Interaction of Di-(2-ethylhexyl)-phthalate (DEHP) with Pentabarbitone and Methaqualone[1]

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The detection of di-(2-ethylhexyl) phthalate (DEHP) and other phthalate esters in water, fish, aquatic invertebrates (METCALF et al. 1973; SANDERS et al. 1973) and animal (TABORSKY 1967; NAZIR et al. 1971) and human tissues (JAEGER and RUBIN, 1972; MES et al. 1974) has established them as widespread environmental pollutants (MAYER et al. 1972; WILDBRETT 1973; WILLIAMS 1973). Reports of their migration from the finished polyvinyl chloride biomedical devices into the blood and other physiological fluids (GUESS et al. 1967; JAEGER and RUBIN, 1970, 1972; NEEDHAM and LUZZI 1973; NEERGAARD et al. 1975) and subsequently their entry into humans are of concern. Subtle toxicity of phthalate esters has been indicated by their effects on lung (SCHULZ et al. 1975), cell culture (JACOBSON et al. 1974; JONES et al. 1974; KASUYA 1974) and reproduction in aquatic (SANDERS et al. 1973) and mammalian (SINGH et al. 1972, 1974) species. Sensitivity of the liver towards dimethyl phthalate (DMP), DEHP and dibutyl phthalate (DBP) has been indicated by the decrease in the activity of energy linked reactions (SETH et al. 1974; SRIVASTAVA et al. 1975a, 1975b) and other enzymes LAKE et al. 1975) in treated rats. These observations have led us to investigate their interactions with xenobiotics, since liver is the organ which handles most of the foreign chemicals. The present communication deals with the effect of DEHP on the biological action of two sedative-hypnotics, pentabarbitone and methaqualone.

METHODS AND MATERIALS

Animals and treatment - Adult male and female albino rats (200-250 g) or mice (25-30 g) of I.T.R.C. stock maintained on pellet diet and water ad lib. under

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standard laboratory conditions were used. The animals were divided into four groups which were treated intraperitoneally as follows: (1) Pentabarbitone, 50 mg/kg (2) methaqualone, 30 mg/kg (3) DEHP, 5 ml/kg and pentabarbitone (4) DEHP, 3.7 ml/kg and methaqualone.

Undiluted DEHP was injected 18 hrs prior to the administration of any drug. The doses of the plasticizer are 1/10 of LD_{50} as reported in the literature (SINGH et al. 1972; LAWRENCE et al. 1975). The interaction of DEHP with pentabarbitone and methaqualone was studied in rats and mice respectively.

Sleeping time studies - Sleeping time was recorded as the time elapsing between the loss and the regain of righting reflex. Time elapsing before the loss of righting reflex was recorded as the induction time.

A group of control and DEHP-pretreated male mice run in parallel were sacrificed at desired time intervals by cutting the jugular vein and blood was collected in oxalated-test tubes for the estimation of methaqualone.

Estimation of Methaqualone - Methaqualone concentration was measured in the blood of male mice at 30 min and upon their awakening as follows: To 1.0 ml of blood in a stoppered tube, 0.5 ml of 0.1 N NaOH was added and after a thorough mixing, 15.0 ml of chloroform was mixed and the contents were shaken for 30 min. The chloroform layer was separated by filtration and the residue was washed twice, using 10 ml of the solvent each time. The combined filtrates were evaporated at 50°C over a water bath to dryness and the residue was suspended in 5.0 ml of 0.1 N HCl. A blood sample from untreated animal run in parallel was used as the reference. The absorbance of methaqualone was read at 234 nm in a UNICAM SP-500 Spectrophotometer.

RESULTS

The DEHP-treated animals did not show any signs of central nervous system depression and appeared to be normal.

Tables 1 and 2 summarise the effect of DEHP on the pentabarbitone and methaqualone-induced sleeping time in rats and mice respectively. The pretreatment of animals with DEHP, significantly prolonged the sleeping time induced by the two drugs and had no effect on the induction time in either sex.

TABLE 1

Effect of DEHP on pentabarbitone sleeping time in rats

Group		Induction time (Min)	Sleeping time (Min)
Male	Control	5.00 ± 0.02	74.5 ± 5.7
	DEHP-treated	5.28 ± 0.44 (N.S.)	107.4 ± 8.8 (P<0.01)
Female	Control	3.42 ± 0.82	187.1 ± 11.9
	DEHP-treated	4.72 ± 0.96 (N.S.)	238.0 ± 10.0 (P<0.01)

All values are mean ± S.E. from 8 animals. Probability was evaluated by student 't' test.

TABLE 2

Effect of DEHP on methaqualone induced sleeping time in mice.

Animal		Induction time (Min)	Sleeping time (Min)
Male	Control	8.6 ± 2.1	45.4 ± 2.4
	DEHP-treated	12.0 ± 1.1 (N.S.)	83.2 ± 7.0 (P<0.001)
Female	Control	9.2 ± 0.85	170.0 ± 31.0
	DEHP-treated	9.6 ± 0.68 (N.S.)	309.0 ± 31.0 (P<0.01)

All values are mean ± S.E. from 8 animals. Probability was evaluated by students 't' test.
N.S.: not significant

The results in Table 3 show that the concentration of methaqualone in the blood of DEHP-treated animals was significantly higher around the middle of the sleeping time and was not different from controls at awakening.

TABLE 3

Blood levels of methaqualone (µgs/ml) in normal and DEHP-treated male mice.

Group	At 30 min.	At awakening
Control	15.1 ± 0.5	8.40 ± 0.8
DEHP-treated	20.2 ± 0.8 ($P<0.001$)	8.30 ± 1.1 (N.S.)

All values are mean ± S.E. from 8 animals. Probability was evaluated by student 't' test.
N.S.: not significant.

DISCUSSION

Our results indicate that DEHP alters the duration of action of two drugs, pentabarbitone and methaqualone in rats and mice respectively.

The prolongation of the pentabarbitone or methaqualone-induced sleeping time may be the result of the effect of phthalate on their biotransformation, fatty deposition, excretion or to increased sensitivity of central nervous system towards them, in the presence of DEHP. Such an effect may also be due to the alterations in their distribution in different organs in the presence of the plasticizer (RUBIN and JAEGER 1973). The possibility that DEHP may accumulate in the peritoneal cavity due to its relative insolubility in aqueous solutions and act as a lipid reservoir for pentabarbitone and other drugs has been ruled out by LAWRENCE *et al*. (1975) since cotton seed and mineral oils failed to prolong the sleeping time.

The significantly higher levels of pentabarbitone (SETH, 1975) and methaqualone in the blood of DEHP-treated animals around the middle of their sleeping time and no significant differences at awakening, as compared to controls, suggests that the prolongation in the sleeping time is not related to the increased sensitivity of the central nervous system towards the test drugs. The increase in the sleeping time induced by the two compounds may thus be the result of their delayed metabolic disposition in the presence of the plasticizer. An inhibitory effect of DEHP on microsomal mixed function oxidases supports such an assumption (LAKE et al. 1975).

The prolongation of the two drugs observed in the present investigation is of significance since man may be exposed to phthalate plasticizers and other xenobiotics simultaneously.

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