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Effects of Chlordecone on the Gonads of Freshwater Catfish, *Heteropneustes fossilis*

Arun K. Srivastava,[1] Anil K. Srivastava[2]

[1]Department of Zoology, S. M. M. Town P. G. College, Ballia-277001, India
[2]Department of Zoology, University of Gorakhpur, Gorakhpur-273009, India

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The pesticide chlordecone (commercial name, kepone; decachloro-octahydro-1,3,4-metheno 2H-cyclobuta pentalen-2-one; CD) is extensively used in controlling agricultural pests. It causes arrest of sperm maturation (Anderson et al. 1976) and blocking of reproductive function (Guzelian 1982) as well as a variety of 'estrogen-like' effects on the female reproductive system in many birds and mammals (Eroschenko and Palmiter 1980; Eroschenko and Becker 1984; Eroschenko and Osman 1986).

No information is available regarding histological impact of CD on fish gonads. The present study deals with the histological changes in the gonads of a freshwater catfish H. fossilis induced by CD on acute, sub-acute and sub-lethal concentrations at different time intervals.

MATERIALS AND METHODS

The fish, H. fossilis (length 15.50±1.75 cm) were collected locally from a freshwater lake and acclimatized for 15 d under laboratory conditions at ambient temperature (20–22°C) and natural photoperiod. (11–12 hr). They were fed daily with flour pellets and ground dried shrimp; each pesticide concentration as well as the water of the fish were renewed daily. Food was withheld 24 hr before the experiments. The physico-chemical properties of the tap water used were: pH 7.1–7.3, DO_2 7.7–7.9 mg/L, CO_2 4.4–4.7 mg/L, alkalinity 125–128 mg/L as $CaCO_3$, hardness 140–145 mg/L as $CaCO_3$ and chloride 7.7–8.2 mM/L.

A static acute toxicity bioassay (APHA et al. 1981) has been performed to determine the 96-hr LC50 value (Litchfied and Wilcoxon, 1949) of CD; This value for the catfish has been found to be 0.24 mg CD/L (Srivastava and Srivastava 1987). A stock solution of CD (1mg/mL) was prepared in acetone. For the study of the effect of the CD on histology of gonads, groups of 20–25 fish (5 fish/15-L glass jar) were exposed to acute 0.048 mg CD/L (1/5th of the 96-hr LC50 value), each of

Correspondence to: Arun K. Srivastava

sub-acute 0.024, 0.012 mg CD/L (1/10th and 1/20th of 96-hr LC50 value) and sub-lethal 0.008 mg CD/L (1/30th of 96-hr LC50 value) concentrations for 96-hr, short (10-20 d) and long (30-60 d) term. Parallel groups of each control 5 fish, kept in tap water and receiving equal aliquots of acetone as the treated fish were sampled at specified time intervals for comparison with the exposed fish. Mortality did not exceed 10% in any case.

Autopsy was performed at the end of the experiments. Fish of both the groups were anesthetized with MS (Tricaine mathanesolfonate). Their gonads were extirpated and fixed in aqueous Bouin's fluid. Sections were cut at 4-6 μm after routine paraffin method and stained with hematoxylin/eosin.

RESULTS AND DISCUSSION

The gonads were taken out during the prespawning period. Testes were in advanced stages of spermatogenesis (Fig.1) and ovaries were characterized by oocytes in various stages of development (Fig.5).

Extensive damage in the testes was caused on exposure of fish, both acute as well as short and long term sub-acute to CD (Fig. 2,4). Seminiferous tubules showed flattened, reduced and at places, degenerated and desquammated germinal epithelium and thin intertubular connective tissue. Spermatids and sperms showed cytolysis. The interstitial tissue showed atrophied and vacuolized Leydig cells. Leucocyte infiltration was observed in various parts of the testes. The sub-lethal concentration of 0.008 mg CD/L failed to elicit any marked change in the testes from 10-60 d. Apparent alteration took place in the ovary during long term higher sub-acute (0.024 mg CD/L) exposure. There was significant reduction in the diameter of oocytes of stages I, II and III. Prominent interfollicular spaces were observed in the ovary which probably formed due to shrinkage of the oocytes. A large number of atretic follicles were also observed (Fig. 6). The ovarian histology was not affected during either the acute or sub-lethal concentrations at short and long terms exposures to CD.

No information is available on the deleterious effects of CD on the testicular architecture or on the steroid-secreting cellular site in teleost fish. The observations pertaining to the impact of CD intoxication on the testis of H. fossilis in the present study are similar to those resulting after CD administration to birds and mammals(Gazelian 1982). CD has been shown to promote estrogenic activity in animals (Eroschenko and Palmiter 1980). Eroschenko and Wilson (1974) reported that the testes from maturing and fully grown CD fed quail were severely affected. Generally, there was a reduction of the germinal epithelium thickness and number of the spermatozoa. In

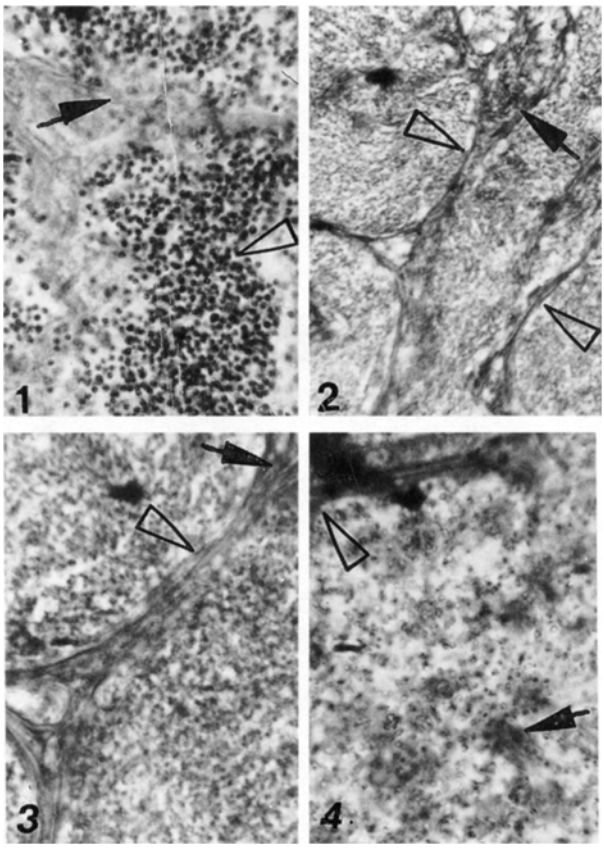

Figure 1. Testis of control catfish showing intense spermatogenic activity (arrowhead) and interstitial Leydig cells (arrow) H/E x 400.

Figure 2. Testis of catfish exposed for 96-hr to acute level (0.04 mg CD/L) showing flattened germinal epithelium (arrowhead), spermateleosis in the lobules; necrosis and vacuolation in interstitial Leydig cells (arrow). H/E x 400.

Figure 3. Testis of catfish exposed for short-term (20 d) to sub-acute level (0.024 mg CD/L) showing flattened germinal epithelium (arrowhead) and necrotic interstitial Leydig cells (arrow). H/E x 400.

Figure 4. Testis of catfish exposed for long-term (60 d) to sub-acute level (0.012 mg CD/L) showing damaged germinal epithelium; necrosis (arrowhead) spermatoleosis and clumping of germ cells in the tubules (arrow). H/E x 900.

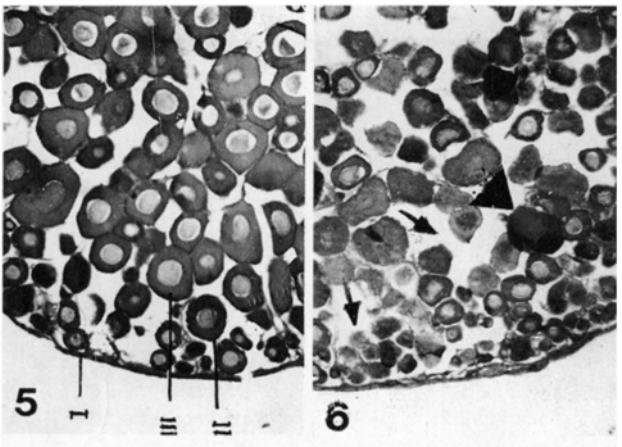

Figure 5. Ovary of control catfish showing various stages (I-III) of oocyte. H/E x 40.

Figure 6. Ovary of catfish exposed for long-term (60 d) to sub-acute level (0.024 mg CD/L) showing atretic follicles (arrowhead) and marked interfollicular spaces (arrow). H/E x 40.

addition, the seminiferous tubules from testes of CD fed birds contained desquammated and abnormal cellular elements. Thus, part of the detrimental effect of CD on the histology of the testes of the catfish could be due to its estrogenic action. It has been shown that estrogen and estradiol benzoate reduced testes size, depressed spermatogenesis and partially inhibited gonadal growth in avian species (Lofts and Murton 1973).

Marked degenerative changes in the ovary of the catfish were observed during the short and long-term exposure to CD. These changes included prominent inter-follicular spaces, reduced growth of oocytes and appearance of atretic follicles. Singh and Singh (1980) reported that cythion/hexadrin (pesticide) arrested the ovarian activity in catfish H. fossilis. The mechanism whereby CD arrests gonadal activity in H. fossilis may be attributed to impaired nucleic acid (RNA and DNA) synthesis (Saxena et al. 1986). CD ingestion by sexually immature quail produced estrogen like effects and increased reproductive tract maturation, cytodifferentiation, ciliation, tubular gland formation and secretary activities in certain cells comprising the magnum and shell gland regions of the oviduct (Eroschenko and Hackman 1981; Eroschenko and Becker 1984). CD has also been shown to alter reproductive capacities of both male and female rats (Desaiah 1984) and it has been

shown to be potent mitochondrial poison in both mammalian and piscine tissues where it inhibits cellular energy production and interferes with toxic transport across memberane (Desaiah 1982). Therefore, mitochondrial damage may also explain the ovarian impairments reported in H. fossilis in this study after CD exposure.

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