Endosulfan-Induced Neurotoxicity in Rats and Mice

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INTRODUCTION

Endosulfan has been classed in literature, among the chlorinated hydrocarbons of the cylodiene group. In a recent report from this laboratory, principal symptoms related to effects of Endosulfan on the central nervous system have been reported in rabbits (GUPTA and CHANDRA, 1975). However, little is known about its toxic effects in other species of animals. Therefore preliminary work on the neurotoxicity of Endosulfan has been undertaken in male and female rats and mice.

MATERIAL AND METHODS

ITRC., Colony bred adult rats and mice of either sex were used for this study. They were maintained on commercial diet while fresh water was supplied ad libitum. The required quantity of Endosulfan dissolved in different vehicles was given intraperitoneally to groups of rats and mice. Control animals were treated with vehicle alone.

LD_{50} was determined according to the method of WEIL, (1952) observing the animals for mortality for a period of 7 days. During the experiment the animals were also observed for cage-side signs of toxicity. Animals dying during the course of the experiment were subjected to post-mortam examination.

For Cholinesterase estimation, the rats were given various doses of Endosulfan disolved in 10% alcohol in ground nut oil and killed 4 hr later. The four hr interval was chosen because at this time the maximum signs of Endosulfan intoxication were expected and no death occurred at this dosage. The animals were killed by decapitation and brain removed Brain was homogenised and a 10 μ litre sample of homogenate was incubated with 4 μ mol of acetylcholine

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in 1 ml of M/15 phosphate buffer, pH 7.2 for 30 min at 37°C as described by AUGUSTINSSON, (1957). A color complex with unhydrolysed acetylcholine was formed by adding 2 ml of 2 M alkaline hydroxylamine, 1 ml of concentrated HCL and 1 ml of 0.37 M ferric chloride, and absorbance recorded at 540 mm. Acetylcholinesterase (AChE) activity was expressed as μ mol of acetylcholine hydrolysed/30 min/10 μl of homogenate.

RESULTS

The mean values of LD_{50} in different sexes and species are given in TABLE 1. It is noted that rats are relatively resistant to the toxic effects of Endosulfan compared to mice. However, in either species the LD_{50} of Endosulfan is about 50% less when alcohol is used as solvent compared to ground nut oil. Further, the sex variation of about the same degree is seen in rats but not in mice. Thus the female rats are about twice as sensitive to the lethal effects of Endosulfan compared to the males irrespective of the vehicle.

TABLE.1.

Influence of Sex and Species on the Acute Toxicity of Endosulfan given intraperitoneally

Species	Sex	Vehicle	LD_{50} mg/kg	95% Confidence limits
RAT	F	Alcohol	22.1	18.6 - 26.9
	M	-do-	46.7	38.9 - 55.6
	F	10% Alcohol in ground nut oil	48.6	36.4 - 51.8
	M	-do-	89.4	73.0 -107.4
MOUSE	F	Alcohol	7.5	5.3 - 10.1
	M	-do-	6.9	5.4 - 8.9
	F	10% Alcohol in ground nut oil	13.5	10.6 - 16.8
	M	-do-	12.6	9.4 - 16.8

Lethality calculations according to Weil (1952) Four dose levels and 16 animals were used for each LD_{50} determination.

The first apparent signs of intoxication observed were: hyperresponsiveness to tactile stimuli, between 2nd and 3rd hr tremors of fore limbs were observed. Initially, respiration was increased followed by depression. The body temperature remained unchanged and sometimes it was even subnormal. Slowly the tremors became more violent and spread to other parts of the body. Occasionaly these tremors were intense and were difficult to differentiate from clonic seizures. None of the animals showed diarrhoea or any discharge from the eyes.

The time course of various neurotoxic effects of six individual female rats who received acute doses of Endosulfan (40 mg/kg) in 10% alcohol in ground nut oil is given in TABLE 2. The table shows that when a single dose of Endosulfan was given to rats the period of death and severity of symptoms varied from animal to animal. Out of six animals, 1st, 2nd and 6th animal

TABLE 2.

Time course of various neurotoxic effects of 6 individual female rats after an acute dose of Endosulfan (40 mg/kg) in 10% alcohol in ground nut oil

No. of animal	Time in hr			
	0-2	2-4	4-8	8-24
1	+++	CCC (D)		-
2	++++	++++(D)	-	-
3	+	++	++++	(D)
4	++++	-	-	-
5	-	-	(D)	-
6	+	++++(D)	-	-

+, hyperresponsiveness to tactile stimuli

++, fine tremors of whole body

+++, moderate tremors

++++, intense tremors which were not distinguishable from clonic convulsions

C, no of epidodes of convulsions.

D, time of death after the administration of Endosulfan.

died within 2 to 4 hr and showed intense tremors which were not distinguishable from the clonic seizures, while the 4th animal survived even after having shown intensive tremors during the first two hr of treatment. The 3rd animal showed seizures at regular or intermittant intervals and died during the night. The 5th animal died during 4 to 8 hr without having shown any tremors or convulsions.

At necropsy, no definite gross pathological lesions were observed in any of the animals.

The values for AChE in brain of control and Endosulfan treated female rats are given in TABLE 3. At lower doses, Endosulfan did not produce any significant change in AChE activity of brain. At higher doses (60 mg/kg) a significant decrease in brain AChE activity was observed.

TABLE 3.

AChE of female rats at 4 hr after various doses of Endosulfan given I/P in 10% alcohol in ground nut oil

Groups	Dose (mg/kg)	AChE[a]	% change	P value
I (Control)	Vehicle	0.63±0.06 ($\bar{6}$)	-	
II (Endosulfan)	10	0.68±0.10 ($\bar{6}$)	7.8(+)	I VS II NS
III (Endosulfan)	30	0.42±0.13 ($\bar{6}$)	33.3(-)	II VS III $P \leq 0.001$
				II VS III $P \leq 0.001$
IV (Endosulfan)	60	0.48±0.02 ($\bar{4}$)	23.9(-)	I VS IV $P \leq 0.001$
				II VS IV $P \leq 0.001$
				III VS IV $P \leq$ NS

Each reading is an average of 5 to 6 rats.

Acetylcholinesterase activity expressed as Amoles of substrate (acetylcholine hydrolysed/30 min/10 µl of homogenate).

The number in parentheses indicate number of animals used ± SE.

DISCUSSION

As expected, this study indicates that the toxicity is dependent on the vehicle used, but it is of great interest to note that the toxicity varies in male and female rats which is yet an another example of well known axiom in the field of toxicology that toxicity depends upon the sex used. The signs and symptoms induced by Endosulfan in rats and mice probably suggest that like other chlorinated hydrocarbons, Endosulfan produces its toxic effects due to CNS stimulation and death may be due to direct depressant effect of Endosulfan on some vital organs of the body. However, hypothermia as observed in this study is in contrast to hyperthemia observed with DDT intoxication in rats (HENDERSON and WODLLEY 1970). Tremors induced by Endosulfan as reported in this study could be due to increase in the rat brain acetylcholine (Ach). Such an increase in Ach has been reported following other tremor producing agents such as tremorine and oxytremorine and (HOLMSTEDT and LUNDGREN, 1966 and COX and POTKONJAK, 1969). Whether the convulsions induced by Endosulfan is due to inhibition of AChE or due to its action on choline acetylase is uncertain. Although Endosulfan produces an increase in whole brain AchE it does not seen likely that the convulssive action is related to this increase. However, after a high dose of tremorine an increase in both free and bound Ach in brain has been reported in the literature (CROSSLAND and SLATER, 1968). Whether there is any change in such free or bound Ach after Endosulfan is still unknown.

In the end it may be pointed out that the sex variation in Endosulfan induced toxicity is important. The question of whether such a sex variation in Endosulfan toxicity also exists in man may be of significance from the point of view of public health problems. Secondly signs and symptoms of Endosulfan poisoning suggested CNS stimulation and these points should be considered in initiating therapy.

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