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An Assessment of the Teratogenicity in the Rat and Mutagenicity in *Salmonella* of Mono-2-ethylhexyl Phthalate

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Mono-2-ethylhexyl phthalate (MEHP) is an intermediary product in the metabolism of di-2-ethylhexyl phthalate (DEHP) (CHU et al. 1978). The toxicological interest in DEHP and its metabolite MEHP centers on the use of DEHP as a plasticizer in the manufacture of polyvinyl chloride; especially plastic containers used to store blood (THOMAS et al. 1978; BAKER 1978; ALBRO & CORBETT 1978; ROCK et al. 1978; SASAKAWA & MITOMI 1978).

Using radiolabelled MEHP, CHU et al. 1978) reported that the distribution of radioactivity was highest in the liver, kidney and urine bladder 20 min. after an intravenous dose to male rats. Twenty-four hours after an oral administration, in this same study, 80% of the radioactivity was eliminated from the body with negligible amounts of the radioisotope in body tissues. It was also reported by these authors that MEHP was further metabolized to 4 other but unidentified products. After a single injection of either MEHP or DEHP, the livers of neonatal rats were found to be relatively free of MEHP two weeks after treatment (THOMAS et al. 1980). An earlier study by THOMAS et al. (1979) reported MEHP to be without fetotoxicity or teratogenicity following intravenous injections.

As part of our general study of the toxicology of MEHP, we report here its teratological assessment in the rat and tests for its mutagenicity in bacteria.

MATERIALS AND METHODS

MEHP was synthesized (98% pure) in our laboratories (CHU et al. 1978). Its identity was confirmed by NMR and GC/MS. Dosing solutions were prepared by dissolving the required amount of MEHP in a minimal volume of 5% $NaHCO_3$ which was then brought to volume with a Sorensen's phosphate buffer (pH 7.4). The control group received a dosing solution which contained the same volume of 5% $NaHCO_3$ in the buffer as was used to prepare the largest concentration of MEHP.

Primparous Wistar rats (Woodlyn Farms, Guelph, Ontario) weighing between 175-200 g were acclimatized upon arrival for one week to a temperature of 21 ± 2°C and a relative humidity of

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40-60%. Female rats then were paired overnight with a proven sire. The presence of a vaginal plug or a positive vaginal smear on the following morning placed the dam in her 1st day of gestation. Pregnant rats were randomly distributed into 4 groups of 15 rats in each. Each rat was caged individually and had free access to food (Master Fox rat cubes) and water. Treatment by stomach intubation began on their 6th day and continued through to their 15th day of gestation. Doses were administered between 9-10 o'clock each morning with dosages of either 0, 225, 450 or 900 mg of MEHP per kg body weight (B.W.). The rate of administration was 1 mL/200 g B.W. and was adjusted daily to the female's changing B.W.

On their 22nd day of gestation the dams were necropsied in order to provide the following information: litter size, individual litter weight, number of deciduomas and maternal weight gain. Maternal weight gain was determined by subtracting the female's weight on the 1st day of gestation from the female's weight at necropsies after removing the pups and uterine horns. Fetuses were examined grossly following which approximately two-thirds of the fetuses from each litter were processed for skeleton examination. The remainder were fixed in Bouin's fluid for a visceral investigation.

Following this experiment and depending on the results obtained, the experiment was repeated using the same protocol but with dosages of 0, 50, 100 and 200 mg of MEHP per kg B.W. A statistical analysis of all data was carried out using either Duncan's multiple range or the chi-square test.

To test for mutagenic potential, MEHP was dissolved in dimethylsulfoxide (DMSO) and applied in a volume of 0.1 mL to plates containing the Salmonella strains, TA1535, TA100, TA1537, TA1538 or RA98. MEHP in amounts of 0.25, 0.5, 1.0 and 2.0 mg/plate were tested twice. Dr. B.N. Ames (Berkley, California) provided the different strains of Salmonella and his procedures for bacterial testing and for preparing Aroclor 1254-induced rat liver for metabolic activation were followed (AMES et al. 1975). Responses to positive control compounds were the same as in a previous study (NESTMANN et al. 1980).

RESULTS AND DISCUSSION

MEHP was lethal to some mothers at dosages of 225, 450 and 900 mg/kg and killed 3, 4 and 11 dams at each dose, respectively (Table 1). There was no set number of doses in any of the treatments which led to death. Some mothers died after only one treatment, while others survived all treatments but died before necropsies. Necropsies on the dead mothers revealed that their deaths could not be attributed to the gavage treatment but were linked to the dose itself. Although the mean maternal weight gain for the 225 and 450 mg/kg treatment groups was somewhat smaller (39.5 and 39.0 g, respectively) than that of the control group (51.6 g), statistically there was no significant

TABLE 1

Maternal and fetal data as a result of the gavage of mono-(2-ethylhexyl) phthalate in the rat on their 6th-15th day of gestation

Dose (mg/kg)	Nos. Females Treated	Died	N.P.*	Maternal Weight Gain[1]	Litters	Mean Litter Weight[1]	Pups	Mean Pups per Litter[1]
0	15	0	2	51.6 ± 5.7	13	5.2 ± 0.1	160	12.3 ± 0.6
225	15	3	3	39.5 ± 7.6	9	5.1 ± 0.1	111	12.3 ± 0.9
450	15	4	3(2)	39.0 ± 9.5	6**	4.8 ± 0.2**	65	10.8 ± 2.8
900	15	11	4		0		0	
0	15	0	2	47.6 ± 3.3	13	5.0 ± 0.1	166	12.8 ± 0.6
50	15	0	0	40.2 ± 3.5	15	5.2 ± 0.1	182	12.1 ± 0.7
100	15	0	5	31.3 ± 3.8**	10	5.1 ± 0.1	126	12.6 ± 0.6
200	15	0	2	31.1 ± 4.5	13	5.2 ± 0.1	161	12.4 ± 0.4

*not pregnant **$p < 0.05$ 1. g, mean ± S.E. ( ) early resorption

TABLE 2

Effect of mono-(2-ethylhexyl) phthalate in Salmonella

mg/plate	His$^+$ reversions/plate[a]									
	TA1535		TA100		TA1537		TA1538		TA98	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
0.0	30	29	124	159	17	26	27	36	28	37
0.25	29	22	144	139	9	13	25	32	31	30
0.5	16	17	115	134	4	13	25	38	20	19
1.0	24	16	75	79	7	10	17	33	20	19
2.0	24	16	18[b]	53[b]	1[b]	6	15	22	15	21

[a]The numbers are average values for duplicate plates.

[b]These plates showed reduced background of His$^-$ cells.

difference. The fact that there was no difference is attributed to the large standard deviations in both treatment groups. One mother gained only 3 g following oral treatment with 450 mg/kg while two female rats on the 225 mg/kg treatment had gains of 15 and 16 g. A chi-square test on the number of litters resulting from the 450 mg/kg treatment and those in the control group, indicated that there was a treatment-related effect ($p < 0.05$). Uteri of two mothers which had received 450 mg of MEHP per kg B.W. were observed, at necropsies, to be edematous with a hemorrhagic fluid. These disturbances were viewed as a possible indication of early resorption and also indicative of the fetotoxic properties of this dose.

Treatment with 450 mg/kg statistically affected the mean litter weight of the live pups ($p < 0.05$). Examination of the fetal skeleton did not reveal any pronounced alterations between treated and control groups other than disturbances in the placement of the sternebrae plates, a 14th and wavy ribs which were observed in both experimental and control fetuses. Visveral anomalies were not seen.

Since it was part of the purpose of the experiment to obtain a no-effect level, the experiment was repeated using levels of 0, 50, 100 and 200 mg of MEHP per B.W. Dosages of 100 and 200 mg/kg reduced the maternal weight gain of dams on the treatment when compared to the control group ($p < 0.05$) (Table 1). All other parameters under examination at these dosages were not affected by the treatment. There were no visceral or skeleton anomalies grossly discernable aside from the even distribution of wavy ribs among the groups. The no-effect level was determined as 50 mg/kg.

MEHP tested in the five strains of Salmonella did not demonstrate a mutagenic effect although lethal responses were induced at the highest amount in TA1537 and TA100 (Table 2).

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