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Effects of Phthalic Acid Esters (PAEs) on the Neonate and Aspects of Teratogenic Actions

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A review of the literature reveals that several different phthalic acid esters (PAEs) are capable of causing testicular damage. Phthalate-induced zinc deficiency is consistent with germinal epithelial damage. Among experimental animals, mice perhaps show the greatest sensitivity to phthalate-induced terata, but high doses/exposure are required. Little toxicologic information is available with regard to phthalate-induced effects upon the neonate.

Introduction

During the past few years there have been a number of review articles pertaining to the biologic effects of the phthalic acid esters (PAE) (*1–3*). In 1981, the National Toxicology Program and the U.S. Interagency Regulatory Liaison Group sponsored a Conference on Phthalates. A host of U.S. governmental bioassays and reports pertaining to the biologic and possibly the carcinogenic actions of some of the phthalates have been cited (*4*). In many respects this International Conference on the Phthalic Acid esters is an outgrowth of early conferences and the continuing research interests in assessing the biological effects of these chemicals. Several laboratories around the world continue to explore the action(s) of the phthalates on a number of physiologic systems. There is very little information about the effects of these chemicals upon the neonate or upon the young. Some information is, however, available as to the possible teratogenic actions of certain of the phthalate. Some phthalates appear to be more fetotoxic or embryotoxic than others. Generally, there also appear to be age-related differences with respect to PAE-induced toxicity, but no studies have focused specifically upon the effects of these plasticizers on the neonate. Of the large number of commercially available PAE, diethylhexyl phthalate (DEHP) seems to have received the most scientific or toxicologic investigation.

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Teratology

Earlier reviews (*1–8*) discussed the teratogenic effects of some of the phthalates and related chemicals. Though PAEs in general seem to require high doses to trigger embryotoxic actions, some appear to be more teratogenic than others.

Some phthalates, in particular DEHP and MEHP, reportedly exert adverse effects on the female reproductive system. While the teratogenic effects of selected phthalates have been more thoroughly discussed by Tomita (*9*), a brief summary of the literature pertaining to DEHP and/or MEHP is depicted in Table 1. Indeed, the rodent has received the most widespread investigation. Although there may be species differences, some of the variation in response to the phthalates could be due to differences in experimental protocols, including routes of administration, and duration of exposure and doses (Tables 2 and 3). Several other investigations, however, have failed to disclose any conclusive teratogenic actions, and based on the incidence of stillborn pups, a comparison of the litters born, the number of litters per female, and the size of the litters. DEHP was concluded to be nonteratogenic in rats (*1*).

Renewed interest in the tertogenicity of PAEs occurred in 1970 when Bower et al. (*11*) reported that dibutoxyethyl phthalate caused congenital malformations in chick embryos. Other laboratories, (*12,13*) also reported an increase in the levels of fetal resorptions in rats after the administration of high doses of DEHP. Different phthalates (dimethyl, diethyl, dibutyl, diisobutyl, dimethoxyethyl, and butylcarbobutoxymethyl

Table 1. Summary of literature on the teratogenic effects of DEHP/MEHP.

Species	No. of studies	Teratogenic	Nonteratogenic
Mouse	9	7[a]	2
Rat	8	4	4
Rabbit	1	0	1

[a] Includes one study reporting decreased fetal weights only.

phthalate) were studied by Singh et al. (*13,14*) who reported an increased incidence of skeletal anomalies in neonatal rats. Singh et al. (*14,15*) later reported that the IP administration of DEHP to male mice caused feto- or embryotoxicity in females. In addition there was an increased incidence of resorption when comparing female controls mated with non-PAE-treated male mice.

Nikonorow et al. (*16*) reported that the oral administration of DEHP caused a significant reduction in placental weights. This finding led the authors to conclude that the effects of orally administered DEHP on reproduction and fetal development were dose-dependent and were influenced by the duration of phthalate administration.

The teratogenic potential of plasma soluble extracts of polyvinyl chloride in rats was studied by Garvin et al. (*17*). A plasma concentration of approximately 185 μg/mL DEHP was obtained by utilizing two different PVC formulations and extracting them with sterile rat plasma. When the DEHP-extracted plasma solutions were administered IV into pregnant rats, no teratogenic or embryotoxic effects were observed. The levels of DEHP leached from plastics posed no teratogenic risks, and only very high doses could induce changes in the reproductive system of laboratory animals (*17*).

Table 2. Effect of DEHP/MEHP on reproduction and fetal toxicity in mice and rabbits[a].

Species	Sex	Route of administration	Principal findings
Mouse	Male	IP	Early fetal death and semisterility
Rabbit	Female	IV	Nonteratogenic
Mouse	Female	PO	Nonteratogenic
Mouse	Female	PO	Neural tube defects
Mouse	Female	PO	Absence of tails
Mouse	Female	PO	Decreased fetal
Mouse	Female	PO	weights
Mouse	Female	PO	Exencephaly, clubfoot Teratogenic

[a] Modified from Thomas (*1*).

Table 3. Effect of DEHP/MEHP on reproduction and fetal toxicity in various studies on female rats.[a]

Route of administration	Principal findings
IP	Fetal resorptions, fetal deaths, and decreased fetal size
PO	No effect upon fertility
PO	Decreased fetal weight and increased fetal resorptions
IP	Reduced conception and increased fetal deaths
IP	Adverse effect upon implantation and parturition, and excessive hemorrhaging and fetal retention
IV	No teratogenic or embryotoxic effects
IV	Nonteratogenic
PO	Nonteratogenic
PO	Nonteratogenic

[a] Modified from Thomas (*1*).

Thomas et al. (*18,19*) reported that monoethylhexyl phthalate (MEHP) injected into previously artificially inseminated female rabbits at critical intervals during gestation caused no significant teratogenic effects. The administration of MEHP did not affect the size of the fetus, as evidenced by no changes in the crown-rump or transumbilical measurements.

The teratogenic potential of plasma-soluble extracts of DEHP-plasticized PVC in rats was also reported by Lewandowski et al. (*20*). No differences were recorded in the growth rates or in the general behavior of either the control or the DEHP-treated groups. The incidence of skeletal, visceral, and gross external defects among offspring was similar for both treated and control groups. Lewandowski et al. (*20*) concluded that during the "critical period" of organogenesis, plasma extracts of PVC plastics were not teratogenic when administered IV to pregnant rats.

Yagi et al. (*21*) examined the teratogenic potential of both DEHP and MEHP in mice. The oral administration of DEHP in doses representing 1/6 or 1/3 of the acute LD_{50} on day 7 of gestation led to 100% fatality of all fetuses. Similar toxic effects were observed with MEHP. The gross abnormalities included exencephaly, open eyelid and club foot. Skeletal abnormalities occurred in the skull, cervical and thoracic bones.

When Ruddick et al. (*22*) evaluated the teratogenic potential of MEHP in the rat, no skeletal defects were noted in the fetuses. Both MEHP-treated and control groups exhibited some disturbances in the placement of the sternebrae plates. No visceral anomalies were noted.

When DEHP or di-*n*-butyl phthalate (DBP) was administered in the diet of pregnant mice, the major teratogenic effects observed were neural tube defects (exencephaly and spina bifida) (*23,24*). DEHP and DBP caused intrauterine growth retardation and delayed ossification. High doses were embryotoxic and possibly teratogenic in mice. The maximum nonembryotoxic doses of these phthalates in mice were more than 2000 times the estimated level of human intake through the food chain (*24*).

The finding that DEHP inhibited hepatic sterologenesis to a greater extent in male than in pregnant female rats led Bell (*25*) to suggest that pregnant rats may be less sensitive than nonpregnant rats to the effects of DEHP because of its partitioning into the fetus. Uptake by the fetus could effectively dilute the DEHP concentration in the maternal liver.

A single IP injection of dimethoxyethyl phthalate (DMEP) to pregnant rats led to embryopathy, including fetal deaths and fetal resorptions (*26*). Hydrocephalus interna, skeletal deformities and appendicular malformations were caused by DMEP. DMEP also caused a

Table 4. Gonadal toxicity of some phthalate acid esters (PAEs)[a].

Species	PAE	Toxicity
Rat	DEHP, MEHP, DOP	+
Mouse	DBP, DEHP	+
Guinea pig	DBP, DEHP	+
Ferret	DEHP	+
Hampster	DBP, DEHP	−

[a] Modified from Gangolli (*30*) and Thomas (*10*).

significant decrease in fetal zinc levels suggesting that this divalent ion may play a role in PAE-induced teratogenesis in the rat (*26*).

In mice, a single injection of DEHP on day 7 of gestation caused a reduction in the body weight of living mouse fetuses. There was no significant change in the number of live fetuses and no gross and/or skeletal anomalies. Higher doses produced some fetotoxicity, a relationship that was dose-dependent (*27*).

Studies by Wolkowski-Tyl et al. (*28,29*) revealed an interesting comparative finding regarding the teratogenicity of DEHP in rats and in mice. DEHP was teratogenic when administered to mice (*28*) in the feed during the entire gestation period at high doses, but DEHP was not teratogenic when administered to rats (*29*).

The majority of investigations of teratogenicity have been undertaken in either mice or rats. PAE-induced teratogenicity seems to occur at high doses in either the nervous system or the skeletal system. Clearly, the amounts that humans could potentially be exposed to bear little relevance to the large doses employed in laboratory animal experiments.

Testicular Effects

Gangolli and his colleagues have not only written an excellent review on the effects of different phthalates upon gonadal function (*30*), but his presentation at this conference (*31*) has shed further insight into how these chemicals affect testicular morphology and zinc levels. At least four different phthalates, studied in at least five different species, have been investigated with respect to gonadal toxicity (Table 4). Table 5 reveals the effects caused by DEHP and/or MEHP while Table 6 depicts the action of certain other phthalates upon gonadal function.

Phthalate-induced testicular injury in animals was initially reported by Schaffer et al. (*32*), wherein dietary concentrations of 0.075, 1.5, and 5.0% DEHP led to tubular atrophy and testicular degeneration resembling senile changes. Harris et al. (*33*) also found occasional incidence of tubular atrophy in rats fed DEHP. Calley et al. (*34*) found that daily intraperitoneal administration (250 mg/kg body weight) of DEHP or di(methoxyethyl) phthalate in mice for 6 weeks significantly reduced the relative testicular weight.

The oral administration of DBP at daily dose levels of 500 and 1000 mg/kg significantly reduced relative testes weight (*35*). The corresponding monoester (MBP) caused even more marked reduction in testes weight (*35,36*). Testicular lesions produced by DBP and MBP resembled

Table 5. Effect of DEHP and MEHP on the male reproductive system.[a]

Species	Agent	Effect
Rat	DEHP	Testicular degeneration
Rat	DEHP	Early fetal death and semisterility
Ferret	DEHP	Testicular degeneration
Rat	DEHP	Testes histological damage
		↓ Testes weight
		↑ Testosterone
		↓ Zinc
Rat	DEHP	Testicular atrophy
Mouse	DEHP	↓ Testosterone
		↑ Testes weight
		↓ Zinc
Rat	DEHP	↓ Testes weight
		↑ Testosterone
		↓ Zinc
Mouse	MEHP	↔ Testes weight
Rat	MEHP	↓ Testes zinc
Rat	DEHP	↓ Testes weight
Rat	DEHP	Changes in testes enzyme

[a] Modified from Thomas (*1*)

those following DEHP treatment. That the mechanism(s) involved in DBP-induced testicular injury resulted not from the accumulation of metabolites or the formation of covalent adducts in testicular tissue is shown by the fact that metabolic disposition studies following oral administration of ^{14}C-labeled DBP showed no evidence of accumulation of radioactivity in the gonads. Testicular atrophy did not appear to be mediated by changes in androgen synthesis or the availability of gonadotrophins (*30*). Urinary zinc levels following DBP treatment were increased. Studies using ^{65}Zn showed that DBP or MBP treatment led to a marked increase in the urinary excretion of radioactivity and a decrease in ^{65}Zn associated with testicular tissue (*30*). Additionally, the loss of testicular zinc in DBP-treated rats was accompanied by a decrease in the activities of alcohol dehydrogenase and carbonic anhydrase.

Gray and Butterworth (*37*) showed in a 90-day toxicity study in rats that DEHP caused testicular atrophy. Sem-

Table 6. Effects of other phthalate acid esters (PAEs) on the male reproductive system.[a]

Species	PAE	Effect
Rat	DOP	Decreased tests weight
Rat	DMP	—↓ Testes weight
		—↑ Zinc excretion
Rat	MEHP, MBP, MIBP	Testicular atrophy
		—↓ Zinc
		—↑ Testosterone
Mouse	MEHP, MBP, MIBP	—↓ Testosterone
		—↑ Testicular weight
Rat	DEHP, DA79P	—↓ Testicular weight
Rat	DBP, MBP	Testes damage
Hamster	DBP, MBP	No gonadal effect
Rat	PA	—↓ Sperm motility

[a] Modified from Thomas (*1*).

Table 7. Effect of DEHP (1000 or 2000 mg/kg daily × 5, PO) on immature (less than 100 g body weight) or mature (greater than 2000 g body weight) rats.[a]

	(% of control)	
Daily dose, mg/kg	Immature rats	Mature rats
Control[b]	100	100
1000	58	94
2000	50	91

[a] Data of K. A. Curto, unpublished observations.
[b] Peanut oil.

iniferous tubular atrophy, comprising a loss of spermatids and spermatocytes, occurred when young (4-week-old) rats were given 10 daily doses of DEHP. Somewhat older rats (e.g., 10- and 15-week-old) showed less testicular damage or none at all. Somewhat comparable findings were obtained in our laboratory by K. A. Curto, wherein she demonstrated that gonadal atrophy was more pronounced in the testes of the immature rat (Table 7). Gray and Butterworth (*37*) studied other phthalates and reported an incidence of gonadal toxicity. Such PAE-induced changes in testicular morphology were not affected by the simultaneous administration of testosterone (*38*). When young male rats were intubated (800 mg/kg daily, ×4) with mono-*n*-butyl esters of *ortho*-phthalic, isophthalic, or terephthalic acid, only the *ortho* ester produced gonadal atrophy. Foster et al. (*38*) indicated that in order for a phthalate to cause testicular atrophy, the ester moiety must be in the *ortho* position and of specific chain length. Only those esters producing testicular damage caused concomitant decreases in gonadal zinc levels.

Later studies by Foster et al. (*36*) sought to explain gonadal toxicity on the basis of species differences, particularly with respect to urinary metabolites. In both rats and hamsters, the major urinary metabolite detected after the oral administration with either di-*n*-butyl phthalate or nono-*n*-butyl phthalate was a glucuronide form of the monophthalate. The urinary levels of unconjugated mono-*n*-butyl phthalate were about three times greater in rats than in hamsters, suggesting that the free form of the PAE may be more gonadotoxic.

Zinc deficiency depresses testicular growth, inhibits sex accessory organ weight, and produces severe atrophy of germinal epithelium (*39,40*). Experimentally induced zinc deficiency in man causes oligospermia and can adversely affect testicular function. Such gonadal toxicities can be reversed by restoring adequate amounts of zinc to the diet (*41*). Some phthalates cause a reduction in reproductive organ zinc. Rat testicular zinc levels are lowered by dietary DEHP or dietary MEHP (*42*) (see Tables 5 and 6). A number of dietary constituents, including calcium and phytate, interfere with zinc absorption (*39*). The exact mechanism of gonadal zinc depletion is unknown, but it may be explained by the formation of a zinc–phthalate complex similar to that described by Foster et al. (*36*). Curto and Thomas (*43*) and Agarwal (*44*), have confirmed the relationship between zinc and testicular atrophy induced by DEHP. DEHP administered orally to rats maintained on a low-zinc diet caused an additive gonadotoxic effect. Interestingly enough, phthalic anhydride reportedly exerts an action upon gonadotropin (*45*).

Last, it should be mentioned that DEHP in the diet (6000 or 12,000 ppm for 103 weeks) fed to rats can actually decrease the incidence of testicular tumors (*46*). These DEHP-treated rats also exhibited lower incidence of pituitary tumors, although the relationship to diet is not known.

Neonatal

Surprisingly little is known about the ability of PAEs to be transferred to offspring (*47*). Radioactivity was recovered from fetal tissues following treatment of maternal rats (*48*). Following injections of MEHP into pregnant rabbits, aqueous homogenates obtained from either the placental or the uterus contained less than μg/mL (*19*). Likewise, MEHP levels in rabbit fetuses were found to contain less than 1 μg/mL. In neonatal rats previously injected with MEHP, very little PAE was detected in their livers (*19*). Bell et al. (*49*) suggested that DEHP can cross the placental barrier, as evidenced by inhibition of sterologenesis in the brain and in the liver of fetal and suckling rats from dams previously fed with this phthalate. Subsequent reports (*50*) indicated that fetuses taken by caesarean section from pregnant rats fed DEHP (beginning 5 to 10 days after conception) exhibited an impairment of sterologenesis in brain and liver. Also, if dams exposed to DEHP during gestation and also during the postnatal period, are permitted to nurse their naturally born offspring, hepatic sterologenesis in pups is reduced (*50*).

Hillman et al. (*51*) attempted to identify DEHP in human neonatal tissues after umbilical catheterization alone or with administration of blood products. Determination

Table 8. Effects of a single IP injection of MEHP in peanut oil administered at 3 days of age to neonatal rats.[a]

	Mean organ weights, mg[b]			
Dose, mg/kg	Liver	Kidney	Cerebral cortex	Lung
Sham	1722 ± 95	485 ± 33	766 ± 29	502 ± 28
Peanut oil vehicle	1807 ± 95	501 ± 31	780 ± 27	480 ± 27
12.3	1788 ± 95	516 ± 31	788 ± 27	482 ± 27
24.5	1659 ± 95	458 ± 31	768 ± 27	474 ± 27
49.2	1734 ± 95	476 ± 31	771 ± 27	492 ± 27

[a] Animals were sacrificed at weaning (21 days of age).
[b] Mean ± SD of at least 10 animals. Data of Thomas and Northup (*52*).

of DEHP levels in neonatal heart and gastrointestinal tissue revealed that those infants that had been previously catheterized or received large amounts of blood products exhibited higher levels of plasticizer than did controls (*51*).

An examination of fetoplacental metabolism in the pregnant rat previously injected with dimethoxyethyl phthalate (DMEP) suggested that there was a rapid transfer of the parent compound to the fetus across the placenta. DMEP caused a significant decrease in the zinc content of the fetus (*26*).

The metabolism and pharmacokinetics of different PAEs has been studied in a number of species (*47*). Little attention has been focused on the neonate with respect to the metabolism of the phthalates. It might be expected that both the developing hepatic and renal metabolic pathways do not possess the same clearance capacities as the adult.

The effects of a single injection of MEHP upon the neonatal rat are depicted in Table 8. It can be seen that under these experimental conditions there were no MEHP-induced changes in the gravimetric response in the liver, kidney, cerebral cortex, or lung. It is noteworthy that despite the three relatively large dose regimens employed, there were no significant differences in hepatic weights.

Summary

Several studies have examined the teratologic potential of DEHP, MEHP, and other phthalates. Teratogenicity is evident in some species, but only at very high doses. Testicular damage can be produced with several different chemical entities of the phthalates. Gonadal zinc deficiency is an observation consistent with phthalate-induced gonadal toxicity.

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