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Dimethoxyethyl Phthalate: Embryopathy, Teratogenicity, Fetal Metabolism and the Role of Zinc in the Rat*

by M. R. Parkhie,†‡ M. Webb† and M. A. Norcross**

A single intraperitoneal injection (0.6 ml/kg) of dimethoxyethyl phthalate (DMEP) was given to groups of Wistar strain rats on day 10, 11, 12, 13 or 14 of gestation. Control rats received 0.6 ml/kg of physiological saline intraperitoneally.

In phthalate-treated rats, embryopathy was manifested by a high incidence (12-79%) of fetal deaths and fetal resorptions. Fetotoxic effects were expressed by a significant reduction in fetal weights. Hydrocephalus interna, a congenital malformation of the brain, was caused by DMEP. Congenital skeletal deformities (66-96%), with multiple skeletal (14-64%) and appendicular malformations (25-57%), were also induced by DMEP. Control rats exhibited no congenital malformations of the brain and no appendicular or multiple skeletal deformities.

DMEP caused a significant decrease in the zinc content of the fetus.

Fetoplacental metabolism 1 and 4 hr after intravenous administration of ^{14}C-DMEP suggested rapid transfer of the parent compound to the fetus across the placenta and that DMEP is a teratogenic moiety. The possible role of zinc in phthalate-induced teratogenesis in rats is also discussed.

Introduction

Plastics have become an integral part of our everyday lives. Phthalate esters are plasticizers widely used in the manufacture of plastics (*1*). In the United States, an estimated one billion pounds of phthalate esters are produced annually (*2*, *3*). There are approximately 20 different phthalate compounds sold as vinyl plasticizers, and one of the most toxic of these is dimethoxyethyl phthalate (DMEP) (*1-3*).

Phthalate esters are found in the composition of floor tiles, various types of home furnishings, waterproof clothing, industrial tubing, food wrapping materials (*4*, *5*), and a variety of medical and paramedical devices, including heart valves, vascular grafting material, intrauterine devices, catheters, dialyzing units, blood sets and disposable syringes. Residues of phthalates have been found in milk (*6*), human tissues and blood plasma (*7*), bovine tissues and the hearts of dogs, rabbits and rats (*8*). Phthalates, in general, are colorless, high-boiling liquids, soluble in organic solvents but immiscible in water, and they are degraded very slowly in the ambient environment. Diethylhexyl phthalate (DEHP), for example, closely resembles organochlorine pesticides (DDT, PCB) in rate of uptake, storage, and biomagnification in a variety of aquatic organisms (*9*).

The U.S. Department of Health and Human Services, particularly the Food and Drug Administration (*4*, *10*) and the National Institute of Environmental Health Sciences (*11-13*) has, therefore, a continuing interest and concern regarding the safety of phthalates. This concern is reflected in the sponsoring of conferences on phthalate esters in

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†Toxicology Unit, Medical Research Council, Carshalton, Surrey, SM5-4EF, England.

‡Present address: Food and Drug Administration, Office of the Commissioner, 5600 Fishers Lane, Rockville, Maryland 20857.

**Bureau of Veterinary Medicine, Food and Drug Administration, Rockville, Maryland 20857.

1972 (*3*, *4*, *9*, *13*) and in 1981. Several reviews (*14-23*) presented the toxicity of phthalate esters. Although DMEP has been shown to be one of the most toxic phthalates, little is known regarding the teratogenicity caused by a single maternal injection of DMEP during the organogenetic period in the rat.

Dietary supplementation of zinc has prevented teratogenic effects in rats induced by the maternal administration of a chelating agent, ethylenediaminetetraacetic acid (*24*). Dietary deprivation of zinc, on the other hand, could induce embryopathic and teratogenic effects in the rat, which was otherwise resistant to the teratogenicity of EM-12, a stable analog of thalidomide (*25*). Although phthalate esters bear similarities to thalidomide in inducing congenital malformations in the chick embryo (*26*), the role of zinc, if any, in phthalate-induced teratogenicity has not been reported.

Metabolism studies on phthalates using radiotracer techniques have been conducted in adult rats (*27-31*). There is only one report concerning maternalfetal transfer of radiolabeled phthalates. It deals with ^{14}C-DEHP and diethyl phthalate (*32*). Fetal metabolism of ^{14}C-DMEP, however, is unknown. The present studies, therefore, were designed to determine whether (a) maternal administration in rats of a single dose of DMEP during the organogenetic period induces embryopathic, fetotoxic or teratogenic effects; (b) multiple congenital skeletal malformations significantly higher than that of the control could be induced by DMEP; (c) uptake of ^{65}Zn by and the zinc content in the fetus are altered by administration of DMEP; and (d) DMEP reaches the fetus as a metabolite or as the unaltered parent compound.

Materials and Methods

Teratological Studies

Outbred female rats of the Wistar strain weighing 240-280 g were used. One female was placed with each male in a separate cage. The dropping of the mating plug from the female on the morning following mating was counted as day zero of the pregnancy, at which time the females were allocated to control and treatment groups. All animals had free access to a high protein pelleted rat diet (supplied by BP Nutrition, Stepfield, Witham, Essex, England) and water and were housed in a temperature-controlled (22 ± 2°C) animal facility.

DMEP of analytical grade was administered intraperitoneally (IP) at a dose of 0.6 ml/kg body weight on day 10, 11, 12, 13 or 14 of gestation. The control animals received the same volume (0.6 ml/kg) of physiological saline intraperitoneally. The number of animals in each treatment group is presented in Table 1.

Pregnant rats were anesthetized by chloroform on day 20, and the fetuses were removed by cesarian operation. More than half of the fetuses were assigned for evaluation of skeletal abnormalities (*33*, *34*); the rest were assigned for detection of organ malformations. The fetuses were prepared for evaluation of soft tissue abnormalities after fixation in Bouins fluid for one week (*35*) and then in 70% ethanol for 5 days. Wilson's technique was used for sectioning the fetuses and to evaluate gross organ pathology (*36*). The fetuses were prepared for evaluation of the skeletal abnormalities by the modified rapid clearing technique using the KOH-Alizarin Red S method of Staples and Schnell (*37*). Modifications included using 70% industrial methylated spirit (ethanol 94.5%; methanol 4.0%, water 1.5% *v/v*) in place of ethanol for fixation, leaving fetuses for 3-4 days in 1% KOH containing 0.01% Alizarin Red S stain for maceration and staining and keeping fetuses in clearing fluid for 2 to 3 days before transferring to an ethanol:glycerol (1:1, *v/v*) mixture for evaluation. The skeletous were evaluated and were classified as normal or retarded (*38*, *39*).

Table 1. Fetotoxicity of DMEP in rats: reduction in fetal weight.

Treatment[a]	No. of animals	No. of fetuses	Mean fetal weight (± SEM), g	d (p = 0.01), g[b]
Normal saline controls	17	157	4.09 ± 0.049	—
DMEP-10	19	49	2.87 ± 0.095[c]	0.156
DMEP-11	14	137	2.78 ± 0.027[c]	0.105
DMEP-12	12	113	3.04 ± 0.062[c]	0.141
DMEP-13	10	123	2.99 ± 0.029[c]	0.136
DMEP-14	15	102	3.34 ± 0.058[c]	0.215

[a]The number after DMEP refers to the day of gestation on which DMEP was injected.
[b]Minimum weight difference required for the mean fetal weight of the treated rats to be significantly different from that of the controls at p = 0.01 using Dunnetts test.
[c]Reduction in fetal weight was highly significant ($p < 0.01$).

Uptake of ^{65}Zn by and Zn Content in the Placenta and Fetus

DMEP or physiological saline was injected IP on day 13 of gestation at 0.6 ml/kg 4 hr prior to the administration of carrier-free $^{65}ZnCl_2$ in the coccygeal vein at a dose of 15 μCi/ml per kilogram of body weight. The animals were sacrificed by decapitation at 15 min, 1 hr and 4 hr after administration of the radiotracer zinc. The uptake of ^{65}Zn was measured with a Packard autogamma counter. The concentrations of zinc in the placental and fetal tissues were measured by atomic absorption spectrometry with a method (*40*) which was reliable and repeatable to 0.2 ppm of zinc. This experiment included 24 pregnant female rats, with four rats at each time period within a treatment group.

Fetoplacental Metabolism of ^{14}C-DMEP

^{14}C-DMEP, specific activity 0.912 mCi/mmole (2.025×10^7 DPM/mmole), was prepared from [carbonyl ^{14}C] phthalic acid (supplied by Radiochemical Centre, Amersham, Bucks, England). Radiochemical purity of the labeled ^{14}C-DMEP was ⩾ 99% as confirmed by gas-liquid and thin-layer chromatography. Chemical identity was confirmed by NMR and GC/MS.

Rats were injected intravenously on day 13 of gestation with ^{14}C-DMEP or physiological saline at 0.6 ml/kg body weight, and the animals were sacrificed by decapitation at 1 and 4 hr after treatment. Each treatment group had three animals. Placentae and fetuses were dissected out. For measurement of total phthalate concentration, pooled portions of placental (0.33 ± 0.10 g) and fetal tissues (0.50 ± 0.12 g) were dissolved in 1 ml Soluene and 10 ml of Instagel (both obtained from Packard Instrument Co.). For acid and neutral extractions of DMEP, portions of the placentae (0.40 ± 0.08 g) and the fetuses (0.42 ± 0.18 g) were homogenized with 1 ml of 0.9% NaCl in a ground glass homogenizer. The homogenates were extracted three times with ethyl acetate (2.5 ml). The extractions were separated into organic and aqueous phases by centrifugation (4000 g, 5 min). The residual aqueous phase was acidified to pH 1.0 with $2N$ H_2SO_4 and then re-extracted with ethyl acetate. The neutral and acid extracts were made to 10 ml with ethyl acetate. A portion (5 ml) of each extract was transferred to a scintillation vial and was evaporated under a stream of N_2 at 37°C to a volume of ⩽ 1 ml before adding 10 ml of Instagel for counting. The chemiluminescence of the samples was minimized by storing the samples in a dark, cold storage room for 4-7 days. Counts, counting efficiency, and the original weight of the sample were used to calculate the radioactivity of each specimen in terms of disintegrations per minute per gram of tissue. These values were used in conjunction with specific activity of injected ^{14}C-DMEP to estimate molar concentrations of the teratogen in the fetus and the placenta.

Statistical Procedures

The reduction in mean fetal weight following administration of DMEP was compared with the normal saline controls by using Dunnett's procedure (*41*). Chi-square with Yates's continuity correction (*42*) was used to test the hypothesis that the percentages of dead and/or resorbed fetuses are equal in treatment and control groups and to compare the percentages of skeletally malformed fetuses with the control. Since the expected frequencies of the congenital deformities of the brain were small, Fisher's exact test was used to test these data. Student's t-test was used to compare the mean uptake of ^{65}Zn by and the Zn content of the placentae and the fetuses.

Results

Embryopathy, Fetotoxicity and Teratogenicity of DMEP

Maternal administration of DMEP in rats caused a significant ($p < 0.01$) reduction in the mean weight of living fetuses as compared to the corresponding value for the physiological saline-treated controls (Table 1). A single injection of DMEP induced a pronounced ($p < 0.01$) fetotoxicity during the organogenetic period regardless of the day of injection. The fetotoxic (Table 1) and the embryopathic (Table 2) effects were greater during the early stages (days 10 or 11 of gestation) of organogenesis than during the later stages (days 12, 13 or 14 of gestation).

Embryopathic effects were evaluated by percentage of dead and resorbed fetuses (Table 2). Reproductive toxicity of DMEP, in this regard, was four to seven times higher upon injection on day 10 of gestation as compared to days 11-14; 79% of the fetuses were dead and resorbed following maternal administration of DMEP on day 10. Intraperitoneal administration of normal saline to pregnant rats resulted in fetal death and resorptions in 7.6% of fetuses.

In comparison to controls, DMEP induced higher rates of congenital deformities of the brain. None of the fetuses from the control group exhibited hydro-

Table 2. Embryopathic effects of DMEP in rats.

Treatment	No. of rats	Total implantations	Number (%) fetuses, dead or resorbed	p values[a]
Normal saline controls	17	170	13 (7.6)	
DMEP-10	19	229	180 (78.6)	<0.0001
DMEP-11	14	175	38 (21.7)	0.0004
DMEP-12	12	141	28 (19.9)	0.0027
DMEP-13	10	139	16 (11.5)	0.3358
DMEP-14	15	121	19 (15.7)	0.0483

[a]The level of probability at which percent of dead or resorbed fetuses following administration of DMEP differed significantly from that of the control by the chi-square test.

cephalus interna, a malformation of the CNS, as opposed to a 13-26% rate of malformation in DMEP-treated animals (Table 3).

The incidence of congenital skeletal malformations caused by DMEP is presented in Table 4. Control rats exhibited an 18% incidence of skeletal malformations whereas DMEP-induced skeletal deformities were found in 66-96% of the fetuses. This rate of skeletal dysmorphogenesis was 3.5- to 5-fold higher than that in the controls. Fetuses in the control group showed no multiple malformations or appendicular dysmorphogenesis, while maternal administration of DMEP caused congenital multiple skeletal malformations in 14-64% of the fetuses and appendicular deformities in 25-57% of the fetuses. Malformations in the appendicular fibula were represented by its retardation, or by its absence. DMEP caused significantly higher ($p < 0.005$) rates of congenital skeletal deformities and multiple malformations in fetuses at all stages of gestation. Malformations in the fibula were observed upon administration of DMEP on day 12 or 13. The skeletal deformities induced by DMEP included complete loss of thoracic ribs, their lack of articulation from the spinal column and forked ribs with bending, cracking and cessation of the vertebral column at the lumbar or at the lower sacral region and the appendicular deformities as represented by the absence or marked shortening of the fibula.

Adverse Effect of DMEP on Maternal-Fetal Zinc Metabolism

The uptake of ^{65}Zn by placentae and fetuses and the zinc content in these tissues 1 hr post administration of ^{65}Zn are presented in Figures 1 and 2. There was no significant difference ($p < 0.05$) in ^{65}Zn uptake of the placentae and fetuses and the placental zinc content between DMEP and physiological saline treated animals. Fetal zinc content of rats treated with DMEP was, however, significantly lower ($p < 0.01$) than of those treated with physiological saline. The uptake and content of zinc in placentae and fetuses in other treatment groups (15 min and 4 hr) were not significantly different from the control.

Fetoplacental Metabolism of ^{14}C-DMEP

^{14}C-DMEP activity associated with the placenta and the fetus differed with respect to extraction (Table 5). Placental activity was associated mostly with the acidic metabolic products, since less than 10% (mean 6.4 ± 0.8) of the total activity was extractable from the neutral homogenates with ethyl acetate; while the remainder was recovered from the tissue residue after acidification. In contrast, 31-44.0% (mean 37.3 ± 4.5) of the fetal activity

Table 3. Congenital malformation of the brain produced by DMEP in rats.[a]

Treatment	No. of rats	No. of fetuses		% fetuses malformed	p values[b]
		Examined	Malformed		
Normal saline controls	17	26	0		
DMEP-10	19	14	2	14.3	0.117
DMEP-11	14	45	6	13.3	0.057
DMEP-12	12	55	13	23.6	0.004
DMEP-13	10	30	4	13.3	0.075
DMEP-14	15	39	10	25.6	0.004

[a]Hydrocephalus interna.
[b]Fisher's exact test was used for these data.

Table 4. Congenital skeletal malformations caused by DMEP in rats.

Variables	Saline controls	Gestational age (days) at administration of dimethoxyethyl phthalate				
		10	11	12	13	14
Fetuses examined	131	35	92	58	93	63
Fetuses malformed	23	31	61	50	89	57
Malformations, %	17.5	88.6	65.9	85.9	96.0	90.6
Significance (p)[a]	–	<0.005	<0.005	<0.005	<0.005	<0.005
Fetuses multiple with malformations (no.)	0	21	13	37	31	27
Multiple malformations, %	0	60.0	14.1	63.7	33.3	42.8
Significance (p)[a]	–	<0.005	<0.005	<0.005	<0.005	<0.005
Fetuses showing absence or retardation of fibula (no.)	0	0	0	33	23	0
% of fetuses showing malformations of fibula	0	0	0	56.9	24.7	0

[a]Significance denotes the level of p value at which percent malformations differs significantly from the control by Yate's continuity correction of chi-square test.

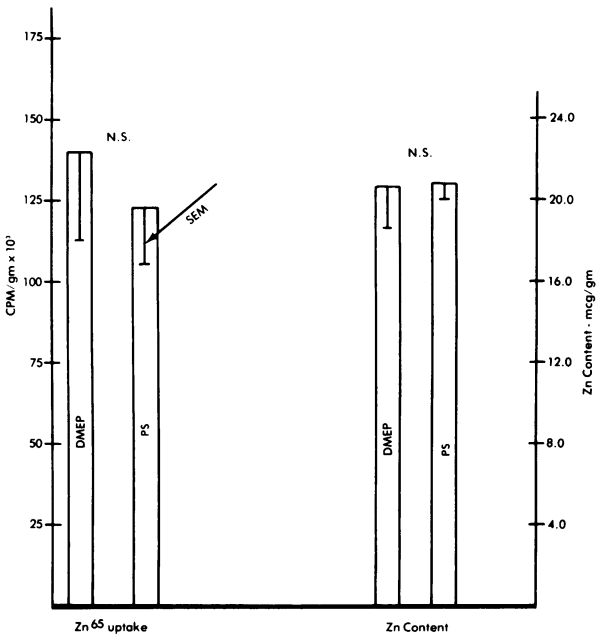

FIGURE 1. Mean uptake of ^{65}Zn by and the mean zinc content in 1 g of placenta 1 hr following administration of ^{65}Zn. Rats were pretreated with CMEP or physiological saline (PS) 4 hr prior to ^{65}Zn administration. Zinc content is in μg/g wet tissue. SEM is the standard error of the mean value based on four rats per treatment group. NS stands for not significantly ($p > 0.05$) different from PS group.

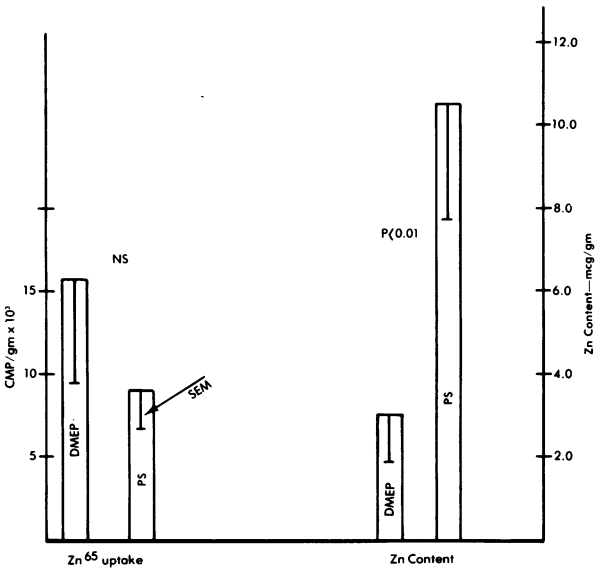

FIGURE 2. Mean uptake of ^{65}Zn by and the mean zinc content (μg/g wet tissue) in the fetus 1 hr following administration of ^{65}Zn.

could be extracted, presumably as the unchanged diester, from the neutral homogenates with ethyl acetate 4 hr following administration of DMEP.

These preliminary results suggest, therefore, that the unmetabolized DMEP is transferred rapidly across the placenta to the fetus and the DMEP may be the teratogenic moiety.

Discussion

Teratogenicity of DMEP

The reported fetal deaths and resorptions caused by a single maternal administration of DMEP on day 10 of gestation in rats (Table 2) are in general agreement with the embryotoxicity resulting from multiple administrations of DMEP (*22*). A significant ($p < 0.01$) reduction in fetal weight (Table 1) has also been observed with other phthalate esters such as DEHP and dibutyl phthalate in rats (*43*) and in mice (*44*).

DMEP has been shown to be the most embryo- and fetotoxic member of the phthalate ester family (*3*,

Table 5. Metabolism of ^{14}C-DMEP in placental and fetal tissues of the rat.

Rat no.	Tissue	Time after administration, hr	DMEP concentration, nmole/g wet tissue		
			Total	Neutral extract	% DMEP
1	Placenta	1	2796.4	100.7	3.6
1	Fetus	1	1640	848.3	51.7
2	Placenta	1	556.2	43.1	7.8
2	Fetus	1	368	101	27.4
3	Placenta	4	396	30	7.6
3	Fetus	4	45.4	14	30.8
4	Placenta	4	326.4	17.6	5.4
4	Fetus	4	23.6	10.4	44.1
5	Placenta	4	376	29	7.7
5	Fetus	4	40.9	13.3	32.5
					$\bar{X}$ = 6.4 ± 0.8
					$\bar{X}$ = 37.3 ± 4.5

16, 20). In part, this may be due to its high aqueous solubility compared to other phthalate esters, although the aqueous solubility of all phthalate esters is extremely low. DMEP, for example, is about 143 times (0.73 ml/100 ml H_2O) more soluble in water than DEHP (0.0051 ml/100 ml H_2O) and is the most soluble phthalate ester (*3, 21*). Fetal deaths and resorptions caused by DMEP were as high as 79% upon a single injection on day 10 of gestation (Table 2).

Congenital malformations of the brain involving hydrocephalus interna similar to those caused by DMEP (Table 3) have also been induced by dietary deficiency of zinc in rats (*45-51*). This is the first report to document hydrocephaly caused by the maternal administration of DMEP in rats.

Phthalate esters have been studied for their congenital skeletal dysmorphogenetic effects in rodents, but in this regard, these studies are the first to report skeletal malformations following a single injection of DMEP. Phthalate esters are considered compounds related to thalidomide (*26*). Congenital multiple skeletal malformations and appendicular dysmorphogenesis, which are considered "typical" of the thalidomide syndrome (*39*), have been difficult to produce in rats. These studies are the first, however, to report congenital multiple skeletal malformations and appendicular dysmorphogenesis following maternal administration of DMEP. Congenital multiple skeletal malformations upon a single intravenous maternal administration of thalidomide were also reported in our laboratory for the first time (*52*).

Intraperitoneal administration of sterile normal saline to pregnant animals resulted in some dead and resorbed fetuses and also caused skeletal malformations in control animals. Such effects have been reported earlier (*53*). The reproductive toxicity of DMEP, however, was several times higher than the normal saline control. The teratogenic effects of DMEP reported here have greater significance than the results where teratogenicity of phthalates is compared to untreated controls (*22*).

The malformations reported here are several hundredfold higher than the sporadic malformations found in rats, and are, therefore, due to the teratogenicity of DMEP. Major skeletal malformations in rats occur at a frequency of 0.41% (*54*), while those caused by DMEP were 161-234 times higher than this background level (Table 4). Craniofacial organ deformities such as hydrocephaly occur sporadically in rats at a frequency of 0.0506% (*54*), while DMEP induced this CNS malformation (Table 3) in 13-26% of the fetuses (257-514 times higher than the sporadic occurrence).

Role of Zinc in DMEP-Induced Teratogenesis

The conclusion that DMEP alters zinc metabolism in rats is based upon significantly ($p \leq 0.01$) decreased zinc content in the fetuses of DMEP-treated rats as compared to that in the controls (Fig. 2).

Pregnant rats cannot mobilize zinc sufficiently from their body stores including that from the liver and bone (*50, 51*), to supply the needs of their fetuses. As a result of this insufficient zinc supply, a transient deficiency of zinc may have been produced following DMEP administration which, in turn, is reflected by the decreased zinc content in the fetal tissue. The teratogenic effects reported here are in agreement with those caused by the decreased zinc content in fetuses when rats were placed on zinc-deficient diets during gestation (*51*).

Permeability of the placenta to zinc increases

after day 18 of gestation (*55*). However, this has not negated the validity of the results reported here because fetal zinc concentrations relate to maternal administration of DMEP or of physiological saline on day 13 of gestation. The fetal zinc concentration (micrograms per gram of wet tissue) reported here is far lower than that reported by Hurley and Swenerton (*51*) in 19- and 21-day-old fetuses. This may be due to the increased permeability of the placenta to zinc after day 18 of gestation and due to maternal supplementation of dietary zinc at 100 ppm, as well as other factors (*54*).

The results of the fetoplacental metabolic studies of zinc (Figs. 1 and 2) are also in agreement with the pharmacokinetic aspects of placental transfer of drugs using a single compartment model for dam and fetus, and in agreement with the first-order kinetics described by Levy and Hayton (*56*).

The adverse effects of DMEP on zinc metabolism and associated fetal anomalies reported here (Tables 1-4, Figs. 1, 2) permit one to draw an analogy between the phthalate-induced testicular damage in rats and the associated adverse effects on zinc metabolism caused by phthalates (*57, 58*). The authors postulate that the protection provided by zinc in phthalate-induced testicular damage (*57, 58*) may be analogous to the protection offered by zinc against certain teratogens in rats (*24*). Singh et al. (*32*) reported that first order kinetics describe the elimination of ^{14}C-labeled phthalates from maternal blood, amniotic fluid and fetal tissue with time. Also, zinc (*59*) and phthalates (*32*) follow, in general, a similar time course and concentration kinetics in maternal blood plasma and fetal tissue. Therefore, the authors propose a role of zinc in DMEP-induced teratogenesis in rats.

The reduced content of zinc in the target organ, the fetus (Fig. 2), following administration of DMEP, may be due to the rapid turnover rate of zinc following administration of phthalates (*57, 58*). Coadministration of zinc was found to offer a substantial measure of protection against testicular atrophy and loss of organ weight caused by dibutyl phthalate (*57*).

The protective effect, if any, of coadministration of zinc and the underlying mechanism of zinc against the fetotoxic and teratogenic effects of DMEP is unknown, as is the protective mechanism of zinc therapy in phthalate-induced testicular atrophy. There are many common parallels, however, between congenital abnormalities caused by zinc deficiency in rats (*45, 47-50, 60, 61*) and those produced by DMEP (Tables 1-4). These include the manifestation of fetotoxicity by a marked reduction in fetal weight (Table 1); embryopathy by fetal resorption (Table 2); congenital malformations of the brain (*45, 47, 48, 50, 60, 61*), namely, hydrocephalus interna (Table 3); and skeletal malformations (Table 4). The incidence of hydrocephalus interna produced by DMEP (Table 3) was similar to that produced by maternal zinc deficiency (*51, 60, 61*), although, in general, its severity was much less.

Fetoplacental Metabolism of ^{14}C-DMEP

Although no specific study has been reported which identifies the nature of DMEP or its metabolites in the rat fetus or placenta, the results reported here are in general agreement (*28-32, 62*) with the metabolism of phthalates in nonpregnant rats as obtained by using radiotracer techniques.

There is a relationship between teratogenicity and carcinogenicity (*63*). Such a relationship for DMEP, is not unequivocally established. Although further studies are required to confirm whether or not DMEP is a carcinogen, there are examples of a carcinogen having teratogenic potential and a teratogen having carcinogenic potential (*63*). This is so because congenital malformations occur through genetic defects, or through embryotoxic effects on prenatal development, or both. For example, methotrexate has carcinogenic properties and possesses teratogenic potential, while 6-mercaptopurine, cyclophosphamide, and nitrosamides are teratogenic and have carcinogenic potential (*63*). Several researchers (*3, 10, 12, 16, 26, 64, 65*) have therefore cautioned about the toxicity and possible health threats of phthalates.

Conclusions

The studies described here establish that the maternal administration of dimethoxyethyl phthalate (DMEP) in a single dose during the organogenetic period (days 10-14) in the rat causes: (a) embryopathic, fetotoxic and teratogenic effects several times higher than reported by the widely studied plasticizer, diethylhexyl phthalate (DEHP); (b) congenital deformities of the brain, such as hydrocephalus interna; (c) congenital skeletal malformations (66-96%), including multiple skeletal (14-64%) and appendicular deformities (25-57%); (d) adverse effects on maternal-fetal metabolism of zinc; and (e) teratogenicity, as mentioned above, due to DMEP in the fetus.

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REFERENCES

1. Thomas, J. A., Darby, T. D., Wallin, R. F., Garvin, P. J., and Martis, L. A review of the biological effects of di-(2-ethylhexyl) phthalate. Toxicol. Appl. Pharmacol. 45: 1-27 (1978).
2. Daniel, J. W. Toxicity and metabolism of phthalate esters. Clin. Toxicol. 13(2): 257-268 (1978).
3. Autian, J. Toxicity and health threats of phthalate esters: review of the literature. Environ. Health Perspect. 3: 3-26 (1973).
4. Shibko, S. I., and Blumenthal, H. Toxicology of phthalic acid esters used in food packaging material. Environ. Health Perspect. 3: 131-137 (1973).
5. Anonymous. Phthalate in food. Nutr. Revs. 32(4): 126-128 (1974).
6. Cerbulis, J., and Ard, J. S. Methods for isolation of di-octylphthalate from milk lipids. J. Assoc. Off. Anal. Chem. 50: 646 (1967).
7. Jaeger, R. J., and Rubin, R. J. Migration of a phthalate ester plasticizer from polyvinyl chloride bags into stored human blood and its localization in human tissues. N. Engl. J. Med. 287: 1114-1118 (1972).
8. Nazir, D. J., Alcaraz, A. P., Bierl, B. A., Beroza, M., and Nair, P. O. Isolation, identification and specific localization of di-2 ethylhexyl phthalate in bovine heart muscle mitochondria. Biochemistry : 4428-4432 (1971).
9. Metcalf, R. L., Booth, G. M., Schuth, C. K., Hansen, J. J. and Lu, P. Y. Uptake and fate of di-2 ethylhexylphthalate in aquatic organisms and in a model ecosystem. Environ. Health Perspect. 3: 27-34 (1973).
10. Anonymous. Phthalate effect on health is still not clear. Chem. Eng. News, 50: 14-15 (Sept. 18, 1972).
11. Peterson, R. V. Toxicology of plastic devices having contact with blood. Final Report, NIH Contract 73-2908, 1973, pp. 35-41.
12. Rall, D. P. The invisible pollution. N. Engl. J. Med. 287: 1146-4147 (1972).
13. NIEHS. Environ. Health Perspect. Exptl. No. 3 (1973).
14. Bower, R. K., Haberman, S., and Minton, P. D. Teratogenic effects in the chick embryo caused by esters of phthalic acid. J. Pharmacol. Exptl. Therap. 171: 314-324 (1970).
15. Calley, D., Autian, J., and Guess, W. L. Toxicology of a series of phthalate esters. J. Pharm. Sci. 55: 158-162 (1966).
16. Dillingham, E. O., and Autian, J. Teratogenicity mutagenicity and cellular toxicity of phthalate esters. Environ. Health Perspect. 3: 81-89 (1973).
17. Guess, W. L., and Haberman, S. Toxicity profiles of vinyl and polyolefinic plastics and their additives. J. Biomed. Mater. Res. 2: 313-335 (1968).
18. Jones, A. E., Kahn, R. H., Groves, J. T., and Napier, E. A. Phthalate ester toxicity in human cell cultures. Toxicol. Appl. Pharmacol. 34: 283-289 (1975).
19. Lawrence, W. H. Phthalate esters: the question of safety. Clin. Toxicol. 13: 89-139 (1978).
20. Lawrence, W. H., and Tuell, S. F. Phthalate esters: the question of safety—an update. Clin. Toxicol. 15: 447-466 (1979).
21. Peakall, D. B. Phthalate esters: occurrence and biological effects. Residue Rev. 54: 1-41 (1975).
22. Singh, A. R., Lawrence, W. H., and Autian, J. Teratogenicity of phthalate esters in rats. J. Pharm. Sci. 61: 51-55 (1972).
23. Singh, A. R., Lawrence, W. H., and Autian, J. Mutagenic and antifertility sensitivities of mice to di(2-ethylhexyl) phthalate (DEHP) and dimethoxyethyl phthalate (DMEP) Toxicol. Appl. Pharmacol. 29: 35-46 (1974).
24. Swenerton, M., and Hurley, L. S. Teratogenic effects of a chelating agent and their prevention by zinc. Science 173-62-64 (July 2, 1971).
25. Jackson, A. J., and Schumacher, H. J. The teratogenic activity of a thalidomide analogus, EM-12 in rats on a low-zinc diet. Teratol. 19: 341-344 (1979).
26. Verrett, M. J., Mutchler, M. K., Scott, W. F., Reynolds, E. F., and McLaughlin, J. Teratogenic effects of captan and related compounds in the developing chick embryo. Ann. N. Y. Acad. Sci. 160: 334-343 (1969).
27. Chu, I., Villeneuve, D. C., Secours, V., Franklin, C., Rock, G., and Viau, A. Metabolism and tissue distribution of mono-2-ethylhexyl phthalate in the rat. Drug Metab. Dispos. 6: 146-149 (1978).
28. Daniel, J. W., and Bratt, M. The absorption, metabolism and tissue distribution of di(2-ethylhexyl) phthalate in rats. Toxicology 2: 51-65 (1976).
29. Ikeda, G. J., Sapienza, P. P., Couvillion, J. L., Farber, T. M., Smith, C. P., Inskeep, P. B., Marks, E. M., Cerra, F. E., and Van Loon, E. J. Distribution and excretion of two phthalate esters in rats, dogs and miniature pigs. Food Cosmet. Toxicol. 16: 409-413 (1978).
30. Schulz, C. O., and Rubin, R. J. Distribution, Metabolism and excretion of di-2-ethylhexyl phthalate in the rat. Environ. Health Perspect. 3: 123-129 (1973).
31. Williams, D. T., and Blanchfield, B. J. The retention, distribution, excretion and metabolism of dibutyl phthalate-^{14}C in the rat. J. Agr. Food Chem. 23: 854-858 (1975).
32. Singh, A. R., Lawrence, W. H., and Autian, J. Maternal-fetal transfer of ^{14}C diethylphthalate in rats. J. Pharm. Sci. 64: 1347-1350 (1975).
33. IRLG (Interagency Regulatory Liaison Group). Recommended guidelines for teratogenicity studies in the rat, mouse, hamster or rabbit. Office of Health Affairs, FDA, Rockville, Md., 1981.
34. Kelsey, F. O. Present guidelines for teratogenic studies in experimental animals. In: Congenital Defects. D. T. Janerick, R. G. Skalko and I. H. Porter, Eds., Academic Press, New York, 1974, pp. 195-202.
35. Beck, F. Evaluation of organs (gross organ pathology). In: Methods in Perinatal Toxicology, D. Neubert, M. J. Merker and T. E. Kwasigroch, Eds. Georg Thieme, Stuttgart, 1977, pp. 103-105.
36. Wilson, J. G. Environment and Birth Defects. Academic Press, New York, 1973, pp. 17, 226.
37. Staples, R. E., and Schnell, V. L. Refinements in rapid clearing technic in the KOH-Alizarin Red S method for fetal bone. Stain Technol. 39: 61-64 (1964).
38. Lorke, D. Evaluation of skeleton. In: Methods in Perinatal Toxicology. D. Neubert, H. J. Merker, and T. E. Kwasigroch, Eds., George Thieme, Stuttgart, 1977, pp. 145-152.
39. Schumacher, H. J., Blake, D. A., Gurian, J. M., and Gillette, J. R. A comparison of the teratogenic activity of thalidomide in rabbits and rats. J. Pharmacol. Exptl. Therap. 160: 189-200 (1968).
40. Willis, J. B. Analysis of biological material by atomic absorption spectroscopy. In: Methods of Biochemical Analysis, D. Glick, Ed., Vol. 11, Wiley, New York, 1963, pp. 2-67.
41. Steel, R. G. D., and Torrie, J. H. Principles and Procedures of Statistics, McGraw-Hill, New York, 1960.
42. Everitt, B. S. The Analysis of Contingency Tables. Wiley, New York, 1977.
43. Nikonorow, M., Mazur, H. and Piekacz, H. Effect or orally administered plasticizers and polyvinyl chloride stabilizers in the rat. Toxicol. Appl. Pharmacol. 40: 355-364 (1973).
44. Shiota, K., Chou, M. J. and Nishimura, H. Embryotoxic effects of di-2-ethylhexylphthalate (DEHP) and di-*n*-butylphthalate (DBP) in mice. Environ. Res. 22: 245-253 (1980).
45. Hurley, L. S. and Shrader, R. E. Congenital malformations

of the nervous system in zinc-deficient rats. In: International Review of Neurobiology, C. C. Pfeiffer, Ed., Academic Press, New York, 1972, pp. 7-51.
46. Hambridge, K. M., Neldner, K. H., and Walravens, P. A. Zinc, acrodermatitis, enteropathica and congenital malformations. Lancet 1: 577-578 (March 8, 1975).
47. Warkany, J. and Petering, H. G. Congenital malformations of the central nervous system in rats produced by maternal zinc deficiency. Teratology 5: 319-334 (1972).
48. Hurley, L. S., and Swenerton, H. Congenital malformations resulting from zinc deficiency in rats. Proc. Soc. Exptl. Biol. Med. 123: 692-696 (1966).
49. Burch, R. E., and Sullivan, J. F. Clinical and nutritional aspects of zinc deficiency and excess. Med. Clin. North Am. 60: 675-685 (1976).
50. Hurley, L. S., Gowan, J., and Swinerton, H. Teratogenic effects of short-term and transitory zinc deficiency in rats. Teratology 4: 199-204 (1971).
51. Hurley, L. S., and Swenerton, H. Lack of mobilization of bone and liver zinc under teratogenic conditions of zinc deficiency in rats. J. Nutr. 101: 597-604 (1971).
52. Parkhie, M. R. and Webb, M. Embryotoxicity and teratogenicity of thalidomide in rats. Teratology, in press.
53. Dubin, N. H., Baros, N. A., Cox, R. T., and King, T. M. Implantation and fetal survival in the rat as affected by intrauterine injection of normal sterile saline. Biol. Reprod. 21: 47-52 (1979).
54. Palmer, A. K. Problems associated with screening of drugs for possible teratogenic activity. Exp. Embryol. Teratol. 1: 17-33 (1974).
55. Feaster, J. P., Hansard, S. L., McCall, J. T., and Davis, G. K. Absorption, deposition and placental transfer of zinc^{65} in the rat. Am. J. Physiol. 181: 287 (1955).
56. Levy, G., and Hayton, W. Pharmacokinetic aspects of placental drug transfer. In: Fetal Pharmacology, L. O. Boreus, Ed., Raven Press, New York, 1971, pp. 29-39.
57. Carter, B. R., Cook, M. W., Gangolli, S. D., and Grasso, P. Studies on dibutylphthalate induced testicular atropy in the rat: Effect on zinc metabolism. Toxicol. Appl. Pharmacol. 41: 609-618 (1977).
58. Foster, P. M. D., Thomas, L. V., Cook, M. W., and Gangolli, S. D. Study of the testicular effects and changes in zinc excretion produced by some *n*-alkyl phthalates in the rat. Toxicol. Appl. Pharmacol. 54: 392-398 (1980).
59. Hansard, S. L. Placental transfer and fetal utilization of absorbed minerals by developing swine. In: Swine in Biomedical Research, L. K. Bustad, and R. O. McClellan, Eds., U.S. Atomic Energy Commission, Washington, D.C., 1966.
60. Warkany, J., and Petering, H. G. Congenital malformations of the brain produced by short zinc deficiencies in rats. Am. J. Mental Deficiency 77: 645-653 (1973).
61. Adeloye, A., and Warkany, J. Experimental congenital hydrocephalus: A review with special consideration of hydrocephalus produced by zinc deficiency. Child's Brain 2: 325-360 (1976).
62. Tanaka, A., Adachi, T., Takahashi, T., and Yamaha, T. Biochemical studies on phthalic esters 1. Elimination, distribution and metabolism of di-(2-ethylhexylphthalate) in rats. Toxicology 4: 253-264 (1975).
63. Neubert, D. Teratogenicity: any relationships to carcinogenicity. In: Molecular and Cellular Aspects of Carcinogen Screening Tests. (IARC Sci. Publ. 27), R. Montesano, H. Bartsch, and L. Tomatis, Eds., International Agency for Research on Cancer, Lyon, 1980, pp. 169-178.
64. Hillman, L. S., Sally, S. L., and Sherman, W. R. Identification and measurement of plasticizer in neonatal tissues after umbilical catheters and blood products. N. Engl. J. Med. 292: 381-386 (Feb. 20, 1975).
65. Bell, F. P. Polyvinylchloride plastics: the enemy within. Artery 2: 384-389 (1976).