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Phthalate Monoesters Levels in the Urine of Young Children

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Phthalates are ubiquitous industrial chemicals used in consumer products, in plastics and in pesticide formulations. Phthalates have been included in pesticide formulations as both active or inactive ingredients. In addition, phthalates are used as plasticizers in certain plastic children's toys and as ingredients in consumer products such as soaps, shampoos, perfumes and cosmetics.

In animal studies, phthalate administration has produced developmental and reproductive toxicity as well as carcinogenicity. Dibutyl phthalate (DBP) and benzylbutyl phthalate (BzBP) exposure *in utero* and through lactation produce altered reproductive development in male rat pups (Mylechreest et al. 1998). Furthermore, di(2-ethylhexyl)phthalate (DEHP) produces testicular toxicity in developing male rats (Gray and Gangolli 1986). Phthalates are rodent carcinogens but via a mechanism not thought to be active in humans (David et al. 1999).

Humans and rodents metabolize phthalate diesters to the corresponding monocarboxylic acid derivative, commonly called the monoester, which is glucuronidated and excreted in urine and/or feces (Albro et al. 1973; Dirven et al. 1993). For example, DEP, DBP, and DEHP are metabolized to the corresponding glucuronidated monoesters, monoethyl (MEP), monobutyl (MBP), and monoethylhexyl (MEHP) phthalate, which are excreted. Unpublished data suggest that BzBP is metabolized predominately to monobenzyl (MBzP) phthalate (*Castle L., personal communication*). A proportion of the monoester undergoes further oxidation to other products (Albro et al. 1973; Dirven et al. 1993).

Widespread phthalate exposure in adults has been demonstrated previously (Blount et al. 2000a, 2000b). However, children were not examined in these earlier studies. Children are generally recognized as being at risk for greater exposure to environmental contaminants because of behavioral and anatomic characteristics (Chance and Harmsen 1998).

The lack of information about phthalate exposure in children led us to include these compounds in a pilot study originally designed to examine pesticide exposure. This

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pilot study was implemented in Imperial County, California, from January to March 2000. We selected Imperial County because of its large agricultural industry where a variety of crops are grown year-round. Residences in Imperial County frequently adjoin agricultural fields and field proximity is a known risk factor for measurable pesticide exposure in children (Simcox et al. 1995). This report ascertains young children's phthalate exposure from multiple sources by measuring urinary phthalate monoesters.

MATERIALS AND METHODS

We recruited families with children aged 12-18 months for this pilot study. Parents were approached during the clinic visit when the child was ready to receive a first measles-mumps-rubella vaccine. A trained bilingual study staff member screened parents for study participation. Parents who were eligible and chose to participate in the study were read a consent form and were interviewed in the language of their choice (English or Spanish). During the clinic interview, the primary caregiver was asked about home pesticide use, occupational exposure, and residential proximity to agricultural fields. Four weeks after the initial clinic interview, the caregiver was re-interviewed at his or her home. Information was updated about recent home pesticide use and occupational exposure; residential proximity to fields was verified. In addition, during the home visit, the caregiver was asked about their use of perfumes and cosmetics as well as about the child's use of soft plastic toys.

Urine samples were collected from the children during the initial clinic contact and again during the second visit about 4 weeks later (27-35 days). Children's urine samples were obtained by use of self-adhesive collection bags (U-Bag, Hollister Incorporated, Libertyville, IL). The collection bags were pre-screened and found to be devoid of phthalate monoester contamination. The study protocol and consent form were approved by Human Subjects Internal Review Boards at the University of North Carolina at Chapel Hill, USEPA, Westat Inc. and CDC.

The analysis method for phthalate monoesters in urine has been described previously (Blount et al. 2000a). All samples were spiked with $^{13}C_4$-labeled phthalate monoesters and 4-methylumbelliferone glucuronide. The samples were then treated with β-glucuronidase to release the phthalate monoesters from their conjugated forms. Deconjugated urine samples were extracted twice with solid phase extraction cartridges (Oasis HLB SPE, Waters Corp., Milford, MA) and resuspended in mobile phase. Chromatographic separation by high pressure liquid chromatography was followed with tandem mass spectrometry on a triple quadrupole instrument using atmospheric pressure chemical ionization (Finnigan Inc., San Jose, CA). Levels of 4-methylumbelliferone were monitored as quality control (QC) for the deconjugation step. Method blanks, QC samples (spiked human urine) and standards were analyzed along with unknown human urine samples. Limited urine volume was available for some samples, and detection limits for those samples were slightly higher than previously reported. (Blount et al. 2000a). Urinary creatinine levels were measured

using an ASTRA analzyer (Beckman Inc., Brea, CA) based on a Jaffe rate reaction (Gunter et al. 1996).

All statistics were performed separately on both unadjusted and creatinine-adjusted monoester levels. Repeated measures analysis of variance models were used to assess the possible effects of gender and 15 other measures from the questionnaire (the parents' cosmetics and perfume use, the presence of a farm or pesticide worker in the household, use of plastic toys, overall child's health, fever, vomiting and diarrhea, cough and pneumonia in the last year, breast feeding, consumption of solid food) on MEP, MEHP, MBzP, and MBP. The small subject population restricted our approach to include only 2 factors (independent variables) in each model. We analyzed models with gender and one of each of the other 15 variables. The dependent variables were the urinary levels of MEP, MEHP, MBzP, and MBP, unadjusted and on a creatinine-adjusted basis.

RESULTS AND DISCUSSION

Twenty children were originally enrolled in this study, but one child was lost to follow-up at the home visit. Nineteen of the original children provided urine samples, were aged 13.4±1.5 months (11.8-16.5 months) and were predominately Hispanic (17 Hispanic, 2 Caucasian). Fourteen were boys and 5 girls. Twelve children had two urine samples, and seven children had one urine sample available for analysis. As expected, the average urinary creatinine level, 28.5 ± 13.8 mg/dL (mean ± s.d.; Table 1) for the children in this study was much lower than the creatinine level in urine from the adults sampled in the National Health Assessment and Nutritutional Examination Survey (NHANES III) study (137.9 ± 77.2 mg/dL; Blount et al. 2000b; Burtis and Ashwood 1999).

All urine samples from the 19 children had detectable levels of MEP, MBzP, and MBP (Table 1). Urinary MEP, MBzP, and MBP suggest exposure to DEP, DBP, and BzBP which are primarily used in consumer products such as fragrance-containing soaps, shampoos and perfumes as well as nail polish and beauty products. These phthalates are also used in some cleaning and car care products. Eight urine samples from six children had detectable levels of MEHP suggesting DEHP exposure. DEHP is used primarily as an additive to plastics such as children's toys and blood bags. Analyses of the urinary free monoester levels were not possible due to the limited amount of sample available from the children in this study. The free monoester levels would have allowed an examination of the metabolism of these compounds in children.

Urinary levels of mono-isononyl, monooctyl, and monocyclohexyl phthalates were also measured but were not detected in any sample. The detection limits for these analytes ranged from 0.9-9.0 ng/mL due to differing volumes of samples available for analyses. Further research on exposure to these phthalates will require much larger sample volumes than were available in this study.

Table 1. Urinary phthalate monoester (ng/mL) and creatinine levels in children

Child	Creatinine (mg/dL)	MEP	MBzP	MBP	MEHP
1	55.7 37.4	210 89.1	12.3 19.7	7.7 16.1	6.8 <6.0
2	21.0 9.7	28.3 17.2	87.6 27.4	22.0 20.3	<4.0 <12.0
3	17.6 29.3	57.1 62.2	11.5 20.1	12.5 17.1	<1.2 <12.0
4	45.4 37.8	330 969	24.1 20.5	30.3 27.8	8.8 6.1
5	25.5	97.6	20.2	112	3.2
6	10.0 42.0	93.7 1110	19.0 145	21.7 52.4	<12.0 <12.0
7	17.8 25.0	78.4 116	13.9 12.7	14.4 2540	<6.0 <12.0
8	10.7 9.1	30.7 28.0	5.4 8.4	4.1 5.7	<1.3 <4.0
9	21.0 19.8	52.6 57.5	6.4 <8.0	16.3 12.9	<4.0 <12.0
10	21.0	30.5	6.8	6.6	<4.0
11	21.5 66.7	45.7 152	77.8 316	287 203	<4.0 15.2
12	16.5 17.7	48.7 228	28.0 39.2	14.7 20.3	<2.4 <2.4
13	11.5	37.1	5.5	14.0	<1.7
14	NA	51.7	28.7	30.4	<4.0
15	25.8 80.9	487 1380	32.4 96.2	68.8 324	<8.0 17.2
16	18.4	14.8	10.5	27.1	<12.0
17	24.5	49.4	16.1	55.3	<12.0
18	51.0	184	48.4	70.6	<6.0
19	23.4 37.5	79.6 86.4	23.7 27.5	34.7 154	47.3 6.1

MEP=monoethylphthalate, MBzP=monobenzylphthalate, MBP = monobutylphthalate, MEHP = monoethylhexylphthalate.

Neither unadjusted nor creatinine-adjusted urinary phthalate monoester levels correlated significantly with any item from the questionnaire including reported pesticide use, parental pesticide-associated occupation, cosmetic use, use of plastics or illness frequency. Determining sources of these phthalate exposures will require further research.

The mean urinary MBP, MBzP, and MEHP levels for the children in this study were above the 50th percentile of the previously reported adult levels (Table 2; Blount et al. 2000). This crude comparison neglects to consider that infants produce about one third of the urine volume of adults with about one seventh the body weight (Greene 1991). Combining this information suggests that DBP, BzBP and DEHP exposure on a body weight basis may be at least twice as high for these children as compared to the adults in NHANES III.

Table 2. Summary urinary phthalate metabolite levels (ng/mL) in children

Analyte	Mean	Stan. Dev.	NHANES III Percentile
MEP	184.1	246.9	25th -50th
MBP	117.4	287.6	75th -100th
MBzP	35.6	44.8	50th -75th
MEHP	4.6	6.4	50th -75th

MEP=monoethylphthalate, MBzP=monobenzylphthalate, MBP = monobutylphthalate, MEHP = monoethylhexylphthalate.

Variation in monoester levels between urine samples from the same child greatly exceeded that expected from the analytical method (±10%) and that found in the QC samples (±13.5%; Table 1). The large variation between samples from the same child at different times suggests that two spot-urine samples alone probably do not adequately describe phthalate exposure for any individual child. Multiple urine samples from each individual child may be required to fully assess the exposure to phthalates.

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