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Short-term Toxicity of Methyl Benzimidazole Carbamate in Dogs

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The systemic fungicide methyl benzimidazole carbamate (MBC) is extensively used to control fungal diseases of food and vegetable crops including orchards. It is absorbed by roots and transported to various parts of the plant (Prasad et al 1979) with residues persisting for periods ranging from 15 to 45 days (Arora et al 1977).

WHO (1977) recommended the desirability of conducting short-term studies for elucidation of its effect on the liver in rats and dogs. Wide species variation in acute toxicity of MBC has been reported (Janardhan et al 1986). Ninety day feeding studies in rats revealed its potential to cause hepatotoxicity and nephrotoxicity (Janardhan et al 1987).

This paper gives the results of similar study conducted with MBC in dogs to assay the possible toxicity to vital organs like haemopoetic system, liver and kidneys. The results were evaluated in relation to data obtained from studies in rats.

MATERIALS AND METHODS

Methyl benzimidazole carbamate was supplied by Regional Research Laboratory, Hyderabad, India. The sample used for this study was 98% pure. It is a grey coloured light crystalline powder with a molecular weight of 290. It is practically insoluble in water.

Apparently healthy young mongrel dogs of both sexes were obtained from Municipal Corporation, Hyderabad, India. The dogs were treated for all acto-and endoparasites and were vaccinated for rabies and kept under observation for a period of thirty days before using as experimental subjects. The dogs were housed at 25°±3°C with relative humidity of 45 to 55 per cent. Tap water and stock standard diet given ad libitum. The light period was from 5 a.m to 6 p.m.

Twentyfour dogs weighing between 7.5 to 11.5 kg were assigned randomly to four groups each of 3 males and 3 females. Care was taken to have approximately similar average body weights. MBC was administered orally suspended in 500 ml of milk. Animals in control group received same quantity of vehicle (milk) alone. Based on the results of the experiments carried out in rats, three dosage levels viz. 20, 40 and 80 mg per kg body weight per day were selected for the short-term toxic studies in dogs.

All dogs were weighed weekly for 13 weeks and observed for toxic signs and mortalities. At intervals of 15, 30, 60 and 90 days blood samples were taken from all animals and examined for haemoglobin concentration, erythrocyte count, total and differential leucocyte counts. At the end of the experiment blood sugar (Nelson-Somogyi 1957), blood urea (Natelson 1957) and whole blood cholinesterase (ChE) activity (Fleisher and Pope 1954) were determined in each sample of blood, and serum was analysed for alkaline phosphatase (Kind and King 1954), GOT and GPT (Reitman and Frankel 1957) activities and bilirubin concentration (Malloy and Evelyn 1937). Urine analysis, including appearance pH, glucose, occult blood, ketones and microscopy of the sediment, was carried out.

At the end of the experimental period all the dogs were sacrificed by injecting large dose of nembutal sodium intravenously. Gross autopsy examination was performed and brain, liver, heart, kidney, lugs, spleen, adrenals, testes/overies, prostate/uterus and bladder were collected and their weights recorded.

Liver and kidney tissues from all dogs in control and treated groups were fixed in 10 per cent formalin. Paraffin-wax sections were stained with haemotoxylin and eosin for histopathological examination.

Statistical analysis of the data was carried out according to the student's 't' test (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

The dogs in all groups remained in good health throughout the experimental period without showing any toxic symptoms. The early decrease in body weight (Fig 1) in males was not made up later in the study although the subsequent rate of weight gain was similar to that in the controls. The results of body weight indicate that MBC did not influence significantly the body weight in dogs.

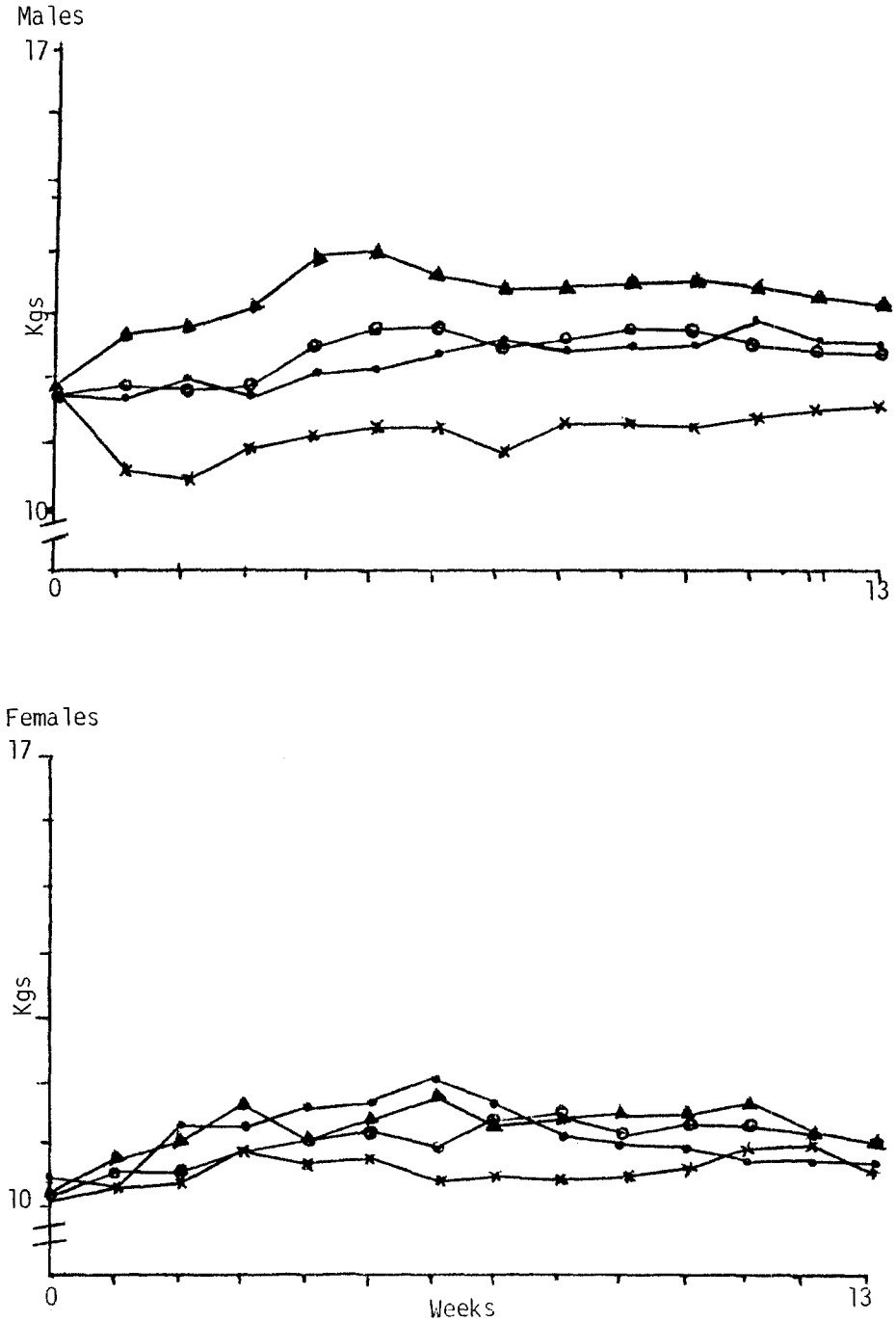

Figure 1 WEEKLY BODY WEIGHTS OF DOGS TREATED WITH MBC DAILY FOR 90 DAYS

▲ Control ✖ 20 mg/kg o 40 mg/kg • 80 mg/kg

Table 1 : Haematology of 3 male and 3 female dogs per groups at different intervals after MBC treatment

Treatment (mg/kg/day)	150 days		30 days		60 days		90 days	
	M	F	M	F	M	F	M	F
Haemoiglobin (mg/100 ml)								
0	15.5	15.1	16.1	16.2	15.8	16.2	16.7	17.4
20	14.2	13.7*	15.0	13.5*	16.0	15.4*	16.6	14.7*
40	15.1	14.7*	15.7	14.1*	17.0	15.6*	15.6	15.5*
80	15.6	13.7*	16.6	14.7*	15.6	16.0	16.4	14.9*
Erythrocytes ($10^6/mm^3$)								
0	6.4	6.0	7.3	7.6	8.0	7.9	9.1	9.8
20	5.2	4.8	5.5	4.8	7.2	6.6	9.8	6.3
40	6.1	6.2	7.4	6.2	8.2	6.6	7.4	7.1
80	7.0	5.1	9.2	6.1	6.9	7.2	8.5	6.1
Leucocytes ($10^3/mm^3$)								
0	11.4	14.6	11.3	14.2	12.1	15.7	11.0	14.9
20	11.1	14.1	10.0	9.1*	10.0*	7.3*	7.5*	7.4*
40	11.7	11.7	10.5	9.2*	11.2*	9.9*	8.6*	8.5*
80	11.6	9.9*	8.8	10.6*	7.1*	8.6*	10.4*	7.9*
Neutrophylls (%)								
0	78	87	81	94	72	71	61	70
20	79	77	87	82	75	74	70	70
40	78	76	81	79	77	70	68	69
80	76	78	83	85	74	70	74	66
Lymphocytes (%)								
0	20	8	18	4	24	22	28	27
20	16	17	9	16	20	22	24	27
40	18	21	17	18	20	27	28	28
80	19	18	15	12	23	24	23	31

Table 2 : Terminal blood chemistry in dogs after 90 days treatment with various levels of MBC

Group (mg/kg/day)	Alk. pase (U/l.)	SGOT (U/l.)	SGPT (U/l.)	Blood sugar (m mole/l.)	Blood urea (m mole/l.)	S.bilirubin (mg %)
Male dogs						
0	5.26±0.00	17.00±1.53	25.00±2.65	3.98±0.34	4.61±1.04	0.35±0.01
20	6.25±0.63	21.67±2.61	27.33±3.06**	4.25±0.25*	7.55±1.79	0.55±0.00**
40	5.23±0.76	20.67±2.41	31.33±1.86**	5.70±0.21*	6.88±0.15*	0.66±0.08*
80	5.23±1.25	26.33±3.39*	29.00±1.73*	4.28±1.21*	13.00±2.12*	0.84±0.13*
Female dogs						
0	4.76±0.94	16.67±3.34	27.67±2.97	5.11±0.27	5.94±0.03	0.37±0.03
20	5.86±0.95	21.33±1.34	31.33±6.85*	4.30±0.42*	5.27±1.70	0.83±0.17*
40	6.70±1.90	23.67±4.71	27.00±2.52*	4.27±0.39*	6.27±0.48	0.57±0.16*
80	5.38±1.35	26.33±1.77*	28.33±5.37**	3.83±0.15*	8.55±0.78*	0.57±0.16*

Values are mean ± S.E.

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

The composition of urine samples collected at different stages showed no changes attributable to the administration of MBC.

Haematological values (Table 1) reveal that there was a reduction ($P < 0.05$) of haemoglobin concentration in females only. An increase of erythrocyte number in males and decrease in females was noted at 15 and 30 day intervals, whereas at 60 and 90 day intervals, erythrocyte number decreased in both sexes. Leukocyte count registered a decrease in all treated groups ($P < 0.01$) especially in females after 30 day treatment. Neutrophil and lymphocyte counts were not affected in any group. Due to the haematological changes that occurred independently of the dosage and without any regularity, we believe that MBC has no significant influence on haemopoeitic system.

MBC did not affect the whole blood cholinesterase activity in any of the groups, which is in further conformity with our earlier observations in rats, showing that MBC has no inhibitory effect on ChE activity in dogs also.

The values of serum alkaline phospohatase activity (Table 2) show no significant difference among the groups. There was a dose-related increase in SGOT activity which was significant ($P < 0.01$) at the high dose. Significantly higher levels ($P < 0.05$) of GPT activity was noted although it was not dose-related. Dose-unrelated increase in males and decrease in females was recorded in blood sugar concentration. Significant increase ($P < 0.01$) in serum bilirubin concentration was observed in all the treated groups.

Liver sections revealed dose-related changes ranging from sparse infiltration by inflammatory cells, fatty infiltration, extensive congestion and inflammatory and degenerative changes.

Though the enzymatic changes point to mild hepatic cell injury the dose-related and marked histopathological achanges were observed in liver sections suggesting severe hepatotoxicity of the pesticide to dogs as compared to rats (Janardhan et al 1987).

Kidney sections from all dogs showed tubular dilatation and hydropic degeneration in low dose group and periglomerular fibrosis in medium dose group. In high dose group, in addition to periglomerular fibrosis, hyalinisation and extensive vascular congestion were noted. The changes in blood urea concentration due to MBC treatment correlate closely with comparable histopathological changes which were predominently

degenerative. These histopathological changes suggest that dogs tend to be more susceptible to nephrotoxic effect of MBC when compared to rats. The organ weight - body weight ratios (Table 3) showed lower values for adrenals in all treated dogs and that testes in males and overies in females showed lower values in high dose group. These changes could be attributable to antimetabolic nature of MBC resulting in cytotoxicity (Bartels-Schooly and MacNeil 1971) to the highly proliferative tissues of these organs. The increase in organ weights in males and decrease in females with regard to heart, liver and spleen is more difficult to interpret as to whether the effect is incidental or otherwise.

Table 3 Organ weight/body ratios in percentages in dogs treated with MBC for 90 days

Organ	0 (mg/kg/day)	20	40	80
No.of Males	3	3	3	3
Liver+	2.600	2.620	2.270	3.370
Kidney	0.370	0.383	0.390	0.393
Heart+	0.530	0.570	0.580	0.520
Spleen+	0.283	0.290	0.377	0.407
Lungs	0.733	0.736	0.759	0.693
Adrenals	0.025	0.019*	0.009*	0.018*
Testes	0.138	0.136	0.146	0.038*
Prostate	0.303	0.287	0.271	0.286
Bladder	0.151	0.143	0.135	0.141
Brain	0.530	0.500	0.480	0.610
No.of Females	3	3	3	3
Liver+	4.190	3.220	3.110	4.180
Kidneys+	0.523	0.477	0.463	0.430
Heart+	0.630	0.560	0.600	0.600
Spleen+	0.690	0.440	0.377	0.327
Lungs	0.809	0.709	0.845	0.721
Adrenals	0.036	0.025*	0.023*	0.022*
Overies	0.023	0.050	0.028	0.018*
Uterus	0.416	0.435	0.458	0.435
Bladder	0.139	0.146	0.153	0.135
Brain	0.560	0.520	0.510	0.610

* $P < 0.01$ (doses) + $P < 0.01$ (sexes)

The results of these studies point to high susceptibility of dog to hepato-and nephrotoxicity of MBC. These results could be extrapolated to higher mammals including man and may serve as a caution against possible damage to vital organs like liver and kidneys on prolonged exposure, hence the need for minimising the health hazards by judicious use of this chemical. In

view of the histopathological changes in liver and kidneys, though mild at low level tested, it was not possible to establish a no effect level and there is need to investigate further this aspect by studying the short-term effect of lower levels of MBC on liver and kidneys.

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REFERENCES

Arora A, Vyas SC, Jain AC (1977) Persistence of systemic fungicides carbendazim and benomyl on plants. PANS 23:430-431

Bartels-Schooley J, MacNeill BH (1971) Phytopathol 61:818

Fleisher JH, Pope EJ (1954) Colorimetric method for determination of red blood cells cholinesterase activity in whole blood. Arch Ind Hyg and Occup Med 9:323

Janardhan A, Bhaskar Rao, A, Sisodia P (1986) Species variation in acute toxicity of monocrotophos and methyl benzimidazole carbamate. Ind J Pharmacol 18:102-103

Janardhan A, Bhaskar Rao, Sisodia P (1987) Subcronic toxicity of methyl benzimidazole carbamate in rats. Bull Environ Contam Toxicol 38:890-898

Kind RRN, King EJ (1954) Estimation of plasma phospatase by determination of hydrolysed phenol with amino-antipyrine. J Clin Path 7:322-326

Malloy HT, Evelyn KA (1937) The determination of bilirubin with the Photoelectric colorimeter. J Biol Chem 119:481-490

Natelson S (1957) Microtechniques of clinical chemistry for the routine laboratory. Ed Thomas CC, Springfield, Illinois p. 381

Nelson N, Somogyi M (1957) Methods in enzymology. Eds Colowick Sp and Naplan, Vol.3, p. 85

Prasad KVV, Vyas SC, Shukla BN (1977) Translocation of systemic fungicides benolate MBC and carboxin in groundnut plant. Pesticides 11:13-14

Reitman S, Frankel S (1957) A colorimetric method for the determination of serum glutamic oxalacetic acid glutamic pyruvic transanienases. Am J Clin Path 28:56-63

Snedecor GW, Cochran WG (1967) Statistical methods. 6th Ed. Iowa State Univ. Press, Ames, Iowa

WHO-Tech Rep Ser (1977) Pesticide residues in food. Report of the 1976 Joint FAO/WHO Meeting. Further work or information required/desired. No.612:129

Wright WA, Stringer A (1973) The Toxicity of thiabendazole, benomyl, methyl benzimidazol-2-yl carbamate and thiophanatemethyl to the earth worm, Lumbricus terristris. Pestic Sci 4:431

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