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Effect of Chlordane on Testicular Tissues of Swiss Mice

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Chlordane is a widely used soil insecticide, particularly useful in the protection of wooden structures from termite damage (Beeman and Matsumura 1981). This chlorinated hydrocarbon compound is highly persistent in the environment, thus its use has been restricted in 1976 by the Environmental Protection Agency because of its long persistance and suspected cracinogenicity (EPA 1976).

Technical chlordane is a mixture of many chlorinated hydrocarbons of the cyclodiene group including mainly cis-and trans-chlordane, heptachlor nonachlor and few others (Cochrane 1976) and because of this complexity very little metabolism work of any significance has been carried out with this material (Schwemmer et al 1970). It is known that organochlorine compounds (OC) are lipophilic environmental pollutatns reaching animal body through food chains and tend to be accumulated in selected body tissues (Kilgore and Li 1970) thus causing a wide range if adverse effects , among them fertility, gestation and viability are known to be affected (Keplinger et al 1968). Fishbein (1979) stated that the effect of cyclodiene insecticides on reproduction are equivocal and vary with the compound and test species employed.

This work represent a contribution to find out the effect of chlordane feeding on the spermatogenesis in swiss mice.

MATERIALS AND METHODS

Thirty sexually mature laboratory bred male swiss mice of an average body weight of 25± 2 gm were used in this work. They were randomly divided into three groups of ten mice each. Two of them recieved doses of 100 and 300 mg/Kg body weight, of technical chlordane dissolved in corn oil, orally by means of stomach tube consequently for 30 days, so that each mouse of group I recieved 0.08 mg of the active ingredient (a.i.) of chlordane as a daily dose, while each mouse in group II recieved 0.25 mg a.i. as daily doses. Group III was considered as Control group recieving the same volume of corn oil (0.1 ml) daily. All animals were kept under the same conditions (room temperature 22°± 2°C, diet and tap water was allowed ad Libitum. Body weight for all groups was recorded at three day intervals.

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Animals were sacrificed 24 hr after the last dose. Testis were removed, fixed in Bouin's fluid and processed in a series of graded ethanol, paraffin sections were cut out at a 6 um thickness and stained with Harris hematoxylin and eosin stain, and examined with a light microscope.

From each single testis five consequative sections (representing the middle part of the testes) were examined. To study the effect of chlordane on the testicular tissues, the following indicies were used in this work; the average diameter of the seminiferous tubules based on 15 regular shaped tubules, percentage of damaged tubules in each section examined and the histological changes from the normal testicular tissues. Results were analysed using F test.

RESULTS AND DISCUSSION

During the period of the experiment, death was recorded in group II only, where 5 mice (out of ten) died on 3rd, 4th, 5th, 7th and 21st days of dosing.

The effect of the two different doses on the body weight was obvious where a slight increase in body weight of the experimental group I (3.91% of the starting weight) was noticed during the course of the experiment (Table 1) however this increase was more marked in group II reaching 4.79% of the starting weight and assumed a gradual pattern to become statistically significant ($p< 0.05$) particularly at the begining of the last week, and continued to increase and to be more significant ($p< 0.01$) at the end of the last week of dosing in comparison with the control and experimental group I (Table 1). On the contrary, group III (control) showed a gradual decrease in body weight reaching 21.6% of the starting average weight (26.3 gm).

Table 1. Changes in body weight of the three animal groups.

Group I		Group II		Group III (control)	
1	2				
Body weight	S.E.	Body weight	S.E.	Body weight	S.E
23.0	0.528	27.1	0.448	26.3	1.003
23.0	0.534	28.0	0.467	26.6	0.896
23.4	0.705	25.6	1.009	25.7	0.979
23.4	0.673	27.2	0.560	26.0	1.068
24.2	0.795	27.1	0.455	25.6	1.013
23.3	0.648	27.5*	0.817	24.2	0.979
23.4	0.298	27.2**	1.032	21.9	1.078
23.8	0.445	28.5**	1.390	20.4	0.867
23.4	0.491	28.5**	1.601	20.5	0.915
23.9	0.486	28.4**	1.738	20.6	1.356

1: Mean values in gm; 2: Standard error; *: Significance at 5% level; ** Significance at 1% level.

The testis in control group did not show any gross or microscopic changes (Fig. 1). The histological changes were only observed in the two experimental groups (I &II), where size reduction was obvious in the seminiferous tubules particularly in group II (Fig. 2A & B). Table 2 shows mean diameter of the tubules of the three groups.

Table 2. Mean diameter (in microns) of the seminiferous tubules of the two treated groups as compared with the control group.

Animal No.	Group I*	Group II*	Control *
1	178.2	157.7	164.3
2	180.5	152.0	157.7
3	175.4	147.2	171.6
4	189.9	110.2	193.1
5	131.4	91.4	182.4
Standard error	± 10.221	±13	±6.686

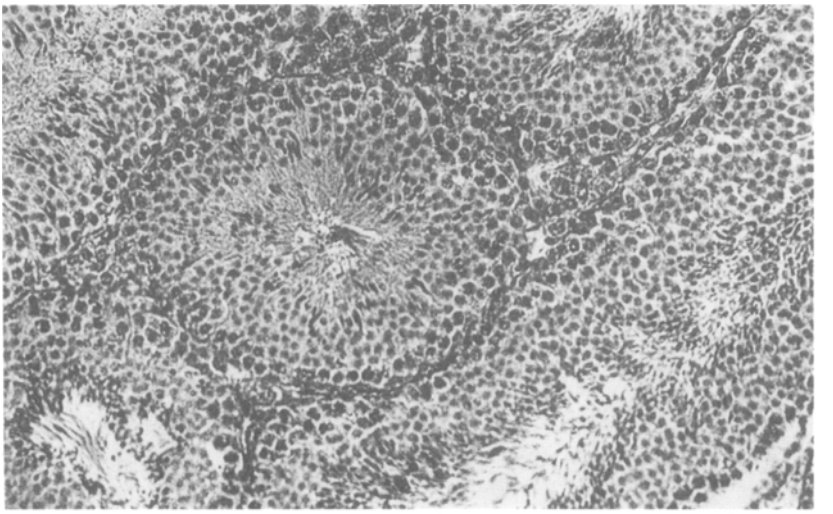
* Values are for the average diameter of 15 randomly selected tubules.

It can be seen from Table 2, that significant difference in the diameter is present at $p < 0.05$ between groupI &II, while no such difference can be noticed between group I and control. On the other hand, degenerative changes in seminiferous tubules was so common that both normal and damaged tubules were present even within the same microscopic field of the two experimental groups. Table 3 represent percentages of the damaged tubules.

Table 3. Percentages of damaged tubules.

Animal group	No. of damaged tubules/section	Total No. of tubules per section	Percentages of damaged tubules
1	26	150	17.33
	32	162	19.75
	38	233	16.30
	48	230	20.86
	38	346	10.98
II	90	290	31.03
	67	152	44.07
	132	224	58.92
	74	180	41.11
	58	240	24.16
Control	16	280	5.71
	13	216	6.01
	5	300	1.66
	3	230	1.30
	1	220	0.45

The degenerative changes were very common among all stages of spermatogenic epithelium. Karyopycnosis and cytoplasmic vacuolation was also present. The tubules were filled with necrotic



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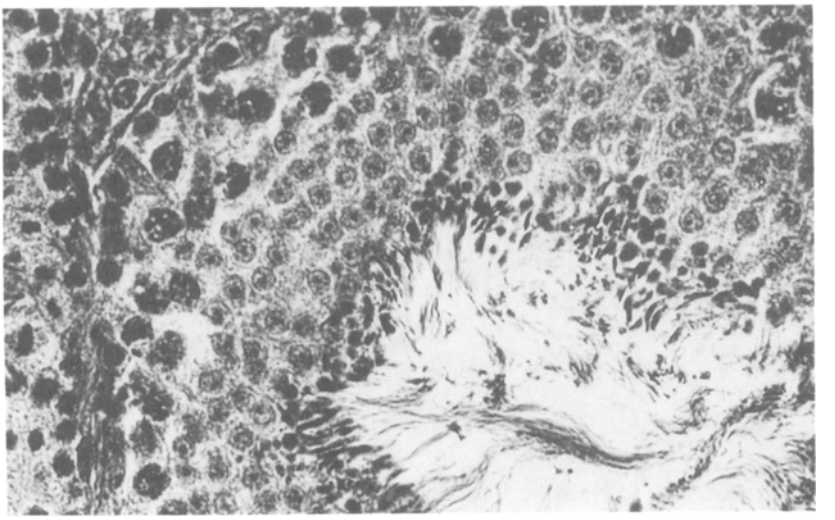

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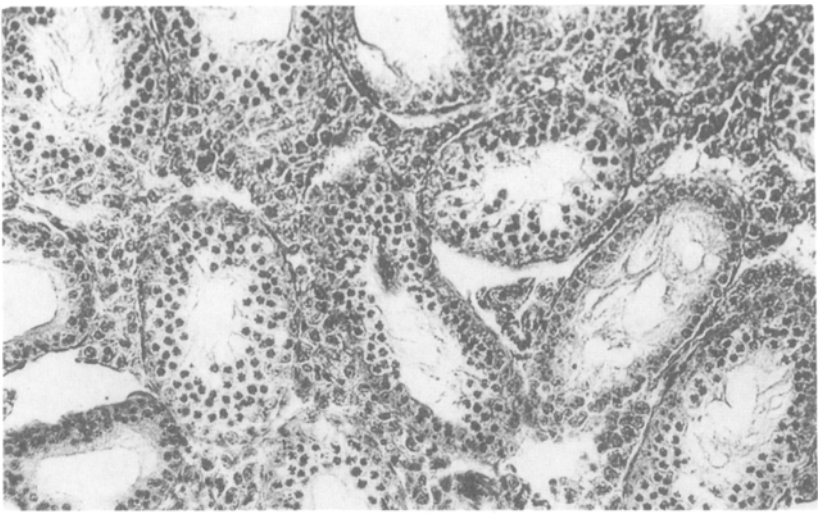
Figure 1.(A & B) Normal histological picture of the testis of a control showing intact seminiferous tubules, orderly arranged spermatogenic cells and normal interstitial tissue, H&E. A.X 200, B. X 400



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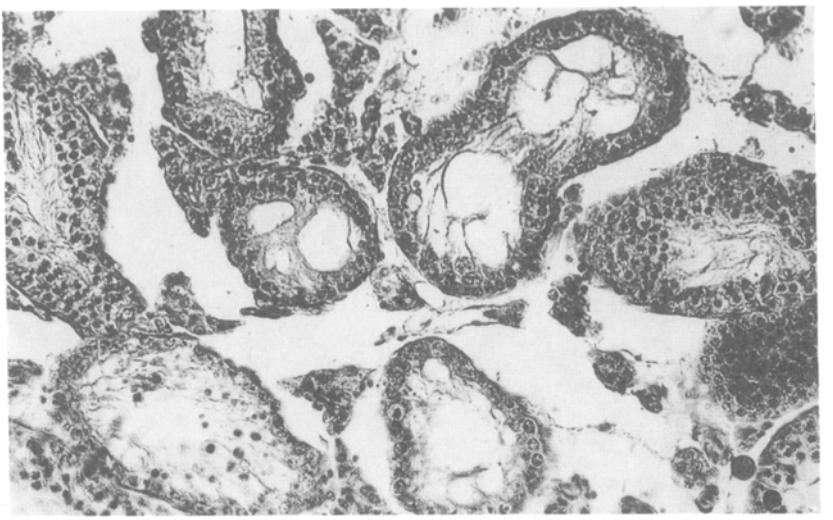

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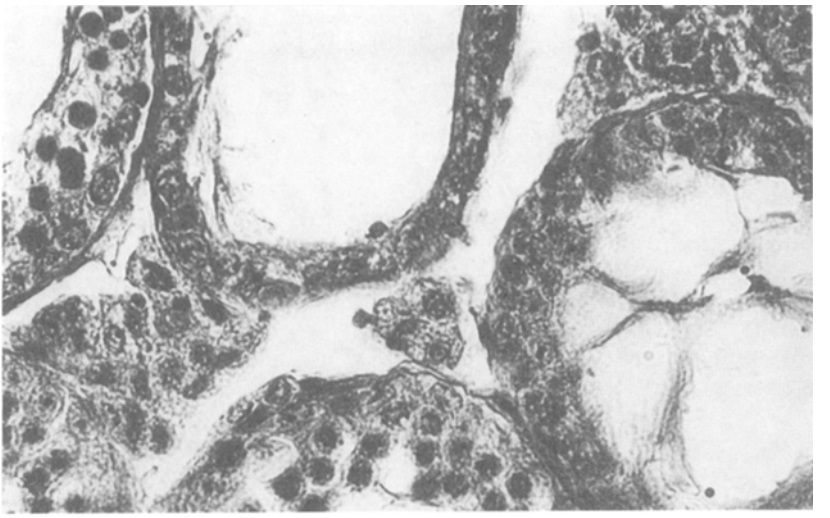
Figure 2A.Testis of mice fed 100 mg/Kg of chlordane showing atrophied seminiferous tubules, H &E, X 200
2B.Testis of mice fed 300 mg/Kg of chlordane showing more reduction in diameter of the affected seminiferous tubules, H &E, X200



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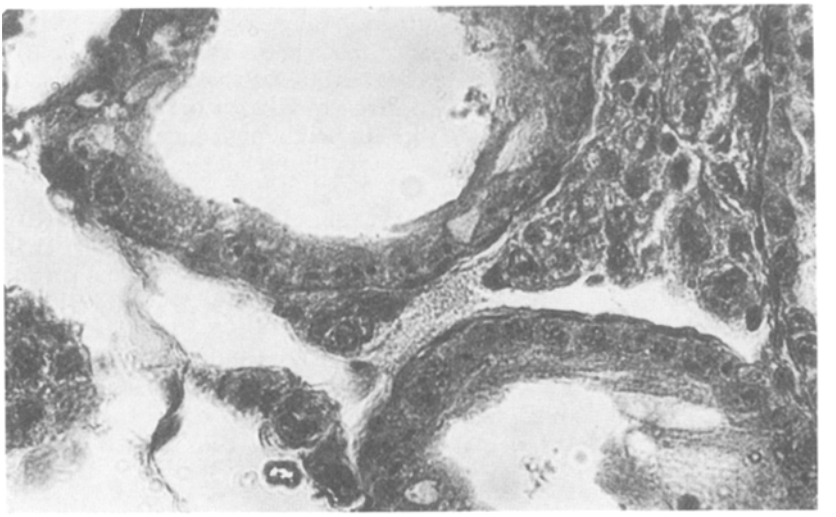

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Figure 3.(A&B) Seminiferous tubules after feeding 100 & 300 mg/Kg of chlordane respectively showing degenerative changes tubular odema, karyopycnosis, cytoplasmic vaculation and proliferation of Leydig cells, H & E, X 400

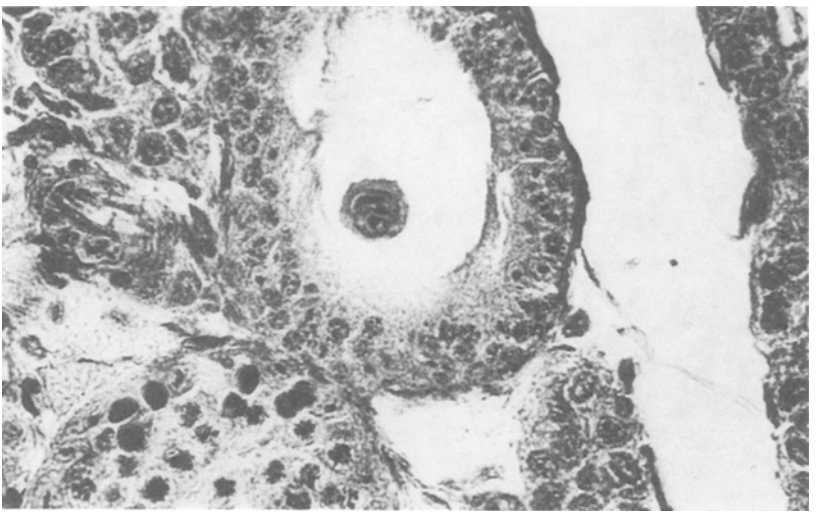

Figure 4. Testis of mice fed 300 mg/Kg of chlordane showing a giant cell in the seminiferous tubules, reduced spermatogenic activity, H & E, X 400

cells, sparse number of spermatocytes. They were also filled with irregularly scattered cell and oedematous fluid (Fig. 3A & B), while the presence of multinucleated cells was more pronounced in the testis of group II (Fig. 4). The interstitial tissue showed a little proliferation of Leydig cells with a slight increase in space (Fig. 3A & B).

The effect of chlordane on the tesicular tissues of the male swiss mice is evident. Results of this study reveals that this compound has a damaging effect on spermatogenesis in the two different doses used (100 mg/Kg and 300 mg/Kg body weight). Similar degenerative changes in testicular tissue induced by other chlorinated insecticides were also noticed earlier by Dikshith and Datta (1972), Krause et al., (1975). Study of Nigam et al., (1979) also showed that oral feeding of chlorinated insecticides produced degenerative changes in the testes of swiss mice.

In the present work, the testicular lesions observed in mice were directly related to doses used, this fact was also mentioned by Datta and Dikshith (1973). Multinucleated cells obsereved in the damaged seminiferous tubules of the two experimental groups in this work was piculiar which was also mentioned by many other workers (Dikshith and Datta, 1973); Datta and Dikshith, 1973; Dikshith et al., 1975; Fishbein 1979; Nigam et al., 1979). On the other hand, it is well known fact that exposure to organochlorine compounds causes reduction in size of the seminiferous tubules. Krause et al., (1975) mentioned that DDT causes reduction in tubular diameter, this was also noticed in the present work, in group II due to feeding of 300 mg/Kg body weight of chordane.

Moreover, it is known that pesticides are considered as an antispermatogenic and antifertility agents which directly affects the germinal epithelium while affecting Leydig cells and gonadotrophin regulation hormones indirectly (Hall, 1970; Gomes, 1970; Krause and Homola, 1974) therefore the slight alteration of Leydig cells after chlordane feeding in the present work could be explained on the basis of this fact.

Conney et al. (1972) and many other workers (Conney et al., 1967; Kuntzman et al., 1967; Welch et al., 1967; Conney et al., 1971) suggested that organochlorine insecticides induce the hepatic-microsomal enzymes and consequently causes alteration in the normal histological picture of the testis through the hydroxylation of androgenes, and according to Lehninger (1976) this induction is the primary step in the metabolism of androgens.

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