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Quantitative Study of Testicular Recrudescence in the Fresh Water Teleost, *Channa punctatus* (Bl.) Exposed to Pesticides

P. K. Saxena* and Kanta Mani

Department of Zoology, Faculty of Basic Sciences, Punjab Agricultural University, Ludhiana-141004, India

The pesticides have been recognised as the environmental pollutants of potential toxicological concern to fishes as diagnosed by their acute and chronic toxicities (Eaton 1970; Lane and Livingston 1970; Bhattacharya et al. 1975; Jackson 1976; Aminikutty and Rege 1977; Koundiya and Ramamurthi 1978; Rao and Murthy 1978; Singh and Singh 1980; Tilak et al. 1980; Konar 1981).

Since fecundity of the fish depends on the number of mature oocytes in the ovaries and the quality and quantity of sperms formed in the testes, studies on the effect of chronic exposure to pesticides on gonadal recrudescence shall be of immense value. The effect of pesticides on the reproduction including gonads are scanty (Carlson 1972; Freeman and Idler 1975; Kapur et al. 1978; Saxena and Garg 1978; Pandey and Shukla 1980; Singh and Singh 1980, 1982; Konar, 1981). Present investigation on the effect of toxicologically safe concentrations of fenitrothion 50% E.C.- an organophosphate and carbofuran 3% G- a carbamate, on the quantitative aspect of testicular recrudescence in terms of the testicular weights and the occurrence of various spermatogenetic stages in the lobules of testes of fresh water teleost, Channa punctatus during exposure for 120 days (mid-April through mid-August) extending over the maturing, prespawning, and the spawning phases of the annual reproductive cycle, is an attempt in this direction.

MATERIALS AND METHODS

Adult specimens of the fish, C. punctatus (20-25 cm) for the present study were collected during March from the ponds situated in the vicinity of Ludhiana city. The fish were acclimatized to the laboratory

*Correspondence and reprint requests

conditions in the glass aquaria (92x46x46 cm) for about a fortnight prior to the initiation of the experiment. The toxicologically safe concentrations of pesticides were calculated by the method of Bask and Konar (1977) and were estimated at 1.5 ppm for fenitrothion 50% E.C. and 5 ppm for carbofuran 3% G. The fish were exposed to each concentration separately on 15th April, 1980 and the exposure was continued for 120 days extending over the maturing, prespawning, and the spawning phases of annual reproductive cycle of this species. The fish were got fed on goat liver ad libitum twice a week. After feeding water of the aquaria was changed and the predetermined quantity of the pesticide was added to the water of each experimental aquarium. The water temperature and the photoperiod in the aquaria were not controlled. However, these conditions were similar in both the experimental and control aquaria.

The results are given in terms of the pesticides' commercial formulation and not in terms of the active ingredient of the pesticides used because only commercial formulations are used in the agricultural practices. Since carbofuran was not fully soluble in the water, it was first dissolved in a small quantity of the acetone before it was added to the experimental aquaria. In the control group, therefore, quantity of the acetone used for dissolving the carbofuran was also mixed in the water. Before exposing the fish to toxicologically safe concentrations of pesticides, five specimens were autopsied and these served as the initial controls. Following exposure of the fish to the pesticides, at least five specimens each from the experimental and control groups were collected at 30 days interval, till the experimental was terminated at 120 days of exposure. The specimens were anaesthesized with MS 222 (1:4000 tricaine methane sulphonate, Sandoz) and the testes were dissected out and weighed on the torsion balance. For comparison, testicular weights were calculated on 100 g body weight basis. The testes were fixed immediately in aqueous Bouin's fixative and cross sections were obtained at 6 µm thickness. The sections were stained with ironalum-heamatoxylin technique. The process of spermatogenesis in the testes was distinguished in six stages i.e., primary spermatogonium(Stage I), secondary spermatogonium (Stage II), primary spermatocyte (Stage III), secondary spermatocyte (Stage IV), spermatid (Stage V) and sperm (Stage VI) according to Hyder (1969). For the sake of comparison between the treated and the control groups, the percentage occurrence of various stages of the spermatogenesis in the lobules of the testes were taken into consideration.

The statistical significance between the treated and the control groups and also in between the fenitrothion and carbofuran treated groups was calculated by the analysisof variance (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

Following exposure to the toxicologically safe concentration of carbofuran/fenitrothion, the testicular weights in the treated fish were lower($p < 0.01$) than in the control fish at 30 days of exposure and this trend in the testicular weights of the treated fish continued up to the termination of the experiment i.e., at 120 days exposure (Table 1). However, maximum per cent decrease in the testicular weights of the treated fish was recorded at 90 days of exposure in carbofuran treatment, but at 120 days of exposure in fenitrothion treatment (Table 1). Further, a comparison between the treatments revealed that the fenitrothion treatment caused more decline in the testicular weights than caused by carbofuran treatment. The low testicular weights in the treated fish has been attributed to the loss of the germ cells in the lobules of testes as a consequence of their degeneration. In Tilapia mossambica too, Pandey and Shukla (1980) have recorded a decrease in the testicular weights following treatment with benzene hexachloride at 2 and 4 ppm for 10 days.

At the initiation of experiment (15th April, 1980) the testes of control fish showed the occurrence of the spermatogonia (primary/secondary) and the spermatocytes (primary/secondary), while occurrence of the spermatids and sperms was hardly observed in the lobules. At this stage, the percentage occurrence of the primary spermatocytes was maximum in the testicular lobules (Table 2). At 30 days of exposure, the percentage occurrence of the primary and secondary spermatogonia were higher ($p < 0.01$) in the testes of the treated fish than in the control. However, among the treated groups, their occurrence was higher in the fenitrothion treated fish. On the contrary, the percentage occurrence of the primary spermatocytes was lower ($P < 0.01$), while that of the secondary spermatocytes was higher ($P < 0.01$) in the testes of the treated fish than in the control. Further, a comparison between treated groups revealed low ($p < 0.01$) occurrence of the primary spermatocytes, but a higher ($p < 0.01$) occurrence of the secondary spermatocytes in the testes of fenitrothion treated fish than in carbofuran treated fish.

At 60 days of exposure, the percentage occurrence of

the primary spermatogonia in the testes of treated fish was higher ($P < 0.01$) than in the control. However, the percentage occurrence of the primary spermatogonia in the testes of fenitrothion treated fish was lower ($P < 0.01$) than in carbofuran treated fish. On the contrary, the percentage occurrence of the secondary spermatogonia in fenitrothion treatment was higher ($P < 0.01$) than in carbofuran treatment and the control, whilst their occurrence in the latter two groups did not vary significantly (Table 2). The occurrence of the primary spermatocytes in the treated groups was higher ($P < 0.01$) than in the control as noticed at 30 days of exposure. However, the occurrence of the secondary spermatocytes in the testes of carbofuran treated fish was higher ($P < 0.01$) than in the finitrothion treated and control fish, while their occurrence in the testes of fenitrothion treated fish was lower ($P < 0.01$) than in the control. At this stage, occurrence of the spermatids in the testes of the control fish was higher ($P < 0.01$) than at 30 days of exposure, while these spermatids could hardly be observed in the testes of the treated fish. The occurrence of the sperms was noticed in the testes of control fish, but not in the testes of the treated fish.

At 90 days of exposure, the testes of the treated fish revealed occurrence of both primary and secondary spermatogonia, with their percentages being higher ($P < 0.01$) in the testes of fenitrothion treated fish (Table 2). On the contrary, these spermatogonia were hardly observed in the testes of the control fish. The testes of the fenitrothion treated fish also revealed higher ($P < 0.01$) occurrence of the primary spermatocytes than in the testes of carbofuran treated and control fish. However, the occurrence of these primary spermatocytes in the testes of carbofuran treated fish was higher ($P < 0.01$) than in the control (Table 2). Likewise, the percentage occurrence of the secondary spermatocytes in the testes of the fenitrothion treated fish was higher ($P < 0.01$) than in the control, while these secondary spermatocytes were not observed in the testes of the carbofuran treated fish (Table 2). The occurrence of the spermatids in the testes of the treated fish was lower ($P < 0.01$) than in the control. A comparison of their occurrence in the treated fish revealed their low ($P < 0.01$) occurrence in the testes of treated fenitrothion treated than in carbofuran treated fish. The occurrence of the sperms waslow in the testes of the treated fish than in the control. Further, a comparison among the treatments, revealed low occurrence of the sperms in the testes

Table 1 Effect of toxicologically safe concentrations of carbofuran (5 ppm) and fenitrothion(1.5 ppm) on testicular weights(mg)/100 g of body weight in C. punctatus (Bl.)

Duration of exposure (days)	Control	Carbofuran treated	Treated as % of control	Fenitrothion treated	Treated as % of control
(Initial) 15th April, 1980	84.61 ±3.11	-	-	-	-
30	113.75 ±2.90	111.77* ±4.49	98	91.80*[1] ±4.49	81
60	125.84 ±8.40	104.97* ±3.20	83	87.40*[1] ±1.28	69
90	110.18 ±6.72	86.09*[1] ±1.25	78	46.15*[1] ±0.46	42
120	94.28 ±5.40	75.93*[1] ±5.10	80	34.57*[1] ±0.51	37

Values are expressed as Mean±S.E. of observations from five different specimens
*$p < 0.01$ significance level with respect to control
[1]$p < 0.01$ significance level with respect to carbofuran treatment

Table 2 Effect of toxicologically safe concentration of carbofuran(5 ppm) and fenitrothion (1.5 ppm) on percentages occurrence of various spermatogenetic stages (except sperms) in the testes of C. punctatus (Bl.)

Duration of exposure (days)	Primary spermatogonia			Secondary spermatogonia		
	C	T_1	T_2	C	T_1	T_2
Initial(15th April,1980)	6.0 ±0.50	-	-	11.0 ±0.20	-	-
30	3.0 ±0.25	6.50** ±0.25	12.00**[1] ±1.64	5.0 ±0.32	13.0** ±1.50	27.0**[1] ±1.55
60	2.0 ±0.22	7.0** ±0.40	6.0**[1] ±0.52	3.0 ±0.55	3.0 ±0.34	14.00**[1] ±1.13
90	-	1.0 ±0.32	9.0[1] ±0.43	-	1.0 ±0.46	5.01[1] ±1.10
120	2.0 ±0.15	1.50* ±0.35	12.0**[1] ±0.17	6.50 ±1.25	1.0** ±0.20	4.0**[1] ±0.30

Values are expressed as Mean±S.E. of observations from five different specimens
*$p<0.05$ **$p<0.01$ significance level with respect to control
[1]$p<0.01$ significance level with respect to carbofuran treatment
- = absence of the spermatogenetic stage
C = control ; T_1 = carbofuran treatment ; T_2 = fenitrothion treatment

Table 2 contd.

Duration of exposure (days)	Primary spermatocytes			Secondary spermatocytes			Spermatids		
	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2
Initial(15th April,1980)	80.0 ±7.63	-	-	3.0 ±0.45	-	-	-	-	-
30	87.0 ±1.40	74.50** ±0.62	53.00**[1] ±2.82	4.0 ±0.13	6.00** ±0.40	8.00**[1] ±0.52	1.0 ±0.52	-	-
60	72.0 ±1.72	83.0** ±3.08	75.0**[1] ±2.22	6.0 ±0.42	7.0** ±0.44	5.0**[1] ±1.47	17.0 ±1.63	-	-
90	5.0 ±0.21	63.0** ±3.77	69.0**[1] ±1.77	2.0 ±0.74	-	8.00** ±0.36	93.0 ±5.42	35.0** ±1.28	9.0**[1] ±0.73
120	15.50 ±1.12	69.50** ±3.12	74.0**[1] ±1.36	1.0 ±0.63	-	7.0** ±1.21	75.0 ±1.48	28.0** ±1.36	3.0**[1] ±0.39

Values are expressed as Mean±S.E. of observations from five different speciments
*$p<0.05$ **$p<0.01$ significance level with respect to control
[1]$p<0.01$ significance level with respect to carbofuran treatment
- = absence of the spermatogenetic stage
C = control; T_1 = carbofuran treatment; T_2 = fenitrothion treatment

of the fenitrothion treated fish. Following 120 days of exposure, the percentages occurrence of the primary and secondary spermatogonia were lower in the testes of carbofuran treated fish than in fenitrothion treated ($P < 0.01$) and control fish ($P < 0.05$), whilst the testes of fenitrothion treated fish revealed higher ($P < 0.01$) occurrence of the primary spermatogonia, but lower ($P < 0.01$) occurrence of the secondary spermatogonia than in the control (Table 2). The trend in the percentages occurrence of other spermatogenetic stages viz., primary spermatocytes, secondary spermatocytes, and spermatids in the testes of the treated and control fish were more or less similar to that observed at 90 days of exposure. However, the sperms occurrence was low in the testes of the carbofuran treated fish than in the control, while these sperms could hardly be observed in the testes of the fenitrothion treated fish.

The effect of toxicologically safe concentration of carbofuran/fenitrothion on the occurrence of various spermatogenetic stages in the testes revealed that both carbofuran and fenitrothion treatments not only arrested, but also delayed the formation of the spermatids and the sperms. Further, it was noticed that the fenitrothion treatment was relatively more effective in inhibiting the formation of the spermatids and sperms than did the carbofuran treatment. While working on survival and reproduction of fathead minnow, Primephales promelas, Carlson(1972) did observe that the continuous exposure to carbaryl at 0.68 mg/l for 9 months prevented reproduction and also decreased the survival of the fish. Recently, Pandey and Shukla (1980) have studied the effect of benzene hexachloride (BHC) at 2 ppm and 4 ppm on the testicular histology of Tilapia mossambica and observed vacuolated cells as well as necrosis in the lobules. Further, these authors concluded that the spermatogenesis was impaired at the spermatid stage, since sperms were hardly observed in the testicular lobules.

In present studies, the low degree of testicular recrudescence in the treated fish has been attributed to the low levels of gonadotropin(s) which in turn may be responsible for the reduced steroidogenic activity in the testes as well as for the low levels of steroid hormones (testosterone and progesterone) in the blood plasma (our unpublished observations). In Salvelinus fontinalis, Freeman and Idler (1975) have also noticed regression of the testes as well as a decrease in steroidogenesis following polychlorinated biphenyl (PCB) treatment. On the mode of action of pesticides

in Heteropneustes fossilis, Singh and Singh (1982) have opined that the organophosphate pesticides like cythion and Paramar M 50 act through the hypothalamus, where they seem to inhibit the secretion of the gonadotropin releasing hormone(GnRH) which, in turn, decreases the synthesis and release of the gonadotropin(s) from the pituitary gland followed by the reduced gonadal activity. On the other hand, the chlorinated hydrocarbon pesticides like aldrin and hexadrin act directly on the gonads to suppress their activity.

The existing literature suggests that the gonadal recrudescence in fishes are regulated by varied titers of the gonadotropic hormone(s) (Hoar, 1965). Since, Wiebe(1970) observed a significant decline in the levels of 3 β -HSDH in the gonads following treatment with methallibure, an antigonadotropic substance, a direct influence of the gonadotropin(s) on the steroidogenesis in the gonads has been visualized. Kapur et al.(1978) have also attributed decline in the levels of 3β HSDH in the testes of mirror carp, Cyprinus carpio following treatment with carbofuran/ fenitrothion may explain the low occurrence and delayed formation of the spermatids and sperms as well as degeneration of the germ cells in both the treatments.

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