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Monocrotophos: Short-Term Toxicity in Rats

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Monocrotophos (MCP) is a broad spectrum organophosphorous insecticide used against a wide range of insect pests of a variety of crops and domestic animals. It is one of the highly toxic agricultural chemicals with wide variation in toxicity between different species (Janardhan et al 1986).

The residual activity of MCP on various parts of vegetable crops was reported to vary from 11.97 to 88.70 ppm (Narkhade et al 1977) which was in excess in comparison with the tolerance limit of 0.2 ppm (Awasthi et al 1977). The residues were shown to last from 9 to 11 days (Puri 1975 and Prasad Rao 1976) on vegetable crops. Since no data on toxicity were reported in the literature, WHO (1977) has recommended the use of short-term toxicity studies of MCP in mammals. Long-term feeding study in rats (Sittinbourne Res Centre, 1983)* revealed the cholinesterase NOEL to be 0.03 ppm (1.3 ug per kg-day). In a separate mouse study (Shell Tox Lab, 1983)* the chronic LOEL was 1 ppm (0.15 mg per kg-day). In a reproduction study in rats (Toxigenics Inc, 1983)* the maternal and fetal NOEL was 0.3 and 1.0 mg per kg-day respectively. Subsequent studies revealed teratogenic potential in pregnant rats and rabbits (Janardhan et al 1984).

MCP, being a relatively non-volatile persistent systemic insecticide (Corey et al 1965), is widely used in India for plant protection programmes on various cash crops like chillies, cotton, sugarcane, pulses, vegetables and fruit orchards. Experiments were therefore designed to carry out short-term toxicity test in rats to assay the possible toxicity

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to vital organs like haemopoietic system, liver and kidneys.

MATERIALS AND METHODS

Monocrotophos (3-hydroxyl-N-methyl-Cis-crotonamide dimethyl phosphate) was synthesized and supplied by Regional Research Laboratory, Hyderabad (India). The structural formula is:

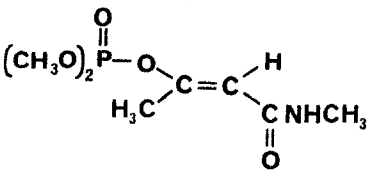

Figure 1. Chemical Structure of Monocrotophos

The sample used for this study was 70% pure. The compound is a reddish brown liquid at room temperature with a molecular weight of 223. It is soluble in water, acetone and alcohol.

Weanling Wistar strain rats weighing between 100 to 200 g were obtained from the breeding unit of the National Institute of Nutrition, Hyderabad. They were housed two per wire cage, in day light at 25 ± 3°C with a relative humidity of 45-55%, light cycle 6.00 am to 6.00 pm under conventional conditions. They were fed with semi-synthetic standard diet and tap water ad libitum. The diet was prepared freshly every day. The animals were acclimated for 7-10 days prior to experimental use.

Five groups each of 3 male and 3 female weanling rats were administered 0, 0.3, 0.6, 1.2 and 2.4 mg MCP per kg body weight per day orally by intragastric intubation for 2 weeks. Animals were observed for toxic signs. Body weights were recorded weekly. At the end of the second week the animals were killed, the liver and kidneys were weighed and the whole blood cholinesterase (ChE) activity was estimated colorimetrically (Fleisher and Pope, 1954). The test provided the basis for the selection of dose levels for the 90 day study.

Four groups of each 10 male and 10 female weanling rats were administered with MCP at dose levels of 0 (control), 0.3, 0.6, 1.2 mg per kg body weight daily for 90 days. Weekly body weights and mortality during the treatment period were recorded. Blood samples were collected from all rats at 15, 30, 60 and 90 day intervals from retroorbital plexes and examined for haemoglobin concentration, erythrocyte count and total and differential leucocyte counts. ChE activity of

the whole blood pooled from two rats of the same sex was determined. At the end of the experiment serum glutamic pyruvic transaminase (GPT), glutamic oxaloacitic transaminase (GOT) (Reitman and Frankel, 1957) and alkaline phosphatase (Kind and King, 1954) activities and bilirubin concentration (Malloy and Evelyn, 1937) were estimated. Glucose (Nelson and Somogyi, 1957) and urea (Natelson, 1957) concentrations in blood were determined in male and female rats of each group. Rats were transferred to metabolic cages. Urinalysis was carried out on 24 hour pooled urine from five rats.

At the end of the experiment, all rats were killed by decapitation. An autopsy was carried out to find out any macroscopic abnormalities. Brain, heart, liver, spleen, lungs, kidneys, adrenals, ovaries/testes, uterus/prostate and bladder of all rats were excised and weighed. Paraffin-wax sections of liver and kidneys were stained with haematoxylin and eosin for microscopic examination.

Statistical analysis of data obtained was carried out employing the Student's t-test (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

In monocrotophos treated rats growth ratardation was observed in both sexes as compared to those of control. The effect was maximum at week 5 in rats in 1.2 and 0.6 mg and at week 6 in rats receiving 0.3 mg per kg body weight. Female rats were relatively more susceptible (20.74%) compared to males (13.73%) with regard to weight loss. The mortality rate at the end of the experiment was 5% in males and 17% in females in highest dose tested. Haemotological examination (Table 1) revealed no consistent or dose related effect. At 90-days a significant decrease in haemoglobin concentration, erythrocyte and leucocyte counts with lowered neutrophil and lymphocyte number was observed. Similar observations have been made with other organophosphate pesticides like parathion and malathion which could be attributed to marked anticholinesterase activity (Casida, 1973). Decrease in haemoglobin concentration with concomitant increase in bilirubin concentration indicates a haemolytic condition which could be the result of direct toxic effect on erythrocytes. Likewise haematological changes are common in toxicity by chemicals in general (Ortega et al 1956; Chakravarthy et al 1978, and Dikshith et al 1980).

Urinalysis showed concentration of urine in about 4-6 weeks which could be the result of dehydration from excessive sweating and diarrhoea observed during the

experiment. This may be attributable to the increased parasympathetic activity.

Per cent inhibition of the wholeblood ChE activity (Table 2) in males was an average of 52.36 in four determinations spread over 90 days. Whereas in females it averaged 66.10. At the end of the experiment the per cent inhibition of brain ChE activity in males and females was 33.23 and 63.90 respectively showing a marked dose-related inhibition of ChE activity in blood and brain. Females exhibited greater susceptibility over males with regard to ChE inhibition by MCP. Similar changes were reported in guinea pigs exposed to different concentrations of quinolphos (Dikshit et al 1980) and rats fed with parathion and malathion (Chakraborthy et al 1978).

Serum analysis at 90-days revealed a trend towards increase ($P < 0.5$) in serum GOT and GPT activity at 0.3 and 0.6 mg per kg dose in females whereas in males at 0.6 and 1.2 mg per kg dose levels (Table 3). Serum alkaline phosphatase activity decreased significantly in all treated groups except in males with 1.2 mg per kg dose. There was a significant increase in serum bilirubin concentration. Blood urea levels raised in all treated groups except in females of high dose group. Blood glucose levels showed an increase which was significant only in rats on 0.6 mg per kg dose.

Organ weights expressed as per cent body weight of male and female rats are shown in Table 4. Significant increase was seen at all levels in the relative weights of the liver, kidneys and bladder, whereas relative weights of spleen increased significantly in both males and females.

Histopathological examination of liver from treated rats revealed dose-related changes ranging from mild degree of degenerative changes to periportal fibrosis and widespread necrosis. In case of kidneys, dose-dependent inflammatory changes ranging from mild swelling of glamerular cells to interstitial inflammatory reactions and marked interstitial nephritis were observed.

The rise in activity of both transminases associated with an increase in serum biliribin concentration is suggestive of acute liver injury (Tokha-El-Sherif, 1970). The rise in alkaline phosphatase may be the result of toxic chemical stress (Murphy and Porter, 1966). Longstanding exposure to agricultural chemicals including organophosphorous compounds may produce adaptive mechanisms expressed by the tendency towards an increased synthesis of various serum enzymes

Table 1. Haematological values in rats treated with MCP for 90 days

M.C.P. (mg/kg/day)	15 days		30 days		60 days		90 days	
	M	F	M	F	M	F	M	F
Haemoglobin (mg/100 ml)								
0	23.2	19.9	21.1	21.8	22.8	21.0	20.6	20.9
0.3	20.6	23.2	20.0	19.7	20.4	23.0	18.8	20.4
0.6	23.5	20.8	18.5	22.8	19.9	20.6	18.0	20.8
1.2	22.5	22.9	21.0	21.7	17.3	23.6	12.0	12.8
Erythrocytes ($10^6/mm^3$)								
0	9.2	9.3	8.7	8.6	10.2	9.5	10.1	9.7
0.3	10.2	9.2	10.3	8.5	9.6	10.7	9.7	9.6
0.6	9.9	8.3	10.2	8.9	10.1	9.5	7.4	8.7
1.2	9.6	9.4	10.3	9.2	8.2	11.1	5.3	8.5
Leucocytes ($10^3/mm^3$)								
0	5515	7065	5775	5835	4650	4430	5990	6120
0.3	3975	5575	6050	6795	5845	6450	2725	4265
0.6	3695	5535	5760	3225	6943	7175	3165	4320
1.2	5833	7515	5575	6920	4985	6550	2550	4750
Neutrophylls (%)								
0	25.0	22.0	14.0	20.0	17.5	22.0	22.0	25.0
0.3	25.0	25.0	24.0	24.0	24.0	26.5	20.5	24.0
0.6	24.0	25.5	15.0	22.0	19.0	23.5	19.5	24.5
1.2	20.5	24.0	23.0	20.0	21.5	24.0	17.0	22.0
Lymphocytes (%)								
0	74.0	77.0	85.0	80.0	82.0	77.0	77.0	74.0
0.3	74.0	75.0	75.0	75.0	76.0	73.0	72.0	71.0
0.6	75.0	74.0	84.0	78.0	80.5	70.0	78.0	73.0
1.2	79.0	75.0	76.0	79.0	78.0	76.0	74.0	73.0

Table 2. Per cent inhibition of cholinesterase activity at different intervals in rats treated with various doses of MCP for 90 days

Treatment (mg/kg/day)	Whole Blood				Brain
	15 days	30 days	60 days	90 days	90 days
Males					
0.3	64.27	52.85	42.68	22.34	32.65
0.6	79.59	70.35	38.59	18.90	25.41
1.2	78.03	81.02	53.43	26.38	41.62
Females					
0.3	74.00	66.76	55.90	72.86	72.22
0.6	68.30	79.89	67.74	57.41	63.53
1.2	76.89	78.53	70.95	33.67	55.96

Table 3. Blood chemistry of rats treated with MCP

Group (mg/kg/day)	Alk.pase (I.U/lit)	SGOT (I.U/lit)	SGPT (I.U/lit)	Ser.bilirubin (mg/per cent)	Blood sugar (m.moles/lit)	Blood urea (m.moles/lit)
Males						
Control	67.47± 5.94	86.60± 2.81	38.40± 2.08	0.43±0.05	4.78±0.48	7.29±0.82
0.3	59.92±15.92	288.50±42.36*	119.63± 0.43*	0.64±0.03	5.34±0.44	11.57±1.80
0.6	23.00±10.01	301.00±88.76*	64.13±15.42	0.69±3.11	5.56±0.30@	11.58±3.11
1.2	49.34±10.15	110.00± 0.60*	20.00± 2.21	0.70±0.05	4.72±0.80	14.08±0.78
Females						
Control	81.52± 4.64	95.25± 9.03	38.10± 0.70	0.83±0.03	4.58±0.09	6.24±1.56
0.3	49.54± 8.51*	329.00±66.62*	95.40±12.21	0.85±0.03	5.75±0.54	9.12±1.70
0.6	27.89± 0.98*	249.25±37.70*	73.60± 2.82*	0.88±0.03	5.80±0.13@	7.29±0.89
1.2	26.89± 2.45	91.28± 0.75	24.00± 0.60	1.08±0.06	5.63±0.49	5.19±0.36

Values are mean ± S.E. * $P < 0.01$ @ $P < 0.05$

Table 4. Organ weight/body weight ratios in parcentage in rats treated with MCP for 90 days

Organ	0 mg/kg/day	0.3	0.6	1.2
No. of males	10	8	8	6
Liver	3.273	2.738@	2.488@	3.212@
Kidney	0.719	0.595@	0.533@	0.686@
Heart	0.333	0.379	0.306	0.303
Spleen	0.125	0.152@	0.107@	0.133@
Lungs	0.659	0.634	0.637*	0.467*
Adrenals	0.006	0.011*	0.009	0.008
Testes	0.978	1.139	1.020	0.806
Prostate	0.037	0.057	0.041	0.035
Bladder	0.009	0.019@	0.027@	0.016
Brain	1.084	0.798	0.943	0.957
No. of females	10	10	9	10
Liver	2.688	2.627	2.687@	2.899@
Kidneys	0.652	0.524@	0.551@	0.713@
Heart	0.287	0.337	0.336	0.294
Spleen	0.091	0.155@	0.139@	0.109
Lungs	0.490	0.555	0.689*	0.530*
Adrenals	0.011	0.013	0.009	0.009
Ovaries	0.013	0.033	0.024	0.017
Uterus	0.021	0.064	0.057	0.050
Bladder	0.011	0.019@	0.018@	0.012
Brain	0.969	1.134	1.197	1.250

* $P < 0.05$ @ $P < 0.01$

(Bushueva, 1970). These enzymatic changes corroborate with dose-related degenerative changes observed on histopathological examination of liver.

Significant rise in blood urea levels can be correlated with dose-dependent inflammatory reactions, evidenced on histopathological examination of kidneys, suggesting nephrotoxicity.

Decrease in liver and kidney weights are generally in conformity with the histopathological changes. Increased spleen weights could be attributed to functional hypertrophy response.

These studies have thus provided evidence that MCP in doses studied had harmful effects on vital organs like the haemopoetic system, liver and kidneys. However, with the doses tested it was not possible to establish a no effect level and there is a need

to investigate this aspect further by studying the short-term effects of lower levels of MCP. In view of its toxic potential to liver and kidneys it would be of interest to determine the long-term toxicity on vital organs.

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