Bull. Environ. Contam. Toxicol. (1990) 45:742–747

Bulletin of Environmental Contamination and Toxicology

Effect of Sublethal Doses of Three Pesticides on the Ovary of a Carp Minnow *Rasbora daniconius*

Anjali Rastogi and S. K. Kulshrestha

Department of Zoology, Motilal Vigyan Mahavidyalaya, Bhopal—462 008, India

The contamination of water resources by pesticides may affect nontarget aquatic organism including fish. Any change in the environment may affect reproductive physiology adversely as ovaries perform duel function of producing eggs and steroid hormones. The pesticides are known to cause various histopathological effects on the overy of fish (Cairns et al. 1967; Kapur et al. 1978; Kulshrestha and Arora 1984; Mani and Saxena 1985; Singh and Sahai 1985; Sahai 1987).

The present study is aimed to investigate comparative histopathological effects induced by sublethal doses of three pesticides of different categories, viz., carbamate compound carbofuran (Furadon 3G Railies India Ltd. Bombay); organochlorine compound endosulfan (Thiodan, 35% EC, Bharat Pesticides Manufacturing Co., Delhi); an organophosphate compound methyl parathion (Paracid, 50% EC, Bharat Pesticides Manufacturing Co., Delhi) on the ovaries of a carp minnow Rasbora daniconius. The carp minnow was chosen for study for its easy availability, small size and tolerance to wide range of environmental factors.

MATERIALS AND METHODS

The live specimens of fish (5 ± 1 cm in length and 6 ± 1 gm in weight) were brought from local lakes and acclimatized for 15 days under laboratory conditions. LC_{50} values were calculated using semilog paper and sublethal values were selected at 1/5th of the LC_{50} values. Static bioassay method (APHA 1985) was used for exposure to pesticides. The physico-chemical characteristics of diluent water obtained from head tank used in aquaria were analysed after APHA (1985) (Table 1). The experiments were conducted in spawning season of the fish from May to July. Groups of about 50 fish were exposed to sublethal doses of 0.001 mg/L endosulfan, 0.1 mg/L carbofuran and 0.1 mg/L methyl parathion for 75 days in rectangular glass aquaria; controls were kept without pesticides. All specimens were fed on alternate days to avoid effect of starvation. The water and pesticide solutions were renewed daily. Three

---

Send reprint requests to S.K. Kulshrestha at above address.

specimens from each treated and control group were sacrificed on days 5, 10, 15, 30, 45, 60, and 75 by severe blow on the head. Each killed fish was weighed, its ovary dissected out, weighed and fixed in alcoholic Bouin's fluid.

Table 1. Physico-chemical characteristics of diluent water.

Characteristic	Range
pH	7.4 - 7.6
Temperature	22°C - 25°C
Total Solids	14 - 17 mg/L
Total Alkalinity	50 - 58 mg/L
Total Hardness	62 - 70 mg/L
Dissolved Oxygen	6 - 8 mg/L

Serial microtomy sections were cut and stained by hematoxylin/ eosin and Mallory's triple stain. The percentage and size of the oocytes of different kinds were calculated by random selection of 300 to 400 oocytes from different regions of the ovary of each treated and control specimen by using an occular micrometer. Observations were made in three samples of each treated and control group to consider variations, if any. The average value was used for preparing histograms.

Average GSI has been calculated in fishes by the following formula:

$$GSI = \frac{\text{Weight of the goand}}{\text{Weight of fish}} \times 100$$

The 't' test was applied to find statistical relation between treated and control groups.

RESULTS AND DISCUSSION

In control specimens the immature ovary contained oogonia and immature oocytes. The oogonia were small with large nucleus, single nucleolus, and clear cytoplasm while the immature oocytes were spherical with clear cytoplasm and large nucleus containing 5 to 10 nucleoli. The maturing ovary contained maturing oocytes with undulated nuclear membranes, some nucleoli in the pockets of nuclear membrane and indistinct vitelline membrane. In mature ovary the extravesicular cytoplasm of mature oocytes contained yolk granules and cortical alveoli.

The exposure of fish to endosulfan, carbofuran, and methyl parathion produced several deleterious effects. The peritoneal lining was severely damaged on prolonged exposure to methyl parathion, but endosulfan and carbofuran exposure did not have any effect. The prolonged exposure to three pesticides produced necrosis and fibrosis in the connective tissue. Blood vessels were dialated. Methyl parathion produced maximum damage followed by carbofuran and endosulfan. The ovigerous lamellae were ruptured on pesticide exposure. Kulshrestha and Arora (1984) reported increase of fibrous layer of tunica albuginea and dilation of blood vessels. Harilal and Sahai (1986) also reported degeneration in ovigerous lamellae.

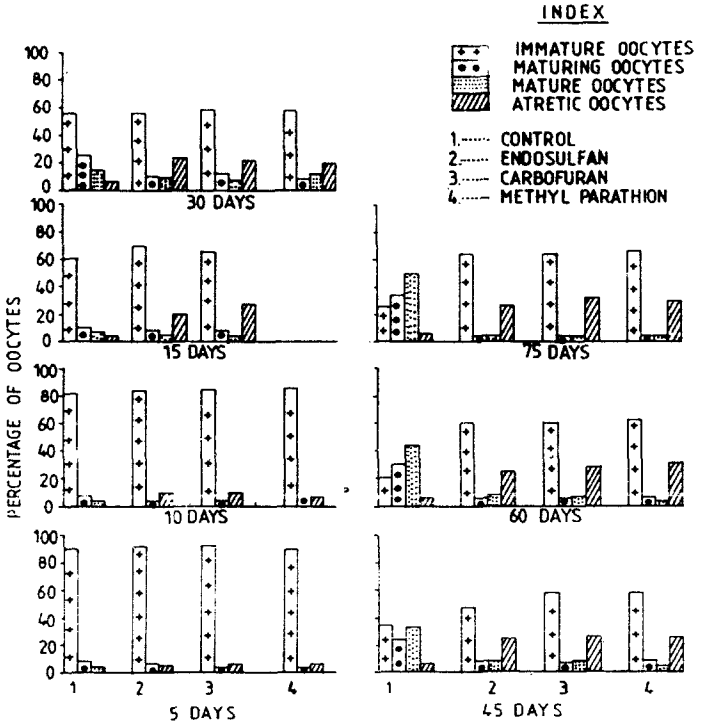

FIG. 1: PERCENTAGE OF OOCYTES IN TREATED AND CONTROL SPECIMENS OF RASBORA DANICONIUS.

Sublethal doses of all the three pesticides produced various effects on the oocytes of different types in the present study. The diameter of the oocytes decreased with progressive duration of the exposure as compared to controls. In 5 days exposure no significant change in diameter was observed in the immature occyte but subsequent exposure produced significant changes ranging from $p > 0.10$ to $p > 0.001$. (Table 2). The percentage of immature oocytes increased while that of mature ones decreased (Fig. 1) from 0 to 75 days. The cytoplasm of immature oocytes developed net-like structure on exposure to endosulfan

Table 2. Analysis by 't' Test and standard deviation showing the egg diameter in control and treated ovaries of Rasbora daniconius.

Duration Exposure Days	oocyte Maturity	Egg diameter in micron			
		Control	Endosulfan 0.001 mg/L	Carbofuran 0.1 mg/L	Methyl Parathion 0.1 mg/L
5	Immature	88.75 ±9.60	60.60(-) ±10.66	61.4(-) ±12.56	68(-) ±13.35
10	Immature	91.87 ±9.90	72.98(++++) ±4.50	57.4(++++) ±7.61	59.35(++++) ±7.61
	Maturing	183.75 ±45.5	136.6(++++) ±16.0	141.77(+++) ±37.9	132.2(++++) ±19.87
15	Immature	96.5 ±5.05	70.53(++++) ±9.33	63.2(++++) ±4.60	55.07(++++) ±8.46
	Maturing	175.05 ±41.36	139.90(+++) ±6.14	133.72(++++) ±9.59	150.71(+++) ±7.37
	Mature	341.38 ±49.41	179.04(++++) ±36.70		
30	Immature	95.65 ±4.41	60.30(++++) ±6.26	69.55(++++) ±12.10	55.19(++++) ±3.00
	Maturing	164.4 ±45.69	143.36(+) ±3.58	133.34(++) ±3.28	147.62(-) ±9.63
	Mature	402.73 ±90.31	252.63(++++) ±13.67	251.12(++++) ±19.12	
45	Maturing	181.69 ±38.1	170.07(-) ±27.56	167.35(-) ±27.44	155.30(++) ±26.97
	Mature	440.45 ±63.56	301.49(++++) ±60.00	265.20(++++) ±32.48	263.82(++++) ±51.60
60	Maturing	190.32 ±29.17	173.87(++) ±3.60		168.99(++) ±13.38
	Mature	522.74 ±8.82	408.35(++++) ±102.64	383.03(++++) ±113.31	441.32(++++) ±20.99

Superscript symbols indicate significance levels with respect to control. (+)$p>0.10$, (++) $p>0.05$, (+++) $p>0.01$ (++++) $p>0.001$, (-) insignificant. Measurements for 75 days not taken due to gravid stage.

and methyl parathion while carbofuran did not produce this effect. The maturing oocytes underwent slight reduction in size and deformity in shape in all treated groups. The yolk vesicles were damaged and interfollicular spaces increased.

The exposure to endosulfan, carbofuran, and methyl parathion produced repture of oocyte walls, and disintegration of cortical alveoli and yolk globules with maximum damage in endosulfan.

The GSI increased with time in all treated and control specimens. Reduction of GSI was observed on exposure to pesticides as compared to controls; maximum reduction was observed in methyl parathion, followed by endosulfan and carbofuran (Fig. 2).

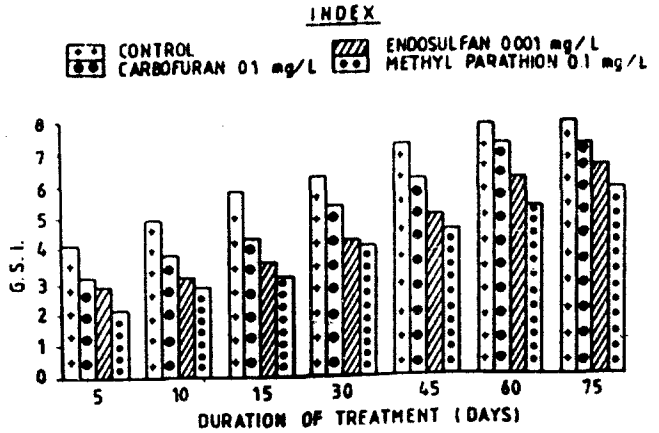

FIG.2 : GONADOSOMATIC INDICES (G.S.I.) IN CONTROL AND TREATED SPECIMENS OF RASBORA DANICONIUS.

Deleterious effects of pesticides have been reported in earlier studies such as delayed maturity (Crandall and Goodnight 1962), abortion in Gambusia (Boyd 1964), reduction in reproductive efficiency (Burdick et al. 1972) and decrease in the percentage of different stages of oocytes alongwith reduction in GSI (Kulshrestha and Arora 1984; Pandey and Shukla 1984; Singh and Sahai 1985). Notable features of the present study include severe damage to the peritoneal lining, vacuolation of cytoplasm in immature oocytes, damage to yolk vesicles in maturing oocytes and disintegration of cortial alveoli and yolk globules in mature oocytes.

Acknowledgments. The authors are thankful to the M.P. Council of Science and Technology, Bhopal, for financing the research project and to the Principal, M.V.M., Bhopal, for laboratory facilities.

REFERENCES

APHA (1985) Standard methods for the examination of water and waste water. Amer Pub Health Assoc New York

Boyd CE (1964) Insecticides cause mosquito fish to abort. Prog Fish Cult 26:138

Burdick GE, Dean HJ, Harris EJ, Seka J, Karcher R, Frisca C (1972) Effect of rate and duration of feeding DDT on the reproduction of salmonid fishes reared and held under control condition. N Y Fish Game J 19:97 - 115

Cairns J Jr., Foster NR, Loos TT (1967) Effect of sublethal concentration of dielbrin on laboratary populations of guppies Foecilia keticulata (Peters). Proc Acad Nat Sci Phil 119:75-91

Crandall CA, Goodnight CJ (1962) Effects of sublethal concentration of several toxicants on growth of common guppy Lebistes reticulatus. Limmon Oceanog 7:233-239

Harilal KS, Sahai YN (1986) Effects of carbaryl on the gonads of Heteropneustes fossilis Bloch. In: Dalela RC, Sahai YN and S Gupta (eds) Environment and ecotoxicology, Academy of Environmental Biology, Muzaffarnagar, pp 277-281

Kapur KK, Kaur K, Toor HS (1978) The effect of fenitrothion on reporduction of teleost fish Cyprinus carpio communis Linn A chemical study. Bull Environ contam Toxicol 20:438-442

Kulshrestha SK, Arora N (1984) Impairments induced by sublethal doses of two pesticides in the ovaries of a freshwater teleost Channa striatus Bloch. Toxicol Lett 20:93-98

Mani K, Saxena PK (1985) Effect of safe concentrations of some pesticides on ovarian recrudescence in the freshwater murrel Channa punctatus A quantitative study. Ecotoxicol Environ Safe 9:241-249

Pandey AK, Shukla L (1985) Ovarian recrudescence in a fresh water teleost Sarotherodon mossambicus J Environ Biol - 6(3):195-204

Singh S, Sahai S (1985) Effect of malathion on the immature ovary of Rasbora daniconius A histopathological study. Intl J Acad Ichthyol 6:11-15

Sahai S (1987) Toxicological effects of some pesticides on the ovaries of Punctius ticto (Teleosti). Proc 8th Annual Acad Environ Biol:21-29

Received April 2, 1989; accepted March 28, 1990.