SP Colowick and NO Kaplan. Academic Press New York. p630-631.

Conklin PJ, Ranga Rao K (1978) Toxicity of sodium pentachlorophenate to the grass shrimp, Palaemonetes pugio, in relation to the molt cycle. In: Pentachlorophenol:Chemistry, Pharmacology and Environmental Toxicology (ed) KR Rao. Plenum Press New York. p181-192.

Domschke S, Domschke W, Classen M (1971) Zum mechanismus der Leberzellschadigung durch Alkylphosphate. Naturwissenchaften 58:575.

Doudoroff P. Anderson BG, Burdick GE, Galtsoff PS, Hart WB, Patrick R, Strong ER, Surber EW, Van Horn WM (1951) Bioassay methods for the evaluation of acute toxicity of industrial wastes to fish. Sewage Industr Wastes 23:1380-1397.

Folch J, Less M, Stanley GHS (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497-509.

Hochachka PW, Somero GN (1973) Strategies of biochemical adaptations. WB Saunders Company Philadelphia.

Khorram S, Knight AW (1977) The toxicity of Kelthane to the grass shrimp, Crongon franciscorum. Bull Environ Contam Toxicol 18:674-682.

Lal B, Singh TP (1986) In vivo modification of fatty acids and glycerides metabolism in response to 1,2,3,4,5,6-hexachlorocyclohexane and cythion exposure in the catfish, Clarias batrachus. Ecotoxicol Environmental Safety 11:295-307.

Lambert M, Neish AC (1950) Rapid method for estimation of glycerol in fermentation solution. Can J Research 28:88-89.

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with Folin phenol reagent. J. Biol Chem 193:265-275.

Matsumura MM, Margulius SP, Saligman AM (1972) Environmental Toxicology of Pesticides. Academic Press New York. p30.

Madhu Ch (1983) Toxic potentials of lindane and lipid metabolism, haematological and histopathological changes in selected tissues of fish, Tilapia mossambica (Peters) at different exposure periods. Ph.D. Thesis. S.V.University Tirupati India.

Murthy AS, Devi AP (1982) The effect of endosulfan and its isomers on tissue protein glycogen and lipids in the fish, Channa punctatus. Pestic Biochem Physiol 17:280-286.

Natelson S (1971) Techniques in Clinical Chemistry. 3rd ed. Thomas, Springfield Illinois.

Oser BL (1979) Hawk's Physiological Chemistry. 14th ed. McGraw-Hill, 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, 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, Rao KVR (1988) Effects of technical and commercial grade phosphamidon on the carbohydrate metabolism in selected tissues of penaeid prawn, Metapenaeus monoceros (Fabricius). Bull Environ Contam Toxicol 40:389-395.

Singh H, Singh TP (1980) Short-term effect of two pesticides on lipid and cholesterol content of liver, ovary and blood serum during the prespawning phase in the freshwater teleost, Heteropneustus fossilis (Bloch). Environ Pollut 22:85-90.

West ES, Todd WR, Mason HS, Van Brucgen JT (1967) Text book of Biochemistry. 4th ed. MacMillan Co., New York. p342-345

Received August 15, 1988; accepted April 3, 1989.