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Toxic Effects of Mercuric Chloride, Methylmercuric Chloride, and Emisan 6 (An Organic Mercurial Fungicide) on Ovarian Recrudescence in the Catfish *Clarias batrachus* (L.)

R. Kirubagaran and K. P. Joy

Centre of Advanced Study in Zoology, Banaras Hindu University, Varanasi–221 005, India

Mercury (Hg) is a ubiquitous, highly toxic heavy metal which is bioconcentrated upthrough the food chain. Despite our extensive knowledge of its toxicity to living organisms and repeated incidences of Hg poisoning, widespread contamination of Hg remains a serious environmental hazard (Wren 1986). In India alone, about 180 tons of Hg are introduced into the environment every year (Chaudry 1980) which ultimately reach the aquatic systems. In fishes Hg is accumulated in the form of methylmercury (World Health Organization 1976). Mercurial toxicity in fishes has been focused mainly on tissue uptake and subcellular distribution (McKim et al. 1976; Olson et al.1978); nephrotoxicity (Trump et al. 1975); development, hatching and survivability of young ones (Armstrong 1979; Sharp and Neff 1980) and teratology (Weis and Weis 1977). Very few studies have been attempted to investigate Hg toxicity on gonadal activity of fishes throughout the breeding season (Ram and Sathyanesan 1983,1986). In a previous investigation we have studied the toxic effects of mercuric chloride ($HgCl_2$), methylmercuric chloride (CH_3HgCl) and emisan 6 (an alkoxyalkyl fungicide) on the survival and histology of the kidney of the catfish, Clarias batrachus (Kirubagaran and Joy 1988). The present report deals with toxic effects of these mercurials on ovarian recrudescence in the catfish, an economically important species in the subcontinent.

MATERIALS AND METHODS

Forty adult female catfish weighing 60 ± 5 g were collected from Gangetic riverine system in and around Varanasi. They were acclimated to laboratory conditions for 14 days before starting the experiment. The fish were

Send reprint requests to Dr.K.P.Joy at the above address.

divided into four groups of 10 each. The group 1, 2 and 3 were exposed to safe concentrations of 0.05 mg L^{-1} mercuric chloride (98% W/W, BDH), 0.04 mg L^{-1} methylmercuric chloride (98% W/W, Wilson Laboratories, Bombay) and 0.5 mg L^{-1} emisan 6 (6% methoxyethyl mercury chloride + 94% inert ingredients, W/W, Excel Industries Ltd., Bombay), respectively. The group 4 fish were untreated and served as control for the experimental groups. The fish were kept in well-water (pH 7.3, hardness 23.2 mg L^{-1}, dissolved oxygen 8 mg L^{-1}) which was replenished daily with the required amount of the pollutants. The fish were fed minced goat liver on alternate days and were maintained under natural photoperiod (11L:13D - 13L:11D) and ambient temperature (16-22°C). The experiments were started in the first week of February when the ovaries were in the resting phase of the ovarian cycle. Five fish from each group were sacrificed after 90 days in the month of April (late preparatory phase). The remaining fish were sacrificed after 180 days in the last week of July (spawning phase). On termination of the experiments, the ovaries were removed, weighed and fixed in Bouin's fluid for 24 h. Paraffin sections (6 µm) were cut in transverse plane and stained with Ehrlich's haematoxylin-eosin. The gonadosomatic index (GSI) was calculated in percentage as

$$\frac{\text{weight of the ovary}}{\text{weight of the fish}} \times 100.$$

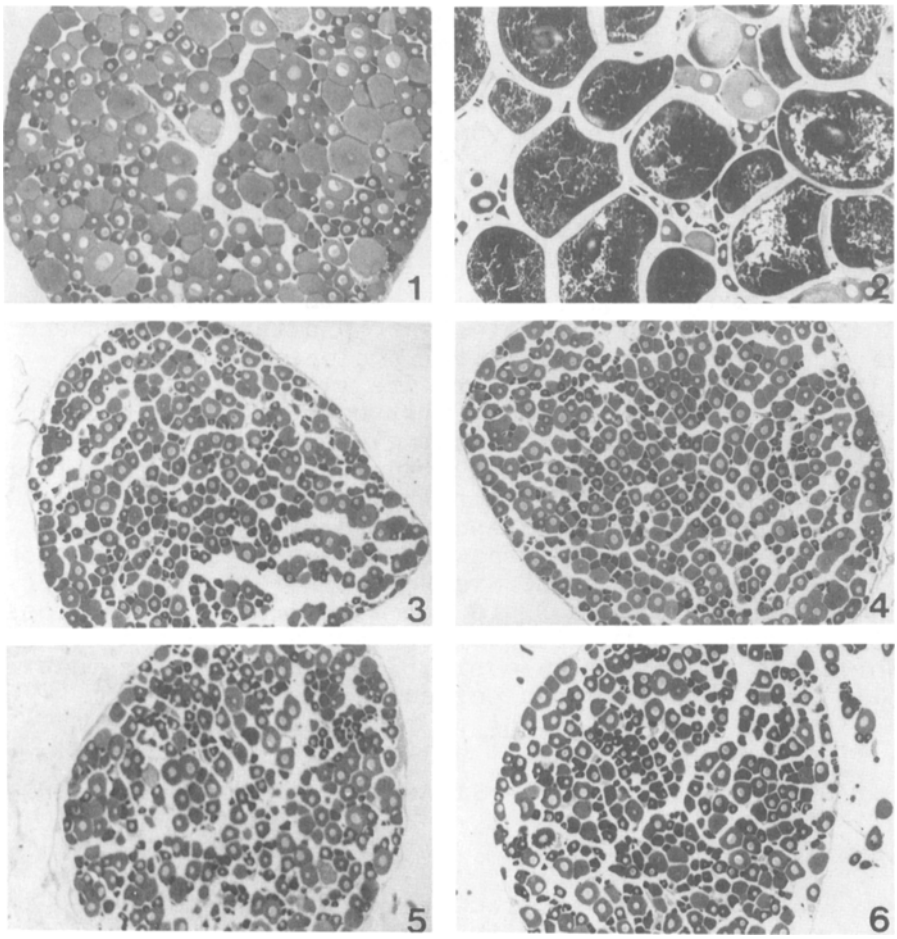
For statistical analysis Student's 't' test was used.

RESULTS AND DISCUSSION

The normal pace of ovarian growth was uninterrupted in control fish maintained in the laboratory. The GSI increased greatly between 90 and 180 days (Table 1). In the 90 day group, the ovary contained mainly early

Table 1. Effect of mercurial compounds on the GSI(%) of female catfish after 90 and 180 days treatment (Mean ± S.D.)

Days	Control	$HgCl_2$	Emisan 6	CH_3HgCl
90	1.96 ± 0.21	1.23 ± 0.12 P < 0.001	1.46 ± 0.11 P < 0.01	1.40±0.09 P < 0.01
180	6.31 ± 0.25	3.25 ± 0.27 P < 0.001	4.34 ± 0.31 P < 0.001	4.56±0.33 P < 0.001

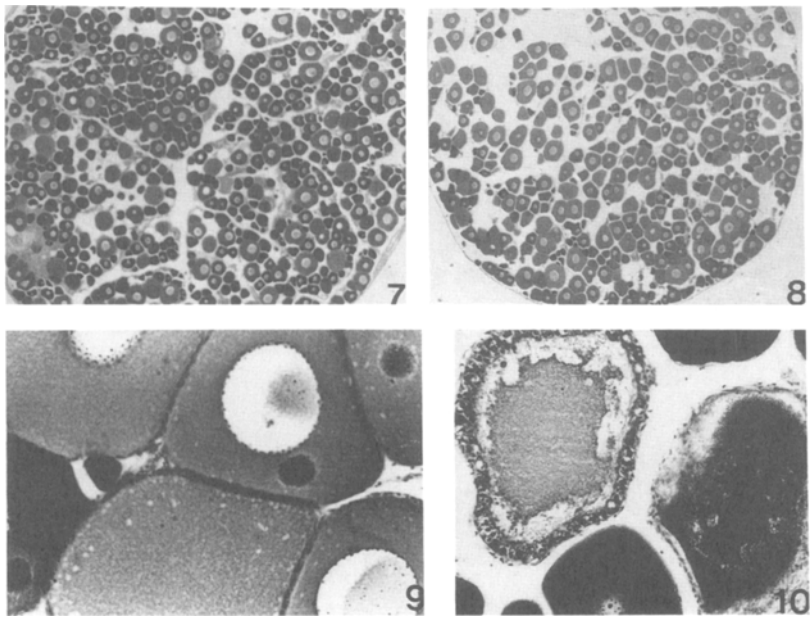


All figures are transverse sections of the ovary. Ehrlich's hematoxylin-eosin.

Figs. 1&2. 90- and 180-day control ovaries with stage I and II, and stage IV oocytes, respectively. X 45.

Figs. 3-6. The ovary of 90- and 180-day $HgCl_2$ (Figs.3&4 respectively), and CH_3HgCl (Figs.5&6) treated fish showing only stage I, non-vittelogenic oocytes. X 45.

stage II oocytes with cortical yolk vesicles around the periphery. The follicular epithelium started differentiation into granulosa and thecal layers (Figs. 1&2). The yolk nucleus was prominently seen with a darkly stained central region and a less stained granular marginal zone (Fig. 9). In 180-day control group, the ovary

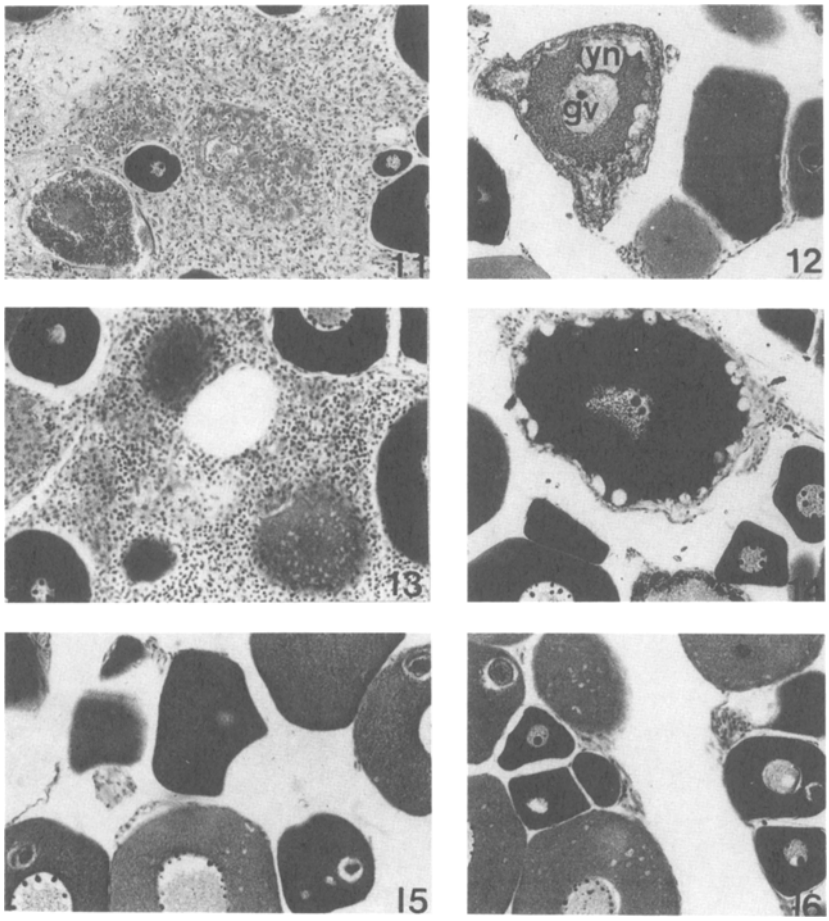


Figs. 7&8. 90- and 180-day emisan 6-treated fish showing stage I oocytes, respectively. X 45.

Fig. 9. 90-day control fish showing late stage I and II oocytes. Note the well developed yolk nucleus. X 200.

Fig. 10. 180-day $HgCl_2$- treated fish showing atretic oocytes. Note hypertrophied and phagocytic granulosa cells and liquefied regions in the cytoplasm. X 200.

contained mostly stage IV vitellogenic oocytes, their cytoplasm being filled with yolk globules (Fig. 2). The follicular layer was further differentiated with a vitelline membrane (zona radiata), thick granulosa and thecal layers. Exposure of fish to $HgCl_2$, CH_3HgCl and emisan 6 for 90 and 180 days completely arrested ovarian recrudescence (Figs. 3-8). The GSI was decreased significantly compared to the control group (Table 1). In all the treated groups, the oocytes were in non-vitellogenic stage suggesting that Hg impairs vitellogenesis at some stage, regardless of their chemical nature. In Channa punctatus a similar inhibition of ovarian recrudescence has been reported after long term treatment with $HgCl_2$ and emisan 6 (Ram and Sathyanesan 1983,1986). In this species, $HgCl_2$ caused a significant reduction of protein, lipid and cholesterol in the liver and ovary (Ram and Sathyanesan 1985). Similarly, Sastry and Agrawal (1979) reported a



Figs. 11&13. 90-day emisan 6- and 180-day $HgCl_2$-treated fish, respectively showing extensive infiltration of fibroblast-like cells. In figure 13, the empty space marks the position of an oocyte. X 200.

Fig. 12. 180-day $HgCl_2$-treated fish showing an atretic oocyte with liquefied cytoplasm and empty yolk nucleus (yn). gv - germinal vesicle. X 200.

Fig. 14. 180-day CH_3HgCl-treated ovary showing vacuoles around the periphery of an oocyte. X 200.

Figs. 15&16. 90-day CH_3HgCl- and emisan 6-treated fish, respectively showing degenerative changes in the yolk nucleus. X 200.

decrease in the activity of glucose-6-phosphatase and alkaline phosphatase in the ovary of the same species. The above studies suggest indirectly an inhibition of vitellogenesis which led to the impairment of ovarian recrudescence. Vitellogenesis is induced by 17B-estradiol under the influence of pituitary gonadotropin (Ng and Idler 1983). In C. punctatus, the gonadotrops were smaller in size and fewer in number after exposing to $HgCl_2$ and emisan 6 (Ram and Sathyanesan 1983,1986), suggesting the involvement of hypothalamo-hypophyseal-gonadal axis. These aspects are being investigated in the catfish.

In the $HgCl_2$- and CH_3HgCl- treated groups, several oocytes were degenerated (Figs. 10, 12 & 14). The cytoplasm was liquefied resulting in empty spaces. The granulosa layer became hypertrophied and thickened greatly, and contained phagocytosed remnants of oocyte cytoplasmic material. Emisan 6 has been reported to induce degenerative changes in the ovary of C. punctatus (Ram and Sathyanesan 1986), but not after exposing to $HgCl_2$. The ovary was extensively infiltrated with fibroblast-like cells in one fish each from the 90-day emisan 6 (Fig.11) and 180-day $HgCl_2$ (Fig. 13) - treated groups. These cells surrounded the oocytes which were in different stages of resorption, leaving even empty spaces (Fig.13). The yolk nucleus or Balbiani bodies are described in most teleosts during the perinucleolus stage of the oocyte development and its function is obscure at present. The yolk nucleus exhibited degenerative changes in the CH_3HgCl (Fig.15) and emisan 6 (Fig.16) - treated groups, and was even resorbed leaving empty spaces in the cytoplasm. In some oocytes, the site of degeneration appeared to have originated from the yolk nucleus region. The degenerative changes were first noticed in the outer zone of the yolk nucleus which was subsequently resorbed (Fig. 15&16). Such changes in the yolk nucleus after Hg treatment are reported for the first time in this study. The atretic changes caused by Hg may be due to its direct action on the ovary.

Our results suggest that a very low concentration of Hg, as used in this study is capable of completely inhibiting ovarian recrudescence in fishes, no matter what form of Hg is used. The toxicological concern of this metal is compounded by the fact that Hg contamination of our environment is unabated as a result of natural weathering processes and anthropogenic activities (World Health Organization 1976).

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