Sub-acute and Sub-chronic Toxicity of Mono-2-Ethylhexyl Phthalate in the Rat

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Abstract. Groups of six male Sprague-Dawley rats were administered single oral doses of mono-2-ethylhexyl phthalate (MEHP) at 50, 100 or 200 mg/kg or di-2-ethylhexyl phthalate (DEHP) at 2,000 mg/kg in 2% gum acacia and were observed clinically for seven days. Body weight and food consumption were not affected by treatment. The liver weight increased in the groups receiving 100 mg or 200 mg mono-2-ethylhexyl phthalate and 2,000 mg diethylhexyl phthalate. Hepatic microsomal aniline hydroxylase activity was not altered by treatment.

In a subsequent 28-day experiment, groups of ten weanling Sprague-Dawley rats were fed diets containing 0, 25, 100, 400, 1,600 or 6,400 ppm of mono-2-ethylhexyl phthalate. Decreased growth rate occurred in the group receiving the highest dose level. Increased heart and liver weights were observed in animals from the 1,600 and 6,400 ppm groups. Minor alterations in serum biochemical values included decreased SDH and calcium levels, and elevated alkaline phosphatase activity in some treated groups. A mild reduction in red cells saturation and hematocrit was noted in some groups.

In 3-month and 6-month feeding studies, groups of ten male and female weanling Sprague-Dawley rats were fed diets containing 1, 5, 25, 125 or 625 ppm of mono-2-ethylhexyl phthalate. Growth rate and food consumption were not affected at any dose level or time interval. Relative organ weights of rats of both sexes were not altered in the 3-month period, but the liver weights of female rats in the 6-month experiment were increased. Changes in clinical chemistry and hematological values were mild. These included lower LDH, SGOT, hemoglobin, and hematocrit values in male rats at the 3-month period and reduced potassium content at the 6-month period. Histological changes were mild in both male and female rats at both time intervals. Treatment-related lesions were found in the liver, heart, and adrenals. Alteration in the liver consisted of midzonal and periportal eosinophilic cytoplasmic inclusions and vacuolations with isolated binucleated and necrotic hepatocytes. There was a mild enlargement of myocardial nuclei and segmental deregistration of myocardial striations in test animals. The adrenal glands exhibited vacuolation of the zona fasciculata.
which was less severe in the 6-month period than the 3-month counterpart. In conclusion, mono-2-ethylhexyl phthalate is similar to its parent compound diethylhexyl phthalate in that it possesses a low order of oral toxicity in rats.

Phthalate esters are used extensively in the manufacture of a variety of plastics including food packaging materials and polyvinyl chloride (PVC) blood bags. Among these, di-2-ethylhexyl phthalate is the most commonly used plasticizer. Toxicity studies of phthalate esters have been reported in the literature for decades (Shaffer et al. 1945; Carpenter et al. 1953). Because of their ubiquitous presence in the environment and, in particular, the detection of DEHP in human blood stored in PVC bags (Jaeger and Rubin 1972), concern has been raised over their toxicity and bioaccumulation. Much of the research to date has been carried out on the toxicity of DEHP, while little information is available on the toxicity of its metabolites. It has been shown that DEHP is extensively metabolized to MEHP and to the acid, alcohol, and ketone resulting from the side-chain oxidation of MEHP (Albro et al. 1973; Daniel and Bratt 1974). Lake et al. (1975) have shown that DEHP fed to rats caused biochemical and ultrastructural changes in the hepatic endoplasmic reticulum and mitochondria which were substantially reproducible by the administration of MEHP. These authors concluded that the partial hydrolysis of DEHP to MEHP was the degradative step which determined the hepatic changes produced by DEHP. Recent studies from our laboratories have revealed that both DEHP and MEHP accumulate in human blood stored in PVC blood bags (Rock et al. 1978). Prompted by these findings, the present investigations were carried out to assess the toxicity of a single exposure, 28-day sub-acute, 3-month, and 6-month sub-chronic toxicity of MEHP in rats.

**Materials and Methods**

DEHP was obtained as a kind gift from Mr. Jos Mes of Health Protection Branch, Ottawa, Canada. MEHP was prepared according to a method described previously (Chu et al. 1978). Chemical identities and purity (>99%) of these compounds were confirmed by nuclear magnetic resonance spectroscopy (Varian A60A Instrument), and gas chromatography (Hewlett Packard model 5830A, with a 60Ni electron capture detector).

**Toxicity of a Single Dose**

Thirty male adult Sprague-Dawley rats weighing approximately 300 g were randomly divided into five groups of six animals each. The groups were administered single oral doses of 2% gum acacia suspension of MEHP at 50, 100 or 200 mg/kg, or DEHP at 2,000 mg/kg. The control groups received an equivalent amount of vehicle only (2 ml/kg). The animals were allowed free access to food (Master Fox) and water, and observed for clinical signs. Initial and final body weights were recorded. After seven days, all animals were anesthetized with ether and exsanguinated via the abdominal aorta. Gross pathological observations were made during the necropsy and the liver, kidney, heart, spleen and brain were excised and weighed. A sample of the fresh liver was taken for the determination of microsomal aniline hydroxylase activity (Fouts 1963). Serum sorbitol dehy-