Assessing Human Exposure to Phthalates Using Monoesters and Their Oxidized Metabolites as Biomarkers

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Phthalates are a group of industrial chemicals with many commercial uses, such as solvents, additives, and plasticizers. For example, di-(2-ethylhexyl) phthalate (DEHP) is added in varying amounts to certain plastics, such as polyvinyl chloride, to increase their flexibility. In humans, phthalates are metabolized to their respective monoesters, conjugated, and eliminated. However, despite the high production and use of DEHP, we have recently found that the urinary levels of the DEHP metabolite mono-(2-ethylhexyl) phthalate (MEHP) in 2,541 persons in the United States were lower than we anticipated, especially when compared with urinary metabolite levels of other commonly used phthalates. This finding raised questions about the sensitivity of this biomarker for assessing DEHP exposure. We explored the utility of two other DEHP metabolites, mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) and mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), as additional DEHP biomarkers. These metabolites are formed by oxidative metabolism of MEHP. In urine from 62 people, both the range and the mean urinary levels of MEOHP and MEHHP were on average 4-fold higher than those of MEHP; the mean of the individual ratios of MEHHP/MEOHP, MEHHP/MEHP, and MEOHP/MEHP were 1.4, 8.2, and 5.9, respectively. These data suggest that MEOHP and MEHHP are more sensitive biomarkers of exposure to DEHP than is MEHP. These findings also suggest a predominant human metabolic route for DEHP hydrolysis to MEHP followed by oxidation of MEHP; they also imply that a similar mechanism may be relevant for other high-molecular–weight phthalates, such as di-*n*-octyl, di-isononyl, and di-isodecyl phthalates. *Key words:* DEHP, exposure, human, phthalate, urine. *Environ Health Perspect* 111:1148–1151 (2003). doi:10.1289/ehp.6074 available via *http://dx.doi.org/* [Online 24 February 2003]

Phthalates, diesters of phthalic acid, are ubiquitous environmental chemicals. They are components of many consumables, including personal-care products (e.g., perfumes, lotions, cosmetics), paints, industrial plastics, and certain medical devices and pharmaceuticals [Agency for Toxic Substances and Disease Registry (ATSDR) 1995, 1997, 2001, 2002; David et al. 2001]. Phthalates are commonly added to these commercial products to hold color or fragrance, to provide a film or gloss, to make certain plastics more flexible, or in the case of some pharmaceuticals, to provide timed releases (ATSDR 1995, 1997, 2001, 2002).

The potential for nonoccupational exposure to phthalates is high given their use in a vast range of consumable products and because they are not covalently bound to the other chemicals in the formulations. After exposure, phthalates are rapidly hydrolyzed to their respective monoesters, which can be further biotransformed to oxidative metabolites, depending on the phthalate; all of these metabolites can be glucuronidated and excreted in the urine and feces (ATSDR 1995, 1997, 2001, 2002).

Phthalates are of concern in environmental public health because of their potential for human exposure and results of animal toxicity studies. Some phthalates and their metabolic products act functionally as antiandrogens during the prenatal period (Moore et al. 2001; Mylchreest et al. 1998; Parks et al. 2000) and cause reproductive and developmental toxicities in animals, especially in males. Some developmental effects include reduction in androgen-dependent tissues (e.g., seminal vesicles, epididymus, prostate, and anogenital distance) (Agarwal et al. 1986; Ema and Miyawaki 2001; Foster et al. 2001; Mylchreest et al. 2000). Gestational and lactational exposures to large doses of dibutyl phthalate (DBP) and its metabolite monobutyl phthalate (MBP) in rats cause male reproductive tract malformations (Ema and Miyawaki 2001; Mylchreest et al. 1998). DBP reduces the production of testosterone by the fetal testis through an antiandrogenic mechanism (Mylchreest et al. 2002). Studies in male rodents exposed to high doses of di-(2-ethylhexyl) phthalate (DEHP) clearly indicate that the testes are a primary target tissue, resulting in decreased testicular weights and tubular atrophy (ATSDR 2002). The active testicular toxicant may be the DEHP metabolite mono-2-ethylhexyl phthalate (MEHP) (Gray and Beamand 1984; Gray and Gangolli 1986).

Few studies of the reproductive effects of DEHP on females have been conducted, but data suggest that long-term exposures of adult female rats also appear to have deleterious effects, including hypoestrogenic anovulatory cycles and polycystic ovaries (Davis et al. 1994). A recent study suggests that DEHP, through MEHP, suppresses estradiol production in the ovary, leading to anovulation (Lovekamp-Swan and Davis 2003). In turn, exposure to high doses of DBP results in spontaneous abortion of rat pups, demasculinization of fetal male rats, and testicular atrophy in rats, mice, ferrets, and guinea pigs (ATSDR 2001; Mylchreest et al. 1998).

Several phthalates also are carcinogenic in rodents (ATSDR 2002; Huber et al. 1996; Kluwe et al. 1982). DEHP, DBP, and their monoester metabolites appear to have the greatest potential toxicity. DEHP is a peroxisome-proliferator hepatocarcinogen in rodents (ATSDR 2002), but the relevance of carcinogenicity by this mechanism in humans is debatable (Doull et al. 1999; Melnick 2001).

Despite the fact that the potential for nonoccupational exposure to phthalates is high, little information is known about the effects of phthalate exposure to humans. In 2000, the first expert panel of the National Toxicology Program's Center for the Evaluation of the Risks to Human Reproduction (CERHR) evaluated evidence that exposure to phthalates may result in reproductive or developmental risks to humans. The CERHR panel concluded that more data regarding human exposure are needed for evaluating the potential health effects associated with phthalate exposures (CERHR 2000a, 2000b, 2000c, 2000d, 2000e, 2000f, 2000g). We are collecting such exposure information primarily through our participation in the National Health and Nutrition Examination Surveys (NHANES), which allows us to have access to urine and serum samples from a representative sampling of the civilian, noninstitutionalized U.S. population. NHANES is an ongoing national survey, conducted by the

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National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC) and is designed to collect data on the health and nutritional status of the U.S. population. NHANES is unique in its ability to examine public health issues that can be addressed through physical and laboratory examinations of the U.S. population, such as the assessment of exposure to environmental chemicals (National Center for Health Statistics 2003).

Measuring Human Exposure to Phthalates

Understanding human exposure to phthalates requires information on the concentration of these toxicants in the nonoccupationally exposed population and on the pharmacokinetics of the various phthalates. However, because phthalates are usual laboratory contaminants, human studies using the parent phthalates as biomarkers of exposure have been primarily limited to highly exposed populations (Ching et al. 1981; Dirven et al. 1993; Faouzi et al. 1999; Mettang et al. 1996; Pollack et al. 1985). In 2000, we reported a novel approach, which uses phthalate monoester metabolites as biomarkers of exposure to phthalates (Blount et al. 2000a). By analyzing urine for the phthalate monoesters, we avoided contamination from the ubiquitous parent compounds, thus allowing for the assessment of phthalate exposure to the general population (Blount et al. 2000b; CDC 2001, 2003).

Recently, we reported the levels of seven urinary phthalate monoester metabolites in three subsets of NHANES participants (Blount et al. 2000b; CDC 2001, 2003); however, because the concentration data in the 2001 report were included in the 2003 report, we hereafter refer only to the 2003 report. In our first study (Blount et al. 2000b), we analyzed a nonrepresentative, call-back adult cohort of 289 urine samples, collected during 1988–1994 for NHANES III. Interestingly, we found detectable levels of several phthalate monoesters in > 75% of the study population. The high-molecular–weight phthalate monoesters [e.g., MEHP, monooctyl phthalate (MOP), monoisononyl phthalate (MINP)] were detected less frequently and at lower concentrations than were the low-molecular–weight phthalates [e.g., monoethyl phthalate (MEP), MBP, monobenzyl phthalate (MBzP)] (Blount et al. 2000b). Because of the small sample size and nonrepresentative nature of the NHANES III call-back subset population, further investigation was warranted to confirm our findings and to allow more detailed analyses to describe exposure levels in population subgroups. In January 2003, we published a detailed analysis of urinary concentrations of these seven urinary phthalate monoesters from a representative subset of a combined group of the NHANES 1999 and 2000 samples corresponding to 2,541 persons 6 years of age and older (CDC 2003). Consistent with the findings in the nonrepresentative NHANES III population, we found that MEP, MBP, and MBzP were the monoesters with the highest urinary levels and most frequently detected in the NHANES 1999/2000 representative population (CDC 2003). However, the means and median urinary concentrations of MEP, MBP, and MBzP in the NHANES 1999/2000 population were lower than in the NHANES III subset population (Table 1). These findings may be related to the small size and nonrepresentative nature of the sampling for the earlier NHANES III study, but it could also be due to a decrease in exposure to diethyl phthalate (DEP), DBP, and butylbenzyl phthalate for the population represented by NHANES 1999/2000 data. Nonetheless, these NHANES data confirmed that human exposure to phthalates is widespread, but that there were unexplained reasons why the concentrations of the lower-molecular–weight phthalates tended to be greater than the higher-molecular–weight phthalates. This could be due to relative differences in the amounts of the phthalates used, the degree of human exposure, the pathways and routes of exposure, and pharmacokinetic factors—absorption, distribution, metabolism (biotransformation), and elimination. We recognize that any or all of these relative differences could contribute to the large relative difference in urinary concentrations between, for example, MEHP and MBP. However, because our primary mission is to assess human exposure through biomonitoring, we are particularly interested in the fate of the chemical after it is absorbed into the body. We therefore concentrated our efforts in examining differences in distribution, metabolism, and elimination, with particular emphasis on differences in metabolism among the various phthalates. The relatively polar and low-molecular–weight phthalates (e.g., DBP) primarily metabolize into their monoesters and are excreted (Albro and Moore 1974; ATSDR 1995, 2001). In contrast, the higher-molecular–weight phthalates [e.g., DEHP, di-*n*-octyl phthalate (DOP), di-isononyl phthalate (DINP)] are hydrolyzed to their respective monoesters, which, in a multistep oxidative pathway (i.e., [ω-1]- and ω-oxidation of the aliphatic side chain), are metabolized to more hydrophilic, oxidative metabolites (Albro et al. 1973; Albro and Lavenhar 1989; Albro and Thomas 1973; ATSDR 1997, 2002; McKee et al. 2002).

Sensitive Biomarkers of Exposure to DEHP

Human exposure to DEHP should be relatively high because this compound is commonly used as a plasticizer to improve the flexibility of polyvinyl chloride (PVC). DEHP is found in such PVC materials as wall coverings, floor tiles, furniture upholstery, packaging film and sheets, blood storage bags, and medical tubing, including that used for administering parenteral solutions and in hemodialysis treatments (ATSDR 2002). The relatively low levels of MEHP in urine raised the question of whether MEHP is the most sensitive biomarker for assessing DEHP exposure and prompted us to assess the utility of other DEHP metabolites as alternative DEHP biomarkers. Mono-(2-ethyl-5-oxohexyl)phthalate (MEOHP) and mono (2-ethyl-5-hydroxyhexyl)phthalate (MEHHP) are known DEHP metabolites in mammals (Albro et al. 1973; Albro and Lavenhar 1989; ATSDR 2002; Egestad et al. 1996). Four major DEHP metabolites—MEHP, MEOHP, MEHHP, and mono-(5-carboxy-2-ethylpentyl)phthalate (MCEPP)—were found in the urine of persons exposed to DEHP (Albro et al. 1982; Dirven et al. 1993; Schmid and Schlatter 1985); the half-life of DEHP was estimated to be about 12 hr (Schmid and Schlatter 1985). In all of these studies, conducted on a limited number of persons, MEHHP and MEOHP concentrations in urine were higher than MEHP concentrations after oral (Schmid and Schlatter 1985), intravenous (Albro et al. 1982), and occupational (Dirven et al. 1993) exposure to DEHP. MCEPP concentrations were higher than those of MEHP in urine only after oral administration of DEHP (Dirven et al. 1993). These results suggested that MEHHP and MEOHP might be useful biomarkers of exposure to DEHP. To test this hypothesis, we evaluated exposure to DEHP in 62 persons, children and adults, by measuring MEHP, MEOHP, and MEHHP in urine using an adaptation (Silva et al. 2003) of our isotope-dilution HPLC–tandem mass spectrometry method (Blount et al. 2000a). The limits of detection in urine were 1.2 ng/mL MEOHP,

Table 1. Median urinary levels of monoester phthalate metabolites in the U.S. population (μg/L).

Analyte	NHANES III[a]	NHANES 1999–2000[b]	NHANES 1999–2000[c]
MEP	305.0	164.0	180.0
MBP	41.0	26.0	23.0
MBzP	21.2	17.0	13.8
MEHP	2.7	3.2	3.0

[a]Nonrepresentative call-back cohort of 289 adults. [b]Representative sample of the U.S. population (2,541 persons 6 years of age and older). [c]Representative sample of the adult U.S. population (1,461 persons 20 years of age and older).

1.6 ng/mL MEHHP, and 0.9 ng/mL MEHP (Silva et al. 2003).

The assessment of exposure to a given chemical using urinary concentrations of its metabolites is a somewhat tenuous process. For example, data suggest that phthalates are rapidly metabolized to their respective monoesters. The metabolism of lower-molecular–weight phthalates ends with the monoester (Albro and Moore 1974; ATSDR 1995, 2001), but the metabolism of the higher-molecular–weight phthalates continues with transformation of the monoester to oxidative products (Albro et al. 1973; Albro and Lavenhar 1989; Albro and Thomas 1973; ATSDR 1997, 2002; McKee et al. 2002). In this study of 62 urine samples, we found that all three DEHP metabolites were frequently detected (MEHP in 50; MEHHP in 59; MEOHP in 60). Because only 50 of the samples had detectable levels for all three metabolites, statistical analyses were limited to those 50. We first analyzed the data on a population basis. The concentration values were not normally distributed. The medians (and ranges) of concentrations were 4.5 ng/mL (1.4–537 ng/mL) for MEHP, 28.3 ng/mL (4.2–1,860 ng/mL) for MEOHP, and 35.9 ng/mL (2.7–2,417 ng/mL) for MEHHP. The urinary mean concentrations of MEHHP and MEOHP were 4.3-fold ($p < 0.02$) and 3.3-fold ($p < 0.03$) higher, respectively, than the mean concentration of MEHP. Furthermore, the concentrations of MEOHP and MEHHP were highly correlated [correlation coefficient of the linear regression (r^2) = 0.984; Figure 1] and correlated to those of MEHP (r^2 ~ 0.9; Figure 1). We also analyzed the data by comparing the ratios of the concentrations of the metabolites within each of the 50 individuals. The respective mean ratios [and relative standard deviations (RSDs)] of metabolites concentrations were MEHHP/MEOHP = 1.4 (22%), MEHHP/MEHP = 8.2 (80%), and MEOHP/MEHP = 5.9 (74%). These data indicate that when MEHP is further metabolized, the two oxidative metabolites are formed very consistently among individuals, as evidenced by the relative low RSD for the MEHHP/MEOHP ratio. The much higher RSDs for the MEHHP/MEHP and MEOHP/MEHP ratios suggest that the degree of oxidative metabolism varies considerably among people (assuming all values reflect steady state).

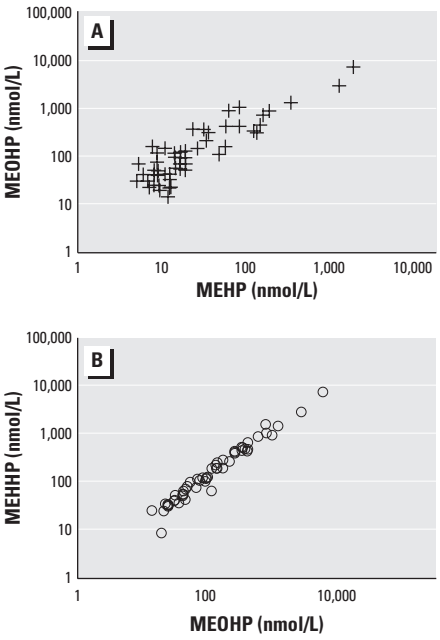

Figure 1. Relationship between the urinary concentrations of the DEHP metabolites MEOHP, MEHHP, and MEHP. (*A*) Correlation between MEOHP and MEHP on a log scale; the correlation coefficients of the linear regressions (r^2) are 0.944 for MEOHP and 0.892 for MEHHP (data not shown). (*B*) The urinary concentrations of MEOHP and MEHHP are highly correlated (r^2 = 0.984).

Future Research Priorities on Phthalates

The use of MEHP as a biomarker for assessing exposure to DEHP is valid, and MEHP certainly can be used for comparing exposure to DEHP among studies, assuming that it is present in detectable concentrations. However, the use of MEHP as the sole biomarker for comparing relative exposures of DEHP to various phthalates in a given study can be misleading. This is especially true when comparing monoester results of the higher-molecular–weight phthalate monoesters, such as MEHP, with the lower-molecular–weight monoesters, such as MEP, because the metabolism of the higher-molecular–weight phthalates is more complex and results in more metabolites, thus decreasing the relative amounts of their monoester metabolites. To properly interpret and model the data (David 2000; Kohn et al. 2000; Koo et al. 2002), one must have knowledge of the pharmacokinetics of each of the phthalates. For example, the higher urinary concentrations and frequency of detection of the oxidative DEHP metabolites MEOHP and MEHHP relative to that of MEHP suggest that the two oxidative metabolites are more sensitive indicators of DEHP exposure than is MEHP alone and that exposure to DEHP may be higher than previously thought based on the NHANES data, which measured MEHP only. Another way to better assess the dose, especially the relative dose, of DEHP is to measure the concentrations of MEHP, MEOHP, and MEHHP. We are currently doing this.

Likewise, we speculate that the lower urinary levels of MOP and MINP found in the NHANES studies may not necessarily reflect lower exposure to DOP and DINP, respectively, although the former may not be highly used. As with DEHP, these phthalates initially metabolize into their monoesters, which then form oxidative phthalate monoesters; these are the major metabolites detected in the urine (Albro and Moore 1974; McKee et al. 2002). Additional research needs to focus on the oxidative metabolites of DOP, DINP, and the other high-molecular–weight phthalates.

It is important to note that MEHP possesses biologically relevant activity. Therefore, MEHP is a valid DEHP biomarker when looking at health end points. To date, no information on the biological activity of the oxidative metabolites is available. It is conceivable that oxidative metabolism reduces the toxicity of the higher-molecular–weight phthalates. Further research to establish the bioactivity of MEHHP and MEOHP is warranted.

Our data support the widespread nature of human exposure to phthalates. However, the measurement of phthalate metabolites in people does not by itself mean that phthalates cause disease. Additional research is required to determine whether exposure to phthalates at the levels found in the general population is a cause for health concern.

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