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ACUTE AND SUBACUTE TOXICITY OF CYCLOHEXENE IN RATS

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SUMMARY

Acute and subacute toxicity studies of the cyclohexene have been carried out in rats (male and female). The acute oral LD50 in rats exceeded 160.4 (119.9-209.8 mg/100 gm body weight) for male and 149.9 (129.6-160.4 mg/100 gm body weight) for female. No adverse effects attributable to the solvent were noted after daily oral doses of (80, 100, 120, 140 and 200 mg/100 gm bw) to rats for 10 days. The death which was observed with the two highest doses (140 and 200 mg/100 gm bw) could be attributed to respiratory failure due to the consequence of inhalation of the vapor of cyclohexene and hemorrhage. An oral doses of (0.0, 15, 50, 150 and 450 mg/kg body weight) of cyclohexene were offered five times per week to rats (male and female) for 4 weeks. The highest dose caused a significant reduction in foetal weight and food intake in the first week. During the rest of the experimental period (4 weeks), the feed intake and body weight increased gradually with the fifth group but still lower than the rest of the treatments. There was no change in the haematological examination. The examination of urine revealed that, there were some ketones of all treatments, also there were no significant differences concerning the thyroid and spleen states between the treated rats and the control.

Keywords: Cyclohexene, toxicity, rats

INTRODUCTION

A variety of solvents have been used since early in the twentieth century for the extraction of edible oils from seeds and the residual meal being used as an animal feed stuff (Fincher, 1958). It is now realized that solvents with a high aromatic content and chlorinated solvents are best avoided on toxicological grounds. For example, the trichloroethylene extract of soybean meal proved toxic to cattle and sheep, causing aplastic anaemia (Pritchard et al., 1952 and Holm et al., 1953). The toxic agent appears to be S-dichlorovinyl-L-cysteine formed by the interaction of the solvent with cysteine in the meal (Mckinney et al., 1957).

Although the amount of solvent remaining in the residual meal or in the extracted oil is very small, since the maximum amount is recovered for re-use, the toxicological properties of solvents such as, cyclohexene or hexachlorobenzene have been examined. Further more cyclohexene was detected to contaminate the ground water in the surrounding of drinking water resource near the petrol areas. Cyclohexene (1, 2, 3, 4 tetrahydrobenzene) could be used for organic synthesis, as catalyst solvent and for oil extraction. Cyclohexene is toxic by inhalation. Kameskii et al., 1973 observed that cyclohexene inhaled at 5-20 mg/L for 2hr decreased the neuromuscular excitability, increased the frequency of cardiac contraction and caused leukocytosis and changes in the protein fractions of the blood serum. They found also a decrease in the activity of acetylcholinesterase in the erythrocytes, besides, mercapto group level in the blood were also observed. There is little information in the literature concerning the toxicology of the cyclohexene or the safety limits could be established. Therefore, it is necessary to carry out an experiment to determine the acute or subacute oral dose with cyclohexene.

MATERIALS AND METHODS

Cyclohexene used in these studies was prepared by dehydration of cyclohexanol at high temperature over various catalysts.

Animals:

25 males and 25 females SPF wister rats weighed (100-110gm). The animals were divided into five groups each included (5 males and 5 females).

Oral administration to rats

In acute single dosing experimental groups, the rats (male and female) were fasted overnight, weighed and the calculated dose was administered by intragastric injection using a ball point needle. five doses of cyclohexene were used (80, 100, 120, 140 and 200 mg/100 gm body weight). Each dose was disolved in 0.5 ml olive oil. All the animals were observed frequently during the immediate post-dosing period and for a further 10 days, after which time they were killed and autopsied. In subacute toxicity study, five groups of ten male and 10 females (100-120 gm) body weigh were administrated by cyclohexene (0, 15, 50, 150 and 450 mg/kg body weight) five times weekly and durated for 4 weeks. It is worthy to note that the control group of rats were given the olive oil during the experimental period while the experimental rats were fed the commercial diet. Body weight and food intake were measured weekly. After the feeding period, the rats were killed in which blood samples were obtained and haemoglobin, packed cell volume and red blood cell count were estimated.

In addition the thyroid gland and spleen were removed and weighed. Statistical analyses were carried out according to Snedecor and Cochran (1976).

RESULTS AND DISCUSSION

Acute oral toxicity in rats

Rats were found to tolerate single doses of cyclohexene of up to 120 mg/kg body weight with little effect, except for some depression of activity. Deaths were occurred in the treated animals of (140 and 200 mg/100 gm body weight) during the first 5 days of experimental period (Table 1). Rats showed signs of respiratory failure due to the consequence of the inhalation of the vapor of the cyclohexne. Autoposy indicated that death was due to anoxian, consequence of the inhalation of the solvent from the pharyngeal region

or even of the vapour itself. The LD50 of cyclohexene was calculated by the method of Litchfied and Wilcoxon (1948) and recorded the value of 160.4 (119.9-209.8 mg/100 gm body weight) for male and 149.9 (129.6-160.4 mg/100 gm body weight) for female.

In this connection, Khera (1974) observed that, maternal toxicity associated with a reduction in foetal weight was caused by 80 or 120 mg hexachlorobenzene/kg of body weight of rats given from day 6 to 21 of pregnancy. The LD50 of cycloheylamine hydrochloride (CHA) in mice after ipinjection was 61.9 mg/100 g body weight (Miyata et al., 1969). On the other hand, Walker and Stevenson (1966) reported that the acute oral LD50 in rats exceeded 20 ml/kg body weight of the hydrocarbon solvent SBP 62/82.

Table 1. The mortality rate of the female and male rats during 10 days of the oral toxic effect of cyclohexene

Cyclohexene levels	No. of rats	Day 1	2	3	4	5	6	7	8	9	10	Total
Female												
80 mg/100 gm bw	5											--
100 mg/100 gm bw	5											--
100 mg/100 gm bw	5											--
100 mg/100 gm bw	5				1	1						2
100 mg/100 gm bw	5		2			2		1				5
Male												
80 mg/100 gm bw	5											--
100 mg/100 gm bw	5											--
100 mg/100 gm bw	5		1									1
100 mg/100 gm bw	5			1								1
100 mg/100 gm bw	5	1	2		2							2

Sub-acute study

No abnormalities were seen in the behaviour of any of the treated rats, but at the highest dosage level (450 mg/kg body weight), the body weight gain of rats of both sexes showed highly significant loss of weight until week three, whereas in the week four the rats tolerate its weight. At the levels of (15, 50 and 150 mg/kg body weight), the body weight gain was unaffected (Table 2). The feed intake was somewhat reduced at the highest dose, the most marked reduction being in the first week. The average feed intake values over the whole experimental period was lowered in rats recieved 450

mg/kg body weight (Table 3).

It could be noticed that the reduction of body weight gain was accompanied by the reduction in the feed intake. The facts that the vapour of cyclohexene prevent the rats to eat hence the feed intake reduced when compared with the control particulary in the first week of experiment these results were confirmed by Gaunt et al. (1974) who reported that, rats which were given diets containing 0, 600, 2000 or 6000 ppm cyclohexylamine hydrochloride (CHA) for 13 weeks recorded a reduction in both body weight gain and feed intake at the two higher dosage levels. Walker and Stevenson (1966) observed no effects attributable to the hydrocarbon solvent SBP 62/82 after: (1) thrice weekly oral doses of 1 or 5 ml/kg body weight of SBP to rats for 13 weeks; (2) once weekly oral doses of 0.5 or 2.5 ml/kg body weight of a 20% emulsion for 26 weeks or (3) daily graded oral doses of 0-0.5 ml/kg body wt of SBP to dogs for 24 weeks. There were no differences between treated and control animals in the haematological findings (Table 4).

Table 2. Body weight gain to the intial body weight during 4 weeks

Cyclohexene levels	Intial wt (g)	Week 1	2	3	4	Total gain
			Male			
Control	100.6	34.4	28.0	34.8	18.0	115.2
15 mg/kg body wt	103.4	29.4	33.8	36.0	16.0	115.2
50 mg/kg body wt	105.2	33.4	30.2	33.2	20.8	117.6
150 mg/kg body wt	105.8	26.0	31.0	27.2	19.0	103.2
450 mg/kg body wt	104.2	-13.0***	39.7	20.0	21.0	80.7
			Female			
Control	102.8	17.2	12.4	17.6	11.0	58.2
15 mg/kg body wt	104.4	15.8	12.6	17.2	06.4	52.0
50 mg/kg body wt	101.6	15.6	09.4	19.0	07.4	51.4
150 mg/kg body wt	106.8	11.0	08.0	16.8	08.0	43.8
450 mg/kg body wt	102.6	-14.4***	22.0	20.6	01.8	44.4

Means on the same row with different superscripts are significantly different ($P<0.05$).

This observation agree with the results observed by Gaunt et al. (1974) by feeding male and female rate on diets containing (0, 600, 2000 or 6000 ppm cyclohexylamine hydrochloride (CHA) for 13 weeks. The examinations of faeces and urine at the two higher

dosage levels indicated the presence of blood in faeces, but not in urine except some ketones in all treatments.

Table 3. Feed consumption (g) during the four weeks experimental period

Cyclohexene levels	Week 1	2	3	4	Total
			Male		
Control	12.17	12.05	13.64	13.90	51.76
15 mg/kg body wt	11.43	11.57	13.83	14.03	50.86
50 mg/kg body wt	12.52	13.28	13.67	15.00	54.47
150 mg/kg body wt	11.05	12.85	12.83	13.53	50.26
450 mg/kg body wt	04.00**	08.74	07.57	09.50	29.81
			Female		
Control	10.12	09.24	10.26	09.03	38.65
15 mg/kg body wt	09.57	09.50	09.93	10.17	39.17
50 mg/kg body wt	09.91	09.29	10.67	10.33	40.20
150 mg/kg body wt	08.41	08.31	07.86	08.17	32.72
450 mg/kg body wt	03.45***	10.09	09.57	09.43	32.54

Means on the same row with different superscripts are significantly different ($P<0.05$).

Table 4. Haematological findings in rats recieved cyclohexen at 15-450 mg/kg body weight for four weeks

Cyclohexene	Hb (g/100 ml)	PCV (%)	RBC (10^6 mm^3)
	Female		
Control	13.6	43	6.60
15 mg/kg body wt	13.7	43	6.59
50 mg/kg body wt	13.9	44	7.05
150 mg/kg body wt	14.0	45	7.22
450 mg/kg body wt	14.0	44	7.20
	Male		
Control	15.3	48	8.40
15 mg/kg body wt	14.8	47	8.14
50 mg/kg body wt	14.9	4.8	7.97
150 mg/kg body wt	14.9	4.6	8.20
450 mg/kg body wt	14.8	4.8	8.20

Hb = Haemoglobin; PCV = packed cell volume

The organs of the treatment rats weighed less than those of the control, but this was attributable to the less body weight when expressed lelatine to body weight (Table 5). These results were not in agreement with those obtained by Walker and Stevenson (1966) who found

no evidence of any differences between the organ weight of the treated or control groups when rats recieved oral doses of 1 or 5 ml/kg of SBP 62/82 for 13 weeks.

Table 5. Feed consumption (g) during the four weeks experimental period

Cyclohexene levels	Thyroid		Spleen	
	Absolute	Relative	Absolute	Relative
			Male	
Control	562.550	0.4193	460.300	0.3424
15 mg/kg body wt	566.200	0.4223	454.600	0.3382
50 mg/kg body wt	468.600	0.3931	362.800	0.3073
150 mg/kg body wt	481.200	0.3986	399.600	0.3003
450 mg/kg body wt	408.800	0.3804	358.200	0.3310
			Female	
Control	394.200	0.2898	349.610	0.2588
15 mg/kg body wt	393.000	0.2891	340.600	0.2521
50 mg/kg body wt	266.000	0.2123	285.600	0.2397
150 mg/kg body wt	193.200	0.1544	254.800	0.2150
450 mg/kg body wt	181.000	0.1472	175.200	0.1461

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دراسة الجرعة المميتة أو الغير مميتة لمركب السيكلوهكسين على فئران التجارب

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اجريت هذه الدراسة لتقدير الجرعة المميتة لمركب السيكلوهكسين على فيران التجارب وذلك باعطاء كل فأر يوميا الجرعات ٨٠ ، ١٢٠ ، ١٤٠ ، ٢٠٠ مللى جرام / ١٠٠ جم من وزن الجسم الحى وذلك لمدة عشرة أيام وكانت الجرعة المميتة للذكور هى ١٦٠ (١١٩,٩ - ٢٠٩,٨ مللى جرام / ١٠٠ جرام) وزن الجسم الحى وللاناث هى ١٤٩,٩ (١٢٩,٦ - ١٦٠,٤ مللى جرام / ١٠٠جرام وزن الجسم الحى . وعند اعطاء الفيران السيكلوهكسين بجرعات ١٥ ، ٥٠ ، ١٥٠ ، ٤٥٠ مللى جرام لكل كيلو جرام من وزن الجسم الحى بمعدل خمس مرات اسبوعيا لمدة أربع أسابيع متتالية لوحظ الاتى : انخفاض شديد فى وزن الفأر للمجموعة التى حصلت على الجرعة ٤٥٠ مللجرام / كم فى الاسبوع الاول وكان ذلك مرتبطا فى نفس الوقت بنقص شديد فى استهلاك الفئران وفى الاسابيع التالية تحسن النمو واستهلاك الفئران ولكن بنسبة أقل من المجاميع الأخرى . ولم تسبب جرعات السيكلوهكسين المستخدمة فى هذه التجربة لم يحدث تغير فى مكونات الدم أو فى أوزان الاعضاء الداخلية وعلى الأخص الغدة الدرقية والطحال .