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Changes in Selected Biochemical Parameters in the Kidney and Blood of the Fish, *Tilapia mossambica* (Peters), Exposed to Heptachlor

V. Radhaiah, M. Girija, and K. Jayantha Rao[1]

Toxicology Division, Department of Zoology, Sri Venkateswara University, Tirupati-517 502 (A.P.), India

Pesticides used in pest control programs seem to produce many physiological and biochemical changes in fresh water organisms by influencing the activities of several enzymes. Alterations in the chemical composition of the natural aquatic environment usually effect behavioral and physiological systems of the inhabitants, particularly of the fish (O'Brien 1967). Although some data are available on the effects of different pesticides on the biochemical aspects of fish gill (Girija 1984), data on heptachlor toxicity on T. mossambica kidney are lacking. Therefore, an attempt has been made to observe certain biochemical parameters of fish, T. mossambica under heptachlor intoxication. Investigation of this nature is useful in understanding the orientation of biochemical changes during sublethal toxicity to ascertain the degree of intensity of the toxicity of heptachlor on the kidney.

MATERIALS AND METHODS

Freshwater fish, Tilapia mossambica (Peters), 15±2 g were collected from the ponds near Tirupati. They were kept in glass aquaria with continuously flowing dechlorinated water to acclimatize them to laboratory conditions (27±2°C, pH. 7.0 ± 0.2, hardness 38 ppm of $CaCo_3$ and light period of 12h). They were fed ad libitum on groundnut cakes daily. The bioassays were conducted in static waters by adopting the procedure of Doudoroff et al (1951) and the LC50 value (0.15mg/L) for 48 h with heptachlor (73% active ingredient) was determined by the method of probit analysis (Finney 1964). One fifth of the LC50 (0.03mg/L) was taken as the sublethal concentration, and the fish were exposed for 5, 10 and 15 days. The control fish were maintained under identical conditions without pesticide in the medium. After the stipulated period, the blood was collected and the kidney was isolated both from control and

[1]To whom correspondence should be addressed

experimental fish. 5% homogenates were prepared for proteins, carbohydrates, amino acids in 10% trichloroacetic acid (TCA), in methanol : chloroform mixture (2:1, v/v) for lipids, in 0.25M cold sucrose solution for SDH, LDH and GDH, centrifuged at 1000 g for 10 min at 4°C to remove cell debris. The clear cell-free exctract was used for all assys.

Protein content was determined with Folin's reagent (Lowry et al 1981) using bovine serum albumin as standard. Anthrone reagent was used to estimate the carbohydrates (Carrol et al 1956) using glucose as standard. The amino acid content was determined by using ninhydrin reagent (Moore and Stein 1954) and tyrosine was used as standard. The lipids were estimated by gravimetric method (Folch et al 1957). The serum non-protein nitrogen (NPN) was estimated colorimetrically as described by Natelson (1972a) using ammonium sulphate as standard. Serum proteins were precipitated with 10% TCA and the protein free supernatant was processed for NPN by the addition of digestion mixture. The blood urea was estimated with diacetyl monoxime procedure described by Natelson (1972b) using urea as standard and the serum creatine was estimated by the reaction with alkaline picrate as described by Folin and Wu (1979) using creatine as standard.

Succinate dehydrogenase (SDH-E.C.1.3.99.1) activity was determined by the amount of formazan formed after 30 min at 37°C incubation by the method of Nachlas et al (1960). Incubation mixture contains 100 µmoles of disodium hydrogen phosphate buffer (pH 7.4), 4 µmoles of INT ((2-p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl) tetrazolium chloride), 40 µmoles of sodium succinate (substrate) and 0.1 ml of supernatant. Lactate dehydrogenase (LDH-E.C.1.1.1.27) activity was estimated by the method of Srikantan and Krishnamurthy (1955). Incubation steps followed are the same as described for SDH, using sodium lactate (40 µmoles) as substrate and 0.1 ml of NAD. Glutamate dehydrogenase (GDH-E.C.1.4.1.3) was estimated by the method of Lee and Lardy (1965). Incubation steps followed are the same as described for SDH, using sodium glutamate (100 µmoles) as substrate and 0.1 ml of NAD. Standard graphs were prepared with formazan, since the activity of enzymes namely SDH, LDH and GDH is dependant upon the formation of formazan. Significance of the differences was assessed through students "t" test (Pillai and Sinha 1968).

RESULTS AND DISCUSSION

Decreased carbohydrate content in heptachlor intoxicated fish (Table 1) may be due to the rapid utilization of carbohydrates by the tissue, possibly to overcome the

pesticide induced stress. Dezwaan and Zandee (1972) reported that anoxic or hypoxic conditions were known to elevate carbohydrate consumption. Decreased protein content (Table 1) in the kidney could possibly be due to protein breakdown leading to increased amino acid pool of tissue. It is also reported that the decrease in protein moiety suggests damage to hepatic tissue and an intensive proteolysis (Rao 1984), resulting in increased amount of free amino acids to be fed into

Table 1. Levels of carbohydrates, proteins, amino acids and lipids in the kidneys of controls and in fish exposed to 0.03 mg/L heptachlor.

Component	Control in days	Exposed fish in days		
	15	5	10	15
Carbohydrates (mg/g wet weight)	3.23 ±0.16	2.04* ±0.08	1.73* ±0.11	1.72* ±0.06
Proteins (mg/g wet weight)	79.2 ±2.19	64.5* ±1.77	58.5* ±6.22	42.0* ±2.32
Amino acids (mg/ g wet weight)	1.50 ±0.04	1.83* ±0.08	1.95* ±0.09	2.97* ±0.25
Lipids (mg/g dry weight)	162 ±26.5	178** ±29.5	182** ±14.2	205* ±39.8

Each value represents the mean of six individual observations. Mean ± S.D., *P value < 0.001; **NS = Not significant.

TCA cycle as keto acids. Increased levels of total lipid content (Table 1) suggests the lipogenesis under pesticidal intoxication. The observed increase of amino acids in the present investigation may be due to decreased utilization of amino acids from other sources like glucose and fatty acids and constant breakdown of proteins, as is also noticed in different organisms exposed to various pesticides (Girija 1984; Rao 1984).

Increased activity of LDH in 10 and 15 days (Table 2) indicates that the fish is favoring anaerobic respiration to meet the energy demands when aerobic oxidation was lowered. This also suggests the forward reaction of LDH, namely pyruvate to lactate, may be more operative during exposure to heptachlor. Consistent increase in GDH activity has been observed under heptachlor impact. This enzyme catalyses the key

reactions, which provide substrates for either protein synthesis (glutamate) or carbohydrate metabolism (α-keto glutamate) of the fish kidney. Since SDH is an important enzyme in TCA cycle, it is logical to assume that with the inhibition of SDH activity, the metabolic pathway might have shifted towards anaerobic side to meet the increased energy demands during pollution stress.

Table 2 Activity levels of SDH, LDH and GDH in the kidneys of controls and in fish exposed to 0.03 mg/L heptachlor.

Component	Control in days	Exposed fish in days		
	15	5	10	15
SDH (µmol formazan formed/ mg/protein/h)	0.78 ±0.05	0.86* ±0.03	0.65* ±0.08	0.59* ±0.04
LDH (µmol formazan formed/ mg/protein/h)	1.85 ±0.14	1.58* ±0.07	1.92** ±0.28	2.19* ±0.34
GDH (µmol formazan formed/ mg/protein/h)	0.23 ±0.03	0.29* ±0.03	0.31* ±0.02	0.35* ±0.02

Each value represents the mean of six individual observations. Mean ± S.D., *P value < 0.001; **NS = Not significant.

Table 3 Levels of urea, serum non-protein nitrogen and creatine in the blood of controls and in fish exposed to 0.03 mg/L heptachlor.

Component	Control in days	Exposed fish in days		
	15	5	10	15
Urea (mg/100 mL)	718 ±65.2	815** ±27.4	835* ±21.5	967** ±15.9
Non-protein nitrogen (mg/100 mL)	50.3 ± 6.1	59.2* ±4.3	64.8* ±1.7	71.9** ±3.9
Creatine (mg/100 mL)	9.8 ±0.52	10.9* ±0.77	11.5* ±0.50	19.1* ±3.91

Each value represents the mean of six individual observations. Mean ± S.D., *P value < 0.001; **NS = Not significant.

The blood parameters like urea, non-protein nitrogen and creatine have shown consistent increase under heptachlor impact (Table 3) possibly be attributed to the failure of kidney clearance with pathological lesions noticed earlier (Radhaiah et al 1986).

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REFERENCES.

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

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

Doudoroff P, Anderson BG, Burdick GE, Gattsoff PS, Hart WB, Patrick R, Strong ER, Surber EW, Ven Horn WM (1951) Bioassay of industrial wastes to fish. Sewage Ind Wastes 23: 130

Finney DJ (1964) Probit analysis, 2nd ed. Cambridge University Press, Cambridge

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

Folin O, Wu H (1979) Serum creatine estimation by alkaline picrate. J Biol Chem 30:98

Girija M (1984) Effect of Heptachlor and Metasystox on functionally active tissue - gill of a freshwater fish. Tilapia mossambica (Peters). MPhil Dissertation, Sri Venkateswara University, Tirupati, India.

Lee YL, Lardy MA (1965) Influence of thyroid hormones on L-glycerophosphate dehydrogeneses in various organs of the rat. J Biol Chem 240:1427

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

Moore S, Stein WH (1954) A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J.Biol Chem 221:907

Nachlas MM, Margulies SP, Seligman AM (1960) A colorimetric method for the estimation of succinic dehydrogenase activity. J Biol Chem 235:499-503

Natelson S (1972a) Non-protein nitrogen procedure with Nessler's reagent. In: Techniques of clinical chemistry, 3rd ed. Charles C Thomas Publishers, Springfield, Illinois, 511-516

Natelson S (1972b) Blood urea - with diacetylmonoxime procedure. In: Techniques of clinical chemistry, 3rd

ed. Charles C Thomas Publishers, Springfield, Illinois, 728-734
O'Brien RD (1967) Insecticides action and metabolism Academic Press, London
Pillai SK, Sinha HC (1968) In: Statistical methods for biological workers. Ramaprasad and Sons, Agra
Radhaiah V, Girija M, Prasadarao P, Jayantharao K (1986) Effect of heptachlor on histology of kidney of a freshwater fish, Tilapia mossambica (Peters) Environ Ecol 4:600-602
Rao KSS (1984) Studies on the toxic impact of carboryl and phenthoate on some metabolic aspects of a murrel, Channa punctatus (Bloch)- A synergistic study. PhD Thesis, Sri Venkateswara University, Tirupati, India.
Srikantan TN Krishnamurthy CR (1955) Tetrazolium test for dehydrogenases. J Sci Indust Res 14C:206-209

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