Bull. Environ. Contam. Toxicol. (2001) 66:306–312

DOI: 10.1007/s00128-001-0006-1

Bulletin of Environmental Contamination and Toxicology

Evaluation of Adverse Effect of Emisan-6 on Male Albino Rats

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Received: 23 February 2000/Accepted: 18 December 2000

Fungicides as a generic class include an array of compounds having diverse chemical structures for controlling fungus growth and related diseases on plants, seeds and grains. These can gain entry into the human body either by accidental ingestion or through cutaneous absorption. Additionally, they can gain entry into the human body inadvertently through the food chain. Furthermore, many of them have been documented to affect reproduction in wildlife and they may also affect humans because of their resistance to degradation and their lipophilic nature that culminates in their accumulation within adipose tissue.

The present investigation evaluates possible *in vivo* reproductive effects of Emisan-6 (methoxy ethyl mercury chloride), an organo-mercurial fungicide, in male rats. Some preliminary data reported from other laboratories have documented that Emisan-6 is associated with systemic effects including altered adrenocortical-pituitary activity and thyroid function as well as ovarian recrudescence (Kirubagaran and Joy, 1988a, 1989,1991).

MATERIALS AND METHODS

Emisan-6 (methoxy ethyl mercury chloride) sold under the trade name Bagalol-6, was purchased from Excel Industries Ltd., Mumbai, India. Chemically it contains methoxyethyl mercury chloride : 50% w/w, inert material and colour: 50% w/w containing 6% organic mercury. It was dissolved in distilled water such that 1 ml contained either 1, 2 or 3 mg of Emisan-6 and was given by gavage. Random-bred male albino rats that were 6-8 weeks old and had an average body weight of between 150 to 200 gm were used for this study. The animals were acclimatized for a week before the start of the experiment. They were maintained in our air-conditioned animal facility (University Central Animal House) with a 12-h light /dark cycle, and provided with standard food pellets (Hindustan Lever Ltd. Bombay, India) and tap water *ad libitium.* Three sets of experiments, each having an different objective, were designed for the present study. In Experiment I, 20 animals were randomly assigned to 4 treatment groups for studying histopathological alterations in the testes and accessory male sexual organs. The 4 groups (5 animals each) were dosed daily with the water vehicle or 1.0, 2.0 or 3.0 mg Emisan-6 / kg body weight for 30 days. Body weights of the rats were recorded weekly during the trial. Diets were withheld from the animals on the night prior to the day of termination of the experiment. Reproductive organs including testes, epididymides, seminal vesicles and prostate glands were weighed. All values are expressed as mean $\pm$ S.E. Body weights and organ weights were subjected to one – way

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ANOVA, and Scheffe's multiple comparison test was conducted when analytic results were significant. The level of significance was taken as $p<0.05$ or 0.01.

For pathological examination, the organs were fixed in Bouin's solution for 24 hr and embedded in paraffin. Sections of 4 μm were stained with haematoxylin and eosin and observed microscopically. In Experiment II, animals were divided into different groups and treated as described above for Experiment I. However, after 30 days of treatment, the male rats were allowed to mate with females of proven fertility in individual cages. The presence of a copulatory plug was taken as evidence of a fertile mating and was counted as the first day of pregnancy. The mated females were lapratomized on the 10th day of pregnancy, and implantation sites in the uterus were recorded. Females that failed to show implantation sites were scored as infertile. In experiment III, animals were divided into the 4 treatment groups as described above. After dosing daily with Emisan-6 for 30 days, dosing was discontinued for the next 30 days. Animals were then allowed to mate with fertile females and implantation sites were assessed as described above for Experiment II.

RESULTS AND DISCUSSION

Body weight gain and genital organ weights measured at autopsy are shown in Table 1. Body weight gain was significantly decreased in the 3 mg/kg group in a dose-related manner. The absolute weights of testes, epididymides, seminal vesicles in the 3 mg /kg group, and prostate in the 2 and 3 mg /kg groups were significantly lower than those of the control group. On the other hand, the relative weights of testes, epididymides, seminal vesicles and prostate did not change due to the suppression of body weight. There were no significant changes in the absolute or relative organ weights of the 1 mg/kg group.

Table 1. The effect of Emisan-6 on body weight gain (gm) and genital organ weight (mg) in male rats.

Parameter	Control	Dose (mg/kg body weight)		
		1.00	2.00	3.00
Initial body weight	171.80±1.80	170.80±6.70	169.00±1.80	172.40±1.8
Final body weight	178.20±1.80	175.20±2.80	172.00±2.10	168.60±1.80*
Testes	787.40±2.20	785.60±1.40	782.60±0.65	776.20±1.05*
Per body weight (%)	0.44±0.014	0.44±0.003	0.45±0.017	0.46±0.02
Epididymides	374.00±6.25	372.40±2.55	363.00±3.52	358.40±0.34*
Per body weight (%)	0.20±0.012	0.21±0.020	0.21±0.019	0.21±0.016
Seminal vesicles	378.60±7.48	374.40±8.72	362.00±0.92	355.20±5.10*
Per body weight (%)	0.21±0.013	0.21±0.013	0.21±0.015	0.21±0.007
Prostate	439.60±5.90	432.00±5.92	423.00±3.49*	416.20±1.05**
Per body weight (%)	0.24±0.013	0.24±0.004	0.24±0.008	0.24±0.015

Data represented as mean ± S.E. ; sample size equals 5.
Values significantly different from the control group, *$p<0.05$; ** $p<0.01$.

There were no specific alterations in the histoarchitecture of testes and accessory reproductive organs evaluated in animals dosed with 1.0mg/kg body weight of Emisan-6. In animals dosed with 2.0mg /kg, there were pathological alterations at some sites, such as a reduced number of germ cell layers and atrophied spermatocytes. In addition, dissolution of tubular basement membranes with resulting leakage of germ cells into the interstitial spaces was observed. Most of the Leydig cells appeared atrophied. With the highest dose of Emisan-6 (3.0mg /kg body weight), the pathological alterations noted above were more marked, with moderate to severe degenerative changes in the

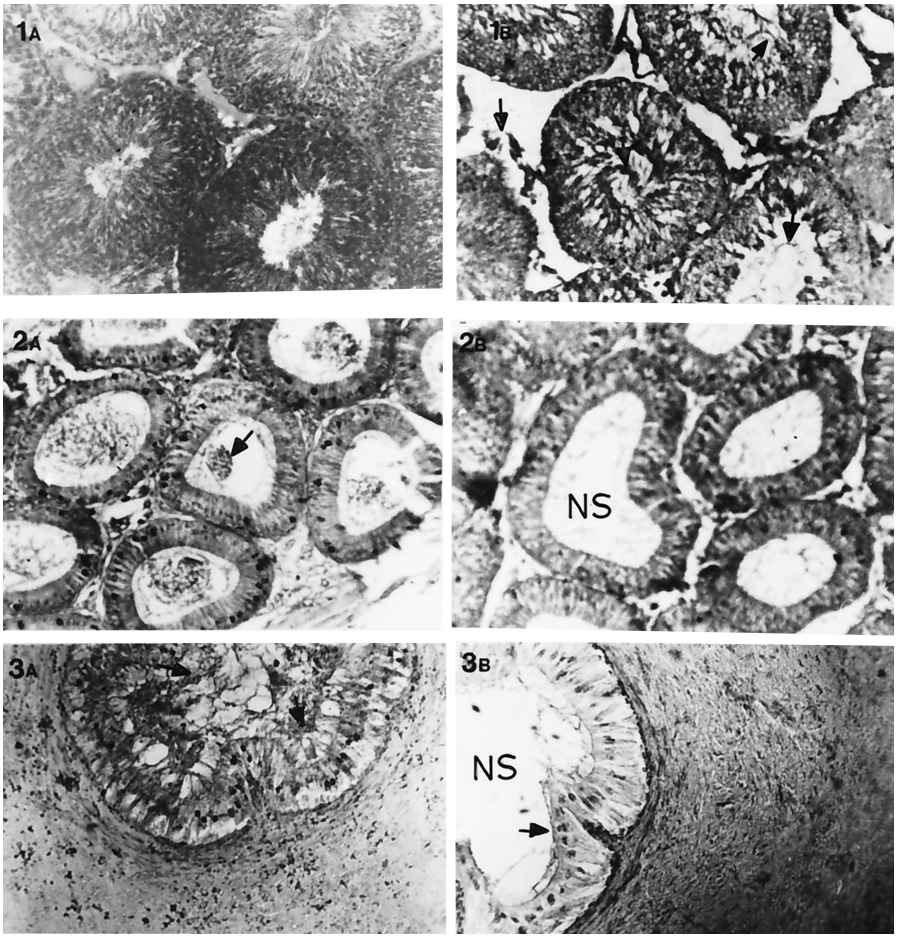

Figure 1A. Testis (Control). Full spermatogenic activity in seminiferous tubules and normal Leydig cells in interstitium (X400)

Figure 1B. Testis (3mg). Sloughing of dead germ cells into tubule lumen. Tubules occluded with debris and degenerated spermatozoa (→) Interstitium contained atrophied Leydig cells (→) (X400)

Figure 2A. Epididymis (Control). Normal histological features with sterocilia and spermatozoa in lumen of ductules (→) (X160)

Figure 2B. Epididymis (3mg).No spermatozoa in the lumen of the ductules (NS) (X250)

Figure 3A. Vas deferens (Control) Normal histological features with sterocilia (→) and spermatozoa in lumen (→) (X250)

Figure 3B. Vas deferens (3mg) Clumped sterocilia (→) and no spermatozoa (NS) (X250)

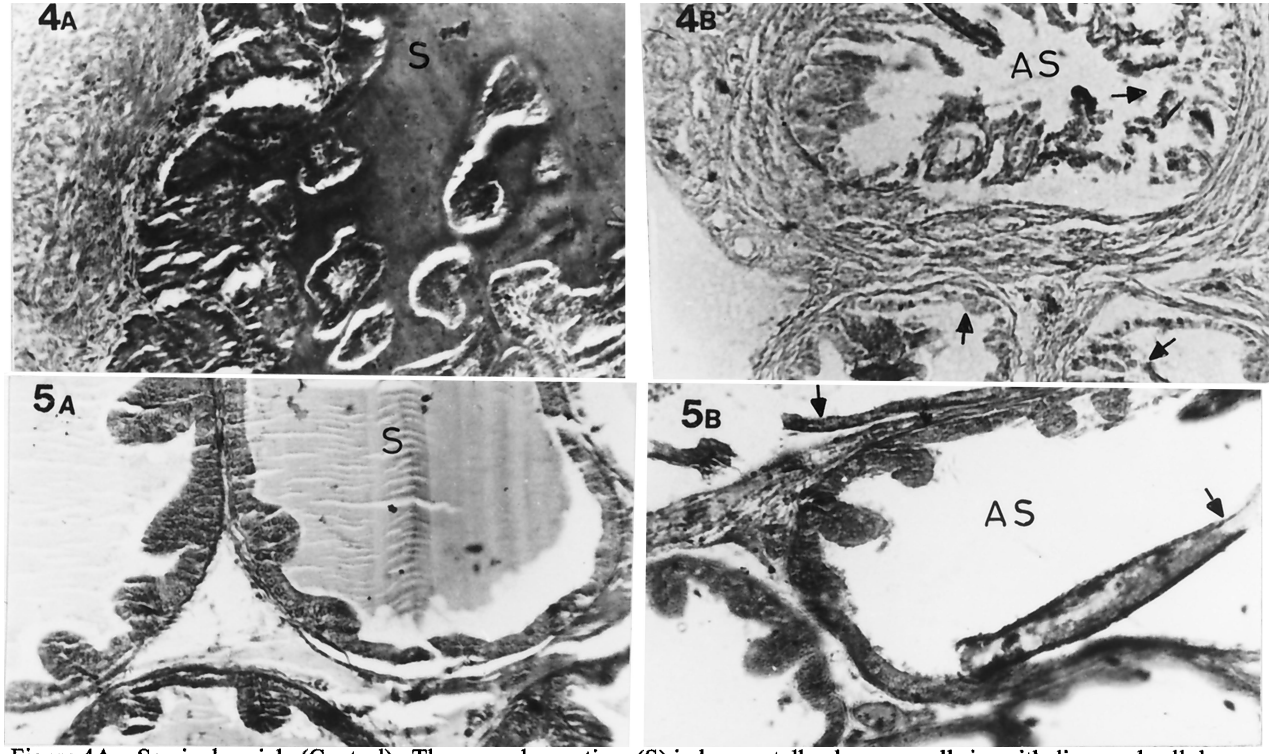

Figure 4A. Seminal vesicle (Control). The normal secretion (S) in lumen, tall columnar cells in epithelium and cellular connective tissue in lamina propria (X400).

Figure 4B. Seminal vesicle (3 mg). Absence of secretion (AS). Damaged lamina propria and muscle layers (→) (X400).

Figure 5A. Prostate gland (Control). Normal histological features with secretion (S) (X400).

Figure 5B. Prostate gland (3mg). Reduced alveoli (→) and no secretion (AS) in alveolar lumen (X400).

seminiferous tubule epithelium resulting in degeneration of spermatids and spermatozoa. At some sites, spermatogenesis arrested at the secondary spermatocyte stage was evident. The lumen of the tubules were filled with sloughed germ cells (Fig. 1B). No specific alterations in histological architecture of the epididymis was observable at the 2.0mg /kg dose, but at 3.0mg /kg, tubules were devoid of spermatozoa (Fig. 2B). Only the highest dose of Emisan-6 (3.0mg /kg) caused an increase in the thickness of the lamina propia of the vas deferens with less sterocilia, additionally, the lumen contained no spermatozoa (Fig. 3B). With 2.0mg /kg, the luminal epithelial cells of the seminal vesicles had an altered shape and nuclear pattern and consequently the luminal space was reduced. With the 3.0mg /kg dose of Emisan-6, these changes were more severe and the lamina propia and muscle layers were severely damaged. The lumen was also totally devoid of secretion (Fig. 4B). Treatment with 2.0mg /kg of Emisan-6 revealed alveoli of the prostate gland lined with low columnar epithelial cells and branched mucosal crypts with disintegrated nuclei in the mucosal folds. At some sites cytolytic lesions were evident. In contrast, with the high dose of Emisan-6 (3.0 mg /kg), alveoli of the prostate glands were found to be in the process of disintegration with reduced or absent mucosal folds. The lumens were devoid of secretion (Fig. 5B).

Table 2. The effect of Emisan-6 on the fertility of male rats after 30 days of treatment.

Dose (mg / kg body wt)	No. of treated males	No. of normal female inseminated	*Fertile matings
0.0	5	5	5 (100%)
1.0	5	5	4 (80%)
2.0	5	5	3 (60%)
3.0	5	5	0 (00%)

*Those that resulted in pregnancy

Thirty days of treatment of Emisan-6 did not affect mating behaviour. However, fertility was greatly affected. Only 20% of the rats that were treated with 1.0 mg Emisan-6 / kg body weight had an infertile mating. On the contrary, the percentage of rats exhibiting infertile matings was as high as 60 and 100% following Emisan-6 treatments at dose levels of 2.0 and 3.0 mg /kg, respectively (Table 2).

Table 3. The effect of Emisan-6 on the fertility of male rats mated 30 days after cessation of dosing.

Dose (mg/kg body wt)	No. of treated males	No. of normal female inseminated	*Fertile matings
0.0	5	5	5(100%)
1.0	5	5	5 (100%)
2.0	5	5	4 (80%)
3.0	5	5	2 (40%)

*Those that resulted in pregnancy

When fertility was evaluated thirty days after discontinuation of Emisan-6 treatment (Experiment III), there was a 20% increase in the 1.0 and 2.0 mg dose groups and a 40% increase in the 3.0 mg dose group compared to immediately after dosing (Table 3).

The use of agrochemicals including fungicides for preventing fungal infections in foodgrains is unavoidable. From the present study the hazards associated with use of such chemicals at high doses are evident and hence of considerable public health concern. Though primarily targeted for inhibiting the growth of fungus, humans can be potentially exposed to fungicides either because of mishandling during spraying operations or as a

consequence of their entry in the human food chain. Furthermore, their lipophilic nature and chemical inertness leads to their accumulation in the body. Grover etal (1989) and Bhatt (1991) reported toxicity of Emisan-6 on the survival of fresh water fish *Puntius ticto*. Kirubagaran and Joy (1995) also reported toxic effects of Emisan-6 on levels of lipids and uptake of P^{32} into phosphoprotein fraction of the ovary and liver in catfish. The results obtained from the present investigation clearly indicated pathological lesions in the testes and accessory sex organs caused by exposure to Emisan-6. There was an absence of spermatozoa in the testis, epididymis and vas deferens as well as an absence of secretion in seminal vesicle and prostate. Our results are in concordance with that of Kirubagaran and Joy (1988) in that Emisan-6 caused ovarian recrudescence in the catfish (*Clarias batrachus)*. Ram and Sathyanesan (1986) also reported inhibition of gonadal development in *Clarias batrachus* by treatment with Emisan-6. Although doses of 1.0 and 2.0 mg/ kg body weight of Emisan-6 given to rats did not cause marked pathological changes, 3.0 mg/kg body weight of Emisan-6 resulted in more pronounced effects including impairment of reproductive performance as indicated by infertile matings that persisted in 60% of rats even after 30 days of discontinuation of treatment with Emisan-6. Furthermore, body weight gain of experimental animals was comparable to that of normal rats, except for group treated with 3mg /kg body weight (Table 1). On the contrary, the absolute weight of selected organs exhibited consistent dose dependent decreases implying some insidious effect due to Emisan-6 treatment. This decline may represent a direct effect on the seminal vesicles and prostate and on the secretions contained therein. As a corollary to our observations, Paul et al (1953) demonstrated a reduction in weights of testes and accessory organs with an absence of spermatids and spermatozoa. Chapparadhali and Kanamdi (1995) reported impaired ovarian steroid production and a significant decrease in ovary and oviduct weights after the administration of mercurial fungicide Emisan-6 to *Rana cyanophnyctis*.

How Emisan-6 affects the testis cannot be deduced from the present investigation although a few speculative mechanism can be cited based on indirect evidences. In the present study the inhibition of spermatogenesis and infertile matings may be due to lack of proper gonadotrophic stimulation or due to imperfect balance of various hormones including steriods. Emisan-6 is reported to alter adrenocortical – pituitary activity (Kirubagaran and Joy, 1991), thyroid function (Kirubagaran and Joy, 1989), and testicular 3- β-hydroxy -Δ5- steroid dehydrogenase, 3 β- hydroxy steroid dehydrogenase) activity (Kirubagaran and Joy, 1988) in catfish. Furthermore, Steinberger and Nelson (1955), Roy etal (1976) and Flicker and Loving (1976) have reported the destruction of the seminiferous epithelium and suppression of spermatogenesis in albino rats using antagonists of follicle stimulating hormone and interstitial cells stimulating hormone as well as those of testosterone. The reduced production of these hormones causes a significant decrease in the weight of testes and accessory organs in male rats (Dorfman, 1963).

In conclusion, a dose of Emisan-6 as low as 3 mg/kg body weight can cause a drastic nonreversible deleterious effect on the reproductive system. It is necessary to further investigate toxicological profiles and other site specific effects associated with Emisan-6 intoxication.

Acknowledgments. We thank to Prof. Asis Datta, Vice-Chancellor and Professor of Molecular Biology, Jawaharlal Nehru University, for providing facilities of the school and Mr. R.N. Saini for excellent photography. We thanks also the reviewers for comments that improved the manuscript.

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