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Environmental Contamination and Toxicology

Dimethoate Toxicity to Gestational Embryonic Ovary of a Live Bearing Fish, *Lebistes reticulatus*

Y. V. Ramana,[1] A. K. Pandey,[2] and Sumati Singh[2]

[1]Jaydee Agrochemicals Private Ltd., Road No. 8, V. K. Industrial Area, Jaipur-302013, India and [2]Endocrinology and Environmental Physiology Unit, School of Studies in Zoology, Vikram University, Ujjain-456010, India

Although reproduction is an important aspect of all living beings for the survival and continuity of race, the increasing environmental pollution acts as an inhibitor of the gonadal activities in fishes (Billard et al. 1981a; Donaldson and Scherer 1982; Pandey and Shukla 1985). Therefore the reproductive toxicity of pollutants pose danger to fisheries and fish propagation as well. The present study is aimed to evaluate the effects of an organophosphorus insecticide, dimethoate, on the gonads of gestational embryos, which were developing intrafollicularly in the female guppy, Lebistes reticulatus - a live bearing fish.

MATERIALS AND METHODS

Gravid adult female guppies, Lebistes reticulatus, 2.4 ± 0.18 cm size and 0.28 ± 0.007 gm weight were collected from local ponds and maintained under laboratory conditions in glass aquaria (46 x 32 x 23 cm) for 10 days. Dimethoate, 30 EC (Rallis India Pvt. Ltd., Bombay, India, under the trade name Rogor), an organophosphorus insecticide having molecular weight 229.2 was dissolved in water for preparing 1 mg/L concentration after calculating LC 50 and LC 100 values as 4.64 and 6.23 mg/L, respectively, at 23°C. The control fish were maintained under similar conditions without addition of dimethoate. Dimethoate forms a clear solution in water without precipitation or suspension. A total of 30 fishes were taken for the present experiment and were fed on commercial fish food every alternate day. The experiment was continued for a period of 21 d only and the embryos were dissected out from the gravid female ovaries of both control and treated adult fishes on 7, 14 and 21 d of

Correspondence author - Y.V.Ramana

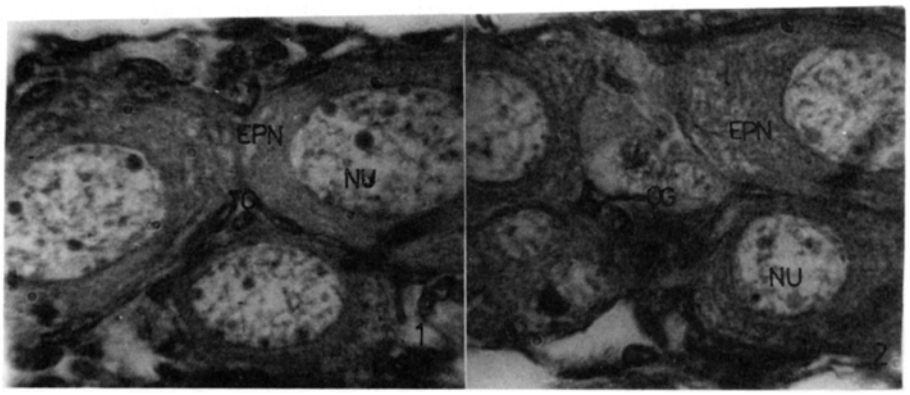
the treatment. The whole embryos were fixed in aquous Bouin's and 10% formalin solutions, dehydrated in graded alcohols, embedded in paraffin wax, sectioned at 5u and stained with Heidenhein's azan and haematoxylin -eosin stains. Statistical analysis of data was done and values were compared with Student's 't' test for significant (P 0.05) changes.

RESULTS AND DISCUSSION

In the embryos of control fish, the ovaries were minute and had different stages of oocytes. Oogonia (OG), chromatin nucleolus (CN) and early peri-nucleolus (EPN) stages were disposed normally. The ovary was small and fully packed with oocytes without any spaces or vacuoles. The ovarian wall was thin. Nuclei were distinct in all the oocytes and oogenetic stages (Fig. 1 and 2). The oocytes of chromatin nucleolus stage were seen clearly with well spread chromatin and a nucleolus. Cytoplasm was clear and without any abnormalities. Ovary appeared normal and healthy in shape, size and contents. The theca cells (TC) appeared normal with clear nucleus. There was no differentiation of seminal receptacle in the embryonic ovaries. The ovarian cavity and ovigerous folds were not seen in these embryonic ovaries.

The ovary of the developing embryos of the fishes exposed to the insecticide was packed with different oocytes. In the oogonia the nucleus was not clear and nuclear wall broken. Spaces appeared in the nucleus of chromatin nucleolus stage and early peri-nucleolus stage oocytes. Nucleoli were not seen in a few oocytes and cytoplasm was seen normal (Fig. 3). The diameters of oogonia, chromatin nucleolus and early peri-nucleolus stage oocytes were significantly reduced ($P < 0.05$). Theca cells increased in size significantly ($P < 0.05$) in these treated ovaries (Table 1). However, there was no space developed in between the different oocytes in the ovary.

The number of oogonia in the ovary of embryos of the 14 d treated fishes decreased. Chromatin nucleolus and early peri-nucleolus stage oocytes were still prominently seen. The nucleus showed spaces due to the disturbance in the chromatin arrangement. The nuclear wall was broken and the cytoplasm showed vacuolization and became thin and granular in appearence (Fig. 4). Of all the stages the oogonia were most severely affected. The diameter of different oocytes was significantly ($P < 0.05$) decreased (Table 2). The thecal cells



Figures 1 and 2. Control ovary with oogonia, chromatin nucleolus and early peri-nucleolus stages with clear nuclei and cytoplasm (x1000).

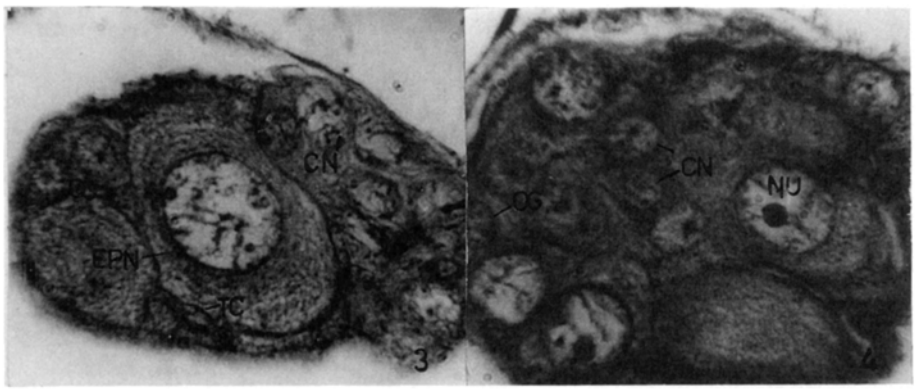


Figure 3. 7 d treated ovary and Figure 4. 14 d treated ovary with vacuolated cytoplasm and nuclei in oogonia and chromatin nucleolus stages (x1000).

increased in their size significantly ($P < 0.05$) with vacuolization in the cytoplasm. However in these cells the nucleus appeared affected. The ovarian wall thick and the ovary was shrunken.

21 days exposed ovaries of the embryos showed severe damages in different oocytes and also in their general structure. The nuclear wall was broken at several places in both the chromatin nucleolus and early peri-nucleolus stage oocytes and cytoplasm was not clear with vast spaces developed in it. Whereas oogonia are not detectable in this treatment due to severe

damages caused by the insecticide. Nucleoli were seen unaffected. The wall of the oocytes was broken and disappeared (Fig. 5). The ovarian wall became thick and ovary was shrunken.

Table 1. Dimethoate effect on the diameter of oocytes of embryo of Lebistes reticulatus (after 7 d)

	Control	Treated
Oogonia (um)	14.52 ± 0.30	13.37 ± 0.39
Chromatin Nucleolus Stage (um)	32.60 ± 0.65	31.10 ± 0.54
Early peri-Nucleolus stage (um)	37.40 ± 0.50	35.10 ± 0.54
Theca cells (um)	2.58 ± 0.06	2.77 ± 0.07

All values are expressed as mean ± SE
Significant level $P < 0.05$.

The diameter of chromatin nucleolus and early peri-nucleolus stage oocytes was significantly ($P < 0.05$) decreased and the theca cells were not seen (Table 3).

Table 2. Dimethoate effect on the diameter of oocytes and theca cells in the embryo of Lebistes reticulatus (after 14 d)

	Control	Treated
Oogonia (um)	14.70 ± 0.50	12.52 ± 0.60
Chromatin Nucleolus stage (um)	33.84 ± 0.66	30.81 ± 0.75
Early peri-Nucleolus stage (um)	38.12 ± 0.62	34.20 ± 0.60
Theca cells (um)	2.63 ± 0.06	3.04 ± 0.05

All values are expressed as mean ± SE
Significance level $P < 0.05$.

The present study indicates that dimethoate at 1 mg/L concentration after 21 d of exposure retards the ovarian activity and development of different oocytes in

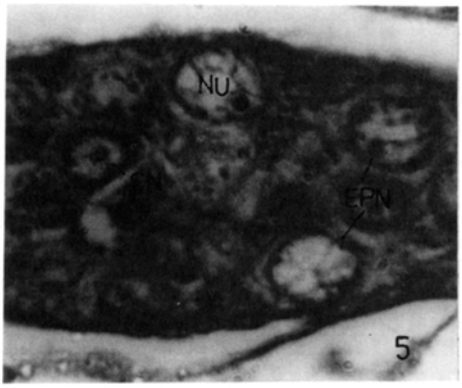


Figure 5. Ovary exposed 21 d with severe damage in various stages of oocytes (x1000).

Table 3. Dimethoate effect on the diameter of oocytes and theca cells in the ovary of Lebistes reticulatus (after 21 d)

	Control	Treated
Oogonia (um)	14.40 ± 0.43	ND
Chromatin Nucleolus stage (um)	34.22 ± 0.78	28.92 ± 0.60
Early peri-Nucleolus stage (um)	39.18 ± 0.63	33.46 ± 0.59
Theca cells (um)	2.62 ± 0.05	ND

All values are expressed in mean ± SE
Significance level $P < 0.05$. ND - Not detectable.

the intrafollicularly developing embryos. The nucleus of the various stages of oocytes was more sensitive to the treatment. It is also revealed that the larger the oocytes, the lesser the effect in the nucleus. In longer exposure the oocyte cytoplasm also was damaged. The ovaries shrunk in the insecticide exposure perhaps due to dehydration. Probably these features developed because of the absense of ovarian steroids which maintain the normal functions of ovary (Yamazaki and Donaldson 1968; Sunderraj and Anand 1970, 72). Shukla et al. (1984) reported that malathion at sublethal concentrations affects the ovarian histophysiology in Sarotherodon mossambicus. Saxena and Arora (1984)

studied the impairments induced by sublethal doses of two pesticides in the ovaries of a fresh water fish, Channa punctatus. Takashi et al. (1986) observed that aminoguanidine when injected into hen's eggs at the earlier incubation stages induced retardation of the development of embryos and aplasia of gall bladder without high mortality. The effect of cadmium on placental structure and its relation of fetal malformations in the mouse was studied by Padmanabhan (1986), and it was reported that subcutaneous administration of cadmium chloride into pregnant mice at 7 d of gestation resulted growth retardation, resorption, death, and morphological abnormalities of fetuses and reduction in the placental weight and suggested that cadmium chloride may exert its effect by interfering with normal placental function. Shahzad and Nasim (1987) reported that dimecron a commonly used insecticide when injected into chick embryos at doses ranging from 0.05 to 2.00 mg/egg resulted in gross morphological abnormalities in kidney and liver. With the available reports on the effect of certain chemicals on the embryos of chick and fetuses of mice, it is evident that dimethoate inhibits and affects the development of ovaries of embryos which were developing intrafollicularly. This study clearly indicates that in Lebistes reticulatus although the development is intrafollicular, no placental barrier to organophosphorus insecticide to prevent such hazardous effects of these insecticides. In view of these observations, the use of such chemicals needs careful manipulation to avoid damages to the fisheries and fish population.

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REFERENCES

Billard R, Bry C, Gillet C (1981a) Stress, environment and reproduction in teleost fish. In: "Stress and Fish", Pickering AD (ed) Academic Press, New York, pp 185-208

Donaldson EM (1975) Physiological and physico-chemical factors associated with maturation and spawning. EIFAC Tech. Pap. 25: 53-71

Donaldson EM, Scherer E (1982) Methods to test and assess effects of chemicals on reproduction in fish. Proc. Workshop Methods Assess. Effects Chem. Reprod. Funct. Biota 1981 (In press)

Padmanabhan R (1986) Effect of cadmium on placental structure and its relation to foetal malformations in the mouse. Z Mikrosk-anat Forsch Leipzig 100 (3): 419-427

Pandey AK, Shukla L (1985) Effects of pesticides on freshwater fishes. Indian Rev Life Sci 5: 3-34

Saxena M, Arora N (1984) Impairments induced by sublethal doses of two pesticides in the ovaries of a fresh water teleost, Channa punctatus (Bloch). Proc 70th Session of Indian Sci Cong pp 90

Shahzad MA, Rubina Nasim (1987) Avian embryo toxicity of dimecron, a commonly used insecticide. Biologia (Lahore) 33 (1): 109-120

Shukla L, Shrivastava A, Merwani D, Pandey AK (1984) Effect of sublethal malathion on ovarian histophysiology in Sarotherodon mossambicus. Comp Physiol Ecol 9(1): 13-17

Sunderraj BI, Anand TC (1970) Ovarian maintenance in the hypophysectomized cat fish, Heteropneustes fossilis with pituitary and steroid hormones. In: Martini L (ed) Inter Cong Hormonal Steroid

Sunderraj BI, Anand TC, Donaldson EM (1982) Effects of partially purified salmon pituitary gonadotropin on ovarian maintenance, ovulation and vitellogenesis in the hypophysectomized cat fish, Heteropneustes fossilis (Bloch) Gen Comp Endocrinol 18:102-114

Yamazaki N, Donaldson EM (1968) The spermiation of gold fish (Carassius auratus) as bioassay for solmon Onchorhynchus tshawytwchia gonadotropin. Gen Comp Endocrinol 10: 383-391

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