In Utero Exposure to Di-(2-ethylhexyl)phthalate and Duration of Human Pregnancy

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Di-(2-ethylhexyl)phthalate (DEHP), the most commonly used plasticizer in flexible polyvinylchloride formulations, is a ubiquitous environmental contaminant. To date, no information exists on the potential health hazards from exposure to DEHP and/or its main metabolite, mono-(2-ethylhexyl)phthalate (MEHP), in high-risk conditions, such as pregnancy and during the neonatal period. The aim of this study was to evaluate prenatal exposure to DEHP and/or MEHP and its possible biologic effects. We measured serum DEHP and MEHP concentrations in the cord blood of 84 consecutive newborns by high-performance liquid chromatography. Relationships between DEHP/MEHP and infant characteristics were tested using Fisher's exact test, unpaired *t*-tests, and univariate linear regression analyses, and significant differences on univariate analysis were evaluated using multiple logistic regression analysis. We found detectable cord blood DEHP and/or MEHP concentrations in 88.1% of the samples. Either DEHP or MEHP was present in 65 of 84 (77.4%) of the examined samples. Mean concentrations of DEHP and MEHP were 1.19 ± 1.15 μg/mL [95% confidence interval (CI), 0.93–1.44, range = 0–4.71] and 0.52 ± 0.61 μg/mL (95% CI, 0.39–0.66, range = 0–2.94), respectively. MEHP-positive newborns showed a significantly lower gestational age compared with MEHP-negative infants ($p = 0.033$). Logistic regression analysis results indicated a positive correlation between absence of MEHP in cord blood and gestational age at delivery (odds ratio = 1.50, 95% CI, 1.013–2.21; $p = 0.043$). These findings confirm that human exposure to DEHP can begin *in utero* and suggest that phthalate exposure is significantly associated with a shorter pregnancy duration. *Key words:* di-(2-ethylhexyl)phthalate, environmental hazards, gestational age, mono-(2-ethylhexyl)phthalate, prenatal exposure. *Environ Health Perspect* 111:1783–1785 (2003). doi:10.1289/ehp.6202 available via *http://dx.doi.org/* [Online 18 August 2003]

Phthalate esters are used widely as plasticizers for polyvinylchloride (PVC) formulations in several applications, including medical devices, toys, food wraps, and building products, to impart flexibility to an otherwise rigid PVC. Di-(2-ethylhexyl)phthalate (DEHP) is the most commonly used plasticizer. Because DEHP does not bind with the plastic, it leaches with time and use from vinyl products, thus becoming a ubiquitous environmental contaminant (Bauer and Herrmann 1997; Bradbury 1996; Giam et al. 1978; Griffiths et al. 1985; Mayer et al. 1972; Mes et al. 1974; Øie et al. 1997; Sharman et al. 1994). In particular, leaching of DEHP from PVC medical devices and deposits in tissue have been well documented (Latini 2000; Tickner et al. 2001). Because the DEHP action depends on dose, time, and age (Latini 2000) and because DEHP effects are influenced by the stage of development at exposure among animals (Akingbemi et al. 2001), the DEHP-related exposure risk is potentially higher for the developing fetus and newborn, particularly preterm. Recently, our preliminary findings indicated that the exposure to these environmental contaminants begins during intrauterine life, that these chemicals are able to cross the placental barrier, and that fetal exposure is closely related to maternal exposure (Latini et al. 2003). The aim of this study was to measure concentrations of DEHP and/or its main metabolite, mono-(2-ethylhexyl)phthalate (MEHP), in a larger population of human neonates and to evaluate possible biologic effects from prenatal exposure to DEHP and/or MEHP.

Patients and Methods

Subjects. Cord blood samples were collected from 84 consecutive newborns (82 singletons, two twins), born at the general-practice Brindisi Hospital, with the following characteristics: 39 male, 45 female; maternal age at delivery, 29.5 ± 5.1 years (range = 18–42); vaginal delivery, $n = 65$ (77.4%); gestational age, 38.4 ± 2.2 weeks (range = 27–42); birth weight, 3,220 ± 680 g, (range = 1,150–4,350); 1-min Apgar score, 7.9 ± 0.9; 5-min Apgar score, 8.8 ± 0.5. Eleven of 84 infants were preterm; only three had very low birth weight. Moreover, four infants who were small for gestational age (SGA) were present in our population. None of the examined infants was born after *in vitro* fertilization pregnancy. The study was approved by the ethics committee of the Brindisi Hospital (Brindisi, Italy), and written informed consent from the parents was obtained. Blood specimens were immediately centrifuged (3,500 × *g*, 7 min), and serum was stored at –20°C until assay. To avoid any contamination from plasticizers in lab equipment, the serum sample collection, preservation, and treatment were performed only with glass devices. The concentrations of DEHP and MEHP were determined by high-performance liquid chromatography, at the Department of Chemistry of the University of L'Aquila, an institution certified in agreement with the International Organization for Standardization 9001 quality system, as described previously (Paris et al. 2003).

Data analysis. Data are expressed as mean ± SD. Pairwise differences between groups were assessed using either Fisher's exact test (categorical variables) or unpaired *t*-tests (continuous variables). The relation between presence of phthalates in the cord blood and potential prenatal risk factors was evaluated using univariate analysis (MedCalc for Windows, version 7.0; MedCalc Software, Mariakerke, Belgium). The effects of potential confounders on the presence of DEHP/MEHP in the cord blood were also examined by using multivariable logistic regression models (SPSS release 6.1 statistical package; SPSS Inc., Chicago, IL, USA). Factors with p-values < 0.25 at univariate analysis were included in the multivariable logistic regression models. The p-values were assessed by using pairwise comparisons of each end point with explanatory variables, excluding the others. A two-sided p-value < 0.05 was considered statistically significant, and the Bonferroni-corrected significance levels were used for multiple *t*-tests.

Results

DEHP, MEHP, or both were present in 74 of 84 (88.1%) of the examined cord serum samples. DEHP and MEHP were each present in 65 of 84 (77.4%) of the examined samples. Mean concentrations of DEHP and MEHP were 1.19 ± 1.15 μg/mL [95% confidence interval (CI), 0.93–1.44, range = 0–4.71] and

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0.52 ± 0.61 μg/mL (95% CI, 0.39–0.66, range = 0–2.94), respectively. MEHP-positive newborns showed a significantly lower gestational age compared with MEHP-negative infants (38.16 ± 2.34 vs. 39.35 ± 1.35; t = –2.163, df = 81, p = 0.033; Figure 1). A comparison of gestational age in different phthalate categories is also shown in Tables 1 and 2 and Figure 2. The results of the logistic regression analysis indicated a positive correlation between absence of MEHP in cord blood and gestational age at delivery [fitted equation: logit(p) = –16.98 + 0.40 × gestational age; overall model fit: chi-squared = 5.45, df = 1, p = 0.019; odds ratio = 1.50, 95% CI, 1.013–2.21].

No statistically significant relations were observed between DEHP or MEHP concentrations and sex of infant, delivery mode, maternal smoking, premature rupture of the membranes, presence of cord loops, neonatal jaundice, or small size for gestational age (< 10th percentile for sex and parity) ($p \geq 0.12$). Furthermore, no significant relations were observed between DEHP or MEHP and birth weight, 1-min or 5-min Apgar scores, or maternal age ($p \geq 0.32$).

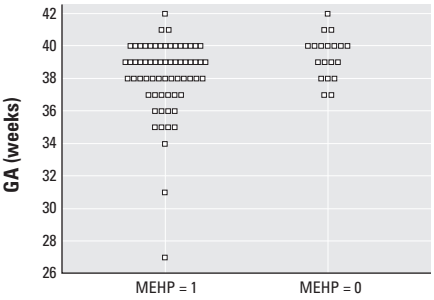

Figure 1. Dot plots of comparisons of gestational age (GA) between MEHP-positive and -negative infants. MEHP = 1, MEHP-positive cord serum samples; MEHP = 0, MEHP-negative cord serum samples.

Discussion

Our findings confirm the presence of detectable DEHP/MEHP in most of the examined newborns at birth, observe phthalate serum concentrations in a wide population of human newborns, and suggest that phthalate exposure is significantly associated with a shorter pregnancy duration. Although the clinical relevance of the observed statistical association deserves further elucidation, this link appears to be plausible, because *a*) exposure to environmental contaminants other than DEHP has been associated with decreased gestation length (Loch-Caruso 2002; Tsai et al. 1997), and *b*) several lines of evidence suggest a possible role for DEHP in the induction and/or potentiation of an intrauterine inflammatory response. A significant ring structure similarity has been observed between DEHP and prostaglandins/tromboxanes, which are proinflammatory mediators (Maroziene and Grazuleviciene 2002); evidence of DEHP-induced interleukin-1 secretion has been reported in mononuclear cells (Calo et al. 1993); and both infants born to mothers with prenatal infection/inflammation (De Felice et al. 1999, 2002) and DEHP-treated experimental animals (Yang et al. 2000) have been reported to undergo a surprisingly similar process of acute thymic involution. Considering that intrauterine infection/inflammation is a well-established risk factor for prematurity (Goncalves et al. 2002), our observation of a shorter pregnancy duration in prenatally exposed newborns suggests that phthalates may play a role in inducing an intrauterine inflammatory process. In addition, no statistically significant differences in other maternal and/or fetal factors potentially affecting pregnancy duration were present in our population.

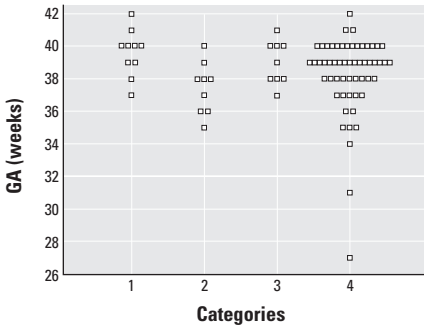

Figure 2. Dot plots of comparisons of gestational age (GA, in weeks) between different phthalate categories: 1, DEHP-negative, MEHP-negative infants; 2, DEHP-negative, MEHP-positive infants; 3, DEHP-positive, MEHP-negative infants; 4, DEHP-positive, MEHP-positive infants.

The potential toxic effects of the observed prenatal exposure to phthalates in human newborns remain unknown to date. However, DEHP-induced anti-androgenic action and abnormalities of the male reproductive system and in sexual behavior have been reported in prenatally exposed animals, likely affecting the normal development of the testes (Arcadi et al. 1998; Foster et al. 2001; Gray et al. 2000; Moore et al. 2001; Tandon et al. 1991). Moreover, DEHP effects on Leydig cell steroidogenesis are influenced by the stage of development at exposure among animals (Akingbemi et al. 2001). The reproductive toxicity mechanism of DEHP may be caused by DEHP's effects on steroid hormone metabolism and sexual development. In fact, recently it has been demonstrated that DEHP altered the expression of genes associated with testis development and steroid hormone synthesis (Wong and Gill 2002). Thus, although the potential adverse effect of prenatal exposure to DEHP on the male reproductive system in humans needs to be investigated in future studies, there is concern that DEHP is a

Table 1. Associations between phthalate presence/absence and birth outcomes for n = 84 infants (range in parentheses).

Infants' characteristics		DEHP$^+$/MEHP$^-$	DEHP$^+$/MEHP$^+$	DEHP$^-$/MEHP$^+$	DEHP$^-$/MEHP$^-$
Mean birth weight (g)		3411.11 ± 597.27 (2,550–4,350)	3,173 ± 706.01 (1,150–4,100)	3008.89 ± 602.7 (1,950–3,700)	3,533 ± 563.44 (3,550–4,350)
Mean gestational age (weeks)		39 ± 1.32 (37–41)	38.27 ± 2.45 (27–42)	37.44 ± 1.59 (35–40)	39.60 ± 1.43 (37–42)
Full-term infants (n = 73)	AGA	9	48	6	10
	SGA	—	—	—	—
Preterm infants ≤ 1,500 g (n = 3)	AGA	—	1	—	—
	SGA	—	2	—	—
Preterm infants > 1,500 g (n = 8)	AGA	—	5	1	—
	SGA	—	—	2	—
Total (n)		9	56	9	10

Abbreviations: AGA, adequate for gestational age; –, negative; +, positive; SGA, small for gestational age.

Table 2. DEHP- and/or MEHP-positive versus -negative infants: comparisons between groups (range in parentheses).

Infants' characteristics	DEHP$^+$	DEHP$^-$	p-Value	MEHP$^+$	MEHP$^-$	p-Value
Mean birth weight (g)	3206.15 ± 692.68 (1,150–4,350)	3284.74 ± 626.50 (1,950–4,350)	NS	3,150 ± 690.68 (1,150–4,100)	3475.26 ± 566.74 (2,550–4,350)	NS
Mean gestational age (weeks)	38.37 ± 2.33 (27–42)	38.58 ± 1.84 (35–42)	NS	38.16 ± 2.34 (27–42)	39.35 ± 1.35 (37–42)	0.033
Total	65	19		65	19	

Abbreviations: –, negative; NS, not significant; +, positive.

human reproductive developmental toxicant. In accordance with a recent report by the Center for Devices and Radiological Health, U.S. Food and Drug Administration, neonates in neonatal intensive care units constitute a population at particularly increased toxicity risk because of multiple medical device–related DEHP exposure (Center for Devices and Radiological Health 2001; Hillman et al. 1975; Latini 2000; Latini and Avery 1999; Loff et al. 2000; Plonait et al. 1993; Tickner et al. 2001). Thus, it is conceivable that prenatal and postnatal exposures may have synergistic and cumulative actions in producing adverse neonatal effects, especially for premature infants with very low birth weight (Roth et al. 1988).

References

Akingbemi BT, Youker RT, Sottas CM, Ge R, Katz E, Klinefelter GR, et al. 2001. Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biol Reprod 65:1252–1259.

Arcadi FA, Costa C, Imperatore C, Marchese A, Rapisarda A, Salemi M, et al. 1998. Oral toxicity of bis(2-ethylhexyl) phthalate during pregnancy and suckling in the Long-Evans rat. Food Chem Toxicol 36:963–970.

Bauer MJ, Herrmann R. 1997. Estimation of the environmental contamination by phthalic acid esters leaching from household wastes. Sci Total Environ 208:49–57.

Bradbury J. 1996. UK panics over phthalates in babymilk formulae [News]. Lancet 347:1541.

Calo L, Fracasso A, Cantaro S, Cozzi E, De Silvestro G, Plebani M, et al. 1993. Plasticizers induced mononuclear cells interleukin 1 production: implications with peritoneal sclerosis [Letter]. Clin Nephrol 40:57.

Center for Devices and Radiological Health. 2001. Safety Assessment of Di(2-ethylhexyl)phthalate (DEHP) Released from PVC Medical Devices. Rockville, MD:U.S. Food and Drug Administration. Available: http://www.fda.gov/cdrh/ost/dehp-pvc.pdf [accessed 5 September 2001].

De Felice C, Latini G, Toti P, D'Addario V, Petraglia F, Bagnoli F. 2002. Small thymus at birth and gestational age. Eur J Pediatr 161:362–363.

De Felice C, Toti P, Santopietro R, Stumpo M, Pecciarini L, Bagnoli F. 1999. Small thymus in very low birth weight infants born to mothers with subclinical chorioamnionitis. J Pediatr 135:384–386.

Foster PM, Mylchreest E, Gaido KW, Sar M. 2001. Effects of phthalate esters on the developing reproductive tract of male rats. Hum Reprod Update 7:231–235.

Giam CS, Chan HS, Neff GS, Atlas EL. 1978. Phthalate ester plasticisers: a new class of marine pollutant. Science 199:419–421.

Goncalves LF, Chaiworapongsa T, Romero R. 2002. Intrauterine infection and prematurity. Ment Retard Dev Disabil Res Rev 8:3–13.

Gray LE Jr, Ostby J, Furr J, Price M, Veeramachaneni DN, Parks L. 2000. Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. Toxicol Sci 58:350–365.

Griffiths WC, Camara P, Lerner KS. 1985. Bis-(2-ethylhexyl) phthalate, an ubiquitous environmental contaminant. Am Clin Lab Sci 15:140–151.

Hillman LS, Goodwin SL, Sherman WR. 1975. Identification and measurement of plasticizer in neonatal tissues after umbilical catheters and blood products. N Engl J Med 292:381–386.

Latini G. 2000. The potential hazards of exposure to di-(2-ethylhexyl)-phthalate in babies: a review. Biol Neonate 78:269–276.

Latini G, Avery GB. 1999. Materials degradation in endotracheal tubes: a potential contributor to bronchopulmonary dysplasia. Acta Pediatr 88:1174–1175.

Latini G, De Felice C, Presta G, Del Vecchio A, Paris I, Ruggieri F, et al. 2003. Exposure to di-(2-ethylhexyl)- phthalate in humans during pregnancy: a preliminary report. Biol Neonat 83:22–24.

Loch-Caruso R. 2002. Uterine muscle as a potential target of polychlorinated biphenyls during pregnancy. Int J Hyg Environ Health 205:121–130.

Loff S, Kabs F, Witt K, Sartoris J, Mandl B, Sartoris J, et al. 2000. Polyvinylchloride infusion lines expose infants to large amounts of toxic plasticizers. J Pediatr Surg 35:1775–1781.

Maroziene L, Grazuleviciene R. 2002. Maternal exposure to low-level air pollution and pregnancy outcomes: a population-based study. Environ Health 9(1):6.

Mayer FL, Stalling DL, Johnson JL. 1972. Phthalate esters as environmental contaminants. Nature 18:411–413.

Mes J, Coffin DE, Campbell DS. 1974. Di-n-butyl and di-2-ethylhexyl phthalate in human adipose tissue. Bull Environ Contam Toxicol 12:721–725.

Moore RW, Rudy TA, Lin TM, Ko K, Peterson RE. 2001. Abnormalities of sexual development in male rats with in utero and lactational exposure to the antiandrogenic plasticizer di(2-ethylhexyl) phthalate. Environ Health Perspect 109:229–237.

Øie L, Hersoug LG, Madsen JØ. 1997. Residential exposure to plasticisers and its possible role in the pathogenesis of asthma. Environ Health Perspect 105:972–978.

Paris I, Ruggieri F, Mazzeo P, Carlucci G. 2003. Simultaneous determination of di(2-ethylhexyl)phthalate and mono(2-ethylhexyl)phthalate in human plasma by HPLC. Anal Lett 36:2645–2654.

Plonait SL, Nau H, Maier RF, Wittfoht W, Obladen M. 1993. Exposure of newborn infants to di-(2-ethylhexyl)-phthalate and 2-ethylhexanoic acid following exchange transfusion with polyvinylchloride catheters. Transfusion 33:598–605.

Roth B, Herkenrath P, Lehmann HJ, Ohles HD, Homig HJ, Benz-Bohm G, et al. 1988. Di-(2-ethylexyl)-phthalate as plasticiser in P.V.C. respiratory tubing systems indications of hazardous effects on pulmonary function in mechanically ventilated, preterm infants. Eur J Pediatr 147:41–46.

Sharman M, Read WA, Castle L, Gilbert J. 1994. Levels of di-(2-ethylhexyl) phthalate and total phthalate esters in milk, cream, butter and cheese. Food Addit Contam 11:375–385.

Tandon R, Seth PK, Srivastava SP. 1991. Effect of *in utero* exposure to di(2-ethylhexyl)phthalate on rat testes. Indian J Exp Biol 29:1044–1046.

Tickner JA, Schettler T, Guidotti T, McCally M, Rossi M. 2001. Overview of patient health risks posed by the use of di-2-ethylhexyl phthalate (DEHP) in PVC medical devices. Am J Ind Med 39:100–111.

Tsai ML, Webb RC, Loch-Caruso R. 1997. Increase of oxytocin-induced oscillatory contractions by 4-hydroxy-2′,4′,6′-trichlorobiphenyl is estrogen receptor mediated. Biol Reprod 56:341–347.

Wong JS, Gill SS. 2002. Gene expression changes induced in mouse liver by di(2-ethylhexyl) phthalate. Toxicol Appl Pharmacol 185:180–196.

Yang Q, Xie Y, Depierre JW. 2000. Effects of peroxisome proliferators on the thymus and spleen of mice. Clin Exp Immunol 122:219–226.