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Teratogenicity of Methyl Benzimidazole Carbamate in Rats and Rabbits

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MBC and its precursor benomyl are being widely used in India as potential systemic fungicides for the control of various plant diseases including those of vegetable crops and fruit orchards, tobaco crops etc. Consequently, the residues are found in various plants of vegetable and food crops (Arora et al. 1977a; Kannaiyan et al. 1975) leading to the constant ingestion of residual quantities of the chemical posing a problem of health hazard for man and animals.

MBC has been demonstrated to interfere with mitosis in different species such as fungi (Hammerschlag and Sisler 1972), viruses (Nishigori et al. 1978), bacteria (Fuchs and Devaries 1978) and the cells of bovine brain (Friedman and Platzer 1978). The chemical has been shown to cause inhibition of DNA synthesis in Neurospora crassa (Clemons and Sisler 1971). It has also been shown to induce nuclear instability in Aspergillus sp. (Hastie 1970).

However, no work has been done on toxicity in mammals (WHO 1977). Hence, the present study was undertaken to evaluate the teratogenic potential, if any, of MBC in rats and rabbits.

MATERIALS AND METHODS

Methyl Benzimidazole-2-Carbamate (Carbendazim) technical grade (98 per cent pure) was synthesized and supplied by RRL, Hyderabad.

Female virgin wistar rats weighing between 150 to 200 gm were obtained from National Institute of Nutrition, Hyderabad. Female virgin albino rabbits 6 months old weighing 1.5-2.0 kg supplied by M/s. Biological Evans were used for the following experiments. The animals were housed at 25°C with access to stock standard diet (Jagannathan 1962) and tap water ad libitum. The light period was from 6.00 a.m. to 6.00 p.m.

Three female rats were mated overnight with a normal male and the morning of finding spermatozoa in the vaginal smear was considered day-o of pregnancy. A suspension in 4 per cent aqueous solution

of gum acacia (BDH) was administered orally by intragastric intubation. The concentration was adjusted to have a maximum injestion volume of 1 ml. Dossages were decided by a preliminary range-finding test with pregnant rats. The maximum dose that was not lethal to any rat, but decreased the weight gain during the gestation period was selected as highest dose (Aruchi et al. 1978). The other dosage levels were fixed in a decreasing order at the rate of one half of preceding dosage. Four groups each consisting of 20 pregnant rats were dosed with 0, 20, 40 and 80 mg/kg/day from 6 to 15 of pregnancy to cover the whole period of organogenesis (Rugh 1968).

In half of the test and control animals pregnancy was interrupted on day-21 of gestation. The uteri were opened by caesarian section. Number of live and dead fetuses and resorptions, if any, were noted. The weights of all live fetuses were recorded and examined for evidence of gross abnormalities under dissecting microscope. Live fetuses were killed by etherisation. Half of them were fixed in 95 per cent ethyl alcohol and cleared for skeletal staining with Alizarin red-S (Dawson 1926). Remaining fetuses were fixed in Bouin's solution for 7 days and then examined for anomalies of the brain and internal organs utilising the free hand razor blade technique as described by Wilson (1965). Other half of treated and control animals were allowed to deliver normally. Neonatal deaths, number of surviving animals, their body weights, gross abnormalities at birth, if any, and size and average body weights of the litters at the time of weighing were recorded.

In case of rabbits double mating technique was adopted to ensure conception rate of 80 to 90 per cent. The doe was placed first with one buck for observed mating and then immediately removed to the second buck for another mating. After observing the copulatin, the doe was returned to her cage. Those confirmed of the mating were considered to be on day-0 of gestation. By conducting a short range-finding study, the highest dose for teratogenic study was chosen which was slightly below the toxic level for non-pregnant rabbits (Gibbson et al. 1966). Two lower doses were also selected, as one half of the preceding dose. Thus, 0, 40, 80 and 160 mg/kg/day of MBC were administered to four groups each consisting of four pregnant rabbits during the period of organogenesis, i.e., 6th to 18th day of gestation (Cook and Fair-Weather 1968). All pregnant females from each group were killed by deep ether anesthesia on day-31 of gestation. Uteri were opened, live and dead fetuses and resorptions were scored. Surviving fetuses were weighed and examined for external malformations. Their viability was tested by maintaining in an incubator at 25°C for 6 hours. Half of them were processed to observe skeletal abnormalities and remaining half for visceral anomalies.

Differences between experimental and control values were analysed for statistical significance according to the methods (Chi^2 test) of Snedecor and Cochran (1967).

Table 1. Effect of Offsprings of Rats Treated with Daily Oral Administration of MBC During 5th to 15th Day of gestation

Treatment (mg/kg/day)	No. of litters	Total No. of conceptuses	Live fetuses		Dead and resorbed fetuses		Mean fetal weight ± S.E	Gross anomalies	Visceral abnormalities	Skeletal malformation
			No.	%	No.	%				
0	8	76 (9.50)	54	71.0	22	29.0	3.23 ± 0.18	0	0	0
20	8	46 (5.75)	24	52.2	22	47.8	3.97 ± 0.19	0	0	0
40	10	77 (7.70)	21	27.3	56*	72.7	3.59 ± 0.08	0	0	0
80	8	53 (6.62)	19	35.8	34*	64.2	3.23 ± 0.17	0	0	0

Numbers in parenthesis are the means
* ($P \leq 0.01$)

Table 2. Effect on Neonatal and Postnatal Mortality and Development of Pups Born to Rats Treated with MBC During 5th to 15th Day of Gestation

Treatment (mg/kg/day)	No. of litters	At birth					Pup mortality at 21 days post partum				
		No. of live fetuses	Mean fetal weight ± S.E	Still births and neonatal deaths		Gross anomalies	Live pups		Mean fetal weight of life pups ± S.E	Dead pups	
				No.	%		No.	%		No.	%
0	6	47 (7.67)	4.035 ± 0.26	0	0	0	45	95.7	18.96 ± 1.82	2	6.4
20	5	30 (6.00)	4.363 ± 0.16	0	0	0	28	93.3	19.31 ± 1.07	2	6.7
40	4	21 (5.25)	4.589 ± 0.18	0	0	0	16	76.2	23.19 ± 1.91	5	23.8
80	4	20 (5.00)	4.592 ± 0.07	0	0	0	16	80.0	21.29 ± 0.31	4	20.0

Numbers in parenthesis are the mean values.

Table 3. Teratological Observations on Fetuses of Rabbit Mothers Treated Orally with MBC Through 6th to 18th Day of Gestation

Treatment (mg/kg/day)	No. of pregnant rabbits	No. of implants	Live fetuses		Dead and resorbed fetuses		Mean weight of live fetuses (g + S.E.)	Survival rate in 6 hours		Gross anomalies	Visceral abnormalities	Skeletal malformations
			No.	%	No.	%		No.	%			
0	4	17 (4.25)	17	100.0	0	0	31.00 ± 2.72	15	88.2	0	0	0
40	4	20 (5.00)	17	85.0	3	15.0	39.00 ± 2.19	15	88.2	0	0	0
80	4	23 (5.75)	18	78.3	5	21.7	33.83 ± 0.23	16	88.9	0	0	0
160	4	15 (3.75)	10	66.7	5	33.3	29.10 ± 0.67	8	80.0	0	0	0

Figures in the parenthesis are the mean values.

RESULTS AND DISCUSSION

The fact that Methyl Benzimidazole Carbamate administration in pregnant rats decreased the number of conceptuses and increased the mortality rate of fetuses in rats ($P \leq 0.01$) (Tables 1 and 2) and rabbits (Table 3) is suggestive of embryotoxic effect of the fungicide.

In the present investigation, methyl benzimidazole carbamate administration through 6th to 15th day of gestation in case of rats, and through 6th to 18th day of pregnancy in rabbits might have possibly affected the embryo either during cleavage or at blastocyst stages resulting in the preimplantation deaths leading to reduced number of conceptuses.

The mean litter weights of live pups at birth and at weaning that were born to treated rats were not affected adversely. Instead there was an increase in their weights, which is in conformity with the findings of sub-acute feeding studies where the chemical has promoted the gain in body weight of young adult rats (Janardhan, 1982).

In fact, agents that interfere with mitosis have been shown to have a teratogenic effect (Nishimura 1964). Benzimidazoles are structurally similar to the DNA bases adenine and guanine and contain a purine ring. The fungicide may act as an antimetabolite competing with purine of adenine and guanine resulting in interference with DNA synthesis. Reduction in DNA synthesis prevents replication of chromosomes interfering with mitotic division (Borisy and Taylor 1967; Friedman and Platzer 1978; Malowista et al. 1968; and Nishigori et al. 1978) resulting in embryotoxicity.

The observation that MBC had not caused any visceral or skeletal malformations suggests that the chemical had no dysmorphogenic effect. The embryolethality might have possibly occurred at the expense of malformations (Wilson 1973) at the doses tested.

The present study indicated that some embryotoxic effects can be expected in live stock due to the persistance of the resitual quantities of MBC in stems and leaves of various food crops which form a major source of fodder. Since fetuses were mainly affected by dosages producing no detectable toxicity in the dam, these observations should also help to establish safety levels rather than the dismissal of a valuable compound.

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