# Effect of Hexachlorocyclohexane Feeding on Testicular Tissue of Pure Inbred Swiss Mice

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It is well known that certain physical and chemical agents cause testicular atrophy and damage of the seminiferous tubules resulting in partial or total arrest of spermatogenesis in animals. The mode of action of the chemicals such as cytotoxic agents (NELSON and PATANELLI 1965), alkylating agents (STEINBERGER and SUD 1970), metals (CHANDRA 1971), organochlorine pesticides like Lindane and Endrin (DIKSHITH and DATTA 1972), Ethyl and Methyl Parathion plus DDT (DATTA and DIKSHITH 1973) and Polychlorinated Biphenyl (DIKSHITH et al. 1975) differs from chemical to chemical. The present study deals with an accidental finding of the changes observed in testicular tissue in an experiment where 500 ppm technical grade BHC was mixed in diet and fed to animals for 10 months to produce liver tumours.

## MATERIALS AND METHODS

Technical grade BHC obtained from Hindustan Insecticides Ltd., New Delhi, was purified and identified by Thin Layer Chromatography.

A total of 150 male pure inbred Swiss mice weighing about 20-24 g were selected for the present experiment. 500 ppm technical grade BHC was mixed in diet prepared in this laboratory. This concentration was taken with an idea to produce liver tumours in shortest possible time of about 30-40 weeks and the dose approximate to highest tolerable limit causing insignificant mortality. Diet was prepared once in a week and kept in cold room. Mice were housed in cages in groups of six in airconditioned room and were given the experimental diet and tap water ad libitum.

6 animals from experimental as well as control groups were killed every month. The experiment was continued for 10 months. All organs were weighed. For histological examinations, the tissues were fixed in

10% formal saline. Sections 5  $\mu m$  thickness were cut and were routinely stained with H & E.

### RESULTS

The testis in control group did not show any change in weight as compared to their body weight throughout the experiment. There were no hypertrophy or atrophic changes in the testis of any of the control animals. Histologically the seminiferous tubules together with interstitial cells were normal (Figs.1, 1a).

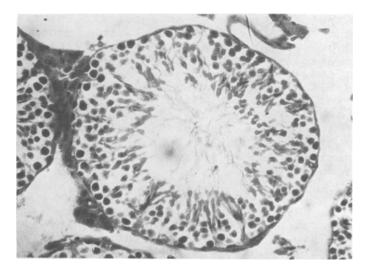


FIGURE 1: Testis of Control Mice showing normal Seminiferous tubule, H and E X 115

There was statistically significant increase in the weight of testis in 3-8 months group. The quantitative difference in the weight of the testis in different groups is given in Table 1. Histopathological changes were observed from third month onward mainly degenerative changes in the seminiferous epithelium and also in different spermatogonic cells (Fig. 2). The seminiferous tubules at times appeared shrunken and were filled with oedematous fluid. A few tubules were completely hyalinized (Fig. 3). In some of the cases there was complete damage of the spermatogonic cells with sparse spermatocyte present in the

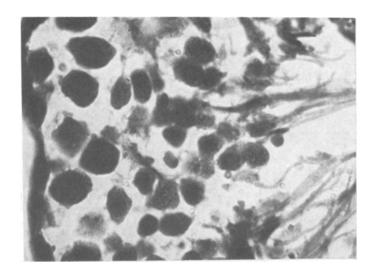


FIGURE 1a: Higher magnification of Figure 1, H and E X 1150

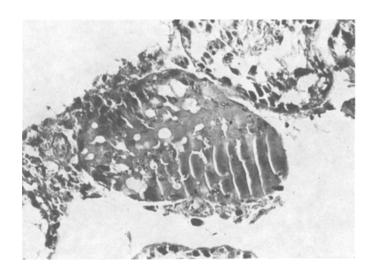


FIGURE 2: Showing complete Hyalinization of Seminiferous tubule of Experimental Group, H and E X 115

TABLE 1 Demonstrates percent increase in the weight of Testis in relation to the Body Weight

Group	Mean	s.D.	T.S.
Control	0.594	0.06	_
1 Month	0.657	0.030	N.5.
2 Months	0.594	0.090	N.S.
3 "	0.757	0.032	**
4 "	0.730	0.077	**
5 "	0.700	0.056	**
6 **	0.702	0.028	**
7 "	0.677	0.053	*
8 "	0.755	0.035	**

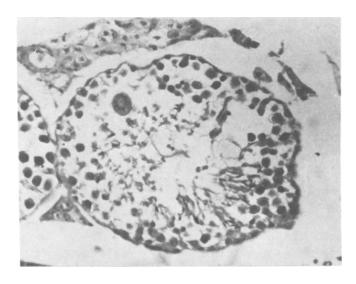


FIGURE 3: Seminiferous tubule showing extensive degenerative changes and Multinucleated giant cell. Hand E X115

<sup>\*</sup> Significant at 5% level \*\* Significant at 1% level

tubules. In extreme cases the tubules consisted of large number of multinucleated giant cells. The number of nuclei observed in some of these cells numbered even 20 (fig. 3, 3a). In some cases there was an active proliferation of interstitial tissue with increase in space.

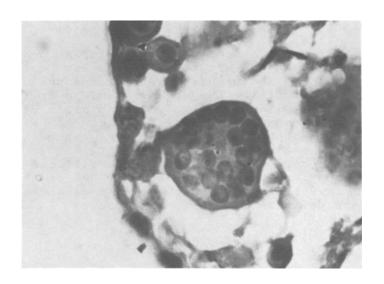


FIGURE 3a: Magnified view of Multinucleated giant cell showing larger number of nuclei, H and E X 1150

#### DISCUSSION

BHC is a persistant type of pesticide and the prolonged and repeated exposure to BHC can produce various ill effects. The recent reports of KELLY et al (1973), COLLINS et al (1971), MARK (1969), and EPSTEIN (1972) have shown mutagenic effect of pesticides in different test systems. Similarly the studies of ESPIR et al (1970) and PECK (1970) have reported impotence among the farm workers, which gives ample scope to visualize the risk involved in the use of these pesticides. DIKSHITH and DATTA (1972) injected intratestically Lindane, which is an important constituent of BHC, and observed degenerative changes in the seminiferous epithelium and also in different spermatogenic cells.

They have also observed a large number of multinucleated giant cells. In the above study as the chemical was injected intratesticularly, effect on the testis was, therefore, severe. Similar changes in the testis were observed when Ethyl Parathion and Methyl Parathion alongwith DDT were orally fed to the rats for 90 days (DATTA and DIKSHITH 1973). The significant degenerative changes like complete hyalinization of the tubules, mild arrest in spermatogenesis and Dedema in tubules have been observed in the seminiferous tubules for the first time in the testis of mice exposed to technical grade BHC. The finding of multinucleated giant cells in extreme cases of degeneration are, however, in agreement with the earlier study(DIKSHITH and DATTA 1972).

These observations are particularly significant since the DDT and BHC are still in use for controlling malaria in India and manufacturedat large scale in the pesticides factories. The workers exposed to these pesticides are either in manufacturing plant of these pesticides or engaged in their spraying. Thus it is very likely that these observations may be of great significance when the results are extrapolated in relation to workers. Although it is very difficult to make any firm commitment on the effects of BHC on the workers. Detailed investigation on the above lines would be needed before any definite conclusion is made.

#### ACKNOWLEDGEMENT

The authors are indebted to Dr.S.K. Chatterjee for his keen interest in the present study. Sincere thanks are due to Mr. A.M. Suthar, Mrs. Rashida Kapadia and Mr. Ashok Kumar for extending technical assistance.

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