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The Effect of Fenitrothion on Reproduction of a Teleost Fish, *Cyprinus carpio communis* Linn: A Biochemical Study

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With the modernization of agricultural operations and the rapid growth of industrial activity, there has been much increase in the manufacture and utilization of insecticides, pesticides and herbicides which ultimately find their way into the rivers, lakes and ponds. Pesticides have been found to be highly toxic not only to fishes (TOOR et al., 1973; TOOR and Kaur, 1974; KAMALDEEP and TOOR, 1977), but also to the organisms which contribute to the food of fishes (ANDERSON, 1960; LOOSANOFF, 1960; DAVIS, 1961; BUTLER, 1969; BUTLER et al., 1970).

In earlier studies in this laboratory, the effect of several pesticides has been seen on the survival of developing eggs and hatchlings, percentage hatchability, frequency of morphological and physiological deformities at different embryonic stages, and survival and behavioral response of the fry/fingerlings of Cyprinus carpio communis Linn. (TOOR and KAUR, 1974; KAMALDEEP and TOOR, 1977). These studies have indicated that fenitrothion is one of the very toxic pesticides being used at present.

The present study deals with the effect of fenitrothion on the reproduction of Cyprinus carpio communis Linn., especially with regard to its effect on the activity of enzymes indicative of steroidogenesis in the gonads. The results regarding the histopathological effects of this pesticide on the gonads, liver, digestive tract and skin shall be presented in a separate paper.

Material and Methods

For the present studies, specimens of Cyprinus carpio comm. Linn., measuring 25-30 cm in length and 100 to 120 g in weight, were collected from Fish Seed Farm, Punjab Agricultural University, Ludhiana. Fish were acclimatized for a week in the laboratory. Safe concentration (0.30 ppm) and sublethal concentration (1.5 ppm) of fenitrothion, 50% EC for C. carpio as determined during earlier studies by TOOR and KAUR (1974), were selected. The dilution technique of SHIVAJI RAO et al. (1976) was followed to prepare the required concentrations. Two replicates for each concentration were made. Ten specimens (both male and female) of C. carpio were exposed to each

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concentration in aquaria measuring 60 X 30 X 30 cm at room temperature ranging from 18-25°C for 6 months (Dec. 76 to May 77). Control experiments were conducted simultaneously. Fish were not fed during the experimental period. The water was renewed once a week and the same concentration of fenitrothion was maintained in each experiment. At the end of experiment, three male and three female specimens each of both treated and controlled groups were sacrificed for biochemical study.

Preparation of Homogenate

Each testis and ovary was weighed and a portion of each was taken for biochemical study. This portion was weighed and homogenized with cold Sørenson buffer (0.1 M Na_2HPO_4 adjusted to pH 7.5 with 0.1 M KH_2PO_4) to make a final homogenate of 1:10. The homogenate was centrifuged at 10,000x g for 20 minutes at 4°C and the supernatant was taken for the study of enzymes.

Determination of Enzymatic Acitivity

The 3-β HSD activity was determined by measuring the rate of NAD reduction at 340 nm during the course of substrate (dehydroepiandrosteron and pregnenolone) oxidation. The measurement was carried out at 37°C with a Unicam S P 800 UV spectrophotometer. The incubation mixture contained:

(1) 0.1 ml of substrate solution (1 mg of substrate in 0.5 ml of dimethyl formamide and 0.5 ml of propylene glycol).

(2) 0.4 ml of the 1:10 extract of testicular or ovarian homogenate.

(3) 2.5 ml of the Sørenson buffer at pH 7.5.

(4) 1.0 mg of NAD.

The NAD was added at the end, after the blank was set with rest of the solutions. The O.D. measurements were made every 15 sec during the first minute and thereafter every minute up to five minutes, when the incubation was terminated. The relative differences in the enzyme activity between the control and the treated specimens were worked out and the analysis of variance was applied to the data. The concentration of the proteins in the sample was found out by the method of Lowry and coworkers (1951).

Results

Table I summarizes the results of the experiments. It can be seen that treatment with the fenitrothion caused a great reduction in the activity of 3-β HSD in the testicular and ovarian extracts. The differences

with regard to activity of this enzyme between the control and treated groups and between the two treated groups are highly significant ($P<0.01$). However, differences among various replicates as well as between the two substrates are also very insignificant. The activity of 3-β HSD is reduced to about 80% and 71.1% in the case of male and about 91.2% and 83.8% in the case of female, as compared to the controls, when treated with lower and higher dosages, respectively.

TABLE I

The effect of safe and sublethal dose of fenitrothion on the activity of 3-β HSD in testicular and ovarian extracts.

Treatment*	Activity of 3-β HSD**(Units)
Male:	
1) Control	2.25±0.0086
2) Safe dose	1.80±0.0086
3) Sublethal dose	1.60±0.0202
Female:	
1) Control	6.25±0.0052
2) Safe dose	5.60±0.0066
3) Sublethal dose	5.24±0.0211

*The results are the mean values with S.E. of means from three different animals and both the substrates, i.e. dehydroepiandrosterone and pregnenolone.

**The activity of 3-β HSD is in terms of the number of units per mg protein, where one unit is equivalent to change in an O.D. of 0.01 per minute.

Discussion

The level of 3-β HSD along with that of various other steroid dehydrogenases is indicative of steroidogenesis in the gonadal tissue (see GURAYA, 1976, for various criteria of steroidogenesis in a tissue). Recent studies in the cold blooded vertebrates (WIEBE, 1970; KAPUR and TOOR, unpublished observations) have indicated that the level of 3-β HSD may be related with the level of gonadotrophins in the circulatory system. The chemicals such as methallibure, which are known inhibitors of

gonadotropins in fishes and other vertebrates (for fish see LEATHERLAND, 1969; PANDEY and LEATHERLAND, 1970; MACKAY, 1971; DADZIE, 1972; BRETON et al., 1973; DeVLAMING, 1974), cause a reduction in the level of 3-β HSD (WIEBE, 1970; KAPUR and TOOR unpublished observations). Hence the decline in the level of the 3-β HSD as a result of the fenitrothion treatment may be because of the action of the pesticide at the gonadotro level. The gonadotropins are also known to stimulate adenyl cyclase enzyme synthesis (FONTAINE et al., 1972). Also they seem to stimulate RNA and protein synthesis in the target tissue (ARMSTRONG and BLACK, 196 REEL and GORSKI, 1968). Hence the action of the fenitrothion on the gonads may be either by inhibition of the adenyl cyclase enzyme or the inhibition of RNA and protein synthesis. It may also have a direct effe on the pituitary by inhibiting the release or synthesis of the gonadotro However, much work needs to be done before we can reach any conclusions to the mode of action of fenitrothion as related to reproduction in fish

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