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Comparative Sublethal Ovarian Pathology of Some Pesticides in the Teleost, *Puntius conchonius* Hamilton

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The literature provides a great deal of information on the acute toxicity of pesticides to non-target organisms, particularly fish. Currently, a greater emphasis is being laid on studying the sublethal effects of pesticides since their concentrations, which are not immediately fatal, are known to interfere with several life processes of fish (Kumar and Pant 1984a, 1985). Although some literature has accumulated on the sublethal histopathology of organochlorine and organophosphate pesticides in fish (Konar 1970; Pimental 1971; Lakota et al. 1978), yet very little is known about their injurious effects on the gonads (Eller 1971; Saxena and Garg 1978). The present paper embodies the comparative ovarian pathology of two organochlorine (aldrin and methoxychlor) and one organophosphate (monocrotophos) pesticides in sublethal concentrations in the freshwater teleost *Puntius conchonius*. The concerned pesticides are some of the most common aquatic pollutants.

MATERIALS AND METHODS

The sublethal ovarian pathology of the above mentioned pesticides was studied by long-term static bioassays, lasting 4 months. Live *P. conchonius*, 5 ± 0.4 cm long, collected from the local lake and acclimatized to laboratory environment for one week in tap water were used in this study. In view of the long duration of experiments, the fish were fed powdered bread cake on alternate days. The sublethal concentrations of the pesticides used in the present experiments (0.0466 µg/l for aldrin; 0.00746 µg/l for methoxychlor and 53 µg/l for monocrotophos) were equivalent to 1/3 of their 96-hr TLm (median tolerance limit) values for the concerned fish, estimated earlier (Pant 1982). Tap water was used as the dilution fluid and the bioassay media were changed every third day. The hardness of the bioassay media was 310 mg/l as $CaCO_3$, measured according to APHA (1975). For each pesticide, a separate group of 24 test fish was used and another group of fish of even number served as the control lot. The ovaries were collected from six specimens each belonging to experimental and control groups for histological examination at the end of 2,3 and 4 months of exposure. These experiments were run concurrent to those meant for evaluating the effects of sublethal heavy metal poisoning on the gonads of the same fish (Kumar and Pant 1984b) and had common controls.

The ovarian histology was examined after fixing the material in aqueous Bouin's fluid and staining 7 µm thick sections with hematoxylin-eosin (H&E). Since the histological changes were not atypical, the effect was studied as the variation in the number of oocytes on percentage basis. The classification of oocytes was the same as in Kumar and Pant (1984b). P-values for the differences between treated and control groups were calculated according to Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The concentrations of aldrin, methoxychlor and monocrotophos used in the present bioassays neither killed the fish nor caused them any visible stress over the entire experimental period of 4 months. The ovaries of *P. conchonius* did not show oocyte maturation beyond stage V in both the control and test specimens. However, in control fish, since there was a consistent fall in the number of oocytes of almost all the stages except stage 0-II with a corresponding rise in the number of atretic oocytes, it appears that active oogenesis did not occur under laboratory conditions (Table I).

The pesticides are reported to hamper reproduction both in piscine and non-piscine species. When the guppies were chronically exposed to endrin, there was a considerable reduction in the size of their second litter (Mount 1962). A concentration of endrin which produced only a partial kill in mosquitofish, resulted into abortion in female survivors when they were again subjected to the same concentration of this pesticide (Boyd 1964). Among the non-piscine forms, there was a significant reduction in egg production by the sublethal concentration of dieldrin in pheasants (Genelly and Rudd 1956) and a reduction in sperm production by DDT in bald eagles (Locke et al. 1966). However, there appears to be a great variability in the extent of gonadal poisoning by pesticides in fish. A chronic exposure of cutthroat trout to endrin, which resulted in an apparent damage to some oocytes, did not produce any change in the testis of this fish (Eller 1971). Lindane, which is toxic to many fish (Brown 1978), was found to be ineffective in inducing pathological derangements in the gonads of rainbow trout (Billard 1978).

The present bioassay experiments have shown that all the three pesticides viz., aldrin, methoxychlor and monocrotophos are severe poisons to the ovaries of P. conchonius even in sublethal concentrations. Although no atypical variations were noticed in the ovaries, yet the populations of oocytes of various developmental stages in all the experimental female fish had been significantly affected (Table I). There was a sharp and progressive increase in the population of atretic oocytes with all the three pesticides, but the maximum atresia level of 22.51% was observed following aldrin treatment for 4 months in contrast to the control value of 9.62% for the same period ($p < 0.01$). Intoxication of the fish Tilapia leucosticta with the sublethal concentration of Lebaycid (an organophosphate pesticide) is reported to induce total atrophy with almost no viable oocytes left in the ovaries of 90% of treated specimens (Kling 1981). Contrary to this condition, in present experiments, the atresia-inducing effects of all the tested pesticides were manifested selectively on different stages of oocytes. Stage V oocytes of P. conchonius appeared to be most vulnerable since, except in case of 2 month monocrotophos treatment, they were found to have totally vanished from the ovaries of all the test fish at all the observation intervals. Stage IV oocytes were more susceptible to methoxychlor and monocrotophos and their populations had been significantly decreased in comparison to their control counts ($p < 0.01$) after 3 and 4 months of exposure. After 4 months, they were not encountered at all in methoxychlor treated fish. On the other hand, the depletory effects on stage 0-II oocytes were exerted only by aldrin and not the other two pesticides. Stage III oocytes were interestingly not affected by any pesticide at any stage. Under similar conditions as in this study, the sublethal exposure of P. conchonius to three heavy metals had resulted in total disappearance of just one of the stages of oocytes (stage V) only in one case i.e. after 4 months exposure to lead (Kumar and Pant 1984b). The pesticides, therefore, appear to be stronger gonadal poisons than the heavy metals for this fish.

In general, the organochlorine and organophosphate pesticides are neurotoxic poisons (Ware 1978) but their mode of action with particular reference to the inducement of gonopathologies in fish is not clearly understood. So far as the gonopathologies of heavy metals in fish are concerned, they have been explained to be the results of either altered lipid metabolism and steroid

TABLE I

EFFECT OF SUBLETHAL PESTICIDE EXPOSURE ON THE OOCYTE POPULATIONS (%) IN *P. CONCHONIUS*

Time of exposure (in months)	Stages of oocyte development				
	Stage 0–II	Stage III	Stage IV	Stage V	Atretic oocytes
Control (2)	51.82±0.56	24.60±0.31	13.21±0.07	3.53±0.14	6.79±0.22
Control (3)	55.80±0.72	19.69±0.51	12.88±0.61	3.02±0.22	8.32±0.35
Control (4)	57.09±0.80	19.67±0.42	11.28±0.52	2.23±0.14	9.62±0.31
Methoxychlor (2)	53.53±0.85NS	25.64±0.97NS	10.24±0.75NS	Nil	10.55±0.60*
Aldrin (2)	48.68±0.91NS	24.64±0.67NS	13.13±0.53NS	Nil	13.51±0.42*
Monocrotophos(2)	54.80±0.84NS	22.57±1.34NS	9.81±0.23NS	2.17±0.32NS	10.59±0.48*
Methoxychlor (3)	57.69±1.51NS	23.70±0.88NS	4.27±0.47**	Nil	14.33±0.98*
Aldrin (3)	45.96±1.17**	22.62±2.80NS	11.60±0.47NS	Nil	19.82±3.12**
Monocrotophos(3)	56.35±1.33NS	22.63±1.10NS	5.92±0.54**	Nil	15.05±0.62*
Methoxychlor (4)	60.04±1.22NS	22.67±1.48NS	Nil	Nil	17.25±1.05**
Aldrin (4)	46.44±0.94**	21.77±1.14NS	9.23±0.41NS	Nil	22.51±1.05**
Monocrotophos(4)	55.17±1.58NS	23.06±1.03NS	3.29±0.73**	Nil	18.40±0.61**

Level of significance of the difference between oocyte number of control and pesticide-treated fish : NS, not significant; *, $p < 0.05$; **, $p < 0.01$

synthesis (Sangalang and O'Halloran 1974) or the suppression of the activity of pituitary gonadotrophs and somatotrophs (Ram and Sathyanesan 1983). However, on the basis of a selective damage of oocytes, a direct adverse action of zinc, copper and lead on the gonads of P. conchonius has been evidenced in our previous study (Kumar and Pant 1984b). The present experiments have also revealed that the populations of the various stages of oocytes are selectively damaged following exposure of the same fish to aldrin, methoxychlor and monocrotophos. The stage III oocytes were found to be totally resistant to these pesticides whereas all other stages were damaged to various degrees. This variability is possibly the result of a direct and selective action of pesticides on the oocytes of P. conchonius. Had the effects of pesticides been mediated by an alteration in steroid synthesis or in the secretion of pituitary hormones, a more or less uniform pattern of changes would have occurred in the ovaries of all the treated fish. The present study also shows that the organophosphate pesticides, which are generally considered to be milder than organochlorines, are also hazardous to fish over long exposures.

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REFERENCES

American Public Health Association (APHA) (1975) Standard methods for the examination of water and waste water, 14th Ed., Washington DC

Billard R (1978) Effect of heat pollution and organochlorinated pesticides on fish reproduction. In : Final reports on research sponsored under the first environmental research programme (indirect action). Commission of the European Communities (publ.), Brussels, Belgium, p 265

Boyd CE (1964) Insecticide causes mosquitofish to abort. Progr Fish-cult 26 : 138

Brown AWA (1978) Ecology of pesticides, John Wiley and Sons, New York

Eller BS Lafayette L (1971) Histopathologic lesions in cutthroat trout (Salmo clarki) exposed chronically to the insecticide endrin. Am J Pathol 64 : 321-336

Genelly RE, Rudd RL (1956) Effects of DDT, toxaphene and dieldrin on pheasant reproduction. Auk 73 : 529-539

Kling D (1981) Total atresia of the ovaries of Tilapia leucosticta (Cichlidae) after intoxication with the insecticide Lebaycid. Experientia 37 : 73-74

Konar SK (1970) Some effects of sublethal levels of heptachlor on rohu Labeo rohita (Ham.) J Inland Fish Soc India 2 : 51-54

Kumar S, Pant SC (1984a) Organal damage caused by aldicarb to a freshwater teleost Barbus conchonius Hamilton. Bull Environ Contam Toxicol 33 : 50-55

Kumar S, Pant SC (1984b) Comparative effects of the sublethal poisoning of zinc, copper and lead on the gonads of the teleost Puntius conchonius Ham. Toxicol Lett 23 : 189-194

Kumar S, Pant SC (1985) Renal pathology in fish (Puntius conchonius Ham) following exposure to acutely lethal and sublethal concentrations of monocrotophos. Bull Environ Contam Toxicol 35 : 228-233

Lakota S, Raszka A, Kupczak I, Hlond S, Stefan J, Roszkowski J (1978) The effect of methoxychlor and propoxur on the health of carp fry (Cyprinus carpio L.). Acta Hydrobiol 20 : 197-205

Locke LN, Chura NJ, Stewart PA (1966) Spermatogenesis in bald eagles experimentally fed a diet containing DDT. Condor 68 : 497-502

Mount DI (1962) Chronic effects of endrin on bluenose minnows and guppies. US Fish Wildlife Serv Res Rept 58 : 1-38

Pant SC (1982) Toxic and histopathologic impact of some pesticides, heavy metallic salts and pH on a teleost, Puntius conchonius Ham., Ph.D. Thesis, Kumaun University, India

Pimental D (1971) Ecological effects of pesticides on non-target species. Exec Off President Off Sci Technol Sup't Doc, Washington, p 220

Ram RN, Sathyanesan AG (1983) Effect of mercuric chloride on the reproductive cycle of the teleostean fish Channa punctatus. Bull Environ Contam Toxicol 30 : 24-27

Sangalang GB, O'Halloran MJ (1974) Adverse effects of cadmium on brook trout testis and on in vitro testicular androgen synthesis. Biol Reprod 9 : 394-403

Saxena PK, Garg M (1978) Effect of insecticidal pollution on ovarian recrudescence in the freshwater teleost Channa punctatus (Bl.). Ind J Exp Biol 16 : 689-691

Snedecor GW, Cochran WG (1967) Statistical methods. The Iowa State University Press, Iowa, USA

Ware GW (1978) The pesticide Book. Freeman WH and Company, USA

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