The Toxicity of Endosulfan in Rabbits

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INTRODUCTION

Endosulfan is one of the important members of the "chlorinated hydrocarbons of the cyclodiene group" and has a widespread use for crop protection in the field of agriculture (MARTIN, 1964 and MILLER, 1965). Modern agricultural practices have reached a stage where a halt in the use of these pesticides would reduce the agricultural production and thereby affect the nation's economy. However, much concern is being shown about the toxic hazards of pesticides in the reports of several workers. Under normal conditions of manufacture, handling and spraying of insecticides, large quantities of the material come in contact with the skin of workers and remain on body surfaces for long periods. This may cause local lesions and systemic manifestations after getting absorbed into the system. Since the most likely route of entry of the compound in case of occupational exposures is through the skin, it was, therefore, considered desirable to study the acute toxicity in the rabbits after dermal application of Endosulfan.

MATERIALS AND METHODS

Industrial Toxicology Research Centre bred female albino rabbits weighing between 1.5 to 2.0 kg were used for this experiment. They were fed a standard pellet diet from Hindustan Lever India Ltd. and water ad libitum. The animals were prepared by clipping the skin of the trunk measuring approximately 1/10th of the total surface area.

Two samples of Technical Endosulfan (indigenous and imported) were referred to us by the National Chemical Laboratory, Poona, India for studying their comparative acute dermal toxicity in rabbits. Both the samples were used for acute LD_{50} studies while only indigenous Endosulfan was used for studying the systemic toxicity. The required quantity of Endosulfan was dissolved in 1 ml chloroform and uniformly painted on the specified areas of

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the skin. The control animals were treated with chloroform alone.

To determine LD_{50} four rabbits were used for each dose and a dose range of 125-225 mg/kg body weight was applied on the skin. The animals were observed for a period of 7 days and the LD_{50} was determined by the method of MILLER and TAINTER (1944). During the experiment the animals were also observed for any signs of toxicity and the animals dying during the course of the experiment were subjected to post-mortem examination.

For histopathological examination four rabbits were painted with Endosulfan (indigenous), the dose being 100 mg/kg body weight, and sacrificed after an interval of 7 days. Controls were treated similarly to the experimental animals and only chloroform was applied on their skin. Liver, kidneys, skin and adrenals were removed and fixed in 10% neutral formalin. Blocks of tissues were processed in a routine way and embedded in paraffin. Five micron sections were stained with haematoxylin and eosin.

To study the effect of Endosulfan (indigenous) on the eye mucosa, six rabbits were divided in three equal groups and aqueous suspension of 5, 10 and 20% of Endosulfan was dropped in the conjunctival sac of each rabbit, the other eye served as control. The animals were observed for a period of 7 days.

RESULTS

Table 1 shows that the acute dermal LD_{50} of indigenous and imported Endosulfan was 167 ± 21 and 187 ± 36 mg/kg body weight, respectively. There was no significant difference in the acute dermal toxicity of two samples of Endosulfan.

After acute dermal application of Endosulfan, the period of death varied depending upon the dose and some of the fatalities were delayed for as long as 7 days. At the higher dose (225 mg/kg), the various neurotoxic effects observed in four individual rabbits are given in Table 2. The table shows that one animal died after a single brief convulsion while others showed outbursts of numerous violent seizures. The fourth animal died without having shown more than hyperexcitability. In general the signs of poisoning varied depending upon dose and somewhat from animal to animal. The onset of symptoms appeared within 3 to 6 hrs. and at earlier stages, hyperexcitability, dyspnea, decreased respiration, discharge from eyes, fine

Table 1: LD_{50} values of Endosulfan in female rabbits

Sample	% Purity	LD_{50} (mg/kg)	Dose range (mg/kg)	P Value
I Endosulfan (indigenous)	>91	167±21	125-225	
II Endosulfan (imported)	90	182±36	125-225	I vs II NS

P > 0.01
NS = Not significant
± Standard deviation

tremors followed by convulsions of a clonic and tonic nature appeared at intermittent or at regular intervals. No change in body temperature was noticed in these animals. The animals preferred to rest on sternum with fore limbs extended. Finally the animals lost the response to painful stimuli first in the hind quarters and then spreading to the fore limbs followed by loss of motility, loss of corneal reflex, deep coma and death.

Table 2: Time course of various neurotoxic effects of four individual rabbits after an acute lethal dose of Endosulfan (225 mg/kg)

Animal No.	Time					
	0-3 hr	3-6 hr	6-12 hr	12-24 hr	1-7 days	Death
1	+	+++	c	-	-	7 hr
2	+	+	cccc	cc	-	3 days
3	++	ccc	-	-	-	4 hr
4	+	++	++	-	-	10 hr

\+ Hyperresponsiveness to sudden sound and tactile stimuli
++ Fine tremors of whoe body
+++ Moderate tremors
c Episodes of clonic convulsions

At necropsy, no definite gross pathological changes were observed in various organs, except that kidneys, peritoneum and muscles underlying the skin were invariably congested. The cause of death could not be ascertained.

Microscopic examination of liver sections of control animals presented normal appearance. After 7 days, the liver of animals treated with Endosulfan showed marked congestion and dilation of sinusoids. In some of the lobules degenerative changes in the hepatocytes were seen around central veins. Nuclei were in the various stages of degeneration and had disappeared from many cells. The

cytoplasm was less eosinophilic and foamy, Kupffer cells showed hyperplasia. Areas of focal necrosis comprising degenerated hepatocytes and small round cells were also seen throughout the section (Fig. 1). Cellularity was increased around portal tracts and bile duct proliferation was evident.

The sections of kidneys of control animals presented normal morphology. After Endosulfan, kidney sections showed groups of glomeruli with shrunken tufts and thickened Bowman's capsule. At places the epithelium of the proximal convoluted tubules was necrotic and desquamated (Fig. 2).

Adrenal cortex of animals of control group presented normal histological appearance. Sections of adrenals of Endosulfan-treated animals showed disruption of oblique cordal arrangement of cells in the zona reticularis; many cells were swollen with foamy cytoplasm and had eccentric nuclei (Figs. 3 & 4), while zonal glomerulosa and zona fasciculata presented normal morphology.

Sections from skin of control and Endosulfan-treated rabbits revealed no adnormality.

DISCUSSION

It is evident from this study that two forms of Endosulfan are not significantly different in their toxicity. The signs and symptoms observed were characteristic of other chlorinated hydrocarbons and the animals most probably died due to neurotoxicity. However, little is known about the biochemical correlates of neurotoxic signs seen during acute poisoning with this insecticide. Furthermore, single dermal application of Endosulfan (100 mg/kg) could produce definite toxic effects on liver, kidneys and adrenals although no cutaneous abnormality was observed in the treated animals. The liver changes as observed in this investigation were in the form of degeneration of the hepatocytes with foamy cytoplasm and bile duct proliferation. Such hepatic changes have been reported following exposure to several other pesticides. The appearance of foamy cytoplasm after administration of biphenyls to rats has been attributed to fatty changes in the liver cells (KIMBROUGH, et al. 1972). Damage to kidney tubules also occurs with wide variety of chemicals and the necrosis of proximal tubules as reported in this study may be due to direct toxic effect of Endosulfan. Sections of adrenals after Endosulfan produced morphological changes only in the inner layer, while various isomers of DDT are known to produce such damage to the inner two zones of adrenal

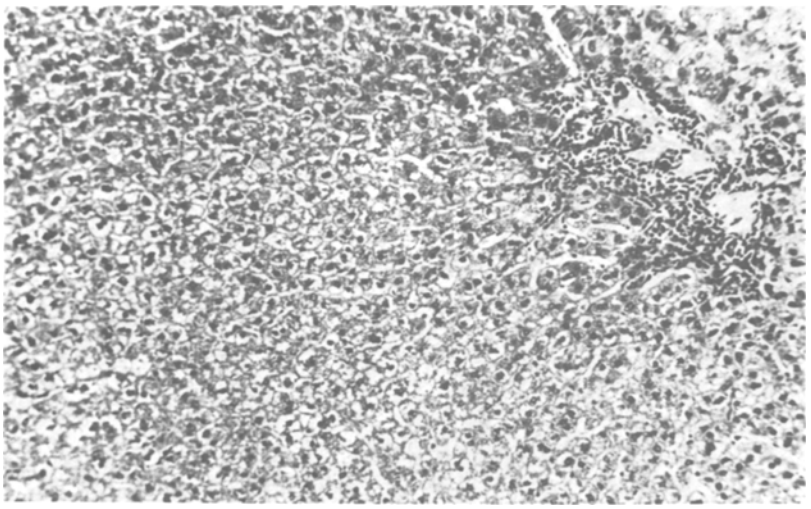

Fig. 1. Liver section of rabbit after 7 days painted with a single dose of Endosulfan (100 mg/kg) showing area of focal necrosis and foamy cytoplasm of the hepatocytes - H & E x 140.

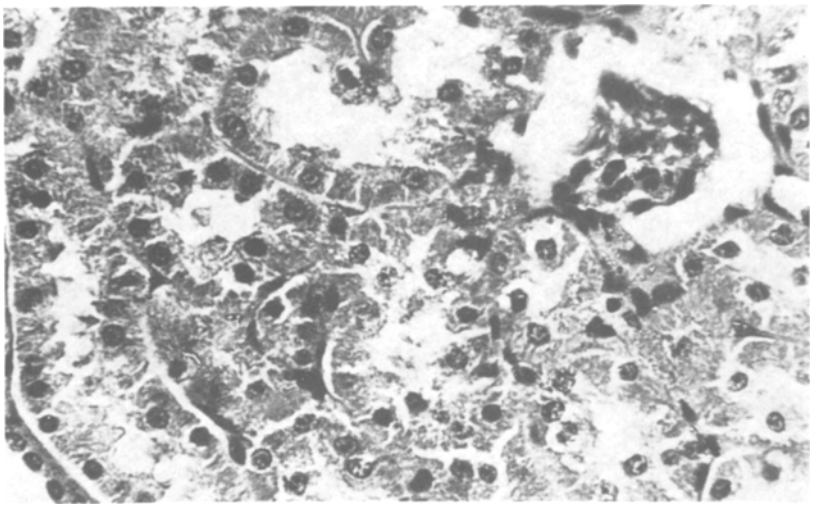

Fig. 2. Kidney section of rabbit after 7 days painted with a single dose of Endosulfan (100 mg/kg) showing shrunken glomerular tuft and necrosis of the epithelial cells of tubules - H & E x 570.

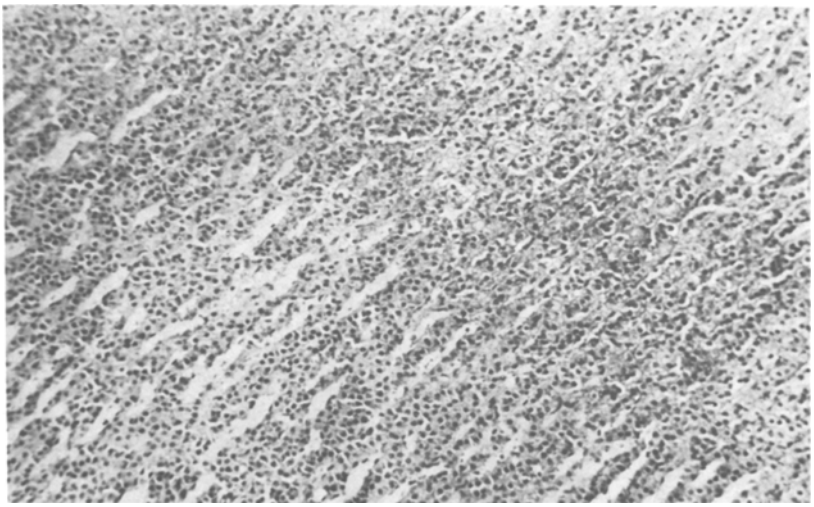

Fig. 3. Section of adrenal cortex of control rabbit showing normal pattern of the three layers - H & E x 140.

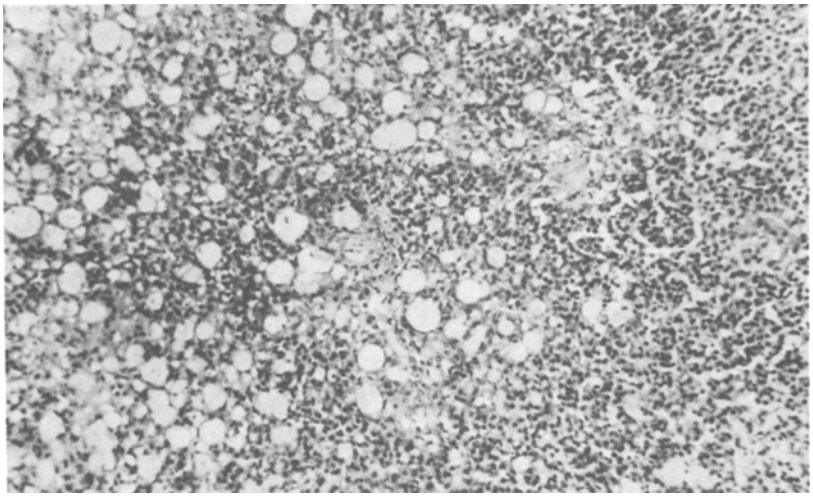

Fig. 4. Section of adrenal cortex of rabbit after 7 days painted with Endosulfan (100 mg/kg) showing zona reticularis with swollen and foamy cells - H & E x 140.

cortex of dog (HART, et al., 1973). The mechanism of such a change in the present experiments need further investigation.

It is well known that most of the pesticides are absorbed through the skin and produce systemic toxicity. However, absence of any skin lesion as observed in this study may be due to the fact that duration of application was long enough to produce skin lesions. Instillation of Endosulfan in the eye mucosa also did not produce any irritation or congestion. This may be due to rapid removal of instilled suspension by the lacrimal fluid. One can therefore conclude that although this compound may not cause acute local dermal toxicity, systemic toxicity cannot be ruled out.

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