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Risk assessment for phthalate exposures in the elderly: A repeated biomonitoring study☆

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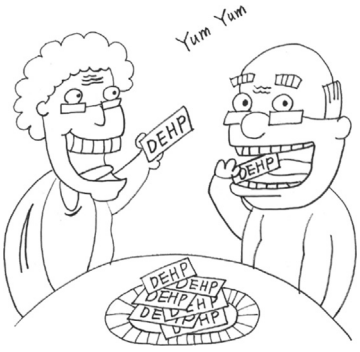
HIGHLIGHTS

- Phthalate exposure affects the development of a variety of diseases in the elderly.
- We repeatedly determined urinary DEHP and DnBP metabolite levels in Korean elderly.
- DEHP and DnBP variations were mainly attributed to intra-individual variation.
- DEHP exposure was strongly correlated to DnBP exposure.
- Urine samples with DEHP >RfD and DnBP >TDI were 20.2% and 3.6%, respectively.

GRAPHICAL ABSTRACT



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ABSTRACT

Recent studies indicated that exposure to phthalates affects the development of a variety of diseases in the elderly population. However, limited information was available about the variability of phthalate daily intakes (DIs) and the proportion of the population that is highly exposed to phthalates. Therefore, we measured the levels of three phthalate metabolites, mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono-n-butyl phthalate (MnBP) in 4014 urine samples repeatedly collected from 1646 elderly people. The DIs of di(2-ethylhexyl) phthalate (DEHP) and di-n-butyl phthalate (DnBP) were calculated using urinary MEHHP, MEOHP, and MnBP levels, and then the proportion of the population that was highly exposed to DEHP and DnBP was calculated. The intra-class correlation (ICC) for MEHHP, MEOHP, and MnBP levels was 0.07, 0.02, and 0.03, respectively, and exposures to DEHP and DnBP were strongly correlated with each other (correlation coefficient = 0.89 and p-value < 0.0001). The geometric mean of estimated DI

Abbreviations: ANOVA, analysis of variance; BE, biomonitoring equivalent; BMI, body mass index; CHMS, Canadian Health Measures Survey; CSTEE, Scientific Committee on Toxicity, Ecotoxicity and the Environment; DEHP, di(2-ethylhexyl) phthalate; DI, daily intake; DnBP, di-n-butyl phthalate; EFSA, European Food Safety Authority; EPA, Environmental Protection Agency; GM, Geometric mean; GSE, geometric standard error; HBM, Human Biomonitoring; ICC, intra-class correlation; KEEP, Korean Elderly Environmental Panel; KFDA, Korea Food and Drug Administration; KNHANES, Korea National Health & Nutrition Examination Survey; KoNEHS, Korea National Environmental Health Survey; LOD, limit of detection; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, mono-(2-ethylhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MnBP, mono-n-butyl phthalate; NHANES, National Health and Nutrition Examination Survey; RfD, reference dose; RV_{95}, reference value at 95th percentile; TDI, tolerable daily intake.

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Daily intake
Risk assessment

was 8.8 μg/$kg_{body\text{-}weight}$/day (range 0.005–3382.0) for DEHP and 1.5 μg/$kg_{body\text{-}weight}$/day (range 0.0002–1076.8) for DnBP. The percentages of urine samples with DEHP > reference dose (RfD, 20 μg/$kg_{body\text{-}weight}$/day) and DnBP > tolerable daily intake (TDI, 10 μg/$kg_{body\text{-}weight}$/day) were 20.2% and 3.6%, respectively. The Korean elderly were co-exposed to DEHP and DnBP, and the variation of DEHP and DnBP metabolite levels was mainly attributed to intra-individual variation, rather than inter-individual variation. Furthermore, Korean elderly were exposed to the hazards of DEHP and DnBP based on the high level of the exceedance rate over TDI or RfD for DEHP and DnBP. Since the elderly are very susceptible to environmental pollutants, the harmful effects of DEHP and DnBP in the elderly population should be further studied in the future.

1. Introduction

Phthalates are known to be exogenous substances that act like hormones in the endocrine system, and disrupt the physiologic function of endogenous hormones to induce a range of problems, including sexual and asexual effects in the body (Hauser and Calafat, 2005; Latini et al., 2006; Park et al., 2013). In particular, recent studies have indicated that exposure to phthalates affected the development of a variety of senile diseases, including diabetes mellitus, insulin resistance, and pulmonary function in the elderly population (Kim et al., 2013; Lind et al., 2012; Park et al., 2013).

People are ubiquitously exposed to phthalates in their daily life through literally thousands of products, such as flooring, medical equipment, paint, plastic bags, cosmetics and air fresheners (Schettler, 2006; Wormuth et al., 2006). Because of their ubiquitous exposure and contamination in the environment, external exposure scenarios to phthalates have always been difficult to evaluate. For this reason, assessments for the exposure to phthalates have been achieved by measuring the internal dose (Pirkle et al., 1995).

Di(2-ethylhexyl) phthalate (DEHP) and di-n-butyl phthalate (DnBP) were widely used phthalates. Their urinary metabolites, mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) and mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) for DEHP and mono-n-butyl phthalate (MnBP) for DnBP, were detected in >99% of the Korean elderly (Kim et al., 2013). This high detection rate may be due to no regulation for adult consumer products except several products for young children and food and beverage containers and cosmetics about the DEHP and DnBP contents in Korea (Korean Ministry of Government Legislation, 2010; Korean Ministry of Government Legislation, 2011; Korean Ministry of Government Legislation, 2013). Therefore, evaluating DEHP and DnBP exposure variations and the proportion of population at risk for DEHP and DnBP with biomonitoring data of the Korean elderly population may be important for elderly healthcare. However, limited evidence was available for variation of DEHP and DnBP exposures and the proportion of the elderly population at risk.

Therefore, in the present study, we measured DEHP and DnBP metabolite levels in urine samples repeatedly collected from the Korean elderly, and estimated the variations of DEHP and DnBP exposures using daily intake (DI) values for DEHP and DnBP calculated from urinary DEHP and DnBP metabolite levels. Furthermore, we evaluated the proportion of the population at risk for DEHP and DnBP in the Korean elderly based on the tolerable daily intake (TDI) and reference dose (RfD) as acceptable oral doses.

2. Materials and methods

2.1. Study population

The Korean Elderly Environmental Panel (KEEP) study was launched to investigate the association of environmental exposure with health outcomes in the elderly population over 60 years old. This KEEP study was composed of KEEP-I (6 surveys for 560 elderly recruited in Seoul between 2008 and 2010) and KEEP-II (3 surveys for 1254 elderly including 137 elderly recruited in KEEP-I and 1117 elderly people newly recruited in Seoul and Asan between 2012 and 2014) (Kim et al., 2013; Kim et al., 2016), and thus the total number of participants was 1677. All participants were citizens who regularly visited community welfare centers and had no difficulty in communicating with interviewers, and there were no other criteria for their participation (Kim et al., 2013; Kim et al., 2016). Participants without urine samples were excluded, leaving 1646 elderly people included in the analyses. The trained interviewers asked detailed questions including demographics, lifestyle habits, medical history, and living area, *etc.* based on a structured questionnaire to participants. Sometimes random questions were repeatedly asked to confirm whether participants answered the question correctly. The study protocol was approved by the institutional review board at Seoul National University Hospital, Seoul, Republic of Korea (ROK) (IRB No. H-0804–045–241 and H-1209-006-424) and conducted in accordance with guidelines laid down in the Declaration of Helsinki. Each study participant provided written informed consent.

2.2. Measuring urinary phthalate metabolites

Participants completed medical examinations (twice every year between 2008 and 2010 and once every year between 2012 and 2014) and provided a maximum of eight urine samples. Morning spot urine samples (50 mL) were collected from each subject, divided into four 12 mL tubes and stored at −20 °C immediately after collection until analyzed within one month.

Humans can be exposed to DEHP and DnBP through ingestion, inhalation, and dermal absorption of literally thousands of products (Schettler, 2006; Wormuth et al., 2006). For this reason, external exposure scenarios to DEHP and DnBP have been difficult to evaluate (Pirkle et al., 1995). Therefore, we measured urinary levels of MEHHP and MEOHP as secondary metabolites of DEHP, and MnBP as a metabolite of DnBP, to evaluate DEHP and DnBP exposure levels. However, we did not measure urinary levels of the primary metabolite of DEHP, mono-(2-ethylhexyl) phthalate (MEHP), to reduce potential exposure misclassification from contamination (Koch et al., 2003).

Phthalate metabolites were analyzed using high performance liquid chromatography tandem mass spectrometry (Agilent 6410 triple Quad LCMS, Agilent, USA; Nexera X2, Shimadzu, Kyoto, Japan) according to previously reported procedures (Hong et al., 2009). Because KEEP-II was conducted after KEEP-I was finished, samples of KEEP-I and KEEP-II were not measured at the same time for phthalate metabolites. However, we repeatedly measured phthalate metabolite levels for some random samples of KEEP-I during KEEP-II for valid comparison among batches, and found inter-day and intra-day coefficient of variations <10%. Furthermore, for the quality assurance and quality control, the accuracy and recovery for the measurement methods were calculated with spiked samples which were either pooled urine purchased from Sigma-Aldrich and found the percent difference between the mean of observed concentrations and the theoretical concentration <5% for both. The limit of detections (LODs) for MEHHP, MEOHP, and MnBP, respectively, were 0.16 μg/L, 0.21 μg/L, and 0.27 μg/L for KEEP-I, and 0.20 μg/L, 0.32 μg/L, and 0.35 μg/L for KEEP-II. MEHHP, MEOHP, and MnBP concentrations under the LODs were assigned as a default value of LOD concentration divided by the square root of two. To capture variation in urine dilution,

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