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Environmental Contamination and Toxicology

Impairment of Thyroid Activity in *Sarotherodon mossambicus* Due to DDT Treatment

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Involvement of fish thyroid in the altered metabolic rate and reproduction in response to organochlorine insecticide has not been well documented. A few reports regarding histopathological alterations in the thyroid gland in fishes due to BHC, gammaxen and folidol in Heteropneustes fossilis (Pandey and Khoche 1975) goitrous thyroid due to organochlorine insecticide in Coho salmon (Sonstegard and Leatherland 1976; Moccia et al. 1977), hypertrophy and goitrogenesis in Sarotherodon mossambicus (Pandey and Shukla 1983), pathological changes in Carassius auratus by endrin (Grant and Mehrle 1973), in Anabas testudineus by endrin (Kumar and Bhattacharya 1977), inhibition of thyroid peroxidase by industrial pollutants in Ophiocephalus punctatus (De and Bhattacharya 1976) and reduced thyrotropic potency of pituitary and serum thyrotropin in Heteropneustes fossilis by hexadrin and cythion (Singh and Singh 1980) are on record. However, this field still requires more work in view of the general application and variety of the insecticides so as to explain the histological and histochemical alterations of the thyroid activity, which may result differently by different chemical constituents. This study therefore was undertaken to know the histology and histochemical responses of the thyroid gland of Sarotherodon mossambicus to DDT (1,1,1-Trichloro-2,2-bis(p-chlorophenyl)ethane) at low concentration (0.001 ppm).

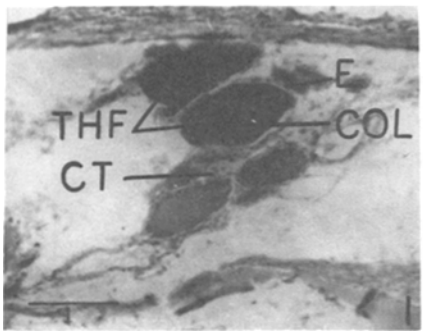
MATERIAL AND METHODS

The healthy and sexually mature, fifty Sarotherodon mossambicus (22.00±0.003 g and 9.2±0.002 cm) were brought from the local fisheries pond near the vicinity of Ujjain (India). The DDT (1,1,1-Trichloro-2,2-bis(p-chlorophenyl)ethane) 50% Tafarol, Rallis Comp. Ltd., Bombay, was dissolved in acetone and added to 20 liter dechlorinated laboratory supply water for

making 0.001 ppm. The solution was prepared in terms of active ingradients as available in the market. The test solution was renewed on every third day so as to maintain the chronic concentration throughout the experiment. On 10th day, the fishes were decapitated and their lower jaw was fixed in to aqueous Bouin's, 10% formalin and calcium formol. Before decapitation the weight and length of fishes were recorded for denoting the condition factor 'K' ($K = \frac{\text{weight of fish}}{(L)^3} \times 100$) after Weatherlay (1972). After decalcification (equal quantity of formic acid and sodium citrate solution), the lower jaw was washed thoroughly in running water and then dehydrated, embedded in paraffin wax, sectioned at 5 to 6 micron thickness and stained by haematoxylin-eosin, Mallory's triple stain, periodic acid Schiff (PAS) reagent (McManus and Mowary 1964), alcian blue (Humason 1967), toluidine blue (Humason 1967), Sudan Black B (McManus and Mowary 1964) and Luxol fast blue (Kuiver and Barrera 1953). The diameter (follicular and nuclear were determined (20 random samples) in control as well as experimental groups and difference if any, in their mean values was calculated by student's 't' test (Bancroft 1972). The E/T ratio (E/T = Height of thyroid epithelium/diameter of thyroid follicles) was calculated after Fortune (1956) in both the groups.

RESULTS AND DISCUSSION

The different thyroid follicles (THF) in DDT (0.001 ppm) treated fish exhibited well marked changes in their organisation and structure. The thyroid follicles became highly goitrous due to hypertrophy (HE) of the follicles whose diameter show a highly significant ($p < 0.001$) rise in comparison to control. The connective tissue (CT) between the thyroid follicles exhibited disintegrating condition. The epithelial cells (E) became low cuboidal, vacuolated and hypertrophied and their diameter was also greater. The nuclei of these cells were mostly inconspecuous due to insecticidal burden however, few of them were seen. Similarly, the colloid (COL) in the lumen of thyroid follicles was not uniformly distributed and appeared thick and vacuolated. However, at places it revealed liquified and thin texture. The acolloidal (ACOL) condition was also prominent in experimental fish. This degenerating colloid lost its characteristic eosin staining (Figure 1 & 2). Vascularization was poor in the treated animals compared with the controls maintained in the laboratory. The E/T ratio



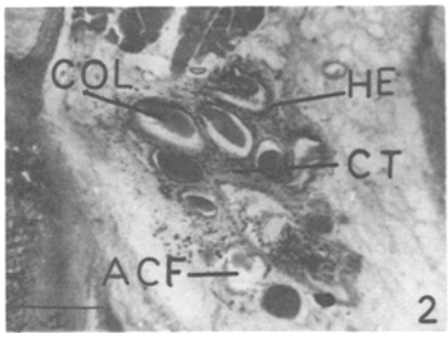


Figure 1. Control group-low epithelium, follicles with evenly distributed colloid (haematoxylin-eosin - X 87).

Figure 2. DDT group-hypertrophied epithelial cells Colloid is liquified and degenerating condition (haematoxylin-eosin - X 87).

of the follicles in treated fish was increased ($p < 0.001$) significantly (Table 1).

Table 1. DDT and thyroid activity in Sarotherodon mossambicus (10 days exposure)

S.No.	Follicular diameter μ	Nuclear diameter μ	E/T Ratio
1. Control	0.237±0.0062	0.0025±0.0035	0.272±0.0032
2. DDT	0.532±0.0002***	0.0049±0.0025***	0.59 ±0.0328***

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

In Mallory's triple stain, the colloid gave varying degree of reaction. Most of the follicles bore red and yellow coloured colloid. A few of them had di-or-tri coloured colloid. At places the colloid had peripheral red colour and the middle region was yellow. In other follicles the colloid was thick and completely red coloured. Multicoloured colloid in a single follicle was an interesting feature after insecticidal exposure of the fish (Figure 3 & 4). The population of these red and yellow colour bearing thyroid follicles were significantly ($p < 0.001$) higher than the control as well as than the blue coloured follicles. The E/T ratio of red and yellow follicles were significantly ($p < 0.001$) higher than that of corresponding follicles in controls. Interestingly, the active follicles had higher E/T ratio in experimental

animals than active ones (Table 2). In the histochemical tests (Table 3) the colloid in treated group

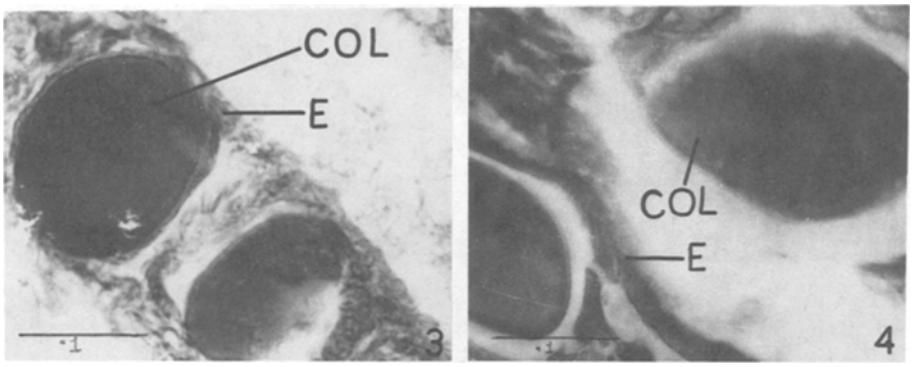


Figure 3. Control group: Mallory's triple stain reaction containing basophilic colloid (X 87).

Figure 4. DDT Group: Mallory's triple stain-enlarged and vacuolated and acidophilic (X 87).

Table 2. Percentage of blue, red and yellow follicles in S. mossambicus (10 days exposure)

S.No.	Type	Control	DDT
1.	Blue	35.00±1.96	9,00±0.0309***
2.	Red	2.00±0.001	28.00±0.327 ***
3.	Yellow	4.85±0.67	12.00±0.09 ***

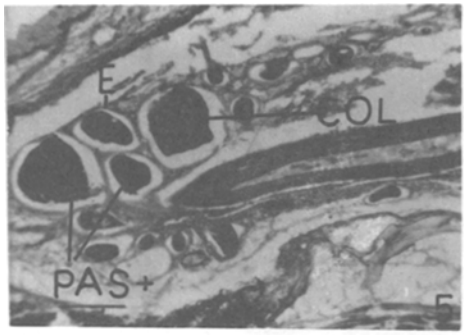
All values are expressed in ± SEM.
Significant level *** ($p < 0.001$).

Table 3. E/T ratio of blue, red and yellow follicles S. mossambicus and condition factor in control and experimental water.

S.No.	Type	Control	DDT
1.	Blue	0.499±0.112	0.21±0.0032 ***
2.	Red	0.012±0.001	0.138±0.0032***
3.	Yellow	0.011±0.001	0.062±0.009 ***
4.	Condition factor	2.68 ±0.0011	2.23 ±0.012

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

was negative to weak whereas the epithelium presented moderate to weak reaction with PAS (Figure 5 & 6) was noted. The alcian blue reaction was also negative both colloid and epithelium (Figure 7 & 8). The toluidine blue test was also negative in both colloid as well as negative in treated fish (Figure 9 & 10).



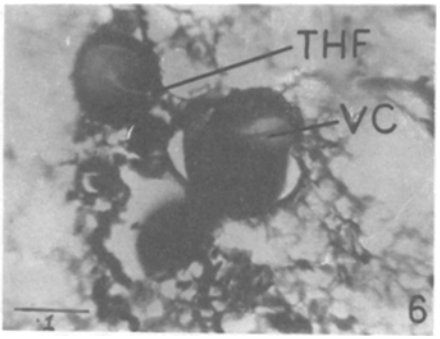
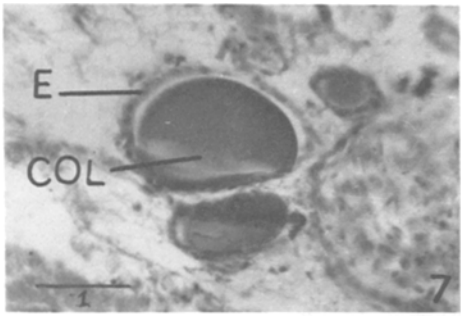


Figure 5. Control group: showing PAS reaction weak intensity in epithelium while strong in colloid (X 87).

Figure 6. DDT group: exhibited negative PAS reaction in epithelium and colloid (X 87).



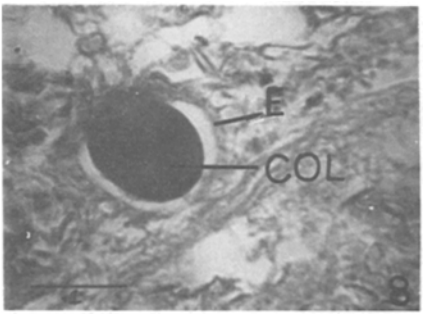
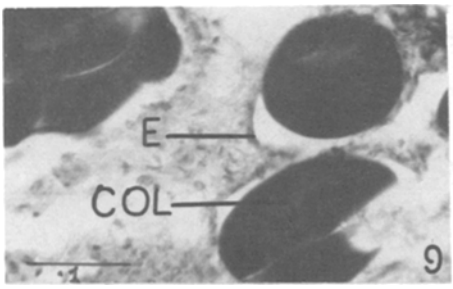


Figure 7. Control group: showing Alcian blue reaction epithelium give weak reaction while colloid exhibits strong reaction (X 87).

Figure 8. Figure 8 DDT group: Showing weak reaction in epithelium and colloid turn yellow (X 87).



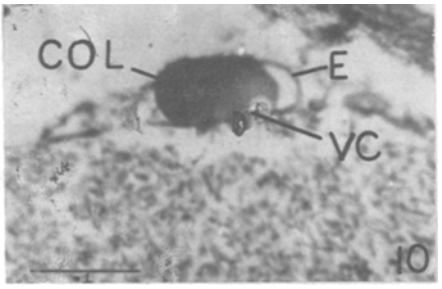
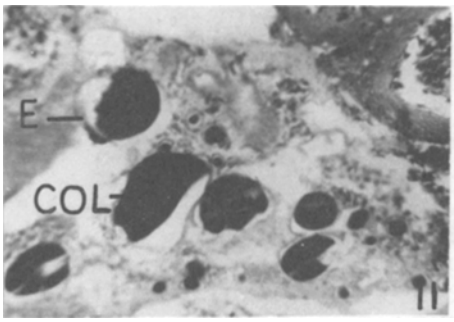


Figure 9. Control group: Showing toluidine blue test epithelium remains negative and colloid gave strong reaction (X 87).

Figure 10. DDT group: Showing negative reaction in epithelium and colloid (X 87).

The DDT treated group exhibited depleted lipid contents compared with controls. The Sudan Black B tests revealed a negative staining in epithelium and colloid (Figure 11 & 12), while Luxol fast blue reaction in



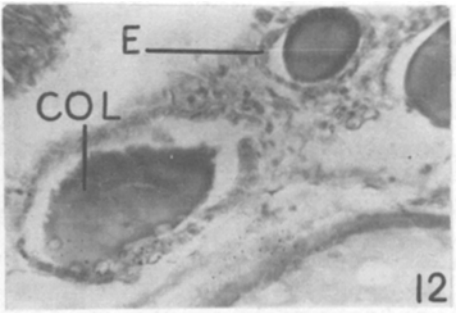
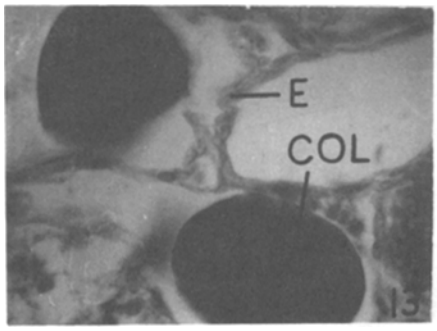


Figure 11 . Control group: Sudan Black B weak reaction in epithelium and colloid gave strong reaction (X 870).

Figure 12 . DDT group: Sudan Black B negative reaction in colloid and epithelium (X 87).

the colloid gave yellow colour instead of blue as in the control and epithelium exhibited absence of phospholipid (Figure 13 & 14).



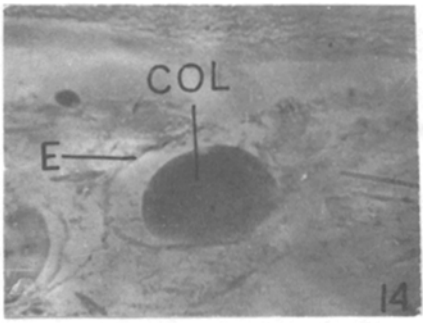


Figure 13 Control group: Luxol fast blue reaction, strong reaction in the epithelium and colloid (X 87).

Figure 14 DDT group: Luxol fast blue reaction weak in the epithelium and colloid turned yellow (X 87).

Although the harmful effects of chlorinated hydrocarbons on the endocrine physiology are well known in fishes (Shukla and Pandey 1978; Pandey and Shukla 1980, 1982, 1983 and Singh and Singh 1980) but studies on the histological and histochemical aspects of

thyroid of teleost in this reference are scanty. Pandey and Sukla (1983) reported that malathion (an organophosphorus insecticide) works as an antihyroidal compound in Sarotherodon mossambicus. In the present study, DDT appears to produce the antagonistic effects in the thyroid in this fish. The results have similarity to the characteristics of thyroidectomy, therefore it is evident that DDT interferes with the thyroid physiology. It is in support of the earlier reports of hypothyroidal activity of some insecticides (Pandey and Khoche 1975). Also industrial pollutants have been recorded to cause the influence on peroxidase activity in thyroid gland of Ophiocephalus punctatus (De and Bhattacharya 1976). Singh and Singh (1980) also reported suppressed thyrotropic secretion from the pituitary gland of Heteropneustes fossilis after cythion and hexadrin exposure. These workers suggested that the insecticides probably retard the hypophysial TSH out put followed by a hyperthyroidal activity. As the histological examination of thyroid gland after insecticidal treatment is sparse, present study clearly demonstrates that lower concentrations where no mortality occurred and condition factor remains unaltered (Table 3). DDT can interfere with the thyroidal structure and physiology of this fish. However, at this stage it is difficult to explain precisely, the role of insecticide in the inhibition of thyroid function, but more histological, histochemical and biochemical studies will depict the facts.

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