CHAPTER 5

SUBACUTE EXPOSURE: EFFECTS FOLLOWING REPEATED ADMINISTRATION OF ENDOSULFAN IN RATS

5.1 INTRODUCTION

Several toxic effects have been observed in animals exposed to endosulfan. Endosulfan is most likely to affect the kidneys, liver, blood chemistry and the parathyroid gland (Farm Chemicals Handbook, 1992). The prominent symptoms of intoxication were observed in animals treated with the higher dose including hypersensitivity such as excitation to touch, noise, trembling, squatting posture and bloody discharge from the eyes before death.

Histopathological examination revealed severe damage to the liver, kidney and testes following oral administration of 10 mg/kg endosulfan in male albino rats (154-160g) for 15 days (Gupta and Chandra, 1976). The study of regional distribution pattern of endosulfan in brain tissues and plasma after rats were fed daily doses of endosulfan (5 or 10 mg/kg) for 15 days revealed that α-endosulfan was highest in the cerebrum followed by the remaining parts of the brain and cerebellum. β-endosulfan was not detected in the "remaining part" of the brain, where as endosulfan sulfate was the only metabolite detected in the rat brain and only a trace amount of it was present in the plasma (Gupta, 1978).

In a study by Nath *et al.* (1978), repeated administration of endosulfan or metapa or their mixture in male albino rats (150-175g) for 30 days did not induce any significant changes in body weight/organ weight ratio, histological changes of enzymatic alterations in the vital organs (liver, kidney, testis and epididymis) of the rats. The residue level of endosulfan in vital organs of rats showed variation with the kidneys showing the highest residue level.

Endosulfan is well known for its hepatotoxic action. Liver enlargement and shortening of pentobarbital sleep occurred following repeated exposure to endosulfan (Gupta and Gupta, 1977). Gupta and Gupta also suggested that endosulfan may shorten the duration of pentobarbital-induced sleep, perhaps by induction of hepatic microsomal enzymes activity, as is evidenced by the changed pentobarbital-induced hypnosis and increased liver weight of endosulfan-treated rats.

At present, there is very little information available on the accumulation of endosulfan in tissues. In addition, only few reports on the effects of endosulfan on reproductive functions are available but are restricted to the adult animals only. Moreover, no report is available on the effect of endosulfan on the testicular maturation (Sinha *et al.*, 1997).

The current study examines the distribution of endosulfan and its metabolite, endosulfan sulfate in plasma, kidneys and liver of juvenile male rats following repeated administration of endosulfan. In addition, animals are also observed for signs of toxicity. Upon termination of treatment, body weight and the weights of selected organs will be determined. Histological examination of the testes was carried out and sera testosterone, T3 and insulin levels were determined.

5.2 EXPERIMENTAL

5.2.1 Materials and chemicals

Technical grade endosulfan was recrystallised to give 88% purity. Polyoxyethylenesorbitan monooleate (TWEEN 80) was obtained from Sigma Chemical, St Louise USA. Rat diet was purchased from the Universiti Kebangsaan Malaysia (UKM) animal house. Other chemicals required in this study are listed in Section 3.5.

5.2.2 Preparation of the endosulfan dosage

For preparation of 10 mg/kg treatment material, 100 mg of technical grade endosulfan was dissolved in a 100 ml mixture of 1:10 TWEEN 80 and distilled water with final concentration of 1 mg/ml. As for the 5mg/kg treatment material, 50 mg of technical grade endosulfan was dissolved in a 100 ml mixture of 1:10 TWEEN 80 and distilled water with final concentration of 0.5 mg/ml.

5.2.3 Animals

Male Sprague-Dawley rats, weighing 40 to 120 g (4 weeks old) and bred by UKM animal house were used in this study. Animals were weight-ranked and assigned to one of the three dosing groups (0, 5 and 10 mg/kg), so that the means and variances among groups were comparable. After assignment to the treatment groups, similarly treated males were housed 3 to 4 per cage. Rats were housed in clear plastic cages (20 x 25 x 47 cm) containing sawdust as bedding and roofed with stainless wire covers. Animals were maintained on a complete and balanced laboratory pelleted diet (UKM animal house) and tap water *ad libitum*, in an animal room in the Department of Pharmacology with room temperature (25-30°C) and a relative humidity of 40 to 50%. The rats were allowed to acclimatise to their new environment for 3 days prior to initiation of endosulfan treatment.

5.2.4 Experimental design and treatments

14 male rats were randomly assigned in each of the three treatment groups. Rats were fed with suspensions of technical grade endosulfan (70α:20β) in TWEEN 80 at a dose level of 5 and 10 mg/kg bw/day for 15 days. A vehicle control group received TWEEN 80 over the same treatment period. Each animal was weighed prior to treatment and daily dosage was adjusted according to their body weight. Treatments were administered daily by oral

gavage beginning at 30 days of age and continuing through 45 days of age. The dose was administered between 0700 and 0900 h using an 18-gauge gavage needle (1 inch length, with a 2.25 mm ball) and a 2.5 ml plastic syringe. Doses were administered on a mg/kg body weight basis which were adjusted daily for weight changes. Body weight (nearest 0.1 g) and the volume of the dose administered (nearest 0.1 ml) were recorded daily.

5.2.5 Necropsy

Half of the animals in the three groups were decapitated on the 16th day and the remaining half on the 30th day (15 days after the last treatment). Blood was collected, centrifuged and stored in siliconized microcentrifuge tubes at -20°C until further residue analysis and hormonal measurements of testosterone, T3 and insulin. At necropsy, the testes, epididymis, liver, kidneys and body weights were recorded. Livers and kidneys were removed for residue analysis. The testes were prepared for histological examination as described in Section 5.2.8.

5.2.6 Residue analysis

α-endosulfan, β-endosuflan, endosulfan sulfate and endosulfan diol were measured in blood plasma and selected tissues using a Shimadzu QP5050A gas chromatograph coupled with mass spectrometer as documented in Section 3.5.8.

5.2.7 Radioimmunoassay (RIA) and hormonal analyses

Assays for sera testosterone, T3 and insulin were carried out using coated tube kits purchased from Diagnostic Products (Los Angeles, CA). The detailed procedures for the preparation of samples prior to hormonal analyses are described in Appendix 4.

5.2.8 Histological evaluation

The testes were prepared for histological evaluation by immersing them in 10% buffered formalin for 24 h. After routine processing, the tissues were embedded in paraffin wax and the sections were cut at 5 μm thickness and stained with haemotoxylin and eosin. The detailed procedures for the preparation of histological evaluation are described in Appendix 5.

5.2.9 Statistical analysis

The SPSS for Windows software package (Release 10.0, SPSS, Inc) was used for all parametric statistical analyses. The body weight, organs weight and hormonal levels were expressed as mean values ± Standard Error of Mean (S.E.M.). All data were analysed by analysis of variance (ANOVA) using General Linear Models (GLM) procedure of the SPSS package. For absolute tissue weights, body weight at necropsy was included as a covariate in the analysis of tissue weights. Significant effects of dose and time post-dosing on hormonal levels were also tested by ANOVA under GLM procedure of the SPSS package, with the α level set at 0.05. Interaction effects were further examined by Student's *t*-test. The least significant difference (LSD) test was used to compare individual treatment means when ANOVA indicated that significant differences were present.

5.3 RESULTS AND DISCUSSION

5.3.1 Body weight and organs weights changes

Body weight changes in rats following repeated oral administration of endosulfan for 15 days and a recovery period of 15 days after cessation of treatment are presented in Table 5.1 and Figure 5.1.

The body weight of rats receiving 5 mg/kg and 10 mg/kg of endosulfan depressed significantly as compared to control animals on the 16th day after treatment. However, most animals were noted to regain their initial body weights by the end of the study, which was the 30th day although the increase was statistically insignificant (Table 5.1).

Absolute and relative organs weights on the 16th and 30th day are presented in Table 5.2, 5.3, 5.5 and 5.6. On the 16th day, no significant increase in the absolute and relative liver weights of the endosulfan-treated groups were observed as compared to the control animals although the mean liver weights were higher in both the treated groups (Table 5.2 and 5.3). Similar pattern was also found when the liver weights were adjusted with body weight at necropsy as covariate (Table 5.4).

Significant increases in the absolute and relative kidneys weights as well as its adjusted weights were seen in the 10 mg/kg group as compared to the control animals (Table 5.2-5.4). However, the testes and epididymis of the treated groups registered a significant decrease in the absolute and relative weights as compared to the control animals, consistent with the results of the adjusted weights of the respective organs.

Similar trend was also observed in all the organs when the animals were sacrificed on the 30th day, except that the absolute and relative liver weights as well as its adjusted weights increased significantly in the 10 mg/kg group as compared to the control group (Table 5.5-5.8). In addition, the relative and adjusted weights of the kidneys were also increased significantly in the 5 mg/kg and 10 mg/kg groups (Table 5.6 and 5.7).

No mortality was observed in control group. However, a mortality rate of 36% was observed with the high-dosed group; one rat died on the 6th and four on the 7th day of the treatment period (Table 5.8), consistent with a previous report that mortality was also found in the high-dosed group when male albino rats weighing 150-158g received endosulfan orally daily by intubation for 15 days (Gupta, 1978). Obvious signs of intoxication were observed in animals treated with the higher dose compared to control animals including hypersensitivity such as excitation to touch, noise and trembling, squatting posture and bloody discharge from the eyes before death. Most animals also exhibited severe diarrhea. Animals treated with the lower dose exhibited essentially normal appearance and behavior although some animals showed signs of mild diarrhea. The cause of death was not determined in this study, hence it was not known whether endosulfan produced lethal liver injury in these animals. However, it was reported that repeated administration of endosulfan produced biochemical effects in the liver and a single dose was unlikely to produce liver injury (Tyagi *et al.* 1984). In 1995, Paul *et al.* demonstrated that repeated exposure of endosulfan at sublethal doses produced hepatic and neurobehavioral toxicities and mortality.

A marked decrease in body weight of the treated groups showed that toxicity was dose dependent with the higher-dosed group exhibiting severe reduction in body weight. The

distinct changes in body weight of rats given 10 mg/kg of endosulfan on the 16th could be due to the toxic effects of endosulfan or the diminished feed-intake resulting in the loss of body weight.

The present data on the increase of the liver weights in the treated groups as compared to the control animals confirms the finding that liver enlargement occurred following repeated exposure of endosulfan (Gupta and Gupta, 1977), especially in the higher-dosed group, although our results show that the increase was statistically insignificant on the 16th day and was significant on the 30th day following 15 days of recovery period after the last treatment.

Gupta and Chandra (1977) also reported that such an increase was associated with pathological changes although the toxic effects of the compound cannot be ruled out when male albino rats weighing 154-160g received endosulfan orally for 15 days. They demonstrated that the morphology of the liver in the 10 mg/kg group was severely damaged as compared to the control and the 5 mg/kg groups, consistent with the claim that endosulfan was known for its hepatotoxic effects (Paul *et al.*, 1995). Since, the morphology of the liver, the hepatotoxic effects and the biochemical effects of endosulfan were not investigated in our study, it was not known whether the increase on the liver weight was actually caused by the toxic effects of the compound or the induction of microsomal enzymes.

The significant decline in the testes and the epididymis weights of the treated groups as compared to the control animals on the 16th and the 30th day may suggest that endosulfan is able to induce damage to gonads attaining sexual maturity. It is also suggested that

testis may be one of the organs appeared to be susceptible to endosulfan. At present, no report is available on the effects of endosulfan in the testicular maturation. This hypothesis is further supported by its histological and morphological profiles of the testes which will be discussed later in the next section.

5.3.2 Residue analysis

The distribution of α-endosulfan, β-endosulfan and endosulfan sulfate in liver and kidneys after repeated exposure is summarized in Table 5.9.

The concentration of endosulfan and its metabolite per gram lipid and % lipid in various parts of the tissues receiving 5 mg/kg and 10 mg/kg endosulfan are summarized in Table 5.10 and 5.11.

On the 16th day, the α and β-isomers of endosulfan in rats receiving the 5 mg/kg dosage were detected only in the kidneys with a mean concentration of 0.95 ± 0.23 μg/g and 0.51 ± 0.15 μg/g respectively (Table 5.9). Endosulfan sulfate was not detected in the kidneys, but was detected in the liver with a mean concentration of 0.09 ± 0.04 μg/g.

On the 30th day, α- and β-isomers were not detected in the kidneys. However, endosulfan sulfate was still detected in the liver, with its mean concentration decreased to 0.05 ± 0.01 μg/g.

At a higher-dosed group (10 mg/kg), the distribution pattern of endosulfan and its metabolite followed similar trends with higher mean concentrations compared to the lower

dosage (Table 5.9). The mean concentrations of the α-isomer and β-isomer in the kidneys on the 16th day were 1.62 ± 0.64 μg/g and 0.64 ± 0.16 μg/g respectively, while the mean concentration of endosulfan sulfate in the liver was 0.09 ± 0.03 μg/g on the 16th day and 0.06 ± 0.01 μg/g on the 30th day.

Table 5.10 and 5.11 show a different pattern of distribution depending upon how the concentrations were expressed. Because of its stability characteristics, it is generally assumed that nearly all the endosulfan present in tissues is in the lipid fraction and therefore, the endosulfan concentration has been expressed per unit weight of lipid. For example, in rats fed with 5 mg/kg of endosulfan for 15 days, the concentration of α-endosulfan per unit kidneys lipid (27.62 μg/g lipid) was 1.9 times higher than the concentration of the β-isomer (14.75 μg/g lipid).

Neither the parent compound nor its metabolite was detected in plasma on the 16th and the 30th day. This was because the half-life ($t_{1/2}$) of α-endosulfan was estimated to be 55 minutes, whereas β-endosulfan, endosulfan sulfate and endosulfan diol were not detected in the plasma as reported earlier in Section 4.3.1. It was known that the β-isomer was cleared rapidly than the α-isomer (EXTOXNET, 1993). Endosulfan diol would not be retained in the plasma and was excreted as one of the metabolites in the urine of rats together with endosulfan-α-hydroxy ether and an unknown water soluble conjugate (Gupta and Gupta, 1979).

Our finding indicated that residue accumulation was greatest in the kidneys followed by the livers after 15 days of administration in the 5 mg/kg group and this was consistent with

previous reports indicated that kidneys also registered the highest residue level of endosulfan when male albino rats (150-175g) received endosulfan orally for 30 days (Dorough *et al.*, 1978; Nath *et al.*, 1978). Only trace amount of endosulfan sulfate residue was recovered from the livers on the 30th day. Rats fed with 10 mg/kg of endosulfan followed the similar profile as in the 5 mg/kg group.

In the present study, since the residue level of endosulfan was not determined in the fatty tissues of rats, we were unable to ascertain its accumulation level. However, Kellner and Eckert (1983) indicated that bioaccumulation did not occur in the fatty tissues of male rats.

Since α-endosulfan has been reported be more stable isomer compared to the β-isomer, and the sulfate formed from both isomers was as toxic as the α-isomer (Brooks, 1975), the death of 5 animals as observed in the higher-dosed group (10 mg/kg) could be attributed to these characteristics.

5.3.3 Hormonal analyses of testosterone, T3 and insulin levels

The levels of sera testosterone, T3 and insulin are summarized in Table 5.12.

Significant changes were also observed in the levels of sera testosterone and T3 of both treated groups compared to the control animals. Animals treated with 5 mg/kg bw/day of endosulfan exhibited significantly lower levels of testosterone and T3 (105.22 ± 6.51 ng/dL and 58.79 ± 1.32 ng/dL respectively) on the 16th day compared to control animals whose concentrations of testosterone and T3 were 226.00 ± 18.00 ng/dL and 72.61 ± 2.56 ng/dL respectively (Table 5.12, Figure 5.2 and 5.3). However, the animals regained their hormonal levels on the 30th day to 143.90 ± 21.22 ng/dL (testosterone) and 70.91 ± 2.93

ng/dL (T3) which were noted to be lower than control animals (Table 5.12, Figure 5.2 and 5.3).

As with the higher-dosed group, animals treated with 10 mg/kg endosulfan, exhibited statistically lower testosterone and T3 levels compared to control animals on the 16th day (31.19 ± 6.72 ng/dL and 49.98 ± 3.45 ng/dL respectively). The hormone levels also showed similar trends as observed with the lower-dosed group (Table 5.12, Figure 5.2 and 5.3).

The insulin levels in treated animals were statistically insignificant compared to control animals for both the treated groups (Table 5.12, Figure 5.4).

The sera testosterone and T3 levels were significantly diminished ($p < 0.05$) in rats treated for 15 days at both dose levels. Both hormone levels remained significantly reduced, although most animals were noted to regain its hormonal levels 15 days following recovery period as compared to the 16th day. Such changes have earlier been reported where an alteration in luteinizing hormone (LH) was responsible for the production of male hormone testosterone (Sinha *et al.*, 1989). It was also postulated that such effects may be induced directly by interference of the compound with reproductive components or indirectly by influencing hormonal regulations (Dalsenter *et al.*, 1997).

Endosulfan was reported to induce hepatic microsomal enzymes as evidenced by decreased sleeping time and decreased blood and brain levels of pentobarbital (Gupta and Gupta, 1977). Since endosulfan induces cytochrome P-450s activities as reported by Gupta and Gupta (1977), the metabolism of thyroid hormones increases, resulting in the

elevation of thyroid-stimulating hormone (TSH) in response to reduced circulating levels of T3 and T4 (Stoker *et al.*, 2000). Since the induction of microsomal enzymes was not determined in this study, we could not confirm whether endosulfan has effects on such induction. Hence, we postulate that the decline in sera T3 level could be attributed to the induction of P-450s activities by administration of endosulfan, resulting in rapid clearance of thyroid hormones (T3 and T4) from the circulation and thus elevate TSH, although not included in the present study, as indicated by Stoker and co-workers (2000).

5.3.4 Histological evaluation

On the 16th day, the absolute and relative testes weights of both treated groups were markedly reduced as reported in Section 5.3.1. The distinct changes were shown on the histological sections where the tubular diameters were smaller with inter-tubular spaces filled with fluid as compared to the control animals.

In a normal testis of a rat, the cells were arranged and packed together with lumens filled with sperm tails (Figure 5.5).

As for the testes of rats exposed to 5 mg/kg of endosulfan, the tubules were smaller as compared to the control animals (Table 5.13, Figure 5.17) with most of the cells had sperm tails. However, they exhibited fluffing of cells which clumped together to form giant-liked cells (Figure 5.8).

For the higher-dosed group (10 mg/kg), the tubules were significantly smaller as compared to the control and 5 mg/kg groups (Table 5.13, Figure 5.17). Most of the testes cells were not filled with sperm tails. The tubules were generally contracted and severely damaged

as compared to the control group (Figure 5.9). Inter-tubular spaces were increased as seen in an overview in Figure 5.9. The cells were loosely arranged where the inter-cellular linkages were weakened. Hence, this phenomenon contributed to the fluffing of cells where immature germ cells were found in the tubular lumens resulting in the formation of giant-liked cells (Figure 5.10).

On the 30th day, the tubules of both treated groups regained its diameter sizes and the changes in size were insignificant as compared to the control animals. However, its tubular diameters were significantly increased as compared to the sizes observed on the 16th day (Table 5.13, Figure 5.17).

Based on the testes' histology of the treated rats from both dose levels, the damage was not severe and insignificant (Figure 5.13 and 5.15) as compared to the control animals (Figure 5.11) although some fluffing of cells resulting in the formation of giant-liked cells was observed (Figure 5.14 and 5.16). Hence, the testicular damage observed on the 30th day following 15 days of recovery period was not as severe as that seen on the 16th day.

5.4 CONCLUSION

The present study shows that the liver and kidneys weights of treated rats remained elevated at the end of the recovery period as compared to the control animals. In addition, the testes and epididymis weights declined significantly in a dose-dependent manner with the higher-dosed group registered the most significant drop. Reduction in body weight and intoxication symptoms were also observed with 5 deaths recorded in the 10 mg/kg group.

Due to the fact that endosulfan is excreted rapidly after oral administration, very low residues were detected in body tissues, with the kidneys of rats registered the highest level in both dose levels followed by the livers. However, its level in the liver was reduced at the end of the recovery period and undetected in the kidneys. It is predicted that the residue level in the liver will disappear if rats were prevented from any ingestion of endosulfan. Hence, we conclude that it is unlikely that endosulfan significantly bio-accumulate in humans.

Reduction in testosterone at both treated groups may suggest that testis is one of the organs most susceptible to endosulfan. A drop in T3 level may also suggest that this compound may also affects thyroid function resulting in the delayed sexual maturation and reduced level of testosterone because thyroid hormones, besides being essential for the growth and development, have been reported to be protective of gonadal functions in male rats (Stoker *et al.*, 2000).

Based on the histology of the testes, the testes appeared to be severely damaged with significant smaller tubules in the higher-dosed group as compared to the control and 5 mg/kg groups. However, at the end of the recovery period, the damage was not as severe as compared to the 16th day and insignificant with its tubules regained to the control size although the formation of giant-liked cells was observed. In measuring the seminiferous tubules, caution should be placed given the immersion fixation of the testes because this may have caused the shrinkage artifacts and therefore perfusion fixation is generally warranted when performing quantitative histometrics. Hence, pubertal development needs to be investigated.

Based on the above findings, it would appear that the effects of endosulfan are reversible and the compound is not as toxic as other organochlorine pesticides such as DDT, which was reported to have persistence and bio-accumulative characteristics on biota and abiota. To date, there is little information available in substantial accumulation of endosulfan in tissues. The kinetic behavior of endosulfan and the mechanism of changes observed in the present experiment need further investigation. Experiments on whether endosulfan alters thyroid function such as the induction of P-450s activities and thyroid gland histopathology need to be investigated because the latter has been proven to be the most reliable parameter for the detection of compounds that alter thyroid function by DeVito *et al.* (1999). In addition, sperm abnormality study such as the sperm morphology, sperm count and biochemical changes in testes should be further investigated to ascertain the reproductive effects of endosulfan in rats. Pair-feeding experiments should be included in the experiment in order to evaluate the possibly sensitive effects of a chemical on the organ weight as suggested by Uemitsu *et al.* (1986). Only further investigation will lead to establishing a complete toxicological profile of endosulfan.

Table 5.1. Body weight of rats (Mean ± S.E.M.) on the 16th and 30th day following administration of endosulfan for a period of 15 days

Dosage	Group	
	Day 16	Day 30
Initial		
Control (0 mg/kg)	91.4 ± 2.4	95.0 ± 2.2
5 mg/kg	100.0 ± 3.2	82.9 ± 7.0
10 mg/kg	105.0 ± 4.6	92.0 ± 4.6
Final		
Control (0 mg/kg)	170.7 ± 4.6	225.0 ± 5.5
5 mg/kg	170.0 ± 5.3	214.3 ± 6.4
10 mg/kg	171.3 ± 11.6	230.0 ± 8.4
% BW gain		
Control (0 mg/kg)	86.9 ± 3.8	137.1 ± 5.0
5 mg/kg	70.1 ± 2.5	170.7 ± 20.4
10 mg/kg	62.9 ± 7.3	151.6 ± 11.0

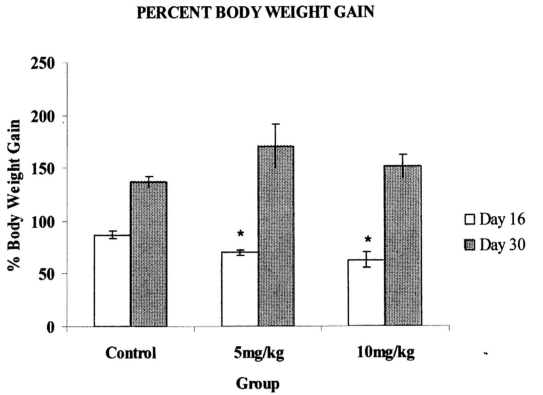


Figure 5.1. Percent body weight gain (Mean ± S.E.M.) following oral administration of endosulfan in rats. Group sizes are as follows: Day 16 - Control (7), 5 mg/kg (6), 10 mg/kg (4); Day 30 - Control (7), 5 mg/kg (7), 10 mg/kg (5).

* indicates that there is a significant difference ($p < 0.05$) between control and respective treated group.

Table 5.2. Absolute organs weights (Mean ± S.E.M.) of control and treated male rats on Day 16

Parameter	Control (0 mg/kg)	5 mg/kg	10 mg/kg
Liver	7.04 ± 0.67	7.15 ± 0.97	7.37 ± 1.39
Kidneys	1.42 ± 0.14	1.41 ± 0.11	1.62 ± 0.30
Testes	1.95 ± 0.11	1.72 ± 0.07*	1.52 ± 0.07**
Epididymis	0.67 ± 0.08	0.51 ± 0.05*	0.37 ± 0.05**

* Significant difference ($p < 0.05$) from the control group

** Significant difference ($p < 0.05$) from the control and 5 mg/kg groups

Table 5.3. Relative organs weights/100 g body weight (Mean ± S.E.M.) of control and treated male rats on Day 16

Parameter	Control (0 mg/kg)	5 mg/kg	10 mg/kg
Liver	4.12 ± 0.05	4.20 ± 0.22	4.28 ± 0.13
Kidneys	0.83 ± 0.02	0.83 ± 0.02	0.94 ± 0.15**
Testes	1.15 ± 0.05	1.02 ± 0.04	0.90 ± 0.07*
Epididymis	0.39 ± 0.01	0.30 ± 0.01*	0.22 ± 0.01**

* Significant difference ($p < 0.05$) from the control group

** Significant difference ($p < 0.05$) from the control and 5mg/kg groups

Table 5.4. Absolute organ weights (Mean ± S.E.M.) of control and treated male rats on Day 16 with final body weight as covariate

Parameter	Control (0 mg/kg)	5 mg/kg	10 mg/kg
Liver	7.03 ± 0.23	7.17 ± 0.25	7.34 ± 0.30
Kidneys	1.42 ± 0.03	1.41 ± 0.03	1.62 ± 0.04**
Testes	1.95 ± 0.03	1.72 ± 0.04*	1.52 ± 0.05**
Epididymis	0.67 ± 0.02	0.51 ± 0.02*	0.37 ± 0.03**

* Significant difference ($p < 0.05$) from the control group

** Significant difference ($p < 0.05$) from the control and 5 mg/kg groups

Table 5.5. Absolute organs weights (Mean ± S.E.M.) of control and treated male rats on Day 30

Parameter	Control (0 mg/kg)	5 mg/kg	10 mg/kg
Liver	7.91 ± 0.92	7.72 ± 0.65	8.91 ± 0.57
Kidneys	1.57 ± 0.12	1.56 ± 0.15	1.70 ± 0.16
Testes	2.58 ± 0.08	2.33 ± 0.07*	2.05 ± 0.14**
Epididymis	0.79 ± 0.11	0.62 ± 0.02*	0.51 ± 0.07**

* Significant difference ($p < 0.05$) from the control group

** Significant difference ($p < 0.05$) from the control and 5 mg/kg groups

Table 5.6. Relative organs weights/100 g body weight (Mean ± S.E.M.) of control and treated male rats on Day 30

Parameter	Control (0 mg/kg)	5 mg/kg	10 mg/kg
Liver	3.51 ± 0.08	3.60 ± 0.06	3.88 ± 0.06**
Kidneys	0.70 ± 0.01	0.73 ± 0.01*	0.74 ± 0.02*
Testes	1.15 ± 0.03	1.10 ± 0.04	0.90 ± 0.06**
Epididymis	0.35 ± 0.02	0.29 ± 0.01*	0.22 ± 0.01**

* Significant difference ($p < 0.05$) from the control group

** Significant difference ($p < 0.05$) from the control and 5 mg/kg groups

Table 5.7. Absolute organ weights (Mean ± S.E.M.) of control and treated male rats on Day 30 with final body weight as covariate

Parameter	Control (0 mg/kg)	5 mg/kg	10 mg/kg
Liver	7.80 ± 0.15	8.04 ± 0.15	8.61 ± 0.18**
Kidneys	1.55 ± 0.02	1.63 ± 0.02*	1.64 ± 0.03*
Testes	2.59 ± 0.03	1.72 ± 0.04*	2.07 ± 0.04**
Epididymis	0.79 ± 0.03	0.51 ± 0.02*	0.51 ± 0.04**

* Significant difference ($p < 0.05$) from the control group

** Significant difference ($p < 0.05$) from the control and 5 mg/kg groups

Table 5.8. Survival rate of rats receiving endosulfan orally for 15 days

Dosage (mg/kg/day)	Survival/number of animals
Control (0 mg/kg)	14/14
5 mg/kg	13/13
10 mg/kg	9/14

Table 5.9. Distribution of α-, β-endosulfan and endosulfan sulfate in tissues after repeated oral administration of endosulfan to rats for 15 days.

Neither endosulfan nor its metabolite was detected in control rats. Each reading is an average of 4-7 rats.

Tissue	Dose 5 mg/kg						Dose 10 mg/kg					
	α-Endosulfan		β-Endosulfan		Endosulfan sulfate		α-Endosulfan		β-Endosulfan		Endosulfan sulfate	
	µg/g Fresh tissue	µg/g Lipid	µg/g Fresh tissue	µg/g Lipid	µg/g Fresh tissue	µg/g Lipid	µg/g Fresh tissue	µg/g Lipid	µg/g Fresh tissue	µg/g Lipid	µg/g Fresh tissue	µg/g Lipid
Liver	*nd	nd	nd	nd	0.09±0.04 (0.02-0.22)	2.80±1.11 (0.73-4.92)	nd	nd	nd	nd	0.09±0.03 (0.04-0.19)	2.33±0.66 (1.08-4.35)
Kidney	0.95±0.23 (0.34-1.86)	27.62±6.41 (9.64-47.96)	0.51±0.15 (0.23-1.19)	14.75±3.95 (6.00-30.63)	nd	nd	1.62±0.64 (0.48-3.11)	53.60±22.24 (18.91-111.92)	0.64±0.16 (0.33-0.97)	21.40±5.33 (9.66-34.82)	nd	nd
Post treatment[a]												
Liver	nd	nd	nd	nd	0.05±0.01 (0.03-0.08)	1.57±0.16 (0.80-2.19)	nd	nd	nd	nd	0.06±0.01 (0.05-0.10)	2.10±0.23 (1.62-3.05)
Kidney	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

* nd = Not detected

[a] 15 days after last administration

Table 5.10. Distribution of α-, β-endosulfan and endosulfan sulfate in selected tissues of rats in relation to lipid contents after 5 mg/kg of orally-administered endosulfan daily for 15 days

No endosulfan and its metabolite were detected in control rats. Each reading is an average of 4-7 rats.

	Liver			Kidney		
Parameter	α-endosulfan	β-endosulfan	Endosulfan sulfate	α-endosulfan	β-endosulfan	Endosulfan sulfate
µg/g fresh tissue	*nd	nd	0.09 ± 0.04 (0.02-0.22)	0.95 ± 0.23 (0.34-1.86)	0.51 ± 0.15 (0.23-1.19)	nd
µg/g lipid	nd	nd	2.80 ± 1.11 (0.73-4.92)	27.62 ± 6.41 (9.64-47.96)	14.75 ± 3.95 (6.00-30.63)	nd
% Lipid in fresh tissue	3.1 ± 0.2 (2.5-4.0)	3.1 ± 0.2 (2.5-4.0)	3.1 ± 0.2 (2.5-4.0)	3.5 ± 0.5 (2.8-3.9)	3.5 ± 0.5 (2.8-3.9)	3.5 ± 0.5 (2.8-3.9)
Post treatment[a]						
µg/g fresh tissue	nd	nd	0.05 ± 0.01 (0.03-0.08)	nd	nd	nd
µg/g lipid	nd	nd	1.57 ± 0.16 (0.80-2.19)	nd	nd	nd
% Lipid in fresh tissue	3.2 ± 0.1 (2.8-3.4)	3.2 ± 0.1 (2.8-3.4)	3.2 ± 0.1 (2.8-3.4)	**na	na	na

Values shown are Mean concentration ± S.E.M. (Range)
[a] 15 days after last administration
*nd = Not detectable
**na = Not available

Table 5.11. Distribution α-, β-endosulfan and endosulfan sulfate in selected tissues of rats in relation to lipid contents after 10 mg/kg of orally-administered endosulfan daily for 15 days

No endosulfan and its metabolite were detected in control rats. Each reading is an average of 4-7 rats.

	Liver			Kidney		
Parameter	α-endosulfan	β-endosulfan	Endosulfan sulfate	α-endosulfan	β-endosulfan	Endosulfan sulfate
μg/g fresh tissue	*nd	nd	0.09 ± 0.03 (0.04-0.19)	1.62 ± 0.64 (0.48-3.11)	0.64 ± 0.16 (0.33-0.97)	nd
μg/g lipid	nd	nd	2.33 ± 0.66 (1.08-4.35)	53.60 ± 22.24 (18.91-111.92)	21.40 ± 5.33 (9.66-34.82)	nd
% Lipid in fresh tissue	3.5 ± 0.3 (3.2-4.4)	3.5 ± 0.3 (3.2-4.4)	3.5 ± 0.3 (3.2-4.4)	3.0 ± 0.2 (2.5-3.5)	3.0 ± 0.2 (2.5-3.5)	3.0 ± 0.2 (2.5-3.5)
Post treatment[a]						
μg/g fresh tissue	nd	nd	0.06 ± 0.01 (0.05-0.10)	nd	nd	nd
μg/g lipid	nd	nd	2.10 ± 0.23 (1.62-3.05)	nd	nd	nd
% Lipid in fresh tissue	2.9 ± 0.1 (2.5-3.1)	2.9 ± 0.1 (2.5-3.1)	2.9 ± 0.1 (2.5-3.1)	**na	na	na

Values shown are Mean concentration ± S.E.M. (Range)
[a] 15 days after last administration
*nd = Not detectable
**na = Not available

Table 5.12. Hormonal levels and tubular diameter (Mean ± S.E.M.) of control and treated male rats during Day 16 and Day 30

Parameter	Day 16			Day 30		
	Control (0 mg/kg)	5 mg/kg	10 mg/kg	Control (0 mg/kg)	5 mg/kg	10 mg/kg
Testosterone (ng/dL)	226.18 ± 31.42	105.22 ± 6.51*	31.19 ± 6.72*	602.56 ± 165.74	143.90 ± 21.22*	71.55 ± 6.71*
T3 (ng/dL)	72.61 ± 2.56	58.79 ± 1.32*	49.98 ± 3.45**	76.37 ± 4.65	70.91 ± 2.93	54.89 ± 3.61**
Insulin (μIU/ml)	7.50 ± 0.68	8.01 ± 0.90	9.42 ± 1.07	9.65 ± 0.97	9.06 ± 0.52	8.92 ± 0.73

* Significant difference ($p < 0.05$) from the control group

** Significant difference ($p < 0.05$) from the control and 5mg/kg groups

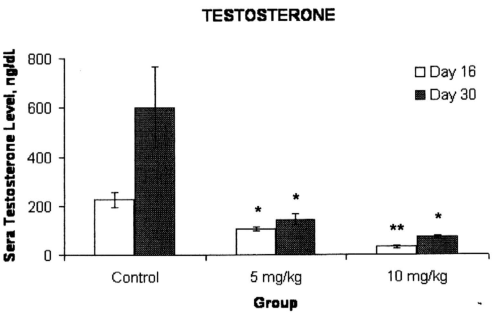


Figure 5.2. Sera concentrations of testosterone (T) following oral administration of endosulfan in rats. Group sizes are as follows: Day 16 - Control (7), 5 mg/kg (6), 10 mg/kg (4); Day 30 - Control (7), 5 mg/kg (7), 10 mg/kg (5). * indicates that there is a significant difference ($p < 0.05$) between control and respective treated group. ** indicates that there is a significant difference ($p < 0.05$) between control and respective treated group and between treated groups.

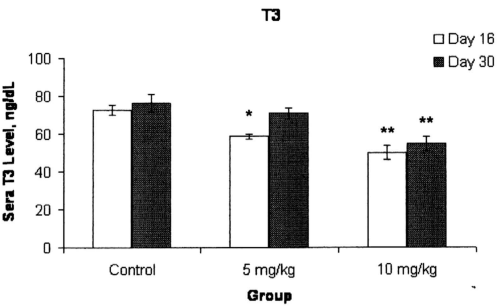


Figure 5.3. Sera concentrations of T3 following oral administration of endosulfan in rats. Group sizes are as follows: Day 16 - Control (7), 5 mg/kg (6), 10 mg/kg (4); Day 30 - Control (7), 5 mg/kg (7), 10 mg/kg (5). * indicates that there is a significant difference ($p < 0.05$) between control and respective treated group. ** indicates that there is a significant difference ($p < 0.05$) between control and respective treated group and between treated groups.

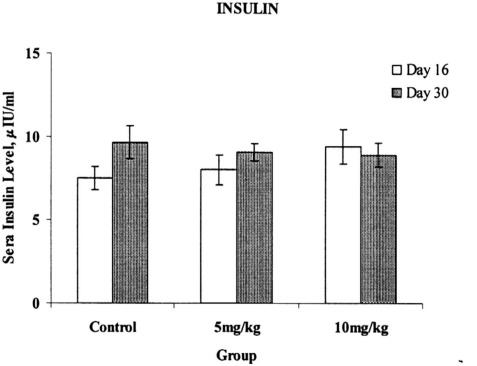
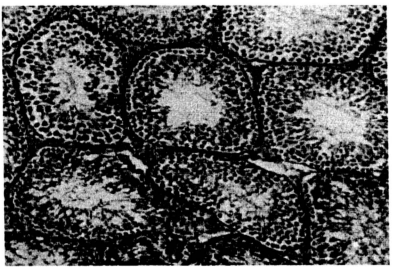
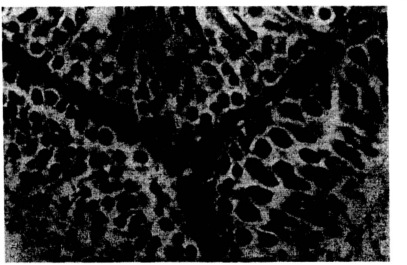
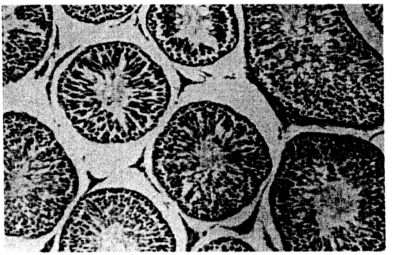


Figure 5.4. Sera concentrations of insulin following oral administration of endosulfan in rats. Group sizes are as follows: Day 16 - Control (7), 5 mg/kg (6), 10 mg/kg (4); Day 30 - Control (7), 5 mg/kg (7), 10 mg/kg (5).







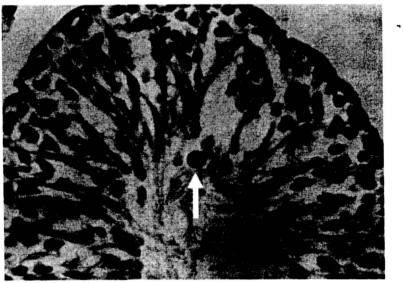
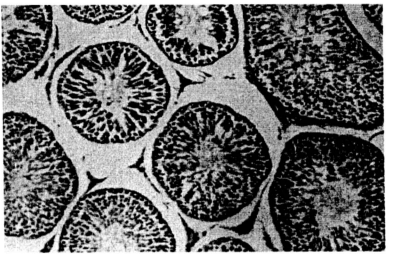


Figure 5.7. Section of rat testis exposed to 5 mg/kg endosulfan for a period of 15 days showing damaged tubules. H & E x 130

Figure 5.8. Section of rat testis exposed to 5 mg/kg endosulfan for a period of 15 days showing damaged tubules. The arrow indicates the formation of giant-like cells. H & E x 550



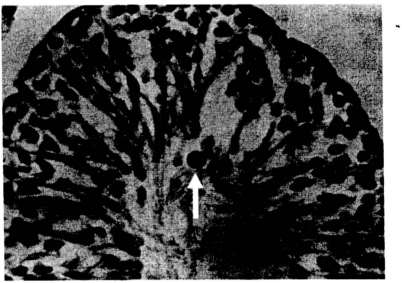
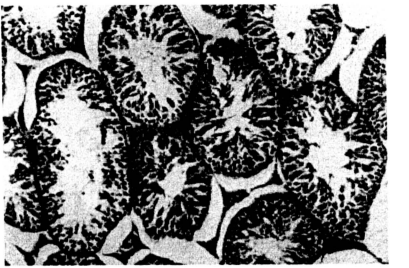


Figure 5.9. Section of rat testis exposed to 10 mg/kg endosulfan for a period of 15 days showing damaged tubules. H & E x 130

Figure 5.10. Section of rat testis exposed to 10 mg/kg endosulfan for a period of 15 days showing damaged tubules. The arrow indicates the formation of giant-like cells. H & E x 550



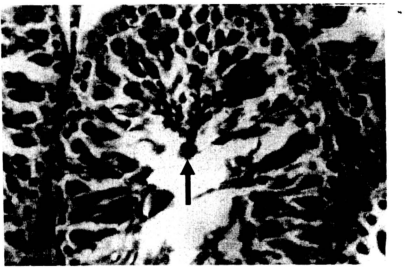
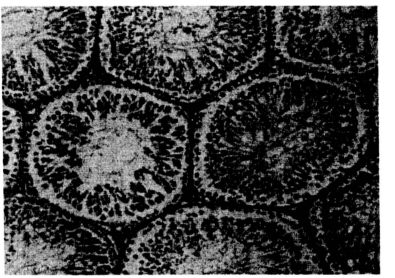


Figure 5.11. Section of rat testis from control group on Day 30 showing normal tubules.

H & E x 130

Figure 5.12. Section of rat testis from control group on Day 30 showing normal tubules.

H & E x 550



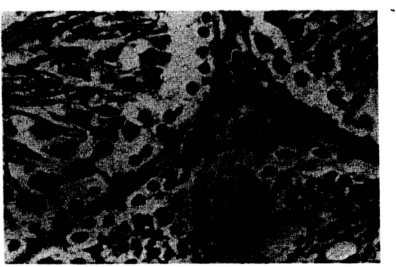
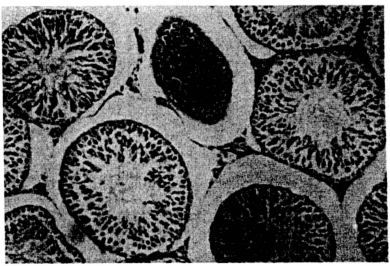


Figure 5.13. Section of rat testis exposed to 5 mg/kg endosulfan for a period of 15 days followed by a recovery period of 14 days showing damaged tubules. H & E x 130

Figure 5.14. Section of rat testis exposed to 5 mg/kg endosulfan for a period of 15 days followed by a recovery period of 14 days showing damaged tubules. The arrow indicates the formation of giant-like cells. H & E x 550



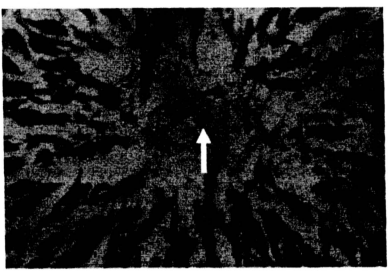
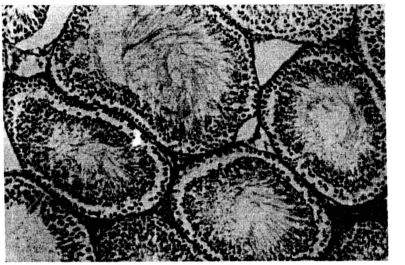


Figure 5.15. Section of rat testis exposed to 10 mg/kg endosulfan for a period of 15 days followed by a recovery period of 14 days showing damaged tubules. H & E x 130

Figure 5.16. Section of rat testis exposed to 10 mg/kg endosulfan for a period of 15 days followed by a recovery period of 14 days showing damaged tubules. The arrow indicates the formation of giant-like cells. H & E x 550



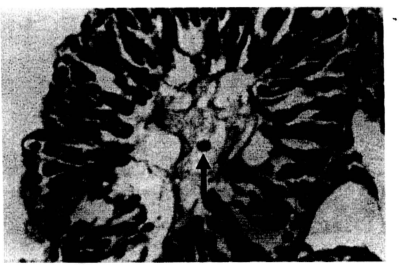


Table 5.13. Tubular diameter of testes on the 16th and 30th day

Concentration	Day 16	Day 30
Control (0 mg/kg)	216.0 ± 3.1	236.7 ± 4.9
5 mg/kg	218.5 ± 5.0	241.6 ± 7.7
10 mg/kg	186.4 ± 4.5**	255.5 ± 11.7

** Significant difference ($p < 0.05$) from the control and 5 mg/kg groups

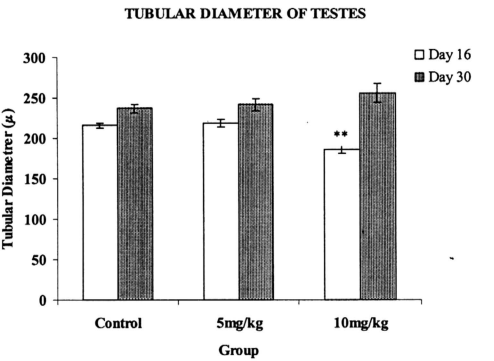


Figure 5.17. Tubular diameters of testes following oral administration of endosulfan in rats. Group sizes are as follows: Day 16 - Control (7), 5 mg/kg (6), 10 mg/kg (4); Day 30 - Control (7), 5 mg/kg (7), 10 mg/kg (5). ** indicates that there is a significant difference ($p < 0.05$) between control and respective treated group and between treated groups.