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 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 with plastic 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; Oie et al. 1997; Sharman et al. 1994). In particular, leaching of DEHP from PVC medical devices and deposits in tissue has 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 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]
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 (\(p \geq 0.32\)).

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

| Infants’ characteristics | DEHP+/MEHP- \(a\) | DEHP+/MEHP+ \(a\) | DEHP-/MEHP+ \(a\) | DEHP-/MEHP- \(a\) |
|--------------------------|------------------|------------------|------------------|------------------|
| Mean birth weight (g)    | 3411.11 ± 597.27 | 3173 ± 706.01    | 3008.89 ± 627.7  | 3533 ± 563.44    |
| Mean gestational age (weeks) | 39 ± 1.32   | 38 ± 2.45       | 37 ± 1.59       | 39 ± 1.43       |
| Full-term infants (\(n = 73\)) | AGA —      | 48               | 6                | 10               |
| Preterm infants ≤ 1,500 g (\(n = 3\)) | AGA —      | 1                | —                | —                |
| Preterm infants > 1,500 g (\(n = 8\))  | AGA —      | 2                | 1                | —                |
| 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+ \(a\) | DEHP- \(a\) | \(p\)-Value | MEHP+ \(a\) | MEHP- \(a\) | \(p\)-Value |
|--------------------------|----------|----------|-----------|----------|----------|-----------|
| Mean birth weight (g)    | 3206.15 ± 692.68 | 3284.74 ± 626.50 | NS        | 3150 ± 620.68 | 3475.26 ± 566.74 | NS        |
| Mean gestational age (weeks) | 38.37 ± 2.33 | 38.56 ± 1.84 | NS        | 38.16 ± 2.34 | 39.35 ± 1.35 | 0.033     |
| Total \(n\)              | 65       | 19       |           | 65       | 19       |           |

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

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 (LochCaruso 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 (Martoziene and Grazuleviciene 2002); evidence of DEHP-induced interleukin-1 secretion has been reported in mononuclear cells (Cao 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.

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
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; Pionait 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).