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Effect of Quinalphos, Organophosphorus Insecticide, on Testicular Steroidogenesis in Fish, *Clarias Batrachus*

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The presence of insecticides in the environment, due to extensive use in agriculture and their low degradation capacity, are of potential toxicological concern for fish (Pickering et al. 1962; Jackson 1960). Insecticides have been found to be highly toxic not only to fish, but also to organisms which constitute the food of fish (Anderson 1960). Catfish, particularly the airbreathing species, are attracting attention of the pisciculturists owing to their high production potential from paddy fields and stagnant shallow ponds (Dehadrai and Mukhapadhayay 1979). Hence, it is necessary to study the immediate and chroniceffects of insecticides on the fish, which form a part of human diet. Although various investigators ovserved the effects of different insecticides on survival and growth rate of fish (Wildish et al. 1971; Jackson 1976) but untoward effects of insecticides on gonads have not yet been studied properly.

In our earlier study we have shown that quinalphos, a widely used insecticide of organophosphorus group in the field of agriculture, has inhibitory effects on the steroidogenic status of testes in rats (Ray et al. 1988). Keeping this in view, an experiment has been conducted with the insecticide quinalphos to study the effects on steroidogenic activity of testes of a dietary catfish, Magur (Clarias batrachus).

MATERIALS AND METHODS

Mature male fish weighing between 95-100 g were obtained from local market and they were acclimatized to laboratory conditions for 10 d in glass aquaria containing sufficient plain potable water (pH 7.54). The water was changed daily and fish were fed tubifex. For the determination of the 96-hr LC_{50} value for quinalphos, a batch of sixteen acclimatized fish were exposed to each dose. The dosages chosen were 0.25, 0.75, 1.25, 1.75, 2.50 mg/L. Preliminary experiments indicated

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that the LC_{50} dose was between 0.75 and 1.75 mg/L. The results are given in terms of commercial formulation of insecticide and not in terms of active ingredients of pure insecticide because only commercial preparations are used in agriculture. The insecticide quinalphos; 0, 0-diethyl-0-quinoxalinyl (2)-thionophosphate, was purchased from Sandoz (India) Limited, Bombay. Sixteen laboratory acclimatized fish were taken at a time in each experimental set. Half of them were exposed to a sublethal dose (0.025 mg/L) for 15 d Remaining half were kept in same water, serving as control, under similar environmental and dietary condition as that of the treated ones. At the day of sacrifice the testes of each fish were dissected out, trimmed and weighed. The experiments were repeated for five times.

Right testis of each fish was used for the estimation of cholesterol content and the study of two steriod biogenic enzymes, 3β -hydroxysteroid dehydrogenase (HSD), 17β -HSD activity were measured by optical measurement (absorbance at 340 nm) of the rate reduction of pyridine, nucleotides (NAD or NADP) according to the method of Talalay (1962) and Jarabak (1969). The assay system contained sodium pyrophosphate buffer, bovine serum albumin and steroid (dehydroepiandrosterone or testosterone). All chemicals were purchased from Sigma Chemicals, St. Louis, Missouri, U.S.A.; except the steroid (Organon India Limited, India). Duplicate samples and blanks (without steroid) were run with each tissue. One unit of enzyme activity is the amount which causes a change in absorbance of 0.001 per min using either of the steroid as substrate. Testicular protein was measured by the method of Lowry et al (1951). The enzyme activity was expressed in unit/mg protein. The cholesterol content of testis was estimated by the following method of Sperry and Webb (1950).

The left testis of each fish was fixed in Bouin's fluid immediately after weighed. Paraffin sections (5 μm thick) were taken from the mid portion and stained with periodic acid Schiff (PAS) - haematoxylin. Seminiferous tubular diameter was measured by occular micrometer from the histological preparation.

The statistical analysis was performed by using an analysis of variance (ANOVA) followed by multiple comparison by the two-tailed 't'-test.

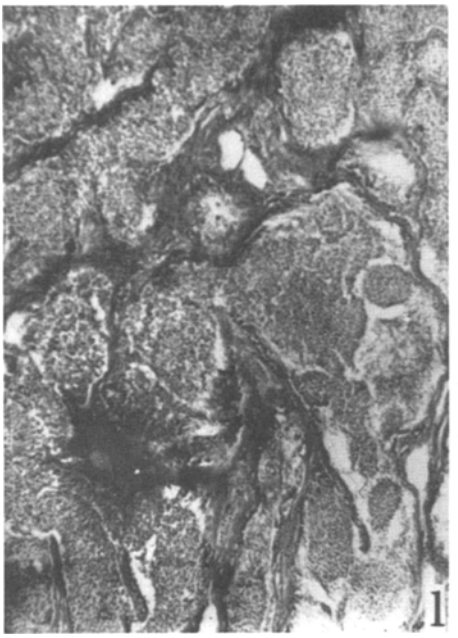
RESULTS AND DISCUSSION

Results of this investigation are summarized in Table 1. The frequent changes of fresh solution of quinalphos in the aquarium significantly decreased the steroidogenic enzymes activity of testes in Clarias batrachus. It has long been known that 3β -HSD and 17β -HSD are the two principal enzymes which are directly related with testicular steroidogenesis (Deane et al. 1962; Baillie et al. 1966) and decrease in activity of this enzymes after quinalphos treatment may suggest an

Table 1. Effect of Quinalphos (0.025/L) on testicular weight, seminiferous tubular diameter, steroidogenic enzyme activity, and cholesterol content in Clarias batrachus. Mean ± SE; n = 8.

Groups	Treatment	Testes weight mg/100g	Seminiferous tubular diameter (μm)	Biochemical activity of steroidogenic enzymes (unit/mg protein) 3β -HSD	17β -HSD	Testicular cholesterol content (mg/g tissue)
I	Control :	40.95	189.17	5.66	3.62	2.00
		±0.79	±5.61	±0.02	±0.06	±0.08
II	Quinalphos :	40.14*	96.37*	4.05*	2.49*	3.20*
		±0.59	±6.35	±0.08	±0.03	±0.01

* $P < 0.001$ (when compared with corresponding control group.)



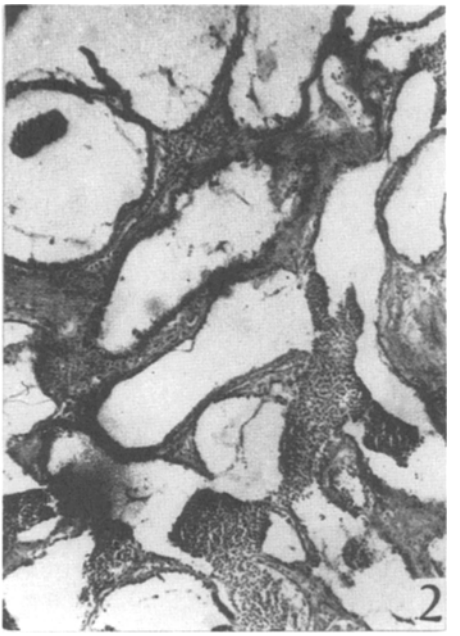


Figure 1. Testis from a control fish showing normal pattern of testicular tissue. PAS-haematoxylin stain (X250).

Figure 2. Testis from a fish treated with quinalphos (0.025 mg/l), showing degeneration and shrinkage of seminiferous tubules. PAS-haematoxylin (X 250).

inhibition of testicular steroidogenesis in Clarias batrachus. This observation is also in agreement with the previous findings in different fish after exposure to polyclorinated biphenyl and fenitrothion (Freeman and Idler 1975; Kapur et al.. 1978). Testicular cholesterol is known as a precursor of steroid biosynthesis (Ryan and Short 1966). An increase in cholesterol content in quinalphos treated testes indicates its accumulation, probably due to inhibition of steroidogenic enzyme activities as well as androgen production (Deb et al. 1977). Moreover, significant decrease in the weights of testes, degenerative changes in seminiferous epithelium and shrinkage of seminiferous epithelium and shrinkage of seminiferous tubular diameter (Figures 1 and 2) further confirms the possible diminition of testicular steroidogenesis and androgen production.

Therefore, from the above facts, it can be concluded that quinalphos supressed the testicular functions in Clarias batrachus, which may be due to the inhibition of steroidogenic enzymes activity.

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