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Environmental Contamination and Toxicology

Effects of Endosulphan on the Hypothalamo Hypophysial Complex and Fish Reproductive Physiology

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Because of the nonspecific action, other than on insects, insecticides have been categorized as serious environmental pollutants (Thomas, 1971; Polunin, 1972; Konar, 1981; Jhingran 1982). There is growing evidence that the runoff insecticides pose problems to the water dwelling organisms (McKim 1975; Spehar, 1979). Hence, it is essential to work out the damages caused by the insecticides at lower concentration in the fish endocrine physiology in relation to fish reproduction and culture. This study therefore aims to assess the histophysiological lesions produced by endosulphan in the hypothalamo-hypophysial complex - ovarian axis in Sarotherodon mossambicus at a dose of 0.001 ppm, a para sublethal concentration.

MATERIAL AND METHODS

From a large samples of adult and healthy fishes a selection of S. mossambicus (9.2± 0.003 cm and 22.00± 0.001 g) was done for this study. Fishes were collected from the Government Fisheries Ponds, Ujjain (India). Fishes were transported and acclimatized for a fortnight in the laboratory after a wash in 0.001% $KMnO_4$ solution. The acclimated fish were seggregated into two batches containing 24 fish each. The insecticide endosulphan (6,7,8,9,10,10 - hexachloro - 1,5,5a,6,9, 9a - hexahydro - 6,9 - methano - 2,4,3 - benzadioxathiepin 3-oxide; Trade Mark: Thiodon '35' E.C., B.L. Industries, Jaipur, India) was dissolved in acetone and added to 20 l aquarium water for preparing 0.001 ppm concentration of insecticide. However, the LC50 for 96 hr was also worked out at 22.5°C and were found to be 0.01 ppm for S. mossambicus under roofed condition. The test concentration was prepared in terms of their commercial formulation available in the market and ready for field use. The equal quantity of acetone was added to control group (water) kept in identical conditions. The water of both these groups was changed on every third day with an aim to keep the fish in constant concentration of this insecticide (as far as possible). The physico-chemical analysis after (APHA 1975) of water of both the groups was done on the renewal days (Table 1).

Table 1. Physico-chemical factor of control and experimental group (20 days treatments)

S.No.	Treatment	Control Gr.	Endosulphan Gr.
1.	pH	8.00 ± 0.01	8.9 ± 0.015
2.	Temp. °C	22.5 ± 0.001	22.5 ± 0.001
3.	Dissolved oxygen mg/1	8.00 ± 0.11	5.4 ± 0.01
4.	Alkalinity mg/lit.		
	CO_3	8.00 ± 0.11	9.1 ± 0.02
	HCO_3	160.00 ± 0.53	165.0 ± 0.387
5.	Hardness mg/1	131.00 ± 0.001	136.0 ± 0.011
6.	Ca mg/1	20.00 ± 0.001	28.0 ± 0.0118
7.	K Factor	2.07 ± 0.001	2.7 ± 0.010

All values are expressed as ± SEM.

The experiment was continued for a period of 20 days. The weight and length of fish was recorded before decapitation for working out the condition factor (K) factor (Weatherlay, 1972) and also the weight of gonads for gonosomatic index (Pickford 1953). Immediately the brain with pituitary gland and ovaries were fixed in 10% neutral formalin, calcium formol and aqueous bouin's fluid for histology and histochemical studies. Routine procedures were followed for these studies. The sagittal paraffin sections of brain and pituitary were stained with Azan, PAS (McManus and Mowary, 1964) AF (Gomori's, 1941) and CAHP (Gomori's, 1950) and transverse section of the ovary were stained with Delafild haematoxylin-eosin and Azan. The 3β and 17β HSDH (steroid dehydrogenase activity) was studied in calcium formol fixed frozen sections of ovaries following the method of Watterberg (1958). The activity of the pituitary gland was assessed by calculating the area of cyanophils and acidophils (20 each in random selection) by Occulometer and tinctorial details. The diameter of nucleus preopticus (NPO) and nucleus lateralis tubaries (NLT) was also recorded. In the ovary, the percentage and diameter of oogonia, immature, maturing and mature oocytes were calculated and comparison of data was made between control and experimental groups by using Student's 't' test (Bencroft 1971).

RESULTS AND DISCUSSION

The pituitary gland in Sarotherodon mossumbicus is of platybasic nature with distinct teleostean organization of proximal pars distalis and rostral pars distalis pars intermedia and neurohypophysis (Figure 1).

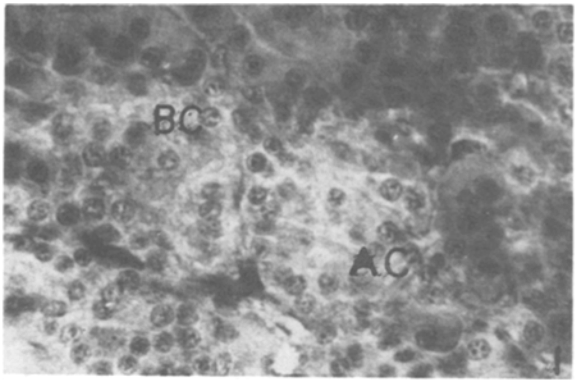


Figure 1 Control pituitary gland - showing basophils and acidophils in active state (X 390 Azan)

The cells of the different areas appeared without any deformities in shape size and cytoplasmic texture (Figure 2)

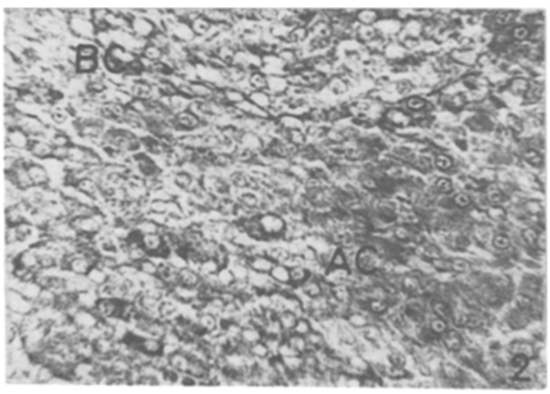
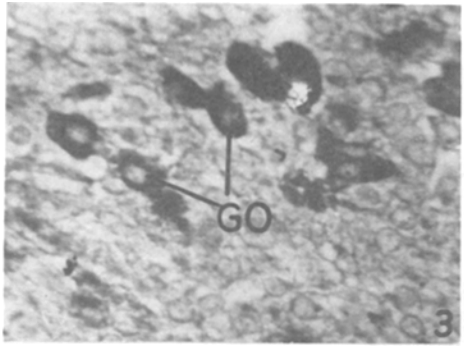


Figure 2 Endosulphan treated fish - showing degenerated basophils and acidophils (X 390, Azan).

The pituitary gland in control group contained well marked gonadotrops (GO) and thyrotrops (TH). These cells were compact. The pars intermedia had fair amount of neurosecretory material. However in endosulphan treated fishes the pituitary contained highly vacuolated gonadotrops (PAS +ve) and thyrotrops (AF +ve). The boundary of these cells were thick than in control animals. A few cells were completely devoid of the cytoplasm and bigger vacuoles were noticed in this gland (Figure 3 and 4). Both PAS +ve and AF +ve cells were affected by this insecticide (Figure 5 and 6). In few fishes the pituitary contained only



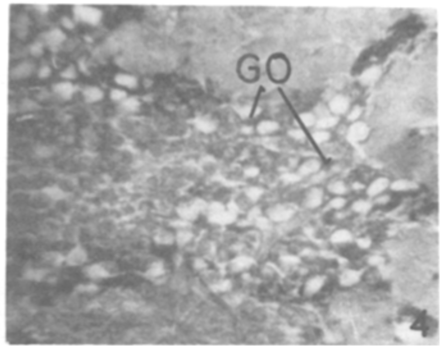
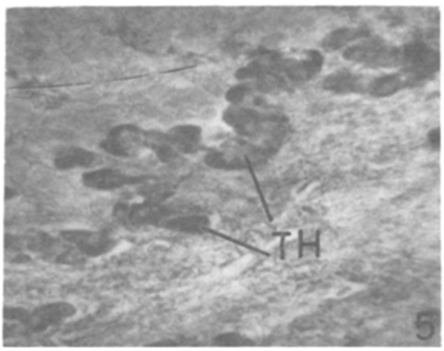


Figure 3 Control pituitary gland - showing PAS +ve gonadotrops (X390).
Figure 4 Endosulphan treated fish - showing vacuolated gonadotrops (X 390).



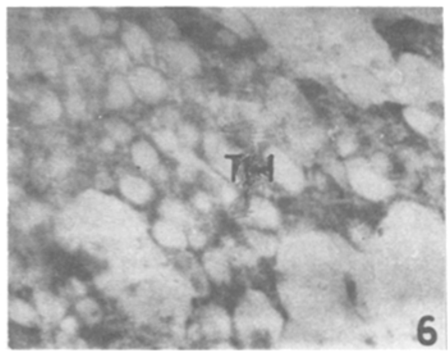


Figure 5 Control pituitary gland - showing AF +ve thyrotrops (X 390).
Figure 6 Endosulphan treated fish - showing vacuolated and degenerated thyrotrops (X390).

aggregate of nuclei. The nuclei of these cells were also damaged structurally and were turning pycnotic. The acidophils (AC) as well as the basophils (BC) exhibited for staining. Hypertrophied (HY) basophils (PAS and AF +ve) cells had significant ($p < 0.001$) size enlargement than those observed in controls (Table 2).

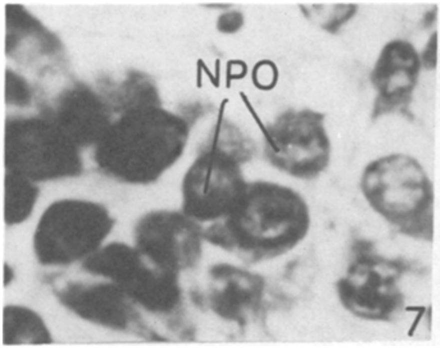
Table 2. Diameter (mean ± SE) of thyrotrops, NPO and NLT in control and endosulphan group.

S. No.	Treatment	Thyrotrops	Gonadotrops	NPO	NLT
1.	Control	6.38±0.09	8.38±0.002	14.50±0.094	13.38±0.01
2.	Endosulphan	21.38±0.01 ***	22.00±0.002 ***	38.00±0.76	20.00±0.38 ***

*** Significant level ($p < 0.001$).

Such changes were in pituitary cell types were observed by Shukla and Pandey (1984) of <u>Sarotherodon</u>

mossambicus after exposing to DDT, BHC and Malathion at 4 ppm concentration. The tinctorial characteristics of different cells were severely affected. The hypothalamic nuclei, the necleus preopticus (NPO) and nucleus lateralis tuberalis (NLT) of all treated fish exhibited deformities in their shape, size and cytoplasmic texture and nuclear structures as well (Figure 7,8,9,10). There was remarkable hypertrophy in these



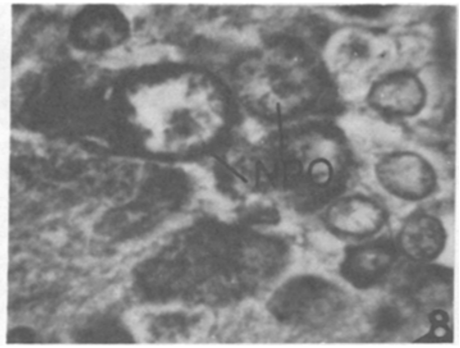
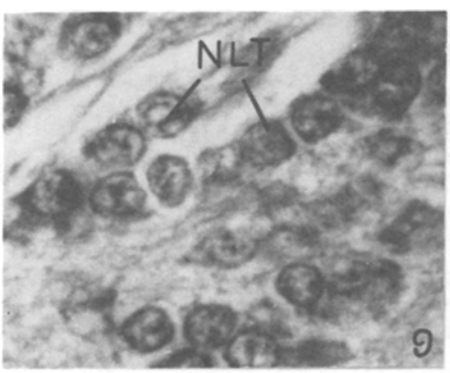


Figure 7.Control - nucleus preopticus with clearly visible cytoplasm and chromatin material (X870, CAHP):

Figure 8.Endosulphan treated fish - showing abnormally enlarged nucleus preopticus with degenerating cytoplasm and chromatin material (X870, CAHP).

cells (Table 2). The cytoplasm was granulated and vacuolated, whereas the nuclear diameter was still greater. The nuclear wall was broken and nuclei were disintigrating, Bare nuclei without cytoplasm were



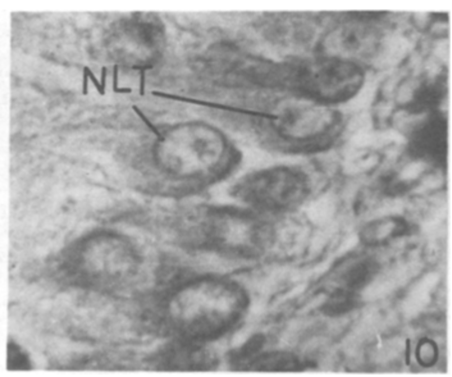


Figure 9 Control showing nucleus lateralis tuberalis in normal appearance (X870 CAHP)

Figure 10 Endosulphan treated fish showing vacuolised nucleus lateralis tuberalis (X 870 CAHP).

also evident especially in NLT. The cytoplasm revealed very poor staining and the axonal parts of these cells were lost. The depletion of the neurosecretion was also quite evident. Moreover, the neurosecretory material in pars intermedia was reduced in the insecticide exposed fishes. The axons of the neurohypophysis were deteriorated after this exposure. The neurosecretory material lost its granularity in the pars intermedia region of the pituitary gland (Figure 9 and 10). These observations add to the report of Pahari and Pahari (1979) in Heteropneustes fossilis, where they recorded the effect of endrin (0.2 ml) injection a clorinated insecticide on NPO.

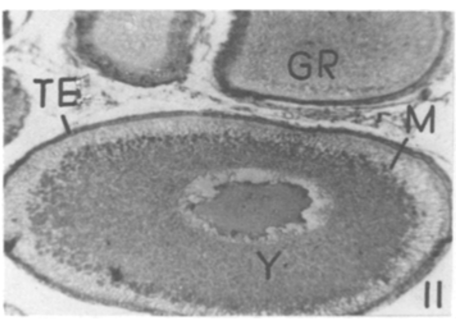
The ovary of endosulphan treated fishes revealed a

Table 3. Diameter of different oocytes in control and Endosulphan treated *S. mossambicus* (20 days treatment)

S.No.	Parameter	Control Gr.	Endosulphan Gr.
1.	peritoneal covering	0.197 ± 0.001	0.68 ± 0.07***
2.	Oogonia	15.87 ± 0.399	6.99 ± 0.33***
3.	Immature	51.00 ± 0.87	17.87 ± 0.187***
4.	Maturing	160.00 ± 20.2	89.2 ± 0.002***
5.	Mature	890.00 ± 41.00	311.00 ± 10.5 ***

All values are expressed in ± SEM; significant level *** ($p < 0.001$).

significant ($p < 0.001$) reduction in the gonosomatic index and showed flaccid and resorptive condition.



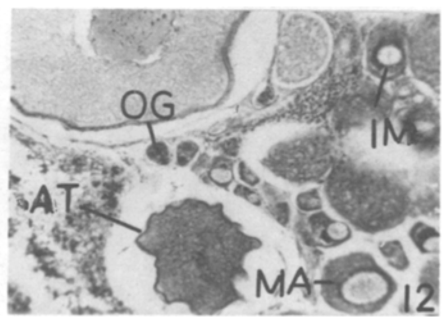


Figure 11 Control ovary - showing strong reaction for 17β HSDH activity (X 87).
Figure 12 Endosulphan ovary - showing negative reduction of 17β HSDH activity (X 87).

In the histological profile the peritoneal layer which gathered fibrous tissue and had thick appearance The spaces between the ovigerous folds were developed. The oogonia (OG), immature (IM) and maturing (MA) oocytes showed structural and cytological deformities.

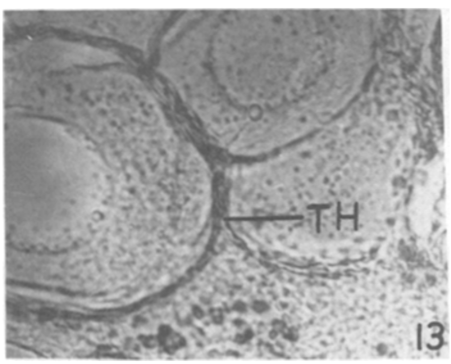
Shape of these oocytes was changed and cytoplasm was degenerated (Figure 11 and 12). The number of mature (M) oocytes was extremely reduced.

Table 4. Percentage of different oocytes in control and endosulphan treated S. mossambicus (20 days treatment).

S.No.	Parameters	Control Gr.	Endosulphan Gr.
1.	Gonosomatic index	1.827±0.75	0.287±0.28***
2.	Oogania	12.00 ±0.837	5.00 ±0.389***
3.	Immature	18.00 ±0.287	21.00 ±1.87(AT)***
4.	Maturing	30.00 ±2.8	18.00 ±0.20(AT)***
5.	Mature	34.00 ±2.11	6.00 ±0.0401(AT)***
6.	Atretic	6.00 ±0.21	50.00 ±2.97***

All values are expressed in ± SEM; Significant level *** ($p < 0.001$). AT = Atretic oocytes.

The yolk (Y) in these oocytes was clumped and cellular masses were more frequent. The diameter of different oocytes also showed a significant ($p < 0.001$) reduction. The theca (TE) and granulosa (GR) cells also exhibited deformities and most of the cells were clumped. The population of atretic (AT) oocytes was significantly ($p < 0.001$) greater than in controls (Table 4). These



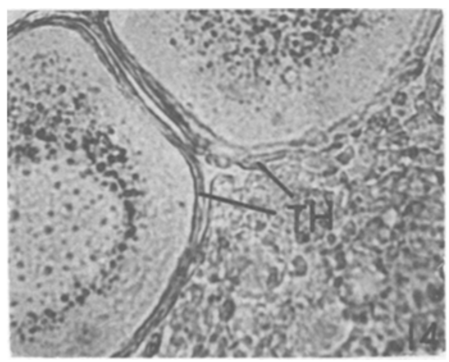


Figure 13 Control ovary - showing reaction for 3β HSDH activity (X 87)

Figure 14 Endosulphan treated ovary - showing negative response of 3β HSDH activity.

changes in the ovaries resemble with the reports on various pollutants in Promephalus promelase by carbaryl (Carlson 1972); in blunt minnow and guppies (Mount 1962) and in Lebistes reticulatus due to certain toxicants (Crandall and Goodnight, 1962); higher percentage of atretic oocytes, resorptive and flaccid ovaries in Channa punctatus after carbaryl and fenitrothion exposure (Saxena and Garg, 1978) and higher incidence of atretic oocytes in *Sarotherodon mossambicus* by malathion (Shukla *et al*., 1984). Due to endosulphan exposure the steroidogenesis was blocked as ovaries were negative to the 3 β or 17 β HSDH.

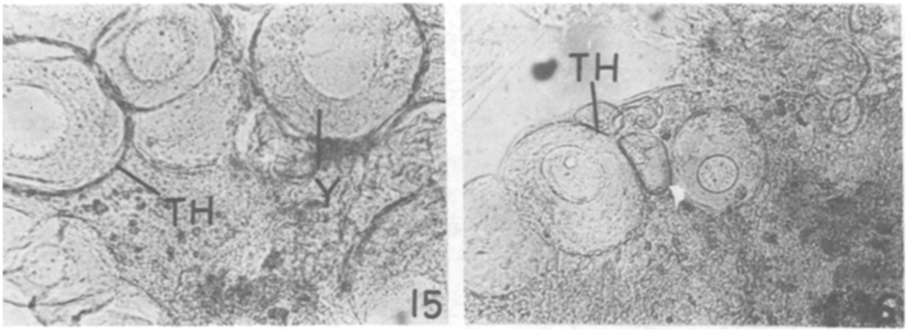


Figure 15 Control ovary - showing strong reaction for 17 β HSDH activity (X 87).
Figure 16 Endosulphan treated ovary - showing negative reaction of 17 β HSDH activity (X 87).

activities (Figure 13, 14, 15, 16). Whereas in unexposed fishes these enzymes gave positive tests. The changes in 3 β HSDH activity in ovary of *Cyprinus carpio* by fenitrothion were reported by Kapur *et al*., (1978), low level of cholesterol in the ovary, reduction in gonadotropin levels of pituitary and blood serum of *Heteropneustes fossilis* by cythion, hexadrin and malathion (Singh and Singh, 1980). The deformities in the hypothalanio - hypophysial complex and ovary may be due to inhibition of neurohormones as Pahari and Pahari (1979) have suggested or inhibition of endogenous gonadotropin(s) which regulate the gonadal physiology in fishes (Yamazaki and Donaldson 1968) as sufficient gonadotropin level is needed for the development and maintenance of mature oocytes (Sundararaj and Anand 1970). In this study it is suggested that the aquatic medium in which the fish lives, when it is contaminated with endosulphan (an organochlorine insecticide) it results in changes in oxygen contents and pH and also in other physico-chemical factors of water, probably these interfere with the reproductive mechanisms in this fish.

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