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Exposure to Di-(2-Ethylhexyl) Phthalate Among Premature Neonates in a Neonatal Intensive Care Unit

Antonia M. Calafat, PhD*; Larry L. Needham, PhD*; Manori J. Silva, PhD*; and George Lambert, MD‡

ABSTRACT. *Objective.* Premature neonates who spend time in a neonatal intensive care unit may be at increased risk of adverse health effects from exposure to di-(2-ethylhexyl) phthalate (DEHP) because of their increased risk of high exposure, their small body size, and their physical condition. DEHP, a reproductive toxicant in animals, is a major component in polyvinyl chloride (PVC) plastics, which are frequently used in medical tubing and blood storage bags. DEHP is not covalently bound to PVC, and it may be easily released from the PVC medical devices. The objective of this study was to determine whether premature infants who undergo medical procedures, such as blood transfusions, intravenous therapy, enteral and parenteral nutrition support, and dialysis, are at increased risk of exposure to DEHP than the general population. Because of their smaller size, children and especially premature and small infants may receive a larger dose of DEHP on a milligram per kilogram basis than adults when the same-size medical device is used for all ages.

Methods. Premature neonates who seemed to have the potential to be on intravenous infusion for >2 weeks and were expected to survive were eligible for enrollment in the study. We assessed exposure to DEHP in 6 premature newborns by measuring in 41 urine samples the levels of 3 DEHP metabolites: mono-(2-ethylhexyl) phthalate (mEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (mEHHP), and mono-(2-ethyl-5-oxohexyl) phthalate (mEOHP).

Results. mEHHP and mEOHP were detected in all 41 urine samples, and mEHP was detected in 33. Because only 33 of the samples had detectable amounts for all 3 metabolites, statistical analyses were limited to those 33. The levels of all 3 DEHP metabolites varied widely, and the urinary mean and median concentrations of mEOHP and mEHHP were 1 order of magnitude higher than those for mEHP. Furthermore, the geometric mean urinary concentrations of mEOHP (1617 ng/mL), mEHHP (2003 ng/mL), and mEHP (100 ng/mL) in these 6 premature infants who underwent intensive therapeutic interventions were found to be severalfold higher than in the US general population (for mEHP, geometric mean in those 6 years and older was 3.43 ng/mL).

Conclusions. This study provides the first quantitative evidence confirming that newborns who undergo intensive therapeutic medical interventions are exposed to higher concentrations of DEHP than the general population. Although the overall benefits of medical procedures using PVC devices outweigh the risks associated with exposure to DEHP, more research is needed to determine whether infants and children who undergo intensive therapeutic interventions using DEHP-containing devices are at higher risk for altered health outcomes than infants and children who undergo similar treatments but are not potentially exposed to DEHP. *Pediatrics* 2004;113:e429–e434. URL: http://www.pediatrics.org/cgi/content/full/113/5/e429; *DEHP, phthalate, plasticizer, urine, biomarker.*

ABBREVIATIONS. PVC, polyvinyl chloride; DEHP, di-(2-ethylhexyl) phthalate; mEHP, mono-(2-ethylhexyl) phthalate; mEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; mEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; IV, intravenous; CERHR, Center for the Evaluation of the Risks to Human Reproduction; RfD, reference dose.

Premature neonates may spend several weeks after birth in a neonatal intensive care unit, where they are exposed to numerous plastic medical devices, many of which may be made from polyvinyl chloride (PVC). Their PVC plastic–rich environment includes a high percentage (up to 40% by weight) of di-(2-ethylhexyl) phthalate (DEHP), which is the only plasticizer approved by the US Food and Drug Administration for medical uses.[1] DEHP, which is not covalently bound to the plastic, is released from PVC medical devices[2–5] at a rate that depends on several factors, including storage and use temperatures, storage time, flow rate of solutions through the tubing, percentage of DEHP in the PVC product, and the lipophilic nature of the solution in contact with the PVC plastic.[6,7] Therefore, patients who undergo medical procedures that involve the use of medical devices that contain PVC may be potentially exposed to much higher levels of DEHP than the general population because these procedures may deliver to the patient via ingestion, intravenous, and dermal absorption considerable doses of DEHP.[2,8–10]

In humans, DEHP is rapidly hydrolyzed to its monoester, mono-(2-ethylhexyl) phthalate (mEHP), which in a multistep pathway is oxidized to other metabolites, including mono-(2-ethyl-5-hydroxyhexyl) phthalate (mEHHP) and mono-(2-ethyl-5-oxohexyl) phthalate (mEOHP).[1,11–14] These metabolites are excreted in the urine and feces, either in their free form or as conjugates, primarily glucuronides. Expo-

sure to DEHP can be assessed by measuring the urinary levels of mEHP, mEHHP, and mEOHP.[15–17]

In male rodent studies, exposure to high doses of DEHP has been shown to lead to decreased testicular weights and tubular atrophy[1,18,19]; similar exposures to adult female rats resulted in anovulatory cycles and polycystic ovaries.[20,21] DEHP and mEHP act functionally as antiandrogens during the prenatal period and cause reproductive and developmental toxicities in rodents[22–25]; however, mEHP may be the active toxicant.[18,19] The testicular toxicity of the oxidative metabolites mEHHP and mEOHP may be lower than that of mEHP.[19]

Critically ill or injured children and infants who undergo medical procedures, such as blood transfusions, intravenous (IV) therapy, enteral and parenteral nutrition support, and dialysis, may be exposed to levels of DEHP much higher than the general population.[2,26] In 2000, the first expert panel of the National Toxicology Program's Center for the Evaluation of the Risks to Human Reproduction (CERHR) suggested that the DEHP intake for infants who undergo intensive therapeutic interventions may be 2 to 3 orders of magnitude higher than for the general adult population.[27] Although the CERHR panel recognized that benefits of medical procedures can outweigh any risks, the panel expressed concern that DEHP exposure in male infants and toddlers, especially in those critically ill, may adversely affect their reproductive tract development. The CERHR panel indicated that additional studies to address human exposure to DEHP, especially in these young male children, are warranted.[27] The US Food and Drug Administration also concluded that children who undergo certain medical procedures may be at increased risk for the effects of DEHP.[2] In June 2003, the American Academy of Pediatrics also expressed concern about the potential harm to children and infants from exposure to DEHP.[26]

No data have been published on exposure to DEHP among critically ill children, and the American Academy of Pediatrics noted that studies designed to evaluate the total exposure from medical procedures to DEHP and its metabolite mEHP would be invaluable.[26] In response to this request, we assessed exposure to DEHP in newborns who underwent medical treatment in a neonatal intensive care unit setting by measuring the urinary levels of 3 DEHP metabolites: mEHP, mEHHP, and mEOHP. This is the first study to determine the levels of DEHP metabolites in newborns who undergo intensive therapeutic medical interventions that typically involve the frequent and intense use of DEHP-containing medical products.

METHODS

The institutional review boards of the University of Medicine and Dentistry of New Jersey/Robert Wood Johnson Medical School and of the Centers for Disease Control and Prevention approved the protocol. Infants who seemed to have the potential to be on IV infusion for >2 weeks and were expected to survive were eligible for enrollment in the study. Six premature newborns (4 girls; average gestational age ± standard deviation [SD]: 24.9 ± 1.6 weeks; average weight at birth ± SD: 665.5 ± 166.5 g) who were born at St Peter's University Hospital and Robert Wood Johnson University Hospital from April 15 through July 8, 2002, were enrolled in the study (Table 1). The race/ethnicity was unknown for 1 of the newborns, 4 were white, and 1 was black. A total of 43 urine samples were collected during July 1 through August 27, 2002. On average, 7 urine samples per infant were collected from enrollment until discharge from the hospital.

A cotton ball or gauze, prescreened for the presence of phthalate monoesters, was used to collect samples from these premature infants[28] and placed inside a urine collection container after collection. The urine containers were labeled, frozen, and shipped on dry ice to the Centers for Disease Control and Prevention's National Center for Environmental Health. On arrival, the gauze or cotton ball used to collect the urine was placed in a 50-mL polystyrene conical tube and centrifuged at 500 rpm for 1 minute to express the urine. In most cases, ~1 mL of urine was recovered; from 2 of the samples, urine could not be recovered. The recovered urine samples were stored frozen at −20°C until analyzed. The analytical method used to measure the DEHP metabolites has been described.[29,30] Briefly, the urine samples were processed using enzymatic deconjugation of the glucuronidated DEHP metabolites followed by solid-phase extraction. To measure the concentration of free metabolites, the enzyme deconjugation step was omitted. The solid-phase extraction eluate was concentrated, and the DEHP metabolites were separated from other components in the extracted urine by reversed-phase high-performance liquid chromatography and quantified by isotope dilution–tandem mass spectrometry. When the levels of the DEHP metabolites were higher than the highest calibration standard, the extraction was repeated using less urine, and the concentrations were calculated after adjusting for the dilution. The urinary concentrations are reported in nanograms per milliliter of urine. Creatinine adjustment was used to correct for urine dilution.[31] DEHP was not measured because of the likelihood of contamination during the steps from sample collection to analysis.

RESULTS

In the 41 samples analyzed for this study, mEHHP and mEOHP were detected in all 41 samples, and mEHP was detected in 33. Because only 33 of the samples had detectable amounts for all 3 metabolites, statistical analyses were limited to those 33 (Table 2). Chemical analyses were performed using less urine than the required 1 mL when the amount of urine was limited. The reduced sample volume resulted in a lower frequency of detection of mEHP than of the oxidative metabolites, which are present at higher levels than mEHP (vide infra).

TABLE 1.   Demographic Characteristics of the Newborns in the Study

Infant	Gender	Ethnicity	Gestational Age at Birth (weeks)	Birth Weight (g)	Age (Days) at Collection of Samples	N*
1	Female	White	26	695	81–83	4 (4)
2	Male	Unknown	26	607	34–80	10 (6)
3	Female	White	23	550	40–92	9 (7)
4	Female	Black	23	440	4–60	11 (9)
5	Male	White	25	821	29–31	2 (2)
6	Female	White	26	880	18–32	5 (5)

* Number of samples analyzed (samples with detectable levels for all 3 analytes).

TABLE 2. Urinary Levels* of mEHP, mEOHP, and mEHHP in 6 Critically Ill Neonates†

Phthalate Monoester	Mean	Geometric Mean	Median	5th Percentile	95th Percentile
mEHP	205 (1875)	100 (800)	129 (668)	6.22 (27.6)	704 (6043)
mEHHP	3419 (24 439)	2003 (16 634)	2221 (14 574)	290 (5707)	13 161 (62 982)
mEOHP	2962 (21 319)	1617 (14 351)	1697 (14 810)	243 (5189)	10 413 (52 189)

* Concentrations are in ng/mL (μg/g creatinine).
† Although 41 samples were analyzed, the parametric results are for the 33 samples (22 for the creatinine corrected measurements) for which all 3 analytes were detected.

In the analysis of the data on a population basis, the levels of all 3 DEHP metabolites varied widely (Table 2). The median concentrations were 2221 ng/mL for mEHHP, 1697 ng/mL for mEOHP, and 129 ng/mL for mEHP. In agreement with previous findings,[15,17] the urinary mean and median concentrations of mEOHP and mEHHP were higher than those for mEHP (Table 2), and the concentrations of mEOHP and mEHHP were highly correlated (correlation coefficient: 0.901). The higher concentrations of the oxidative metabolites than mEHP suggest that the predominant metabolic route for DEHP is hydrolysis to mEHP followed by oxidation of mEHP,[1,11–14] even in these premature newborns. As mentioned, the toxicity of the oxidative metabolites may be lower than that of mEHP.[19]

The creatinine corrected concentrations are shown in Table 2. However, because of the limited amount of urine, creatinine was measured in only 22 of the 33 samples. The creatinine values ranged from 2.3 to 48.6 mg/dL; the mean (and SD) values were 17.0 mg/dL (11.5 mg/dL). The World Health Organization recommends that urine samples with creatinine concentrations $<$30 mg/dL or $>$300 mg/dL be excluded in occupational exposure monitoring[32]; however, no such standards have been recommended for children or neonates. Only 2 (9%) of this study's urine samples had creatinine values $>$30 mg/dL. If the World Health Organization's recommended exclusionary criteria are also to be applied to infants, then most of the samples of this study would have been excluded. Similar situations are likely to occur

in the future as the interest in assessing exposures to environmental chemicals in children increases.[33]

We compared the mEHP levels in these neonates with the levels of mEHP in children from the only 2 published studies on children's environmental exposure to phthalates.[34,35] The geometric mean mEHP concentration (100 ng/mL) for these premature infants was significantly higher than the mean urinary concentration of mEHP (4.6 ng/mL) in 19 toddlers aged 12 to 18 months, mostly Hispanic, in Imperial Valley, California, near the Mexico border,[34] and the median mEHP concentration (129 ng/mL) for these premature infants was ~26-fold higher than the US median for children 6 to 11 years of age.[35,36] Although the background levels of mEHHP and mEOHP in the US general population have not been determined, the median concentrations of mEHHP (2221 ng/mL) and mEOHP (1697 ng/mL) in these premature infants were ~1 order of magnitude higher than those in a nonrepresentative population of 62 children and adults[15] (Fig 1).

We also compared the concentrations of the DEHP metabolites within each of the 6 neonates. The mean and SD values for the urinary concentrations of mEHP, mEHHP, and mEOHP and the ratios of the concentrations of the metabolites for each newborn are shown in Table 3. The mean levels of all 3 DEHP metabolites varied widely among the 6 infants. Furthermore, the mean concentrations of the DEHP metabolites varied significantly within samples for each infant, as evidenced by the high values of the SD (Table 3). We also compared the ratios of the concen-

Fig 1. Median concentrations of DEHP metabolites in 6 critically ill neonates, in 328 children between 6 and 11 years of age from the 1999-2000 National Health and Nutrition Examination Survey,[35,36] and in a demographically diverse group of 62 children and adults.[15]
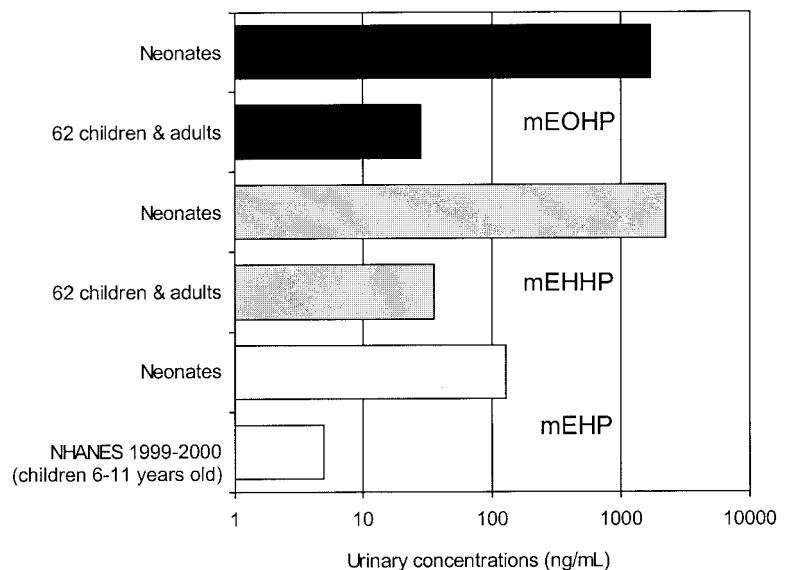
TABLE 3. Mean DEHP Metabolite Urinary Concentrations (ng/mL) and Ratio of Concentrations (SD) for Each Neonate

Infant	mEHP	mEHHP	mEOHP	mEHHP:mEHP	mEOHP:mEHP	mEHHP:mEOHP
1	163 (71.3)	1519 (1044)	1772 (1259)	8.6 (2.2)	10.0 (2.6)	0.9 (0.1)
2	50.8 (49.7)	1707 (1671)	1218 (1212)	46.3 (28.8)	31.5 (19.1)	1.5 (0.3)
3	348 (261)	3198 (2610)	1774 (1145)	9.4 (3.6)	5.5 (1.7)	1.7 (0.5)
4	146 (217)	2973 (3946)	2851 (4361)	71.8 (108)	60.3 (84.7)	1.2 (0.2)
5	433 (354)	7789 (6077)	6545 (5134)	18.4 (1.0)	15.4 (0.7)	1.2 (0.0)
6	241 (235)	6360 (2427)	6437 (2809)	56.1 (50.3)	57.7 (56.1)	1.0 (0.2)

trations of the metabolites within each of the 6 neonates. The mean ratios of metabolite concentrations ranged from 8.6 to 71.8 (mEHHP:mEHP), 5.5 to 60.3 (mEOHP:mEHP), and 0.9 to 1.7 (mEHHP:mEOHP). The SD values for the mEHHP:mEHP and mEOHP:mEHP ratios varied considerably more and were higher than the mEHHP:mEOHP ratio (Table 3). In agreement with our previous findings,[15] when mEHP was metabolized further, the 2 oxidative metabolites were formed consistently in all 6 infants, as evidenced by the relatively low SD values for the mEHHP:mEOHP ratio. The much higher SD values and wider range for the mEHHP:mEHP and mEOHP:mEHP ratios suggest considerable variation in the degree of oxidative metabolism both within each newborn and among them.

DISCUSSION

DEHP can be present in products such as medical examination and surgical gloves; medical tubing including the flexible tubing used for extracorporeal membrane oxygenation, for hemodialysis, and for administering parenteral solutions; umbilical catheters; nasogastric and enteral feeding tubes; respiratory masks; endotracheal tubes; and blood, plasma, IV, and total parenteral nutrition storage bags.[2] Therefore, we examined whether a specific medical procedure and the urinary concentrations of the DEHP metabolites were correlated. Several medical procedures were performed on all of the neonates during their stays at the hospital (Table 4), and >1 of these procedures was done on the days the urine samples were collected. Before or during the collection period, all infants were intubated and received blood transfusions and hyperalimentation, a periph-

TABLE 4. List of Medical Procedures Performed*

Procedure	Neonate					
	1	2	3	4	5	6
Blood transfusion	√	(√)	√	√	(√)	√
Intubation	√	(√)	√	√	(√)	(√)
Hyperalimentation	√	√	√	√	(√)	(√)
Orogastric tube	√	√	√	√	√	√
Intralipids feeding	√	(√)	√	√		(√)
Nasal cannula		√	√	√		√
Continuous positive airway pressure		√	√	√	√	√
IV nutrition	(√)	√	√	√	(√)	(√)
Umbilical vessel catheterization	(√)	(√)	√	(√)		(√)
Isolette		(√)	√	√	√	√
Chest tubes		(√)				
Nasogastric tube feeding		(√)				

* Procedures performed the day of collection of at least 1 urine sample are indicated with a check mark. A check mark in parentheses indicates that the procedure was done but on a different day than sample collection.

eral IV, and an orogastric tube. After extubation, all but 1 infant (infant 1) also received continuous positive airway pressure, and each of 4 newborns (infants 2, 3, 4, and 6) had a nasal cannula when samples were collected. Three of the neonates (infants 2, 3, and 4) had at least 1 IV, 3 (infants 1, 2, and 3) received a sterile aqueous suspension of lipid droplets by IV feeding, and 1 (infant 3) was receiving umbilical vessel catheterization at the time of sample collection. One infant (infant 2) had chest tubes for pneumothorax, but they were removed 8 days before sample collection. Because blood transfusions have been suspected to result in high exposures to DEHP,[2] we examined the effects of this procedure on the levels of DEHP metabolites. Three of the infants had 1 urine sample collected on the day of a blood transfusion. For 1 of the neonates (infant 1) but not the others (infants 3 and 6), this sample had the highest concentration of mEHP; the levels ranged from 143.5 ng/mL to 258.1 ng/mL. We observed a similar pattern when we compared the levels of DEHP metabolites among infants who underwent the same medical procedure on the day the urine samples were collected. These findings suggest that among these 6 neonates, a particular medical procedure and increased concentrations of DEHP metabolites were not correlated. Similarly, we found no correlation between the mean urinary levels of DEHP metabolites and gestational age, age at the time the samples were collected, or birth weight.

Glucuronidation facilitates urinary excretion of phthalate metabolites including those of DEHP, and because the putative biologically active species is the free monoester metabolite, glucuronidation reduces their potential biological activity. Although we would have preferred to have measured free and total DEHP metabolite levels, because of the limited amount of urine, the amounts of free metabolites could not be determined in all urine samples; free mEHHP, mEOHP, and mEHP were detected in 27, 26, and 10 samples, respectively. The median percentages (and SDs) of the free DEHP oxidative metabolites in the neonates' samples (mEHHP: 22% [17%]; mEOHP: 21% [18%]) were higher than those found in 127 urine samples collected during 2001 from a demographically diverse population group (mEHHP: 5% [21%]; mEOHP: 12% [23%]).[37] The percentage of free mEHP in the urine of the neonates was not calculated because of the low frequency of detection of free mEHP. Additional studies are warranted to confirm the percentages of glucuronidation of the DEHP metabolites in individuals, including infants and young children, who are exposed to elevated levels of DEHP.

The DEHP reference dose (RfD), the daily oral DEHP intake estimated to be safe for the human population (including sensitive subgroups), has been established by the US Environmental Protection Agency to be 20 μg/kg/day.[38] The average daily intake of DEHP to healthy Canadian infants younger than 6 months was estimated to be 9 μg/kg/day, based on limited available data on concentrations of DEHP in food, indoor and ambient air, drinking water, soil, and children's products.[39] More recently, the estimated median DEHP exposure in a subset of the US adult population, based on extrapolated intake from the urinary levels of mEHP, was 0.71 μg/kg/day.[40] Although the creatinine clearance is lower in infants than in adults,[41] the average daily intake of DEHP by the premature newborns in this study is probably higher than the RfD because the median concentration of mEHP (2.7 ng/mL) used to calculate the DEHP exposure in the US adult population[40] was almost 50 times lower than the median in this study. Additional data, such as the creatinine clearance normalized by body weight and the percentage of the DEHP dose eliminated in the urine in these neonates, need to be determined so that the average daily intake of DEHP can be calculated more accurately.

This study provides the first quantitative evidence confirming that newborns who undergo intensive therapeutic medical interventions are exposed to higher concentrations of DEHP than the general population and probably higher than the RfD. These results are important because premature infants in intensive care units, who are immature both developmentally and physiologically, may be at highest risk to adverse health outcomes after exposures to DEHP. In addition to increased susceptibility, these infants may receive the highest "relevant doses" for several reasons: 1) the exposure scenario of living in a plastic-laden environment increases their potential dose of DEHP; 2) the activity of gastric lipases, which metabolize DEHP to mEHP, is high in infants to aid in the digestion of fats in milk,[42] and, therefore, infants may be able to convert DEHP to the more toxic mEHP more efficiently than older children or adults; 3) children have a lower capacity than adults for glucuronidation,[43] which could result in delayed excretion of DEHP and its metabolites; 4) the higher permeability in children than adults of the blood-testis barrier may result in increased dosage to the testis to DEHP and its metabolites; and 5) as with the case with lead, children may be able to absorb larger amounts of DEHP from the gastrointestinal tract than adults.[2]

Although the overall benefits of medical procedures using PVC devices outweigh the risks associated with exposure to DEHP, more research is needed to determine whether infants and children who undergo intensive therapeutic interventions using DEHP-containing devices are at higher risk for altered health outcome than infants and children who undergo similar treatments but are not potentially exposed to DEHP.

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