Bull. Environ. Contam. Toxicol. (1996) 57:223-228

Bulletin of Environmental Contamination and Toxicology

Alterations in Protein Metabolism of Muscle Tissue in the Fish *Clarias batrachus* (Linn) by Commercial Grade Dimethoate

G. Begum,* S. Vijayaraghavan

Department of Zoology, Osmania University, Hyderabad 500 007 (A.P.), India

Received: 1 August 1995/Accepted: 18 March 1996

Pollution of aquatic environment by pesticides, bring changes in the metabolic activities and alters physiological state thereby changing the biochemical constituents of aquatic organisms. It is important to examine the toxic effect of pesticides on fish, as they constitute an important link in food chain and their contamination by pesticides imbalances the aquatic ecosystem Fish also form an important part of human food. Due to the persistent nature of organochlorine pesticides, organophosphorus compounds are favored and are used in agricultural pest control. The effects of organochlorine pesticide on fishes were reported by Rao 1989; Deoray and Wagh 1991. The present study has been undertaken to examine the sublethal toxic effects of the organophosphorus (OP) insecticide dimethoate EC 30% on biochemical parameter of protein metabolism in the muscle tissue of fresh water air breathing teleost fish, *Clarias batrachus* (Linneus). Due to its air breathing habit, this fish is an excellent material for laboratory maintenance.

MATERIALS AND METHODS

Healthy, living specimens of *C. batrachus* in the weight range of 35-40 g and length 20-22 cm were collected from local market. Prior to experimentation the fish were allowed to acclimate to laboratory conditions for 2 wk. The fish were fed twice daily with commercial balanced fish feed in the form of pellets at the rate of 3% of body weight during acclimation and experimentation. Fish were starved for 1 d before the initiation of experiments. Commercial dimethoate (EC 30%) was purchased from local market (Rallis India Ltd, Bombay, India). Its IUPAC name is 0,0-dimethyl S-(N-methyl carbamoyhnethyl) phosphorodithioate. It is commonly known as rogor. All the chemicals and reagents used were Analar grade. Commercial grade (EC 30%) dimethoate was first dissolved in 100% ethanol and then diluted with laboratory water so that the final concentration contained 0.0 1mL/L ethanol.

**Present address:* H.N. 16-3-380, Chanchalguda, Hyderabad 500 024 (A.P.), India
Correspondence to: G. Begum

The LC_{50} value determined by probit analysis was found to be 65 mg/L for 96 hr (Begum 1993). One third of LC_{50} value (21.66 mg/L) was chosen as a sublethal concentration in which the fish survived for 192 hr without physical symptoms and mortality. Fish were divided into two groups of 24 each. Group one was exposed to commercial formulation of dimethoate (21.66 mg/L) while the other group was maintained as control in dimethoate free laboratory water containing the same ethanol concentration (0.01 mL/L). Experimental and control water media was renewed every 24 hr and fresh concentration of dimethoate was added to experimental group in order to maintain the concentration of dimethoate constant during 192 hr of exposure. Six fish each from control and experimental groups were killed by severe blow on the head at the end of 24, 48, 96 and 192 hr. Muscle tissue was isolated and used immediately to analyse total, soluble, and structural protein; aspartate and alanine aminotransaminases by the procedures described below. Total, soluble and structural proteins were estimated by Folin phenol reagent (Lowry et al 1951) using bovine serum albumin as standard.

The isolated muscle was homogenized (100 g/L) in 0.25 M ice-cold sucrose solution in an ice-jacketed homogenizer with a motor-driven teflon coated pestle. The homogenate was centrifuged in cold centrifuge machine at 3000 X g for 20 min at 4°C to remove nuclei and cell debris. A clear cell-free extract was used for assay -of transaminases enzyme. Measurements of enzyme activities were performed at 37°C with appropriate enzyme and reagent blanks using a spectrophotometer (Carlzeiss Jena TK 2830). The conditions of the enzyme assays are outlined below.

Aspartate aminotransaminase (EC 2.6.1.1) was assayed by the method of Rietman and Frankel(l957) in a medium (1 mL) containing phosphate buffer (100 µmoles pH 7.4), Gaspartic acid (100 µmoles pH 7.4), L-ketoglutaric acid (2 µmoles pH 7.4) and 0.2 mL of freshly prepared homogenate.

Alanine aminotransaminase (EC 2.6.1.2) was assayed by the method of Rietman and Frankel(l957) in a medium (1 mL) containing phosphate buffer (100 µmoles pH 7.4), DL-alanine (100µmoles pH 7.4), L-ketoglutaric acid (2 µmoles pH 7.4) and 0.2 mL of freshly prepared homogenate. The statistical significance of the difference between the means of control and experimental data was analysed by Student's 't' test.

RESULTS AND DISCUSSION

During the exposure to a sublethal concentration of dimethoate, observations were made to detect the physical symptoms of poisoning on the experimental fish. No visible symptoms or mortality by toxic reaction were observed in exposed fish. Biochemical profiles of control and experimental muscle tissue are presented in Table 1 and Fig. 1. Total protein content of muscle tissue of exposed fish was significantly depleted during 192 hr of experimental span. Maximum depletion was observed at the end of 48 hr; thereafter the depletion declined at the end of 96 hr and 192 hr. A

remarkable reduction in soluble and structural proteins of muscle tissue was observed in fish exposed to dimethoate. The pattern of depletion of soluble and structural protein is similar as that of total protein content. The activity levels of transaminases were elevated throughout the exposure period.

In organisms proteins are of importance as structural components, as biocatalysts, as hormones for control of growth and differentiation. Protein depletion observed in the present study indicates the physiological strategy played by the fish when it is in need of more energy to adapt itself to the changed metabolic system. This leads to the stimulation of degradative processes like proteolysis and utilization of degraded products for increased energy metabolism The soluble protein fraction represents the activity level of enzymes in general (Lehninger 1978). The depletion in soluble protein observed in dimethoate exposed *Clarias batrachus* reveals that the functional aspects of muscle tissue has been affected. The structural protein fraction forms the structural moiety of a cell (Lehninger 1978). It is clear from the present results that the dimethoate has interfered with the structural and functional sites of the muscular unit in clarias.

The pattern of depletion in total, structural and soluble protein observed in the present study shows that the effect is more at the end of 48 hr and then declining. These results

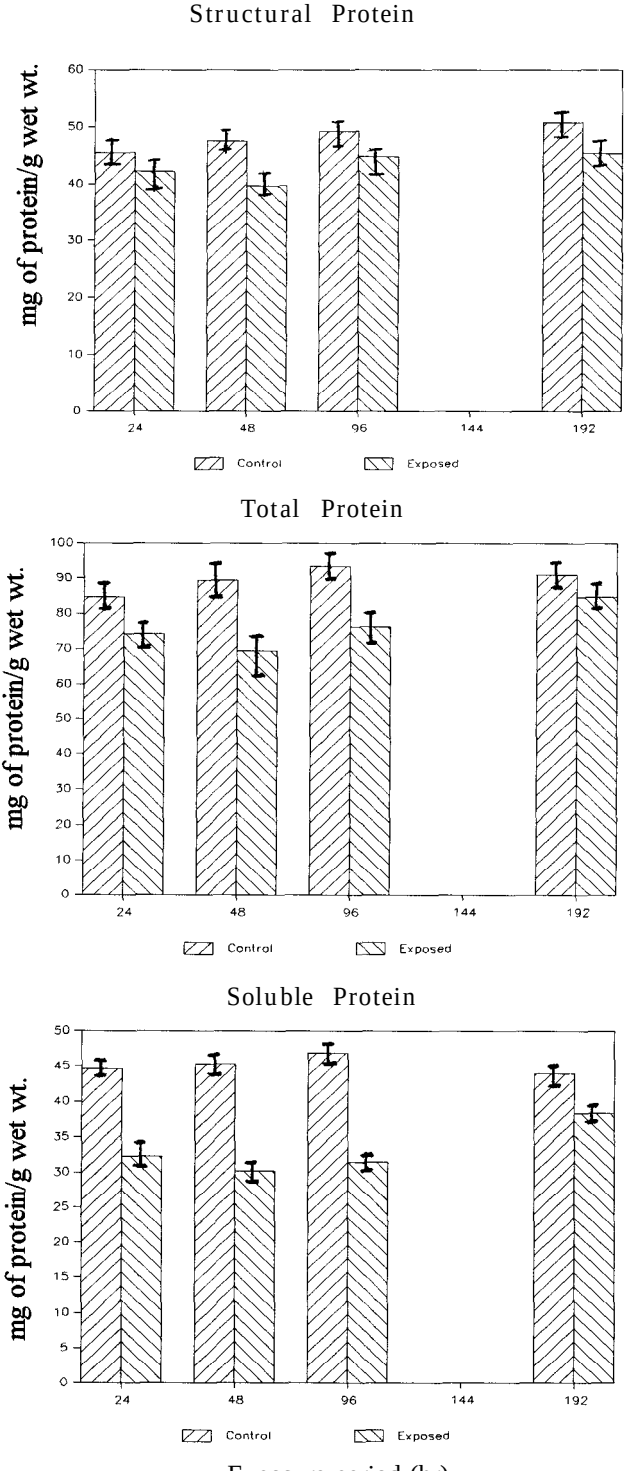
Table 1. Aspartate and alanine aminotransaminases activity levels (µmoles of pyruvate formed/mg protein/ hr) in muscle of control and exposed fish.

Parameters		Exposure period (hr)			
		24	48	96	192
Aspartate amino-transaminase	Control X	0.97	0.88	0.96	0.86
	SE	±0.09	±0.02	±0.04	±0.02
	Exposed X	1.10^{NS}	1.08^{**}	1.21^{**}	0.98^{*}
	SE	±0.01	±0.03	±0.05	±0.03
	% Variance	(+12.82)	(+22.17)	(+25.93)	(+13.92)
Alanine amino-transaminase	Control	1.15	1.01	1.20	1.02
	SE	±0.06	±0.02	±0.02	±0.08
	Exposed	1.86^{***}	1.55^{***}	2.00^{***}	1.79^{***}
	SE	±0.01	±0.06	±0.05	±0.05
	% Variance	(+61.66)	(+53.81)	(+66.66)	(+75.07)

Values are mean ± SE of six observations. Values in parenthesis are % increase (+) over control

Significant at $P < 0.05^{*}$, $P < 0.01^{**}$, $P < 0.001^{***}$, NS : Not significant.

Fig. 1. Muscle proteins in Clarias following dimethoate exposure



can be correlated closely with our earlier reports on accumulation of dimethoate in the fish tissues (Begum et al 1994). The decline in protein contents at the end of 96 hr may be due to the degradation or metabolism of dimethoate to less toxic products and subsequent elimination of de degraded product from the fish tissue via excretory route. Similar pattern of reactions by dimethoate in liver tissue of this fish was also observed earlier (Begum and Viayaraghavan 1995).

The enzymes aminotransaminases are the strategic link between carbohydrate and protein metabolism as they interconvert the metabolites such as *a* -ketoglutarate, pyruvate and oxaloacetate on one hand and alanine, aspartate and glutamate on other hand. Aminotransaminases play an important role in the utilization of aminoacids for the oxidation and/or for gluconeogenesis (Rodwell 1988). Aminotransaminases activity levels were found to increase in muscle tissue of Clarias indicating increased tissue utilisation of aminoacids through these reactions and the formation of oxaloacetate, glutamic acid *a*-ketoglutarate and pyruvate to meet the energy demands warranted by dimethoate stress. Seshagiri et al (1983) recorded a similar increase in aminotransaminases in the muscle tissue of *Sarotherodon mossambicus* exposed to benthiocarb, which gives support to present findings.

Analysis of tissue proteins in the muscle tissue of Clarias was mainly done as this tissue of the fish is important from the point of view of human consumption. The biochemical analysis of the tissue will reveal its nutritive value. The increased rate of protein catabolism as indicated by the elevation of the enzymes aminotransaminases levels in this tissue reveals the poisoning effect of dimethoate. Such studies will help in finding out the remedial measures at appropriate time in polluted organisms.

Acknowledgments. A part of this paper was presented in young scientist award session of IX Annual Conference of National Environmental Science Academy. One of the author (Dr Ghousia Begum) thanks the organizers for awarding 1st prize.

REFERENCES

Begum G (1993) In vivo toxicity and accumulation of dimethoate in freshwater fish, *Clarias batrachus* (Linn) PhD thesis, Osmania University, Hyderabad, A.P., India

Begum G Vijayaraghavan S, Sarma PN, Husain S (1994) Study of dimethoate bioaccumulation in liver and muscle tissues of *Clarias batrachus* and its elimination following cessation of exposure. Pestic Sci 40:201-205

Begum G Vijayaraghavan S (1995) In vivo toxicity of dimethoate on proteins and transaminases in liver tissue of fresh water fish *Clarias batrachus* (Linn). Bull Environ Contam Toxicol 54: 370-375

Deoray BM, Wagh SB (1991) Effect of thiodon on muscle contents of the cyprinid fish *Barilius bendelisis* (HAM). J Environ Biol 12:307-312

Lehninger, AL(1978) Biochemistry Ralyani publications, Ludhiana, India. 223-236

Lowry OH, Rosebrough NJ, Far AL, Randall RJ (1951) Protein measurement with

Folin-phenol reagent. J Biochem 193:265-275
Rao DMR (1989) Studies on the relative toxicity and metabolism of endosulfan to the Indian major carp *Catla catla* with special reference to some biochemical changes induced by the pesticide. Pest Biochem Physiol 33:220-221
Reitman S, Frankel S (1957) A calorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminase. Am J Clin Pathol 28:56-63
Rodwell VW (1988) Metabolism of protiens and ammo acids. In: Martin DW, Mayes PA, Rodwell VW (ed) Harper's Review of Biochemistry. Lange Medical Publications, California, P 265-319
Seshagiri RR, Sreenivasa MR, Dhananjaya MM, Sreeramulu, Cheety C, SwamiKS (1983) Changes in nitrogen metabolism in tissues of fish (*Sarotherodon mossambicus*) exposed to benthiocarb. Bull Environ Contam Toxicol 30:473-478