Bull. Environ. Contam. Toxicol. (1991) 47:918–924

Bulletin of Environmental Contamination and Toxicology

Acute and Chronic Toxicity of Endosulfan to Crab: Effect on Lipid Metabolism

G. Md. Rafi, T. Srinivas, S. J. Reddy, D. C. Reddy,* and R. Ramamurthi

Pesticide and Industrial Toxicology Centre, Department of Zoology, Sri Venkateswara University, Tirupati—517 502, India

The endosulfan (Thiodan (R) 6,7,8,9,10 -hexachloro 1,5,5a,6,9a-hexahydro-6, 9 - methano 2,4,3 - benzodioxathiepine-3 oxide), a broad spectrum nonsystemic organochlorine insecticide is widely used in India due to ban on endrin and the decline in the use of other organochlorine pesticides due to their longer persistence and higher toxicity to mammals. Endosulfan is toxic to fish and its toxic effects have been studied in several freshwater fish (Rao and Murthy 1982; Rangaswamy 1985). However, information regarding toxicity of endosulfan to many freshwater invertebrates is fragmentary. Though few reports are available on the toxic effect of endosulfan on carbohydrate and protein metabolisms of freshwater field crab, Oziotelphusa senex senex (Rajeswari 1989), another nontarget organism of aquatic ecosystem. The work on lipid metabolism under organochlorine insecticide (OCI) stress is scant. The OCI tend to accumulate in the lipid rich tissues of the biosystem due to their lipophilic nature (Agarwal 1981; Bhakthavasthalam and Reddy 1981). The changes in lipid profiles under OCI stress reported to cause profound changes in the metabolism and physiology of animals (Bhakthavasthalam and Reddy 1981; Madhu 1983; Sanyal et al. 1986). Therefore, this paper presents the effects of endosulfan on lipid metabolism in O. senex senex.

MATERIALS AND METHODS

Freshwater crabs, O. senex senex were collected from the local paddy fields under ground (well) water irrigation. Crabs in the weight range of 32±1 g were acclimated to laboratory conditions for about a week during which they were fed with ad libitum minced meat. The water used in laboratory acclimation of crabs was unchlorinated ground water pumped from a deep well within the campus.

---

* Send reprint requests to D.C. Reddy at above address.

Feeding was stopped 1 d before commencing the experiment to overcome differences, if any, due to differential feeding. Sublethal (6.2 mg/L, i.e., 1/3 of 96h LC_{50}) concentration of technical grade endosulfan (99%) dissolved in acetone was chosen to study acute and chronic effects. Only uninjured intermolt (stage C_4) crabs were separated from laboratory crab tanks and divided into four batches of six each and were exposed for 2,4,8 and 15 d to sublethal concentration of endosulfan, respectively. Acetone was added to the medium containing control animals in an amount equal to the largest aliquot (1.6 ml/L) of stock used in the experimental batches.

The following lipid profiles were estimated in the hepatopancreas (HP) and the claw-muscle (CLM) of the crab, O. senex senex. Total lipids were estimated according to Folch et al. 1957. The isolated tissues were weighed and homogenized in 2:1 chloroform-methanol mixture. The supernatant after centrifugation was separated and transferred into corning tube (weight was previously determined). The contents were evaporated at 60-65° C. The residue, left behind was weighed and the difference between initial, final weight of tube represents the amount of total lipids. Lipase activity was measured by Bier (1957) method using p-nitro phenyl acetate as substrate. Free fatty acids were estimated after extraction of lipids with chloroform-methanol mixture. The extract was dissolved in ethanol and titrated with potassium hydroxide according Natelson (1971). Glycerol content was estimated spectrophotometrically by developing color with chromotropic acid according Korn (1959). For measuring iodine number, the lipids after extracting with chloroform-methanol mixture were titrated with sodium thiosulphate according to Hanus iodobromide method as described by Winton and Winton (1947). Phospholipids were estimated spectrophotometrically by developing color with ANSA (1-amino-2-naphthol-4-sulphonic acid) after digesting tissue residue with perchloric acid according to Zilversmit and Davis (1950). Cholesterol content was estimated by Liebermann-Buchard reaction as described by Natelson (1971) spectrophotometrically by using acetic anhydride mixture. The significance of the data was assessed through students "t" test.

RESULTS AND DISCUSSION

The results presented in Table 1 show a significant and gradual increase in total lipid (TL) content of the HP and CLM of crabs as a function of exposure period. This may be due to the increased lipogenesis thereby causing an increased total lipid content in the tissues during endosulfan toxicity. Organochlorine insecticides alter lipid metabolism because of their affinity towards

lipids (Agarwal 1981; Bhakthavasthalam and Reddy 1981; Madhu 1983). The increased lipid content in hepatopancreas might be either channeled to derive energy due to decline in the levels of glucose and glycogen (Rajeswari 1989) or utilized in the synthesis of structural components of cells under endosulfan toxic stress. The percent increase in TL content is high at chronic than at acute exposure periods. It may be attributed to the accumulation of toxic molecules more at chronic periods, favoring lipogenesis, possibly to meet cellular, structural and functional necessities (Surendranath 1989).

Lipases are heterogenous group of hydrolytic enzymes, that aid in the hydrolysis of fats and lipids. Lipids serve as an alternate source of energy in crustaceans, particulary during stress (Gilbert and O'Connor 1970; Chang and O'Connor 1983). The increase in the lipase activity at all exposure periods suggests breakdown of lipids possibly to meet extra energy demands. Further the increased tissue lipase activity coincides with an increase in the free fatty acid content. Lipase activity was found to be more at chronic exposure periods suggesting higher requirement of lipids which are degraded to produce more free fatty acids, to meet the extra energy demands.

The free fatty acid (FFA) content increased and glycerol (GL) content decreased as a function of exposure period (Table 1). The lipase acts on triglycerides to form FFA and GL. FFA's contribute energy through β-oxidation by activating acetyl CoA in crustaceans (Gilbert and O'Conner 1970). The increased FFA's are possibly utilized for lipogenesis with glycerol moiety through esterification to meet energy demands under stress condition because of shortage of carbohydrates. The lipid turnover during stress condition suggests that both lipogenesis and lipolysis are occurring simultaneously possibly to maintain metabolic homeostasis. The decreased GL content may be due to their ready incorporation into neutral lipids or their diversion for the formation of triose phosphates and ketoacids to be used in energy production during toxic stress.

Iodine number indicates the degree of fatty acid unsaturation. High iodine number indicates high amounts of unsaturated fatty acids (Martin et al. 1981). Iodine number increased in the tissues during exposure periods (Table 2). This may be considered as an index of lipogenesis because of their high reactivity.

Phospholipids are predominant circulating lipids in crustaceans (Gilbert and O'Conner 1970, Chang and O'Conner 1983). They serve as energy resources and have

Table 1. Variations in the total lipids (mg/g wet weight), lipase activity (μmoles of p-nitrophenol formed/mg protein/h), free fatty acids (mg/g wet weight) and glycerol (mg/g wet weight) in the hepatopancreas (HP) and claw-muscle (CLM) of freshwater crab, O. senex senex exposed to endosulfan.

Parameter	Control	Exposure periods (days)			
		2	4	8	15
HP Total lipids	196.24 ±18.73	223.46 ±19.74	238.76 ±24.06	279.43 ±31.19	312.65 ±34.78
% Change		13.87	21.67	42.39	59.32
CLM Total lipids	46.83 ±4.47	49.98 ±4.96	53.62 ±5.82	60.59 ±6.98	67.37 ±8.85
% Change		6.73	14.50	29.17	43.86
HP Lipase activity	8.172 ±0.89	8.846 ±0.95	9.511 ±1.35	10.640 ±1.47	13.389 ±2.05
% Change		8.25	16.39	30.20	66.84
CLM Lipase activity	3.627 ±0.38	3.802 ±0.41	3.944 ±0.41	4.284 ±0.49	4.885 ±0.66
% Change		4.82	8.74	18.11	34.68
HP Free fatty acids	21.19 ±2.85	24.89 ±3.93	26.33 ±3.72	31.23 ±4.86	37.40 ±6.39
% Change		17.46	24.26	47.38	76.50
CLM Free fatty acids	7.34 ±0.86	8.16 ±0.97	8.73 ±0.99	9.89 ±1.52	11.27 ±1.86
% Change		11.17	18.94	34.74	53.54
HP Glycerol	0.385 ±0.039	0.336 ±0.039	0.295 ±0.037	0.230 ±0.031	0.131 ±0.026
% Change		-12.73	-23.38	-40.26	-65.97
CLM Glycerol	0.096 ±0.014	0.088 ±0.013	0.075 ±0.01	0.064 ±0.009	0.047 ±0.007
% Change		-8.33	-21.88	-33.33	-51.04

HP = Hepatopancreas CLM = Claw-muscle
Values are mean ±SD of six individual observations.
All differences are significant at 0.05 level.

important role in regulation of permeability properties and serve for the synthesis of structural elements (Madhu 1983; Sanyal et al. 1986). Cholesterol is a predominant sterol in crustaceans (Surendranath 1989) and is used for membrane synthesis and for biosynthesis of several compounds. The phospholipid (PL) content decreased significantly and total cholesterol (TCL)

Table 2. Variations in the iodine number (g I absorbed 100 g lipid), phospholipids (mg/g wet weight), total cholesterol (mg/g wet weight) in the hepatopancreas (HP) and claw-muscle (CLM) of freshwater crab, O. senex senex exposed to endosulfan.

Parameter	Control	Exposure periods (days)			
		2	4	8	15
HP Iodine number	74.68 ±8.17	84.23 ±9.34	93.99 ±13.58	104.21 ±15.64	118.22 ±17.94
% Change		12.79	25.86	39.54	58.47
CLM Iodine number	113.47 ±11.59	124.32 ±13.07	132.12 ±13.76	139.74 ±16.58	158.42 ±19.61
% Change		9.56	16.44	23.15	39.61
HP Phospho-lipids	19.34 ±2.35	16.20 ±2.04	14.66 ±1.88	11.08 ±1.65	7.04 ±1.03
% Change		-16.24	-24.20	-42.71	-63.60
CLM Phopho-lipids	8.79 ±0.94	7.79 ±0.88	7.08 ±0.85	6.09 ±0.81	4.68 ±0.72
% Change		-11.38	-19.45	-30.72	-46.76
HP Total cholesterol	1.372 ±0.112	1.389 ±0.144	1.426 ±0.125	1.461 ±0.143	1.586 ±0.187
% Change		4.67	7.46	10.69	19.52
CLM Total cholesterol	0.435 ±0.042	0.451 ±0.041	0.458 ±0.041	0.471 ±0.045	0.494 ±0.054
% Change		3.68	5.29	8.28	13.56

HP = Hepatopancreas CLM = Claw-muscle
Values are means ±SD of six individual observations.
All differences are significant at 0.05 level.

content increased as a function of exposure period (Table 2). The decrease in PL content may be due to accumulation of toxic molecules, which effects structural, physiological and metabolic functions. Therefore to counteract toxic impact more energy may be derived by utilizing PL content in the tissues. In contrast, the TCL content increased in tissues suggesting that these compounds may help in the synthesis of cellular compounds and to detoxify the toxic molecules to reduce toxic impact (Martin et al. 1981; Surendranath 1989).

Since hepatopancreas is considered to be metabolic center for synthesis, transport and storage of lipids, it experienced maximum changes in lipid profiles during stress condition compared to claw-muscle, whereas in

claw-muscle interaction with toxic molecules may be less. Thus the alterations in lipid profiles as a function of endosulfan toxicity is to tide over the prevailed energy crisis.

REFERENCES

Agarwal N (1981) Effect of DDT on Rhesus monkeys: The effect on lipid metabolism. Second congress of Federation of Asian and Oceanic Biochemists, Bangalore

Bhakthavasthalam R, Reddy YS (1981) Lipid kinetics in relation to the toxicity of three pesticides in the climbing perch, Anabus testudines (Bloch). Proc Ins Nat Sci Aca B 47(5): 670-676

Beir M (1957) Lipases. In:Colowick SD, Kaplan NO(ed) Methods in enzymology, vol 1. Academic press, New York, pp631-634

Chang ES, O'Connor JD (1983) Metabolism and transport of carbohydrates and lipids. In:Mantel LH(ed) The Biology of Crustaceans, vol V. Academic press, New York, pp263-287

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

Gilbert LI, O'Connor JD (1970) Lipid metabolism in arthropods. In:Florkin M, Scheer BT(ed) Chemical Zoology, vol 5. Academic press, New York, pp229-254

Korn AD (1959) Determination of glycerol. In:David Glick (ed) Methods of biochemical analysis, vol VII. Interscience publishers Inc limited, London, p179

Madhu CH (1983) Toxic potentials of lindane on lipid metabolism in fish, Tilapia mossambica. Ph D thesis, S V University, Tirupati, India

Martin DW, Mayes PA, Rodwell VW (1981) Metabolism of lipids. In:Harper's review of biochemistry. 18th edn. Lange Medical publications, California

Natelson S (1971) Techniques of clinical chemistry, 3rd edn. Charles C. Thomas publishers, Springfield, Illinois, USA

Rajeswari K (1989) Effect of low concentrations of enodosulfan on certain metabolic aspects of crab. Ph D thesis, S V University, Tirupati, India

Rangaswamy CP (1985) Impact of endosulfan toxicity on some physiological properties and aspects of energy metabolism of a fish, Tilapia mossambica. Ph D thesis, S V University, Tirupati, India

Rao DMR, Devi AP, Murthy AS (1982) Toxicity and metabolism of endosulfan in three freshwater catfishes. Environ Pollut (Ser A) 27:223-231

Sanyal S, Agarwal N, Subramanyam D (1986) effect of acute sublethal and chronic administration of DDT (Chlophenotane) on brain lipid metabolism of Rhesus monkeys. Toxicol Lett 34(1):47-54

Surendranath P (1989) Studies on toxic impact of

kelthane on lipid metabolism of the panaeid prawn, Metapenaeus monoceros (Fabricius) under sublethal acute and chronic exposures. Ph D thesis, S V University, Tirupati, India
Winton AL, Winton KB (1947) Analysis of foods. John Wiley and Sons Inc, Chapman and Hall Ltd, London
Zilversmit DB, Davis AK (1950) Micro determination of plasma phospholipids. J Lab Clin Med 35:60

Received June 4, 1990; accepted May 9, 1991.