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Teratogenicity/Fetotoxicity of DEHP in Mice

by I. Tomita,* Y. Nakamura,* Y. Yagi* and K. Tutikawa†

The embryonic/fetotoxic effects of DEHP on pregnant mice (ddY-Slc ♀ × CBA ♂) were studied. DEHP was administered orally in dosages of 0.05 ml/kg to 30.0 mg/kg on day 6, 7, 8, 9 or 10 of gestation. A single administration of DEHP over 0.1 ml/kg (1/300 of LD_{50}) on day 7 of gestation decreased the numbers and the body weight of living fetuses, whereas no significant changes in the numbers of living fetuses (with no gross and skeletal abnormalities) were observed compared with those of the control group, when 0.05 ml/kg (1/600 of LD_{50}) of DEHP was administered. The fetotoxicity (fetal death) was dose dependent. The LD_{50} and the nonfetolethal maximum dosage of DEHP in its single, oral administration was 592 mg/kg and 64 mg/kg, respectively. The latter value is much higher than an estimated DEHP intake from commercial foodstuffs in men, which is approximately 0.03 mg/kg/day.

Introduction

The deleterious effects of di(2-ethylhexyl) phthalate (DEHP) on developing embryos and/or fetuses in animals have been reported by a number of investigators (*1-14*).

The present reports are the summaries of our studies (*15, 16*) carried out to determine the embryo toxicity and fetotoxicity of DEHP on ddY-Slc(SPF) (♀) × CBA(SPF)(♂) mice. DEHP was administered orally once during the period of pregnancy. The dosages administered were from 1/30 to 1/1 of LD_{50} (high-dose study) and from 1/600 to 1/30 of LD_{50} (low-dose study).

Through these studies, the nontoxic maximum dose of DEHP in mice was determined, and steps were taken to assess the risk of DEHP for human reproduction.

Experimental

Virgin female mice of the ddY-Slc (SPF) strain (Shizuoka Agricultural Cooperative Association for Laboratory Animals), weighing 27-28 g (8-9 weeks old), were housed with one male mouse of CBA strain (National Institute of Genetics). The atmosphere was maintained at 25 ± 2°C and 50 ± 5% RH. Laboratory diet (Funahashi Farm Co.) and tap water were provided *ad libitum*. DEHP (purity over 99%) was obtained from Tokyo Kasei Co. Ltd. MEHP was synthesized according to the method of Kenyon (*17*) and was more than 99% pure as determined by ECD-GLC analysis. The experimental procedure is detailed in our previous paper (*16*).

Results

Figure 1 shows the body weight changes of pregnant mice. The increase in body weight of the mice was suppressed when 10 ml/kg of DEHP was given PO once on day 7, 8, 9, or 10 of gestation. The suppression was especially clear when DEHP was administered on day 7 or 8 of gestation. A decrease in the body weight of mice, however, was not observed when a low dosage of DEHP (0.05 ml and 0.1 ml/kg) was given, even on day 7 of gestation (see inset in Fig. 1).

Table 1 summarizes the effects of DEHP on the fetuses of mice. The study consisted of four groups: an untreated control group, two DEHP-treated groups (high-and low-dose groups) and an ethylurethane-treated group (positive control). As shown in Table 1, the number of implantations per pregnant mouse was not significantly different among the control and DEHP-treated groups, but the numbers of embryos which suffered an early or late death varied greatly, depending on the day and the

*Laboratory of Health Science, Shizuoka College of Pharmaceutical Sciences, 2-2-1 Oshika, Shizuoka (422), Japan.
†Department of Induced Mutation, National Institue of Genetics, 1111 Yata, Mishima (411), Japan.

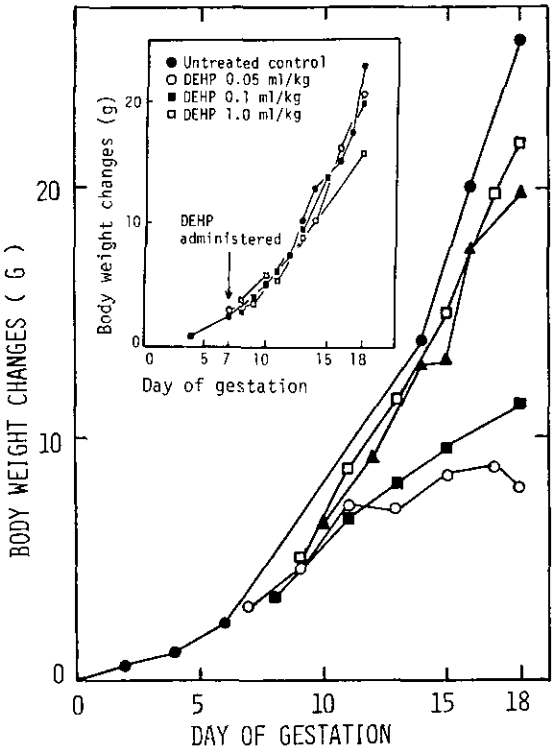

FIGURE 1. Effects of di(2-ethylhexyl) phthalate (DEHP) on the body weight changes of pregnant mice. DEHP was given PO in one dose of 10 ml/kg on (○) the 7th, (■) 8th, (□) 9th, or (Δ) 10th day of gestation; (●) untreated control.

amount of DEHP administered. A high incidence of dead embryos was found in mice which had received DEHP on day 7 or 8 of gestation, whereas a low incidence of dead embryos was found in mice which had received DEHP on days 6, 9, or 10. It must be noted that a higher incidence of early and late deaths among embryos occurred with the higher and lower dosages of DEHP administration, respectively. There was a high rate of gross and skeletal abnormalities among the two groups of mice which had received 2.5 or 7.5 ml of DEHP/kg on days 7 or 8 of gestation, respectively. No abnormality was observed in the other two groups of mice receiving 0.05 or 0.1 ml DEHP/kg although the average body weight of viable fetuses was significantly lower ($p < 0.01$) than that of the control group.

The details of the gross and skeletal malformation in mouse fetuses due to DEHP administration are shown in Table 2. Exencephaly, open eyelid, club foot and bent or no tail were often found in the malformed fetuses, while thoracic lumbar, sacral and caudal vertebrae were often found in an abnormal shape. There was no case of a cleft palate.

Figure 2 shows the correlation between the death and abnormalities (gross and skeletal) of fetuses versus the dosage of DEHP administered on day 7 of gestation. From a straight line ($y = 51.9 \log x + 61.6$, where y = % of fetal death, x = ml/kg of DEHP administered) obtained with 5 log dosage of DEHP, the fetal LD_{50} was determined to be 592 mg/kg on assuming the specific gravity of DEHP to be 0.986. The dosage producing 2% of fetal deaths, i.e., the same mortality rate as found in the control group, was about 64 mg/kg. The exact values of the ineffective maximum dosage for gross and skeletal abnormalities could not be determined because of limited data, but these values are probably less than 0.80 ml/kg (789 mg/kg) and 0.68 ml/kg (670 mg/kg), respectively, as judged from their dose-response curve.

Our trial for the safety evaluation of DEHP with respect to embryo/fetal toxicity is shown in Figure 3. As was demonstrated and stated above, the maximum nonteratogenic (both gross and skeletal) dose and median fetolethal dose (fetal LD_{50}) in mice are approximately the same, around 600 mg/kg, while the maximum nonfetolethal dose in mice is about 64 mg/kg. In humans, approximately 4 mg/kg would be a possible maximum intake of DEHP by blood transfusion or hemodialysis (*18*). The level (unconditional) of the acceptable daily intake for humans (ADI) is about 1 mg/kg/day (*19*).

The maximum intake of DEHP from foodstuffs with which we are most concerned would not exceed 0.03 mg/kg/day, assuming a daily food intake as 1.5 kg/50 kg adult and an average content of DEHP in our foodstuffs as 1 ppm (*20*).

A safety margin of approximately 21-fold thus exists between the nonfetolethal dose of DEHP and the maximum amount of DEHP from foodstuffs, if a 100-fold margin is simply set to extrapolate the data from mice to humans.

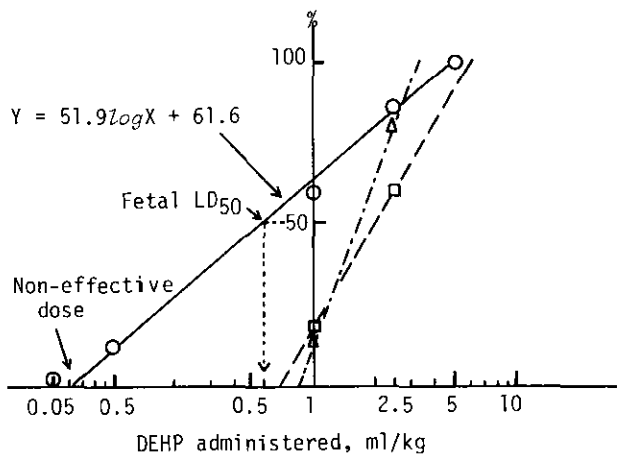

FIGURE 2. Fetotoxicity of DEHP in mice: (○) fetal death; (Δ) gross abnormalities; (□) skeletal abnormalities.

Table 1. Effects of DEHP on mouse fetuses.

Group	Day of gestation	Dose administered, ml/kg[a,b]	Number of implantations (no. pregnant mice)	Number of dead embryos (% live fetuses)		Number of living embryos (% live fetuses)[c]	Numbers of abnormalities[d]		Average body weight of fetuses, g
				Early death	Late death		Gross	Skeletal	
(High dose) untreated control	–	–	56(5)	0	0	56(100)	0	0	1.41 ± 0.06
DEHP (high dose)	6	2.5(1/12)	60(6)	1(1.7)	7(11.7)	52(86.7)	1(1.9)	3(5.8)	1.31 ± 0.12[e]
	7	1.0(1/30)	54(6)	13(24.1)	19(35.2)	22(40.7)	3(13.6)	4(18.2)	1.20 ± 0.09[e]
		2.5(1/12)	36(5)	21(58.3)	10(27.8)	5(13.9)	4(80.0)	3(60.0)	1.17 ± 0.18[e]
		5.0(1/6)	43(4)	27(62.8)	16(37.2)	0			
		10.0(1/3)	59(5)	44(74.6)	15(25.4)	0			
	8	7.5(1/4)	66(6)	8(12.1)	29(43.9)	29(43.9)	19(65.5)	24(82.8)	1.07 ± 0.07[e]
		10.0(1/3)	83(8)	15(18.1)	53(63.9)	15(18.1)	7(46.7)	5(33.3)	1.23 ± 0.14[e]
	9	7.5(1/4)	30(3)	2(6.7)	1(3.3)	27(90.0)	1(3.7)	0	1.28 ± 0.08[e]
		10.0(1/3)	48(5)	2(4.2)	2(4.2)	44(91.7)	9(20.5)	0	1.32 ± 0.09[e]
		30.0(1/1)	48(5)	2(4.2)	16(33.3)	30(62.5)	9(30.0)	5(16.7)	1.08 ± 0.12[e]
	10	10.0(1/3)	65(7)	2(3.1)	1(1.5)	62(95.4)	0	0	1.37 ± 0.13[f]
		30.0(1/1)	63(7)	3(4.8)	3(4.8)	57(90.5)	4(6.5)	0	1.28 ± 0.16[e]
(Low dose) untreated control	–	–	301(31)	2(0.7)	4(1.3)	259(98.0)	0	0	1.39 ± 0.06
DEHP (low dose)	7	0.05(1/600)	200(22)	0	5(2.5)	195(97.5)	0	0	1.31 ± 0.11[e]
		0.1(1/300)	98(11)	2(2.0)	9(9.2)	87(88.8)	0	0	1.27 ± 0.13[e]
		1.0(1/30)	94(10)	1(1.1)	55(58.5)	38(40.4)	3(7.9)	7(18.4)	1.22 ± 0.10[e]
Ethyl-urethane (IP)	7	1.5	34(4)	1(2.9)	5(14.7)	28(82.4)	4(14.3)	4(14.3)	1.08 ± 0.22
	8	1.5	44(5)	3(6.8)	0	41(93.2)	1(2.4)	1(2.4)	1.20 ± 0.10
	9	1.5	41(4)	1(2.4)	25(61.0)	15(36.6)	13(86.7)	15(100)	0.85 ± 0.12
	10	1.5	22(3)	0	0	22(100)	20(90.9)	22(100)	0.87 ± 0.06

[a]Oral administration.
[b]Numbers in parentheses denote fraction of acute LD_{50} (30 ml/kg).
[c]Numbers in parentheses indicate percent of live fetuses based on total number of implantations.
[d]Numbers in parentheses indicate percent abnormalities based on total numbers of live fetuses.
[e]Significantly different from untreated control at 99% level ($p < 0.01$).
[f]Significantly different from untreated control at 95% level ($p < 0.05$).

Table 2. Incidence of gross and skeletal malformations in the fetus of mouse after di(2-ethylhexyl) phthalate (DEHP) administration at various days of gestation.

		Day 6	Day 7		Day 8		Day 9			Day 10	
	Untreated control	2.5 (ml/kg)	1.0 (ml/kg)	2.5 ml/kg	7.5 (ml/kg)	10.0 ml/kg	7.5 (ml/kg)	10.0 ml/kg	30.0 (ml/kg)	10.0 ml/kg	30.0 (ml/kg)
No. fetuses examined	56	60	54	36	66	83	30	48	48	63	65
No. live fetuses	(56)	(52)	(22)	(5)	(29)	(15)	(27)	(44)	(30)	(62)	(57)
No. gross abnormalities	0	1	3	4	19	7	1	9	9	0	4
(%)[a]		(1.9)	(13.6)	(80.0)	(65.5)	(46.7)	(3.7)	(20.5)	(30.0)		(6.5)
Exencephaly	0	0	1	0	7	2	0	0	0	0	0
Open eyelid	0	0	1	0	5	7	0	8	3	0	0
Abdominal hernia	0	0	0	0	1	0	0	0	0	0	0
Club foot	0	0	1	1	5	3	1	1	2	0	0
Poly-dactylia	0	0	0	0	1	0	0	0	0	0	1
Bent tail or none	0	1	1	4	5	4	0	0	0	0	5
Subcutaneous bleeding	0	0	0	0	0	0	0	0	4	0	0
No. skeletal abnormalities	0	3	4	3	24	5	0	0	5	0	0
(%)[a]		(5.8)	(18.2)	(60.0)	(82.8)	(33.3)			(16.7)		
Skull vertebrae	0	0	1	0	7	2	0	0	0	0	0
Cervical vertebrae	0	2	0	0	0	0	0	0	0	0	0
Thoracic vertebrae	0	2	4	2	21	5	0	0	1	0	0
Lumbar vertebrae	0	0	1	3	8	4	0	0	3	0	0
Sacral vertebrae	0	0	1	1	6	4	0	0	1	0	0
Caudal vertebrae	0	1	1	5	3	0	0	0	0	0	0
Ribs	0	1	4	2	20	5	0	0	0	0	0

[a]Percent gross or skeletal malformations based on total number of live fetuses examined.

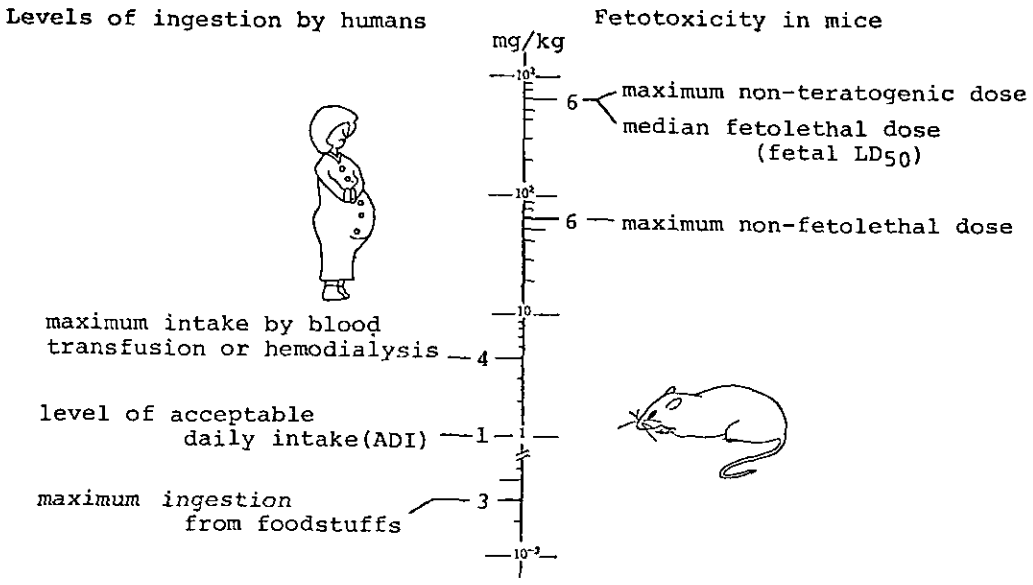

FIGURE 3. Safety evaluation of DEHP in respect to fetotoxicity.

Discussion

Consistent results have not always been obtained in the reproductive/fetotoxic studies of DEHP, due mainly to differences in the dosage of DEHP, the duration and the route of administration. The animal species employed in the experiments is another crucial factor in determining the toxic effects. The effects of DEHP on reproductive function or fetal toxicity in several animal species have been performed by several investigators and are well reviewed (*9*, *12*). The toxic effects of DEHP on the mouse fetuses which have been demonstrated in recent examinations (*7*, *15*, *16*) should be added. It must be also added that the metabolic fate of DEHP in mice seems to be somewhat similar to that in man when solely the metabolic formation of MEHP is considered (*21*). As MEHP is known to be a principal—and possibly the active—metabolite of DEHP (*12*, *16*, *22*), the similarity between mice and humans may have some advantage in extrapolating the mouse data to men.

As shown in Figure 3, the nonfetolethal maximum dose of DEHP in mice by a single oral administration is estimated to be about 64 mg/kg. This value is approximately 2100 times that (0.03 mg/kg) which is a conceivable level of DEHP intake from commercial foodstuffs. Judging exclusively from the above experimental results and estimation, it seems to be unlikely that DEHP will produce any immediate threat to human reproduction, though the conclusive assessment of DEHP should await further study.

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