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# Sub-Chronic Toxicity of Methyl Benzimidazole Carbamate in Rats

A. Janardhan,[1] A. Bhaskar Rao,[2] and P. Sisodia[3]

[1]College of Veterinary Science, Rajendranagar, Hyderabad 500 030, India; [2]R.R.L., Hyderabad, India; and [3]Dr BR Ambedkar Medical College, Bangalore, India

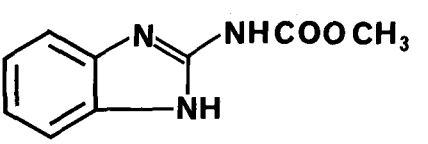
Increasing use of agricultural chemicals for pest control programmes resulted in constant ingestion of their residues present in food chain by man and animals. Consequently the information regarding their harmful effects to health in general has gained importance.

Methyl benzimidazole carbamate (MBC) is a broad spectrum systemic fungicide effectively used against a wide variety of pests of vegetable crops and fruit orchards. On seed treatment MBC was found to be actively absorbed by the seedlings and transported to aerial parts (Prasad et al. 1979). The residual activity of the chemical on various parts of vegetable and food crops was reported to vary from 3.5 to 6.3 ppm (Bandopadyaya and Mukhopadyaya 1975) persisting from 15 days to 45 days in different parts of the plant (Kannayan et al. 1975, and Arora et al. 1977).

However, WHO (1977) has recommended the desirability of conducting short-term and long-term toxicity studies of this fungicide in mammals. Subsequent studies revealed its embryotoxic potential in rats and rabbits (Janardhan et al. 1984). This paper gives the results of sub-chronic administration of MBC in rats designed to assay the possible toxicity to vital organs like the haemopoetic system, liver and kidneys.

## MATERIALS AND METHODS

Methyl benzimidazole-2-carbamate (carbendazim) was supplied by Regional Research Laboratory, Hyderabad (India). The structural formula is:



The sample used for this study was 98% pure. It is a grey coloured light crystalline powder with a molecular weight of 290. It was practically insoluble in water.

Weanling wistar rats weighing between 150-200 g (at the start of the experiment) were supplied by the National Institute of Nutrition, Hyderabad. The rats were housed in wire cages, five in a cage in conventional circumstances. The rats were housed at 25° ± 3°C with relative humidity of 45-55%. Tap water and stock standard diet were given ad libitum. The light period was from 6 a.m. to 6 p.m.

MBC suspended in 4% gum acacia (BDH) was administered orally by intragastric intubation. The concentration was adjusted to have a maximum ingestion volume of 1 ml per animal. Control group received appropriate quantities of vehicle.

Five groups each of 3 male and 3 female rats were administered with 0, 16, 32, 128 and 256 mg/kg body weight daily. Animals were observed for toxic signs. Body weights were recorded weekly. Blood samples were collected and examined for haemoglobin concentration, total erythrocytes and leucocytes count. At the end of two weeks serum was examined for alkaline phosphatase (Kind & King 1954), GPT (Reitman and Frankel 1957) and blood glucose (Nelson-Somogyi 1957) levels. The animals were killed, the liver and kidneys were weighed and examined histologically. This test provided the basis for the selection of dose levels in the 90-day study.

Methyl benzimidazole carbamate at dose rate of 0, 16, 32, 64 mg/kg body weight per day was administered orally for 90 days to four groups each of 10 male and 10 female littermate rats. Littermates were chosen to avoid large variation in results due to genetic effects.

Animals were weighed weekly and behaviour and mortality were recorded. Blood samples from all rats were examined for haemoglobin concentration, erythrocyte count and total and differential leucocyte counts at 0, 15, 30, 60 and 90 days. At the end of the experiment blood and serum pooled from two rats were collected. Serum was assayed for alkaline phosphatase, GOT, GPT activities and bilirubin (Malloy and Evelyn 1937) concentration. Whole blood cholinesterase activity was determined colorimetrically (Fleisher and Pope 1954), blood urea (Natelson 1957) and blood glucose (Nelson-Somogyi 1957) concentrations were estimated. Serum bilirubin was estimated colorimetrically (Malloy and Evelyn 1937). 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. An autopsy was carried out and any macroscopic abnormalities were noted. Brain, heart, lungs, liver, spleen, kidneys, bladder, adrenal, testes/ovaries and prostate/uterus were collected and their weights recorded. Liver and kidneys of all rats in control and treated groups were fixed in 5% formalin. Paraffin-wax sections were stained with haemotoxylin and eosin for microscopic examination.

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

RESULTS AND DISCUSSION

The mean body weights recorded weekly are given in Figure 1. The behaviour of the test animals was normal. MBC in all doses affected body weights adversely during the first five weeks but in succeeding weeks body weights of all treated groups registered a gradual increase. Rats appeared to have adopted to the chemical resulting in steady gain in weight. Increase in body weight indicates that the chemical at the doses studied has been well tolerated by rats when compared to control rats.

The main results of the hematological examination are given in Table 1. Erythrocytes number increased as the period of treatment increased with initial decrease. This increase was more significant ($P < 0.01$) in males when compared to females. There was concomittant decrease in leukocyte count. Both sexes showed increased neutrophil and decreased lymphocyte counts on 30 days.

The hematological findings reveal that the hemopoeitic system might probably be adversely affected. Lower hemoglobin concentration coupled with low erythrocyte population, depression of leucopoesis with a rise in the number of circulating neutrophils and lymphopenia may be the toxic manifestation (Bushby 1970) of MBC on haemopoesis.

Due to highly proliferative nature of blood forming organs, it is probable that antimetabolic nature of MBC (Bartels-Schooley and MacNeil 1971) interferes with DNA synthesis (Clemons and Sisler 1971) resulting in inhibition of mitotic division (Freidman and Platzer 1978) and suppression of haemopoesis in bone marrow and spleen. However, more work is needed to confirm the actual observations and to support the hypothesis of the working mechanism. No significant effects attributable to methyl benzimidazole carbamate were noticed on urinalysis.

No change in activity of whole blood cholinesterase was noted in treated rats. This is in agreement with the earlier findings of Wright and Stringer (1973) who have demonstrated that MBC though a carbamate is devoid of any inhibitory effect on ChE activity. Serum alkaline phosphatase and GPT in high dose group, blood urea and bilirubin in all treated groups were significantly higher (Table 2). High dose of MBC significantly ($P < 0.01$) increased alkaline phosphatase activity in male rats. It may be indictive of an adaptive rise in enzyme activity to the persistent stress (Murphy and Porter 1966). After 90 days of tretment blood sugar concentration at 20 mg dose and blood urea level at 80 mg dose were significantly increased.

Increased serum bilirubin concentration is attributable to parenchymal cell damage as evidenced by increased GPT and GOT activities, affecting the hepatocytes capacity to handle bilirubin. In spite of the high levels of transminases in rats which are indicative of hepatic cell injury, liver sections showed only a marked congestion of central vein with hydropic degeneration. And, histological changes in liver revealed dose-related changes ranging from sparse infiltration by inflammatory cells to inflammatory and degenerative changes.

Kidney sections revealed tubular dilatation and hydropic degeneration in low dose group. Periglomerular fibrosis with medium dose and hyalinisation and extensive vascular congention with high dose were noted. These changes suggest mild hepato, and nephrotoxicity of the pesticide.

The difference in the relative organ weights occurred independently of the dosage and without any regularity (Table 3).

In MBC treated rats, increase in weights of lungs can be correlated with the bronchopneumonic changes observed in lung sections. For the deviation (decrease) in liver and (increase) heart, prostrate, uterus, bladder and brain weights, no plausible explanation could be given and be regarded as incidental. Increase in weights of heart, liver and spleen in females as compared to males could be indicative of a work hypertrophy response.

Though MBC has no ChE inhibiting property, the data presented in this 90-day study provide valuable information regarding its toxicity to liver and kidneys. The information made available through this study could form a suitable basis for further investigation of risk assessment. It could also be utilised for

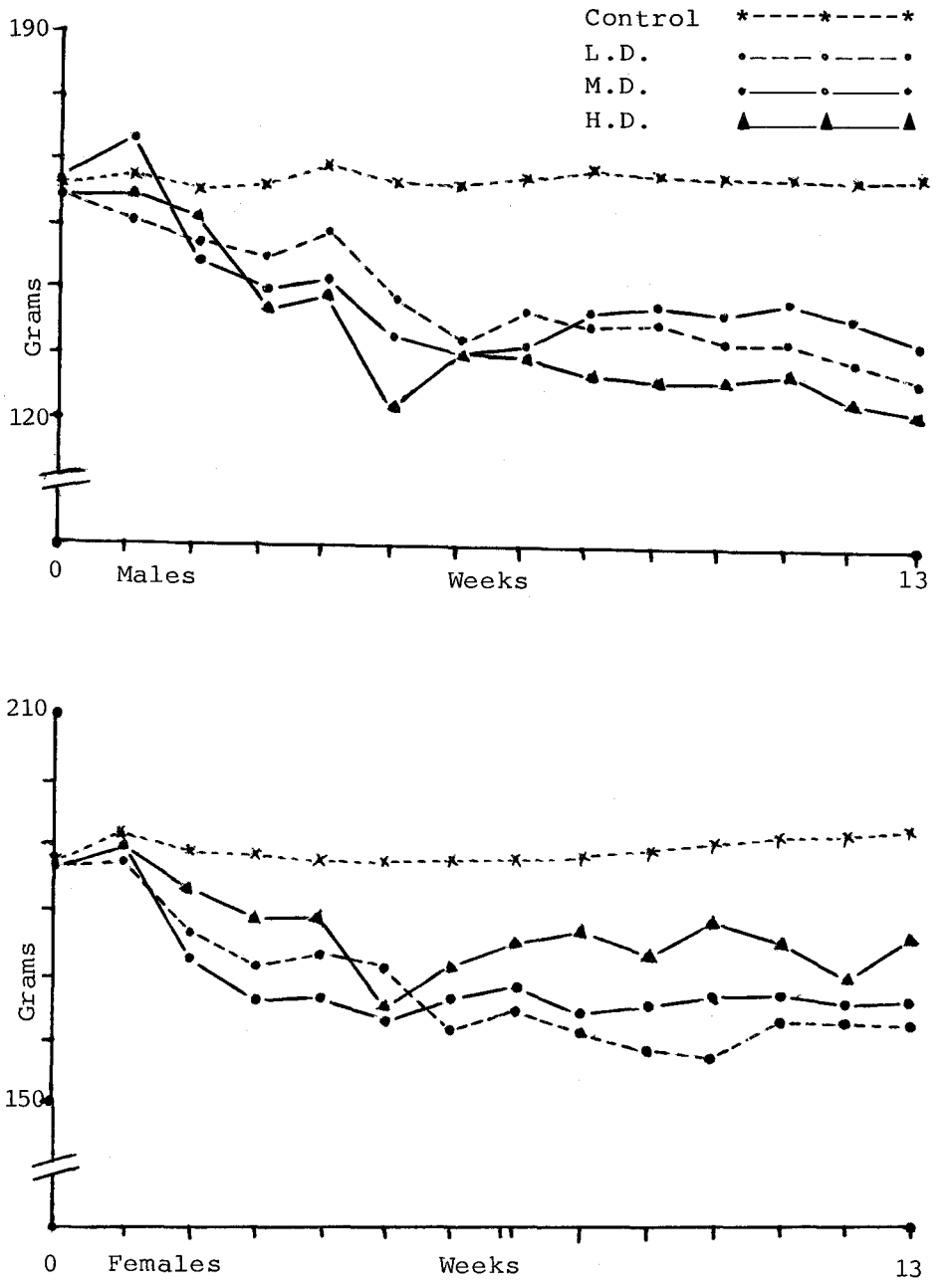



Figure 1 - BODY WEIGHTS OF RATS TREATED WITH MBC

Table 1:Hematology of 10 male and 10 female rats per group at different intervals

| Treatment (mg/kg/day) | 15 days | | 30 days | | 60 days | | 90 days | |
|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F |
| Hemoglobin in mg/100 ml: | | | | | | | | |
| 0 | 14.5 | 15.0 | 10.7 | 8.7 | 12.6 | 12.6 | 13.7 | 12.7 |
| 16 | 10.1 | 9.7 | 12.3 | 12.8 | 12.5 | 12.1 | 13.1 | 13.4 |
| 32 | 13.6 | 13.0 | 12.7 | 11.6 | 12.4 | 11.9 | 13.5 | 13.3 |
| 64 | 13.8 | 13.5 | 12.9 | 13.3 | 12.9 | 13.2 | 12.1 | 13.8 |
| Erythrocytes ($10^6/mm^3$): | | | | | | | | |
| 0 | 10.7 | 10.5 | 10.7 | 8.7 | 8.5 | 8.9 | 8.7 | 8.2 |
| 16 | 10.1 | 9.7 | 8.3 | 8.7 | 8.3 | 7.6 | 8.9 | 8.4 |
| 32 | 9.4 | 8.9 | 8.9 | 8.1 | 7.4 | 7.9 | 8.4 | 8.2 |
| 64 | 9.8 | 9.5 | 8.8 | 9.4 | 7.1 | 8.0 | 8.1 | 8.7 |
| Leucocytes ($10^3/mm^3$): | | | | | | | | |
| 0 | 8.3 | 7.1 | 7.2 | 10.2 | 10.4 | 7.5 | 11.5 | 8.6 |
| 16 | 8.2 | 6.9 | 10.8 | 10.2 | 8.3 | 7.9 | 9.3 | 8.1 |
| 32 | 6.0 | 7.7 | 7.2 | 6.5 | 7.6 | 8.4 | 7.5 | 7.3 |
| 64 | 6.1 | 5.9 | 8.3 | 7.4 | 9.4 | 8.7 | 6.6 | 7.8 |
| Neutrophylls %: | | | | | | | | |
| 0 | 25 | 22 | 115.0 | 6.0 | 25 | 24 | 48 | 41 |
| 16 | 25 | 37 | 25.0 | 23.0 | 41 | 44 | 47 | 51 |
| 32 | 24 | 24 | 10.0 | 22.0 | 42 | 43 | 44 | 42 |
| 64 | 20 | 25 | 24.0 | 17.0 | 41 | 32 | 41 | 40 |
| Lymphocytes 9%: | | | | | | | | |
| 0 | 74 | 77 | 84 | 94 | 74 | 74 | 50 | 61 |
| 16 | 74 | 62 | 53 | 75 | 58 | 55 | 52 | 47 |
| 32 | 75 | 75 | 87 | 78 | 57 | 55 | 54 | 56 |
| 64 | 80 | 74 | 75 | 83 | 58 | 67 | 57 | 58 |

Table 2: Clinical bioichemistry in rats after 90 days treatment with 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.) | Serum bilirubin (mg per cent) |
|---|---|---|---|---|---|---|
| Male rats: | | | | | | |
| 0 | 69.51 ± 4.00 | 95.20 ± 1.32 | 26.56 ± 1.63 | 5.95 ± 0.15 | 6.12 ± 0.18 | 0.45 ± 0.05 |
| 16 | 74.40 ± 11.50 | 80.00 ± 1.27* | 36.86 ± 1.40* | 4.63 ± 0.05* | 6.62 ± 0.12* | 0.63 ± 0.03 |
| 32 | 76.31 ± 2.84 | 95.12 ± 1.32* | 26.00 ± 1.08 | 5.74 ± 0.05 | 4.37 ± 0.10* | 0.73 ± 0.13* |
| 64 | 126.02 ± 14.70* | 75.30 ± 2.54* | 40.10 ± 1.43* | 3.92 ± 0.06* | 2.99 ± 0.11* | 0.82 ± 0.03* |
| Female rats: | | | | | | |
| 0 | 82.06 ± 4.26 | 82.64 ± 0.29 | 24.56 ± 1.42 | 4.95 ± 0.02 | 5.71 ± 0.61* | 0.82 ± 0.03 |
| 16 | 70.96 ± 3.36 | 79.40 ± 1.41* | 22.50 ± 1.08* | 5.44 ± 0.04 | 4.34 ± 0.11* | 0.65 ± 0.04 |
| 32 | 79.90 ± 15.06 | 105.00 ± 2.44* | 26.40 ± 1.40 | 5.54 ± 0.06* | 5.83 ± 0.04* | 1.19 ± 0.04* |
| 64 | 77.69 ± 5.03 | 90.40 ± 2.19* | 37.30 ± 1.48* | 5.01 ± 0.07 | 4.62 ± 0.13* | 1.03 ± 0.05* |

Values are means ± S.E.

* $P < 0.01$

Table 3: Organ-weight/body-weight ratios in percentage in rats treated with MBC for 90 days

| Organ | 0(mg/kg/day) | 16 | 32 | 64 |
|---|---|---|---|---|
| No. of males | 10 | 10 | 10 | 10 |
| Liver | 3.260 | 3.410 | 2.750* | 3.720 |
| Kidney | 0.740 | 0.730 | 0.710 | 0.740 |
| Heart | 0.233 | 0.514* | 0.491 | 0.488* |
| Spleen | 0.270 | 0.320 | 0.190 | 0.460 |
| Lungs | 0.410 | 1.020* | 0.870* | 0.970* |
| Adrenals | 0.026 | 0.027 | 0.027 | 0.037* |
| Testes | 1.321 | 1.162 | 1.189 | 1.029 |
| Prostate | 0.020 | 0.083* | 0.068* | 0.078* |
| Bladder | 0.010 | 0.052* | 0.026* | 0.025* |
| Brain | 0.860 | 1.370* | 1.320* | 1.370* |
| No. of females | 10 | 10 | 10 | 10 |
| Liver | 3.740 | 2.950 | 2.660 | 3.220 |
| Kidneys | 0.770 | 0.710 | 0.670 | 0.830 |
| Heart | 0.236 | 0.584* | 0.520 | 0.532* |
| Spleen | 0.260 | 0.220 | 0.220 | 0.280 |
| Lungs | 0.440 | 0.700* | 0.820* | 0.960* |
| Adrenals | 0.027 | 0.024 | 0.018 | 0.031 |
| Ovaries | 0.039 | 0.044 | 0.047 | 0.066 |
| Uterus | 0.021 | 0.178* | 0.165* | 0.137* |
| Bladder | 0.013 | 0.033* | 0.027* | 0.024* |
| Brain | 0.970 | 1.390* | 1.570* | 1.660* |

* $P < 0.05$ (doses)

studies to establish maximum tolerated dose or for setting up a long-term study to find out no effect level.

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