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Effect of Phthalic Acid Esters on Drug Metabolizing Enzymes

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Health hazards of phthalic acid esters, widely used as plasticizers, have been reported recently (THOMAS et al. 1978; LAWRENCE and TUELL 1979). In spite of their low order of toxicity, di-(2-ethylhexyl)phthalate (DEHP) and other phthalates have been shown to exert wide variety of subtle toxic effects. Of concern is the property of phthalates to modify the biological responses of some pharmacological agents and other xenobiotics. Earlier studies have reported interaction of DEHP with parathion (SRIVASTAVA et al. 1976) pentobarbitone and methaqualone (SETH et al. 1977), carbon tetrachloride (SETH et al. 1979), and ethanol (SETH and AGARWAL 1978). The modification of biological response of these chemicals may be related to their altered rates of biotransformation in the presence of DEHP. These observations prompted studies to investigate the effects of DEHP, dimethyl phthalate (DMP), and di-butyl phthalate (DBP) on the activity of aminopyrine-N-demethylase, aniline hydroxylase, and tyrosine aminotransferase in rat liver after single and repeated exposures.

MATERIALS AND METHODS

Adult male albino rats of Industrial Toxicology Research Centre Colony, maintained under standard laboratory conditions on ad libitum pellet diet (Hind Lever Laboratory Animal Feed, India) with free access to water were used. DEHP, DMP, and DBP were given 5.0, 3.6, and 3.05 mL/kg, respectively, by intraperitoneal route. These doses are 1/10 of LD_{50}, dose of the respective compound and were selected on the basis of their tetratogenic (SINGH et al. 1972), pharmacologic (LAWRENCE et al. 1975), and biochemical effects (LAKE et al. 1975; SETH et al. 1976; SRIVASTAVA et al. 1978).

The animals were weighed and watched throughout the study. Control and treated animals were fasted overnight and sacrificed 18 hr or 7 days after the daily treatment with phthalates. Liver was quickly removed and a portion was homogenized in ice-cold 0.25 M sucrose with the help of a Potter-Elvehjem type glass homogenizer fitted with a teflon pestle to yield suitable homogenates. The assay of aminopyrine-N-demethylase and aniline hydroxylase activities was performed in 9000 x g supernatants according to MAZEL (1971). The activity of tyrosine aminotransferase was estimated by the method of DIAMONDSTONE (1966).

Total protein content was estimated in trichloroacetic acid precipitate by the method of LOWRY et al. (1951) using bovine serum

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albumin as standard. Statistical significance of the results was evaluated by Student's 't' test according to FISCHER (1950) where 'P' values less than 0.05 were considered as significant.

RESULTS AND DISCUSSIONS

Table 1 shows the effect of DMP, DEHP, and DBP on some microsomal enzymes of rat liver. These phthalate esters inhibited the activity of aminopyrine-N-demethylase and aniline hydroxylase but had no effect on glucose-6-phosphatase and NADPH-cytochrome-c-reductase. DMP produced maximum inhibition and this was followed by DEHP and DBP. DBP produced a low inhibitory effect on aminopyrine-N-demethylase activity. However, on repeated intraperitoneal administration of any of these phthalates daily for 7 days no decrease was observed in the activity of these enzymes. DEHP is known to increase the liver weight, microsomal protein, and cytochrome P-450 content after prolonged oral administration in rats (LAKE et al. 1975). These animals also showed an increase in alcohol dehydrogenase activity after an initial decrease and persistent inhibition of aniline hydroxylase activity. An increase in barbiturate sleeping time after a single intraperitoneal or intravenous administration and a decrease of that on repeated oral administration of DEHP has also been observed (DANIEL and BRATT 1974). Perhaps due to such a biphasic action of phthalates no decrease in the activity of aminopyrine-N-demethylase and aniline hydroxylase was evident at 7 days of the treatment.

Effect of phthalate esters on the activity of hepatic tyrosine aminotransferase is shown in Table 2. Single intraperitoneal administration of DMP, DEHP, or DBP produced no significant effect on the enzyme activity. Daily administration of these plasticizers for seven days caused increase in the enzyme activity where significant increase of 61% was observed with DEHP treatment.

These results have provided support to previous observations that phthalic acid esters prolong the barbiturate sleeping time (LAWRENCE et al. 1975; SETH et al. 1977) by interfering with their metabolic disposition.

Phthalic acid esters were found to exhibit no structure-activity-relationship in affecting these enzymes. Similar lack of any role of the molecular structure of phthalate esters in prolonging the pentobarbitone sleeping time (LAWRENCE et al. 1975) or inhibiting the growth of nerve cell fibroblasts (KASUYA 1980) have also been observed.

Tyrosine aminotransferase has recently gained significance as a model for accessing stress factors due to characteristic adaptive-increase in its activity on exposure to xenobiotics (BHATIA et al. 1971; STEFAN et al. 1974; AGARWAL et al. 1978). Activity of the enzyme is also altered on administration of therapeutic agents (CANAL et al. 1963; FERRI et al. 1975). The significant increase in the activity of tyrosine aminotransferase suggests the possibility of stress due to phthalate exposure. Phthalate esters may

TABLE 1

Effect of DMP, DBP, and DEHP on Rat Liver Microsomal Enzymes

Group	Aminopyrine-N-demethylase (nmoles HCHO formed/min/mg protein)		Aniline hydroxylase (pmoles p-amino phenol formed/min mg protein)		Glucose-6-phosphtase (nmoles Pi liberated/min/mg protein)		NADPH-Cytochrome c reductase (nmoles ferricytochrome-c-reduced/min/mg protein)	
	18 hrs	7 days	18 hrs	7 days	18 hrs	7 days	18 hrs	7 days
Control	6.78 ± 0.42	6.78 ± 0.42	818 ± 45	760 ± 140	256 ± 17	282 ± 36	164 ± 13	158 ± 17
DMP-Treated	5.19 ± 0.10[b] (-23.4)	5.81 ± 0.45 (-14.3)	513 ± 24[a] (-37.2)	809 ± 111 (+6.4)	225 ± 33 (-12.1)	251 ± 34 (-10.9)	149 ± 12 (-9.1)	195 ± 19 (+23.4)
DBP-Treated	6.24 ± 0.28 (-7.9)	6.74 ± 0.48 (-0.5)	631 ± 44[c] (-22.8)	763 ± 134 (+0.3)	253 ± 10 (-1.1)	256 ± 24 (-9.2)	150 ± 11 (-8.5)	159 ± 40 (+0.6)
DEHP-Treated	5.22 ± 0.36[b] (-23.0)	6.84 ± 0.50 (+0.8)	581 ± 43[b] (-28.9)	758 ± 103 (-0.2)	315 ± 46 (+23.0)	304 ± 34 (+7.8)	145 ± 8 (-11.5)	186 ± 34 (+17.7)

All values are mean ± S.E. for five observations. Figures in parentheses indicate percent change as compared to controls: (+) indicates an increase and (-) a decrease.

[a] $p < 0.001$; [b] $p < 0.01$; [c] $p < 0.02$

presumably be stimulating the adrenal activity like other xenobiotics to increase the enzyme activity (KATO and GILLETTE 1965; SCHIMKE and DOYLE 1970; BHATA et al. 1971).

TABLE 2
Effect of DMP, DBP, and DEHP on Rat Liver Tyrosine Aminotransferase

Group	Tyrosine aminotransferase pmoles of p-hydroxy benzaldehyde formed/min/mg protein	
	18 hrs	7 days
Control	10.8 ± 0.7	9.2 ± 0.2
DMP-Treated	10.2 ± 0.1 (-4.8)	11.6 ± 0.5[a] (+26.6)
DBP-Treated	10.6 ± 0.4 (-1.2)	12.3 ± 0.9[a] (+33.6)
DEHP-Treated	11.5 ± 0.6 (+6.9)	14.8 ± 1.2[a] (+61.4)

All values are mean ± S.E. for five observations.
Values in parentheses indicate percent change in comparison to controls: (+) indicates an increase and (-) a decrease.
[a] $P < 0.01$

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