CHAPTER 4

ACUTE EXPOSURE: TOXICOKINETICS AND EFFECTS OF A SINGLE ADMINISTRATION OF ENDOSULFAN IN RATS

4.1 INTRODUCTION

The LD_{50} of endosulfan (isomeric mixture) varies widely depending on the mode of administration, species, dosing vehicle and sex of the animal. It was apparent from the studies on oral LD_{50} of endosulfan (Gupta, 1976; Gupta and Gupta, 1977) that female rats (8 - 49 mg/kg) were more susceptible than male rats (47 – 89 mg/kg) to its acute lethal action, often by one order of magnitude or more. Stimulation of the Central Nervous System (CNS) is the major characteristic of endosulfan poisoning (Farm Chemicals Handbook, 1992). The clinical signs of intoxication include piloerection, salivation, hyperactivity, respiratory distress, diarrhea, tremors, hunching and convulsions.

The isomers of endosulfan also show acute oral toxicity profiles similar to that of technical endosulfan. Like endosulfan, the toxicity of metabolites varied depending upon dosing vehicle and species used. Its toxicity was similar to or lower than the parent compound, except for endosulfan diol which has low acute oral toxicity (NRA, 1998). The absorption of undiluted endosulfan through the digestive tract of mammals was observed to be slow and incomplete, but was more rapid in the presence of alcohols, oils and emulsifiers (Gupta and Gupta, 1979).

Phenobarbital is an effective therapeutic measure against an absolute lethal dose of endosulfan in rats, reducing the clinical signs of poisoning and the mortality rate. However, diazepam did not possess any therapeutic effect against endosulfan intoxication in rats (Ebert and Weigand, 1984).

Metabolism studies in rats, after an intraperitoneal injection of 20 mg/kg of technical endosulfan in an oil solution revealed the presence of endosulfan diol and an unknown

compound in the urine as a water soluble conjugation metabolite (Gupta and Gupta, 1979). In a report by Kellner and Eckert, 1983, following administration of ^{14}C endosulfan via oral route to male Wistar rats at doses of 2 mg/kg in cooking oil, excretion was extensive, with greater than 90% of the administered dose eliminated in the urine and feces within the seven days after dosing. The highest tissue concentrations were found in the kidneys (1.8 ppm) followed by the liver (0.23 ppm). The highest blood concentrations were found between 3 and 8 hour, with a mean of the highest observed blood concentrations of 0.25 ± 0.06 μg/ml.

Since contamination occurs in human and animal system either directly or indirectly as environmental pollutants, the present chapter elucidates the toxicokinetics profile and the acute effects of endosulfan following single oral administration in rats.

The present study examines the toxicokinetics of endosulfan and its metabolites following a single oral administration of endosulfan in rats. In addition, animals were also observed for signs of toxicity. Upon termination of treatment, body weight and the weights of selected organs as well as its sera testosterone level were determined.

4.2 EXPERIMENTAL

4.2.1 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.

4.2.2 Preparation of 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.

4.2.3 Animals

Male Sprague-Dawley rats, weighing 40 to 120 g (4 weeks old) and bred in the colony of Universiti Kebangsaan Malaysia (UKM) animal house were used in this study. Animals were weight-ranked and assigned to one of the two dosing groups (0 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.

4.2.4 Experimental design and treatments

7 male rats were arbitrarily assigned (3 control and 4 treated rats) to 13 groups (0.5h, 1h, 2h, 4h, 8h, 10h, 24h, Day 2, 3, 4, 5, 6 and 7). Rats were fed once with suspensions of technical grade endosulfan (70α:20β) in TWEEN 80 at a dose level of 10 mg/kg. A vehicle control group received TWEEN 80 alone. Each animal was weighed prior to treatment and daily dosage was adjusted for body weight. Treatments were administered,

by oral gavage, 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.

4.2.5 Necropsy

Group of rats were decapitated at intervals of 0.5, 1, 2, 4, 8, 10, 24 hr and seven days following administration. Blood was sampled at each interval and analysed for α-endosulfan, β-endosulfan, endosulfan sulfate and endosulfan diol and testosterone measurement. At necropsy, the testes, epididymis, liver, kidneys and body weights were recorded. Livers and kidneys were removed for residual analysis.

4.2.6 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.

4.2.7 Radioimmunoassay (RIA)

Assay for sera testosterone was carried out using coated tube kits purchased from Diagnostic Products (Los Angeles, CA). The detailed procedures for the preparation of samples prior to hormonal analysis are described in Appendix 4.

4.2.8 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 using Student's *t*-test.

4.3 RESULTS AND DISCUSSION

4.3.1 Toxicokinetics profile of endosulfan

Throughout the experiment, only α-endosulfan was detected in blood whereas β-endosulfan, endosulfan sulfate and endosulfan diol were not detected. It has been reported that the β-isomer was metabolised or excreted rapidly than the α-isomer (EXTOXNET, 1993). Endosulfan diol is not retained in the plasma and for any interval of time is excreted as one of the metabolites in the urine of rats besides endosulfan-α-hydroxy ether and an unknown water soluble conjugate (Gupta *et al.*, 1979). The profile of α-endosulfan was plotted as the concentration of α-endosulfan in plasma against the interval time from 0 to 24 hour (Table 4.1, Figure 4.1)

Table 4.1. Profile of α-endosulfan in plasma after single oral administration in rats

Time, hr	Concentration, ng/ml	S.D.	S.E.M.
0	0	0	0
0.5	75	7	4
1.0	63	13	6
2.0	19	2	1
4.0	7	4	2
8.0	*nd	**na	na
10.0	nd	na	na
24.0	nd	na	na

*nd : Not detectable (Below detection limit). Detection limit for the chemicals were as follows: 3 ng/ml (α-endosulfan), 10 ng/ml (β-endosulfan), 30 ng/ml (endosulfan sulfate) and 100 ng/ml (endosulfan diol). **na : Not available

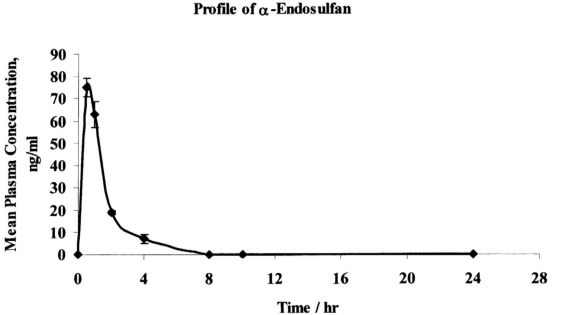

Figure 4.1. Profile of α-endosulfan in plasma after single oral administration in rats.

Values are expressed as Mean ± S.E.M.

Table 4.2. Profile of α-endosulfan plotted as log mean plasma concentration against time after transformation from Figure 4.1. Values are expressed as Mean ± S.E.M.

Time, hr	Log Concentration	S.D.	S.E.M.
0.5	1.8749	0.0435	0.0218
1.0	1.7908	0.0900	0.0450
2.0	1.2672	0.0532	0.0266
4.0	0.7751	0.2530	0.1265

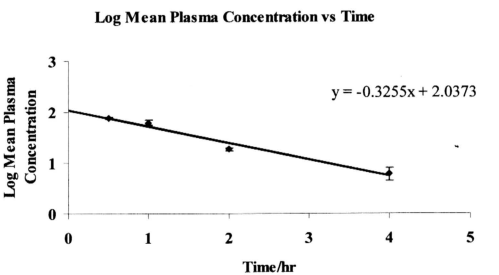

Figure 4.2. Profile of α-endosulfan plotted as log mean plasma concentration against time after transformation from Figure 4.1.

In this study, the plasma concentration of α-endosulfan as a function of time was found to best fit a one-compartment model, consistent with a first order process. Following oral administration, the highest blood concentration was observed 30 minutes after treatment, with a mean value of 75 ± 4 ng/ml. The biological half-life was estimated to be 55 minutes. α-endosulfan was not detected four hours following administration.

4.3.2 Body weight and organ weights changes

Body weight changes in rats receiving single oral administration of endosulfan are summarized in Table 4.3 and Figure 4.3.

No significant changes in body weight gain of rats were observed ($p = 0.30$) although the body weight gain of treated rats was reduced as compared to the control animals (Figure 4.3).

Table 4.3. Body weight gain of rats following a single oral administration of endosulfan for seven days. Values are expressed as Mean ± S.E.M.

Day	% Body weight gain	
	Control	Endosulfan-treated
1	40.9 ± 9.0	39.6 ± 2.1
2	64.3 ± 11.1	47.9 ± 7.1
3	64.3 ± 8.3	57.5 ± 3.6
4	81.1 ± 15.2	106.3 ± 6.3
5	141.7 ± 16.9	80.5 ± 8.0
6	97.2 ± 7.4	96.0 ± 14.8
7	130.9 ± 17.7	113.7 ± 12.5

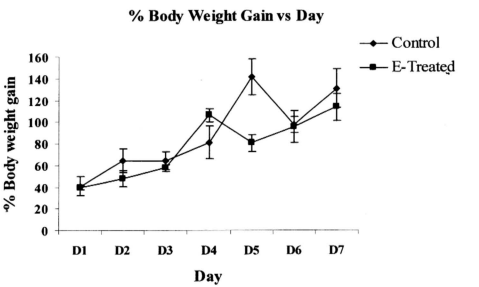

Figure 4.3. Body weight gain of rats following single oral administration of endosulfan for seven days. Group sizes are as follows: Day 1 (D1) - 3 Controls (C), 4 Treated (T); D2 - 3C, 4T; D3 - 3C, 3T; D4 - 3C, 4T; D5 - 3C, 4T; D6 - 3C, 4T; D7 - 4C, 4T. Values are expressed as Mean ± S.E.M.

#E-Treated: Endosulfan treated.

Absolute and relative weight of the liver, kidneys, testes and epididymis are summarized in Table 4.4 - 4.7 and Figure 4.4 - 4.11. All values are expressed as Mean ± S.E.M.

Table 4.4. Absolute weight of various organs (Mean ± S.E.M.) for control rats following single oral administration of TWEEN 80 for seven days

Day	Liver (g)	Kidneys (g)	Testes (g)	Epididymis (g)
1	4.40 ± 0.20	0.97 ± 0.03	0.43 ± 0.07	0.20 ± 0.00
2	3.52 ± 0.35	0.96 ± 0.02	0.60 ± 0.02	0.14 ± 0.02
3	4.75 ± 0.12	0.96 ± 0.07	0.58 ± 0.01	0.13 ± 0.02
4	4.15 ± 0.13	0.93 ± 0.02	0.76 ± 0.08	0.12 ± 0.01
5	4.79 ± 0.20	0.96 ± 0.02	0.89 ± 0.04	0.16 ± 0.01
6	4.47 ± 0.25	0.99 ± 0.08	0.78 ± 0.13	0.13 ± 0.02
7	5.68 ±0.30	0.96 ± 0.05	0.82 ± 0.04	0.14± 0.01

Table 4.5. Absolute weight of various organs (Mean ± S.E.M.) following single oral administration of endosulfan for seven days

Day	Liver (g)	Kidneys (g)	Testes (g)	Epididymis (g)
1	3.88 ± 0.24	1.08 ± 0.09	0.50 ± 0.10	0.20 ± 0.00
2	3.55 ± 0.61	0.98 ± 0.09	0.59 ± 0.05	0.12 ± 0.01
3	4.20 ± 0.43	0.95 ± 0.09	0.71 ± 0.05	0.13 ± 0.01
4	4.35 ± 0.24	1.00 ± 0.08	0.80 ± 0.08	0.17 ± 0.01
5	4.78 ± 0.28	0.99 ± 0.03	0.84 ± 0.08	0.15 ± 0.01
6	4.83 ± 0.22	0.99 ± 0.08	0.89 ± 0.09	0.16 ± 0.01
7	5.34 ± 0.17	1.12 ± 0.06	0.91 ± 0.11	0.14 ± 0.01

For Table 4.4 and 4.5, group sizes are as follows: Day 1 (D1) - 3 Controls (C), 4 Treated (T); D2 - 3C, 4T; D3 - 3C, 3T; D4 - 3C, 4T; D5 - 3C, 4T; D6 - 3C, 4T; D7 - 4C, 4T.

Table 4.6. Relative organ weight / 100 g body weight (Mean ± S.E.M.) for control rats following single oral administration of TWEEN 80 for seven days

Day	Liver (g)	Kidneys (g)	Testes (g)	Epididymis (g)
1	4.71 ± 0.13	1.04 ± 0.02	0.47 ± 0.08	0.21 ± 0.01
2	3.84 ± 0.33	1.06 ± 0.05	0.64 ± 0.01	0.15 ±0.02
3	4.15 ± 0.24	0.84 ± 0.02	0.51 ± 0.02	0.12 ± 0.01
4	4.10 ± 0.20	0.92 ± 0.03	0.75 ± 0.08	0.12 ± 0.01
5	4.29 ± 0.12	0.86 ± 0.01	0.80 ± 0.03	0.14 ± 0.01
6	4.01 ± 0.09	0.88 ± 0.02	0.69 ± 0.09	0.12 ± 0.02
7	4.80 ± 0.28	0.81 ± 0.01	0.70 ± 0.04	0.12 ± 0.01

Table 4.7. Relative organ weight / 100 g body weight (Mean ± S.E.M.) following single oral administration of endosulfan for seven days

Day	Liver (g)	Kidneys (g)	Testes (g)	Epididymis (g)
1	4.62 ± 0.25	1.38 ± 0.15	0.54 ± 0.10	0.24 ± 0.01
2	3.76 ± 0.42	1.05 ± 0.03	0.64 ± 0.04	0.13 ± 0.01
3	4.18 ± 0.16	0.95 ± 0.05	0.72 ± 0.09	0.13 ± 0.02
4	4.06 ± 0.08	0.93 ± 0.03	0.74 ± 0.05	0.15 ± 0.01
5	4.35 ± 0.11	0.90 ± 0.04	0.77 ± 0.08	0.14 ± 0.01
6	4.16 ± 0.02	0.85 ± 0.05	0.78 ± 0.10	0.14 ± 0.01
7	4.41 ± 0.12	0.92 ± 0.04	0.75 ± 0.07	0.12 ± 0.01

For Table 4.6 and 4.7, group sizes are as follows: Day 1 (D1) - 3 Controls (C), 4 Treated (T); D2 - 3C, 4T; D3 - 3C, 3T; D4 - 3C, 4T; D5 - 3C, 4T; D6 - 3C, 4T; D7 - 4C, 4T.

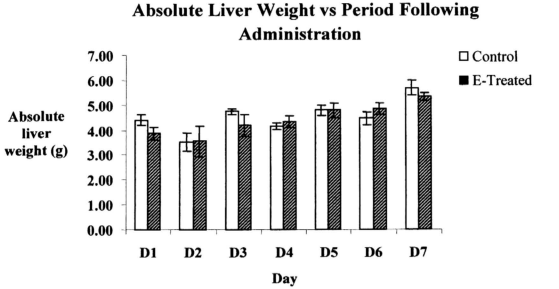

Figure 4.4. Absolute liver weight (Mean ± S.E.M.) of rats versus period following single oral administration of endosulfan for seven days. Group sizes are as follows: Day 1 (D1) – 3 Controls (C), 4 Treated (T); D2 - 3C, 4T; D3 - 3C, 3T; D4 – 3C, 4T; D5 - 3C, 4T; D6 - 3C, 4T; D7 - 4C, 4T. $p = 0.41$.

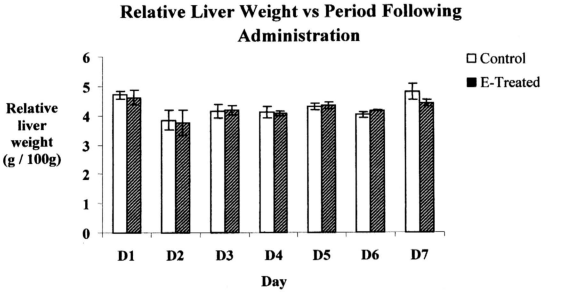

Figure 4.5. Relative liver weight (Mean ± S.E.M.) of rats versus period following single oral administration of endosulfan for seven days. Group sizes are as follows: Day 1 (D1) – 3 Controls (C), 4 Treated (T); D2 - 3C, 4T; D3 - 3C, 3T; D4 – 3C, 4T; D5 – 3C, 4T; D6 - 3C, 4T; D7 - 4C, 4T. $p = 0.48$.

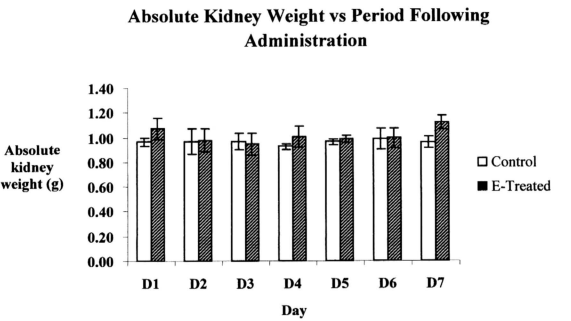

Figure 4.6. Absolute kidneys weight (Mean ± S.E.M.) of rats versus period following single oral administration of endosulfan for seven days. Group sizes are as follows: Day 1 (D1) - 3 Controls (C), 4 Treated (T); D2 - 3C, 4T; D3 - 3C, 3T; D4 - 3C, 4T; D5 - 3C, 4T; D6 - 3C, 4T; D7 - 4C, 4T. $p = 0.07$.

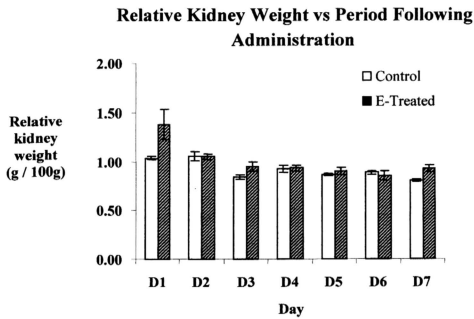

Figure 4.7. Relative kidneys weight (Mean ± S.E.M.) of rats versus period following single oral administration of endosulfan for seven days. Group sizes are as follows: Day 1 (D1) – 3 Controls (C), 4 Treated (T); D2 - 3C, 4T; D3 - 3C, 3T; D4 - 3C, 4T; D5 - 3C, 4T; D6 - 3C, 4T; D7 - 4C, 4T. $p = 0.14$.

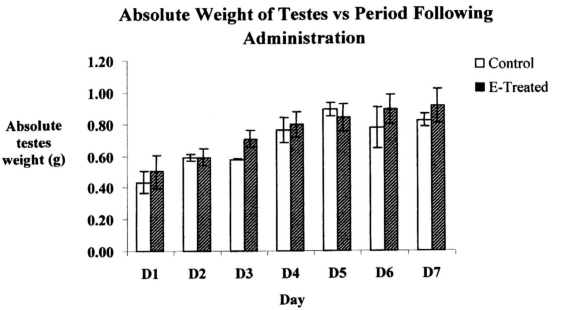

Figure 4.8. Absolute testes weight (Mean ± S.E.M.) of rats versus period following single oral administration of endosulfan for seven days. Group sizes are as follows: Day 1 (D1) – 3 Controls (C), 4 Treated (T); D2 - 3C, 4T; D3 - 3C, 3T; D4 – 3C, 4T; D5 - 3C, 4T; D6 - 3C, 4T; D7 - 4C, 4T. $p = 0.06$.

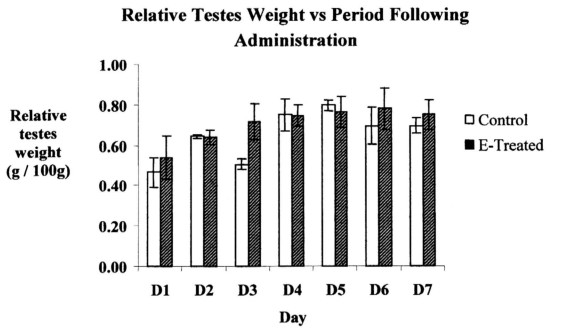

Figure 4.9. Relative testes weight (Mean ± S.E.M.) of rats versus period following single oral administration of endosulfan for seven days. Group sizes are as follows: Day 1 (D1) – 3 Controls (C), 4 Treated (T); D2 - 3C, 4T; D3 - 3C, 3T; D4 – 3C, 4T; D5 - 3C, 4T; D6 - 3C, 4T; D7 - 4C, 4T. $p = 0.13$.

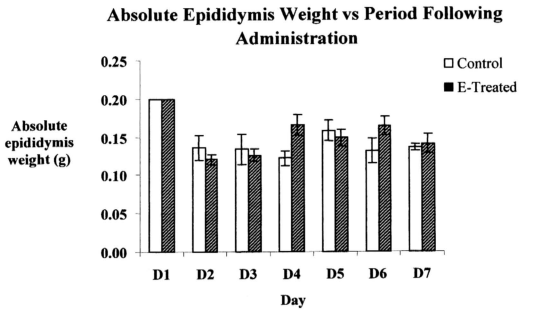

Figure 4.10. Absolute epididymis weight (Mean ± S.E.M.) of rats versus period following single oral administration of endosulfan for seven days. Group sizes are as follows: Day 1 (D1) - 3 Controls (C), 4 Treated (T); D2 - 3C, 4T; D3 - 3C, 3T; D4 – 3C, 4T; D5 - 3C, 4T; D6 - 3C, 4T; D7 - 4C, 4T. $p = 0.45$.

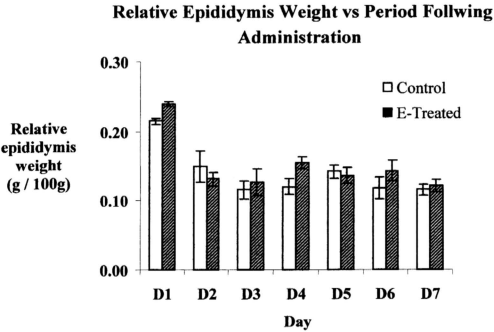

Figure 4.11. Relative epididymis weight (Mean ± S.E.M.) of rats versus period following single oral administration of endosulfan for seven days. Group sizes are as follows: Day 1 (D1) - 3 Controls (C), 4 Treated (T); D2 - 3C, 4T; D3 - 3C, 3T; D4 – 3C, 4T; D5 - 3C, 4T; D6 - 3C, 4T; D7 - 4C, 4T. $p = 0.12$.

Table 4.8. p values obtained from Student's *t*-test for both absolute and relative organ weights

Parameter	p Value	
	Absolute weight	Relative weight
Liver	0.41	0.48
Kidneys	0.07	0.14
Testes	0.06	0.13
Epididymis	0.45	0.12

From Table 4.4 - 4.7, the absolute and relative weights of the liver, kidneys, testes and epididymis in treated rats were insignificant as compared to the control rats ($p > 0.05$). In general, the absolute and relative weights of the liver, kidneys, testes and epididymis in treated rats did not show any discernible trend as compared to the controls.

No mortality and symptoms of intoxication was observed throughout the study.

4.3.3 Residue analysis

The distribution of α-endosulfan, β-endosulfan and endosulfan sulfate in selected tissue (kidneys) after single oral administration of endosulfan is summarized in Table 4.9.

Table 4.9. Residue levels of endosulfan in kidneys of rats in relation to wet weight and lipid content at the end of the 7th day following single oral administration of 10 mg/kg endosulfan. #

Parameter	Kidneys		
	α-Endosulfan	β-Endosulfan	Endosulfan sulfate
μg/g fresh tissue	0.39 ± 0.17	0.35 ± 0.19	*nd
μg/g lipid	7.66 ± 2.87	6.62 ± 3.23	nd
% Lipid in fresh tissue	4.9 ± 0.3	4.9 ± 0.3	**na

*nd: Not detectable

**na: Not available

#No endosulfan and its metabolite were detected in control rats. Each reading is an average of 4 rats. Values are expressed as Mean ± S.E.M. For Table 4.9, group sizes are as follows: Day 1 (D1) - 3 Controls (C), 4 Treated (T); D2 - 3C, 4T; D3 - 3C, 3T; D4 - 3C, 4T; D5 - 3C, 4T; D6 - 3C, 4T; D7 - 4C, 4T.

At the end of the 7th day, the kidneys and the liver were examined for the presence of the parent compound and metabolites. α-endosulfan and β-endosulfan were detected in the kidneys averaging 0.39 ± 0.17 μg/g and 0.35 ± 0.19 μg/g respectively. Endosulfan sulfate

and endosulfan diol were undetected in both the kidneys. Neither the parent compounds nor its metabolite was detected in the livers.

Our finding also indicated that kidneys have the ability and potential to accumulate endosulfan compared to the liver. This was consistent with the previous report, in which Kellner *et al.*, (1983) indicated that kidneys registered the highest residue level of endosulfan (1.8 ppm) compared to the liver which registered approximately 0.23 - 0.48 ppm.

4.3.4 Hormonal analysis of testosterone

The level of sera testosterone is summarized in Table 4.10.

Table 4.10. Sera testosterone levels (Mean ± S.E.M.) of control and treated rats following single oral administration of 10 mg/kg endosulfan. Group sizes are as follows: Day 1 (D1) - 3 Controls (C), 4 Treated (T); D2 - 3C, 4T; D3 - 3C, 3T; D4 - 3C, 4T; D5 - 3C, 4T; D6 - 3C, 4T; D7 - 4C, 4T.

	Sera testosterone level, ng/dL	
Day	Control	Endosulfan Treated
1	10.91 ± 0.39	7.33 ± 3.67
2	102.64 ± 4.58	9.62 ± 4.81
3	80.53 ± 3.54	21.03 ± 12.14
4	156.63 ± 13.72	38.35 ± 19.17
5	46.60 ± 2.08	15.50 ± 7.75
6	66.09 ± 1.37	19.98 ± 9.99
7	31.44 ± 1.50	2.88 ± 1.44

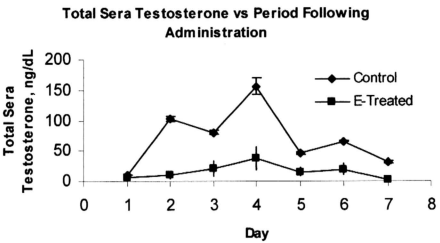

Figure 4.12. Sera testosterone levels (Mean ± S.E.M.) of control and treated rats following single oral administration of endosulfan for seven days. Group sizes are as follows: Day 1 (D1) - 3 Controls (C), 4 Treated (T); D2 - 3C, 4T; D3 - 3C, 3T; D4 – 3C, 4T; D5 - 3C, 4T; D6 - 3C, 4T; D7 - 4C, 4T.
*p = 0.01.

From Table 4.10 and Figure 4.12, sera testosterone levels in treated animals were found to be significantly lower than control animals ($p < 0.05$). Reduction in testosterone at the treated group may suggest that testis is one of the organs most susceptible to endosulfan.