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이 논문을 보건학석사 학위논문으로 제출함

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Abstract

Dose reconstruction from urinary biomarkers in a human panel exposed to bis (2-ethylhexyl) phthalate via inhalation

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While oral consumption has been considered as the main exposure route of bis (2-ethylhexyl) phthalate (DEHP) for general public, dermal and inhalation cannot be ignored as alternative ones especially for consumer products. However, there were very limited information on quantitative contribution of exposure and metabolic profiles via inhalation after using consumer products. The purposes of the present study are: 1) quantitation of inhaled amounts of DEHP after use of a spray-type of consumer product, and 2) suggestion of fractional urinary excretion amounts of key

metabolites of DEHP after inhalation exposure in humans. In order to do so, seven young male Korean volunteers were recruited for an inhalation experiment, where they were located at a closed office room before single exposure to deuterium-labelled DEHP (DEHP-d_4) with a compressible spraying anti-fog solution of DEHP-d_4 at 500 mg/kg. Personal air monitoring of DEHP-d_4 at breathing-zone was made and total excreted urine samples for forty eight hours after exposure were collected to measure key metabolites of DEHP-d_4. Most aerosols of DEHP-d_4 was clear out in about five minutes, and its air concentration was 1.73 ± 0.42 mg/m^3 for the first five minutes. The total cumulative excreted amount of the metabolites was 0.8 ± 0.5, 1.6 ± 0.4 and 3.3 ± 0.9 μg for mono-(2-ethyl-5-hydroxyhexyl) phthalate-d_4 (MEHHP-d_4), mono(2-ethyl-5-oxohexyl) phthalate-d_4 (MEOHP-d_4) and mono-(2-ethyl-5-carboxypentyl) phthalate-d_4 (5cxMEPP-d_4), respectively. The estimated inhaled amounts were 70.8 ± 17.3 μg (AM ± SD) based on an exposure model using the air measurements. MEHHP-d_4, MEOHP-d_4 and 5cxMEPP-d_4 were determined as key metabolites of DEHP in urine as in other studies for exposure through ingestion; however, the relative production of metabolites among the analytes and fractional urinary excretion (F_{ue}) relative to absorbed dose were different: 1.54 ± 0.74 %, 3.26 ± 1.49 % and 6.42 ± 3.42 % for MEHHP-d_4, MEOHP-d_4 and 5cxMEPP-d_4, respectively, which was about 2~20 folds lower than those though ingestion of DEHP.

While personal exposure amounts after single spraying action appeared about 3% of total released amounts of DEHP, inhalational exposure contributed to internal dose of DEHP, it would increase in multiple actions of spraying or continuous exposure. The fractional urinary excretion of DEHP metabolites indicate relatively low metabolism through exposure via inhalation, and dose estimation from urinary metabolites of DEHP with oral F_{ue} could underestimate actual exposure as in occupational places where the main exposure route is inhalation.

Keywords: Bis (2-ethylhexyl) phthalate, inhalation, exposure model, intake dose, fractional urinary excretion

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Ⅰ. Introduction

Phthalates are widely used as plasticizers in polyvinyl-chloride products such as personal care products, toys, building materials, textiles, food containers, cosmetics, medical devices and clothes (IARC, 2000; ATSDR, 2002; Schettler, 2006; US EPA, 2007 and EU, 2008). Because phthalates are ubiquitous in our surroundings, they have been exposed general population and their metabolites were widely detected in human urine (Anderson et al., 2011). Phthalates are classified as endocrine disruptors and bis (2-ethylhexyl) phthalate (DEHP) is the most well-known toxic chemical among phthalates. Moreover, DEHP has been reported as a probably human carcinogenic chemical by the United States Environmental Protection Agency (US EPA, 2007) and the International Agency for Research on Cancer (IARC, 2000). However, there are no regulated the legal or guidance levels of chemical substances in personal care products. Personal care products contain a large number of various chemicals and they exposed general people as well as worker (Duty et al., 2005; Sathyanarayana et al., 2008; Just et al., 2010; Koniecki et al., 2011; Romero-Franco et al., 2011).

Although the major exposure route of DEHP for the general population is generally known to be dietary uptake, recently, many studies argued the

various DEHP exposure sources of indoor environments need to be given more importance. Specifically, literature has said that phthalates exposure via inhalation and dermal contact could be as significant as ingestion in the general population (Bekö et al., 2013). If there is possibility of exposure to phthalates, especially to DEHP, via inhalation, it is difficult to determine the accurate exposure information such as floating amount in air, intake dose (inhalation amount), levels of retention, absorbed amount in human body and pattern and levels of excreted metabolites. Previous studies of pharmacokinetic models to DEHP exposure were mostly ingestion pathway. Although there are some exposed studies via intravenous injection by ordinary medical treatment (given an injection of medical solution), it can't be known how many exposed actual DEHP dose, which was estimated using fractional urinary excretion (F_{ue}) calculated by ingestion of DEHP. To date, previous studies that estimated the intake dose of DEHP using exposure biomarker such as mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) and mono-(2-ethyl-5-carboxypentyl) phthalate (5cxMEPP) in urine have used F_{ue} calculated by ingestion of DEHP regardless of exposure route (Kohn et al., 2000; Calafat et al., 2006; Marsee et al., 2006; Itoh et al., 2007; Guo et al., 2011; Fong et al., 2014 and Cao et al., 2016). As we know, no study on DEHP exposure was conducted on humans or animals via inhalation for the development of fractional urinary excretion and absorption rate from air to the

human body. The studies on DEHP exposure via inhalation were only conducted for cytotoxicity, developmental toxicity and effect to target organ limited in-vivo and in-vitro studies (Merkle et al., 1988; Klimisch et al., 1922; Kurahashi et al., 2005 and Ma et al., 2006).

In previous studies (Fong et al., 2014 and Cao et al., 2016), comparison of daily intake dose of DEHP estimated from the exposure model using environmental data and the dose-reconstruction with exposure biomarkers concentration in biological samples. The intake dose estimated from the biomarker should be higher than the one estimated from environmental monitoring on the assumption that biomonitoring is the reflected all exposure pathway (NRCNA, 2006), but not always in those studies. There were many uncertainty and we suggested the one that was to use F_{ue} calculated by ingestion of DEHP because of no information about absorption factor depending on exposure route such as ingestion, inhalation and dermal contacts.

The purposes of this study are: 1) Estimation of the intake dose with an exposure model after inhalation of sprayed DEHP solution to human and 2) suggestion on F_{ue} calculated by inhalation of DEHP.

Ⅱ. Materials and methods

1. Chemicals and reagents

Bis[(±)-2-ethylhexy] Phthalate-3,4,5,6-d_4 (DEHP-d_4, >99 %) was purchased from C/D/N Isotopes Inc. (Canada) for measurement of concentration in air sample and di-n-Butyl phthalate-d_4 (DnBP-d_4 as internal standard was purchased from AccuStandard® (USA). For analysis of urinary excreted metabolites, we purchased mono(2-ethylhexyl) phthalate-d_4 (MEHP-d_4, >98 %) in C/D/N Isotopes Inc. (Canada); $^{13}C_4$-mono(2-ethyl-5-oxohexyl) phthalate ($^{13}C_4$-MEOHP, >99 %), $^{13}C_4$-mono-(2-ethyl-5-hydroxyhexyl) phthalate ($^{13}C_4$-MEHHP, >99 %), $^{13}C_4$-mono-(2-ethyl-5-carboxypentyl) phthalate ($^{13}C_4$-5cx-MEPP, >99 %) and $^{13}C_4$-mono-n-butyl phthalate ($^{13}C_4$-MnBP, >99 %) in Cambridge Isotope Laboratories, Inc. (USA). We purchased acetonitrile, methanol, and water (HPLC grade) from J.T.Baker; formic acid (98+ %) from ACROS Ordanics; and ammonium acetate (≥97 %), β-Glucuronidase (from Helix pomatia) and Sulfatase (from Helix pomatia) from Sigma-Aldrich (USA).

2. Subjects and exposure condition

The present study was approved by the institutional Review Board of Seoul National University (No. 1408/002-006). We recruited seven subjects by posting on off- and on-line board at Seoul national university. All subjects participated in this study on their own volition. They have not any health problem (such as allergy, asthma and skin disease etc.) and are all male and Korean. Subjects were recommended to wear protective clothing and glove in exposure room because we wanted to only expose via inhalation pathway. Subjects were allowed free access to food, water and beverage and filled in the questionnaire for lifestyle and chart of living pattern during experiment period.

Temperature and humidity of exposure room was 10.4 ± 0.2 °C (10.1 - 10.8 °C) and 46 ± 4 % (40 - 49 %), respectively (AM ± SD (range)). Before subjects entered, the exposure room was naturally ventilated. When subjects entered the exposure room, all window and door was closed and indoor air was circulated by fan. Exposure solution was added standard chemical of 500 mg in 30 mL mixture of anti-fog product and ethanol (16.7 mg/mL) and all sprayed using compressible spray. Subjects are exposed by single spraying for one minute.

3. Samples

For finding proper air sampling time, we collected particulate matters (PMs) time-profile using Portable Aerosol Spectrometer 1.109 (Grimm Aerosol Technik, Ainring, Germany) and SidePak personal aerosol monitor (TSI, model AM510, data logging only $PM_{1.0}$). Personal and area air sample were collected at breathing-zone and center of table (OSHA, 1994), which was placed in center sprayed exposure solution, respectively. Area air sample was collected at the following time points: 0, 5, 10, 15, 30 and 60 minutes after spraying. We collected air sampling at 1 L/min with an OVS-tenax sampling tube (Cat No. 226-56, SKC, USA) that contains 140 mg tenax resin and glass fiber filter in the front section and 70 mg tenax resin in the back-up section. Air sample were stored at -20 °C until analysis.

All excreted urine samples were collected in screw-capped polypropylene bottles and excreted volume of urine was measured. Cumulative excreted amount of the metabolites was calculated to multiply measured concentration of the metabolites in urine using instrumental analysis by excreted volume of urine. Collected urine samples were stored at -80 °C until analysis.

4. Preparation and analysis of samples

Preparation of air sample was referenced from OSHA method 104 (1994). Air samples were defrosted at room temperature for one hour before preparation. Air sample was divided up the front and the back-up sections in 4 mL amber vial with screw-capped. Each air sample vial was added 4 mL of the desorbing solvent, toluene, capped and mixed well. Blank sample of laboratory and field wasn't detected of DEHP-d_4 and all air sample wasn't observed breakthrough. Blank samples of laboratory and field weren't detected of DEHP-d_4.

DEHP-d_4 in air samples was analyzed by a Agilent 6890N gas chromatograph equipped with a Agilent 7683 automatic liquid sample coupled to a Agilent 5975N mass selective detector. Concentration of DEHP-d_4 was quantitated using DnBP-d_4 as internal standard. GC analysis was performed on a HP-5MS (30 m × 0.25 mm ID, 0.25 um film thickness, 5 % phenylmethyl silicone, Agilnt technologies, USA) with helium as carrier gas (1 mL/min). Gradient condition of oven temperature was applied; 0 - 1 min, 60 °C; 1 - 9 min, 60 - 220 °C and 9 - 25 min, 220 - 300 °C and 25 - 28 min, 300 °C. Post run time and temperature were two minutes and 300 °C, respectively.

Preparation of urine samples was referenced from Kato (2006) and Lee (2013). Urine samples were defrosted at room temperature for two hours. 980 μL of urine was add 20 μL of internal standards (MnBP-d_4 0.5 μg/mL), 100 μL of ammonium acetate (1 M), 20 μL of enzyme mixture (β-glucuronidase 926 unit/mL and sulfatase 926 unit/mL). Enzyme base buffer solution (1 M ammonium acetate) was adjusted pH 5.0. Urine samples incubated at 37 °C for two hours. After incubation, we added 2 mL 0.1 M formic acid to each sample. We extracted the DEHP metabolites in urine on solid phase extraction (SPE) manifold using Oasis HLB 60 mg/ 3 mL SPE cartridges (Waters Corp., Milford, MA) and compressed with 2 to 3 psi pressure. The cartridges were conditioned with 3 mL of methanol and 3 mL of water. The treated sample was loaded onto the SPE cartridge. The conical tubes contained samples were washed by 1 mL water and loaded in SPE cartridges. The cartridge was rinsed with 10 % methanol (3 mL), completely dried for two hours and extracted with 3 mL of methanol in new 15 mL conical tube. Extracted solution in conical tube was dried using nitrogen gas (99.999 %) purging. Empty conical tube was spiked 70 % methanol (100 μL) for reconstitution and mixed well. Reconstitution samples were centrifuged (10000 rpm, 10 min) and taken 80 μL of supernatant liquid.

The levels of DEHP-d_4 metabolites were measured using liquid chromatography – tandem mass spectrometry with an AB Sciex 4000 tandem

mass spectrometer (Framingham, MA, USA) coupled to the Shimazu HPLC system (Kyoto, Japan). HPLC analysis was performed on a Shicheido CAPCELL PAK C18 ACR column (2.0 x 150 mm ID, 3 μm) and the column maintained at 35 °C in a thermostatic column oven. The mobile phase was 0.1 % acetic acid in water (solvent A) and in acetonitrile (solvent B) with flow rate of 0.2 mL/min. Gradient condition was applied; 0.0 - 2.5 min, 10 % B; 2.5 - 3.0 min, 10 - 30 % B; 3.0 - 5.0 min, 30 - 40 % B; 5.0 - 7.0 min, 40 % B; 7.0 - 11.0 min, 40 - 55 % B; 11.0 - 12.0 min, 55 - 100 % B; 12.0 - 16.0 min, 100 % B and 16.1 - 20.0 min, 10 % B. The MS/MS system was used in ESI- mode. An injection volume of 5 μL was used and data acquisition was performed using Analyst 1.5.2 software (AB Sciex, USA).

5. Method validation and QA/QC

The method validation of analytical methods was performed at three days because of confirming reliability to each method and was made into low and high concentration (LQ and HQ, 1 and 10 ng/mL in urine) spiked standard chemical working solution. The precision and accuracy ranges were 2.5 - 4.2 % and 93.8 - 103.3 %, respectively.

We used experimenter urine for QA/QC because of not effected exposure to DEHP-d_4. The QA/QC urine samples were prepared the same as validation and real samples and analyzed each batch. The precision and accuracy ranges were 9.7 – 17.2 % and 94.1 – 104.3 %, respectively. In this study, because results of MEHP-d_4 weren't statistical confidence, it excluded from data interpretation. All method validation and QA/QC results represent supplement information including air and urine data (Table S1 and S2).

6. Exposure model and fractional urinary excretion

Estimated intake dose through external data in this case air monitoring data was calculated using exposure model (Eq. 1). Other exposure route was not considered because of the assumption that subjects were only exposed DEHP-d_4 via inhalation.

$$ID_a = C_a \times IR \times ET \qquad \text{(Eq. 1)}$$

Parameters of Eq. 1 means that ID_a (μg) is estimated intake dose (absorption amount) via inhalation using personal air sample; C_a (μg/m^3) is measured concentration of DEHP-d_4 in air using personal air sample; IR (0.49 m^3/h) is inhalation rate of 20's male at rest (KEFH, 2007) and ET (0.08 h, 5 min) is exposure time.

The fractional urinary excretion (F_{ue}) of the excreted amount of the metabolites relative to the DEHP-d_4 was calculated by Eq. 2. It simply means that a molecular dose ratio of the metabolites related to the intake dose of DEHP-d_4.

$$F_{ue}(\%) = \frac{ID_a}{A_m} \times \frac{MW_m}{MW_D} \times 100 \qquad \text{(Eq. 2)}$$

Parameters of Eq. 2 means that F_{ue} (%) is molecular fractional urinary excretion of DEHP-d_4 and the metabolites measured in this study; A_m (μg) is total cumulative excreted amount of the metabolite in urine and MW_D

(μg/μmol) is molecular weight of DEHP-d_4 and MW_m (μg/μmol) is molecular weight of metabolites.

Ⅲ. Results

1. Subjects

The demographic characteristics for seven subjects are summarized in Table 1. The subjects aged greater than 18 years, with a body mass index between 18.4 and 24.0 kg/m^2.

Table 1. Demographic characteristics of seven subjects

Subject	Age (yr)	Body weight (kg)	Height (cm)	BMI (kg/m^2)
A	27	72	184	21.3
B	25	80	183	23.9
C	22	50	165	18.4
D	19	69	165	25.3
E	25	58	170	20.1
F	20	71	176	22.9
G	25	76	178	24.0
Mean ± SD	23 ± 3	68 ± 10	174 ± 8	22.3 ± 2.5

Note: All subjects were male and Korean.

2. Time-profile of particulate matters and concentration of DEHP-d_4 in air

Figure 1 illustrates the time-profile of $PM_{1.0}$ and $PM_{2.5}$ mass concentration in exposure room measured using Portable Aerosol Spectrometer (PAS). Five minutes before spraying (5 min), subjects entered the exposure room and sat. After two minutes of spraying (12 min), PMs concentration was the highest level. About seventy percent of PMs concentration was decreased after spraying for five minutes by adjusting background level (15 min). The trend of the time profile of $PM_{1.0}$ using SidePak was similar to PAS results (data not shown).

As shown in the Figure 1, single spraying of exposure solution was the major determinants for the amount of DEHP-d_4 in air sample. Time-weighted average concentrations of DEHP-d_4 in air calculated by area air sample were dropped with time (Figure 2). Therefore, exposure time was determined as five minutes to calculate the concentration of DEHP-d_4 in air using personal air sample on the basis of time-profile of PMs and DEHP-d_4 concentration of area air sample.

Table 2 represents the DEHP-d_4 concentration of air measured using personal and area air sample for five minutes after spraying of DEHP-d_4 solution. The air concentration measured using personal and area air sample was 1.73 ± 0.42 mg/m^3 and 0.78 ± 0.03 mg/m^3, respectively (Table 2).

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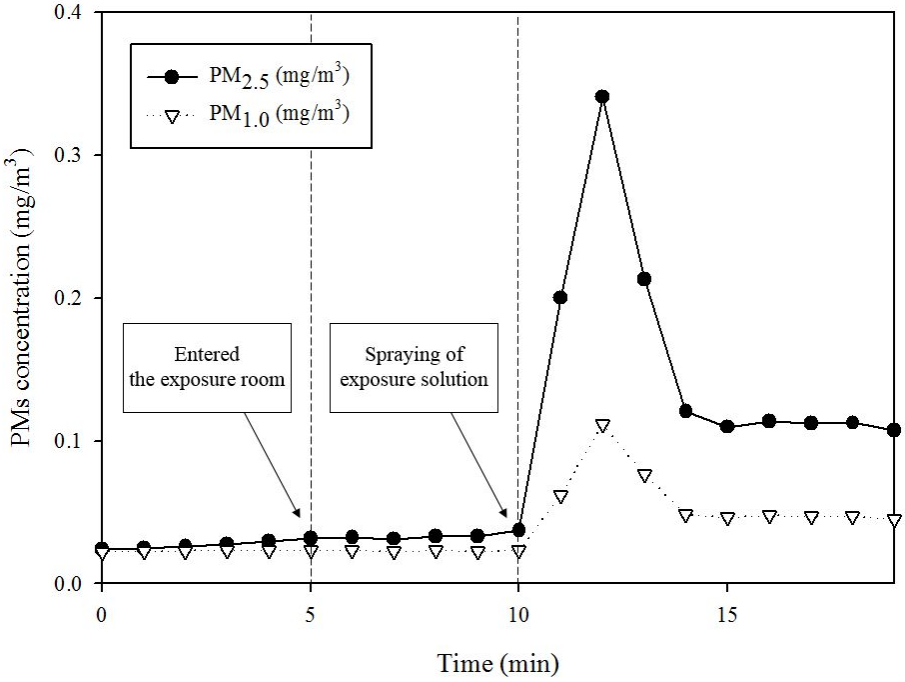

Figure 1. Time-profile of particulate matters concentration in air of exposure room for spraying of DEHP-d_4 solution.

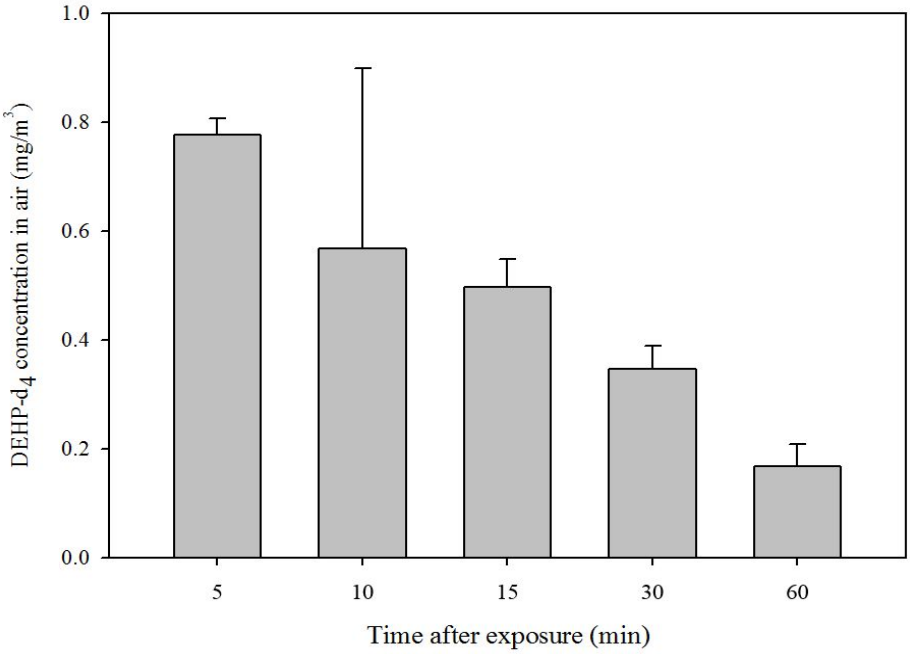

Figure 2. Time-weighted average concentration of DEHP-d_4 in air after spraying of DEHP-d_4 solution. Each data point represents the arithmetic mean and error bar denotes standard deviation (n=2).

Table 2. Air concentration of DEHP-d_4 in personal and area air sample for the first five minutes after spraying

Subject	DEHP-d_4 concentration in air (mg/m^3)
Personal air sample (n=7)	
A	0.94
B	1.58
C	1.96
D	1.97
E	1.61
F	1.78
G	2.28
AM ± SD	1.73 ± 0.42
Area air sample (n=2)	
AM ± SD	0.78 ± 0.03

3. Cumulative excreted amount in urine

Cumulative urinary eliminated amounts were derived multiplying urinary volume by the concentration of each metabolite - MEHHP-d_4, MEOHP-d_4 and 5cxMEPP-d_4. For 48 hours after exposing, the arithmetic mean and the standard deviation were 0.8 ± 0.5, 1.6 ± 0.4 and 3.3 ± 0.9 μg (0.003 ± 0.002, 0.005 ± 0.002 and 0.011 ± 0.003 μmol) for MEHHP-d_4, MEOHP-d_4 and 5cxMEPP-d_4, respectively (Table 3).

4. Estimation of intake dose and fractional urinary excretion

Table 3 shows that intake dose of DEHP-d_4 via inhalation estimated by exposure model (Ep. 1), which is used air concentration calculated by air sampled at subjects' breathing-zone. Estimated intake dose of DEHP-d_4 was 70.8 ± 17.3 μg (0.180 ± 0.044 μmol, AM ± SD).

The fractional urinary excretion calculated by bio-sample in this study (Eq. 2). Because the level of estimated intake dose of DEHP-d_4 and cumulative excreted amount of the metabolites in urine differed from individual to individual, the fractional urinary excretion of the metabolites were different from each person. The range of MEHHP-d_4, MEOHP-d_4 and 5cxMEPP-d_4 were 0.56 – 2.70 %, 2.13 – 6.47 % and 3.79 – 13.9 %, respectively.

Table 3. Air DEHP inhaled, urinary metabolites of DEHP, and the fractional urinary excretion

Subjects	Inhaled DEHP[a] (μmol)	Cumulative excreted amount in urine[b] (μmol)			Fractional urinary excretion[c] (F_{ue}, %)		
		MEHHP-d_4	MEOHP-d_4	5cxMEPP-d_4	MEHHP-d_4	MEOHP-d_4	5cxMEPP-d_4
A	0.098	0.002	0.006	0.014	2.06	6.47	13.9
B	0.164	0.001	0.004	0.010	0.56	2.59	6.18
C	0.203	0.003	0.005	0.008	1.67	2.25	3.89
D	0.204	0.002	0.006	0.011	0.77	3.00	5.50
E	0.167	0.002	0.004	0.006	1.30	2.13	3.79
F	0.185	0.003	0.005	0.011	1.72	2.95	5.79
G	0.236	0.006	0.008	0.014	2.70	3.40	5.91
Aver	0.180	0.003	0.005	0.011	1.54	3.26	6.42
SD	0.044	0.002	0.002	0.003	0.74	1.49	3.42

[Notes] [a]: calculated by Eq. 1; ID_a = (DEHP-d_4 concentration) × (inhalation rate) × (exposure time) / (molecular weight),

[b]: sampled for 48 hr after exposure to DEHP-d_4 and [c]: calculated by Eq. 2; F_{ue} = (ID_a /total cumulative excreted amount of the metabolites in urine) × (molecular weight of DEHP-d_4 / molecular weight of the metabolite) × 100.

Ⅳ. Discussion

We performed the first human study about DEHP exposure via inhalation to suggest fractional urinary excretion (F_{ue}) of inhalation exposed to DEHP. Inhalation exposure route avoids first-pass effect, which was degradation of a chemical in the liver before it reaches the systemic circulation, unlike ingestion (Kwon et al., 2008 and Antosova et al., 2009). We expected that F_{ue} of inhalation was higher than F_{ue} of ingestion. However F_{ue} of inhalation was about 2-to-20 times lower than previous studies exposed via ingestion. Nevertheless, F_{ue} of present study should simply utilize to conduct exposure assessment even though it included many uncertainty such as unknowingness of true intake dose and assumptions.

If DEHP mostly exposed via inhalation, to apply F_{ue} of oral exposure may not calculate intake dose reflected to reality. Therefore, we recommend to use the F_{ue} of inhalation exposed to DEHP instead of using F_{ue} in certain cases. Fong (2014) was conducted the study that comparison of intake dose using exposure- and bio-model to PVC production workers who were mainly exposed to DEHP via inhalation. The result was shown that estimated intake dose using bio-model was lower than exposure model in some case. Representatively, it may not be proper using of F_{ue} calculated by oral exposure of DEHP because biomonitoring is the reflected all exposure pathway

(NRCNA, 2006). The dose-reconstruction with exposure biomarkers concentration were conducted dividing total excreted molar amount of metabolites by F_{ue}. To use of F_{ue} calculated by oral exposure means that we should be possible to underestimate the intake dose of exposed DEHP via inhalation because of lower F_{ue} of present study than others.

In the present study, type of excreted metabolites was same with previous studies and the dominantly excreted metabolite in human urine was 5cxMEPP-d_4 followed by MEOHP-d_4 and MEHHP-d_4. The total cumulative excreted amount of 5cxMEPP-d_4 was four times higher than MEHHP-d_4. On the contrary, many biomonitoring studies including studies of artificial exposure to DEHP via ingestion and intravenous reported that the predominant metabolites were MEHHP or 5cxMEPP followed MEOHP and MEHP in order (Koch et al., 2004 , 2005 and 2011; Becker et al,. 2004 and 2009; Boas et al., 2010; Anderson et al., 2011; Guo et al., 2011a and 2011b; Kasper-Sonnenberg et al., 2012; Kim et al., 2014 and Cao et al., 2016). Furthermore, the ratio of each metabolites was not similar with the literature value. However, the excreted pattern of the metabolites in Kessler et al. (2012) was not accord with many previous studied, although same exposure route (ingestion). These difference wasn't clearly explained. Anderson et al. (2011) showed that three metabolite of DEHP had no statistically significant effects of gender and MEHHP and 5cxMEPP were detectable effects of exposure dose of DEHP. We supposed that the excreted pattern of DEHP metabolites

could be effected on many factors such as exposure route and concentration, sex and race etc. and need to further study.

According to the results for time-profile of particulate matter data (Figure 1), sprayed aerosol using the compressible spray was settled on surface (table and floor) of enclosed exposure room in five minutes. We supposed that sprayed DEHP-d_4 droplets almost existed as particle phase in air and were exposed to the subjects in a short time (about 2 - 3 min) and it confirmed time-weighted average concentrations of DEHP-d_4 in air calculated using area air sample were dropped with time as seen Figure 2.

We investigated intake dose of inhaled DEHP-d_4 and fractional urinary excretion of the metabolites at single spraying. We could find that inhalation exposure to DEHP was not higher than expected. These results needed to carefully use on exposure assessment of inhalation exposed to DEHP, because there were a lot of uncertainties and assumptions. Limitation of present study was only performed on 20's Korean male. Therefore, further study demand to conduct study of exposure various condition such as sex, age and exposure duration, frequency, concentration and phase of DEHP. In conclusion, we recommend to utilize the F_{ue} of inhalation exposed to DEHP instead of using F_{ue} of ingestion in limited condition.

Ⅴ. Conclusions

We conducted human study of DEHP exposure via inhalation. When single spraying of DEHP, aerosols were mostly particle phase and settled down in five minutes. We calculated 70.8 μg of estimated intake dose using environmental monitoring data (ID_a) and suggested fractional urinary excretion (F_{ue}) of inhalation, which was about 2 - 20 times lower than F_{ue} of ingestion. It means that dose estimation of DEHP with F_{ue} of ingestion might underestimate the actual intake amount via inhalation. Therefore, we recommended that the dose-reconstruction with exposure biomarkers concentration in biological samples should use F_{ue} of inhalation when was similar condition with present study. In conclusion, the F_{ue} of present study should be utilizable as basic data to conduct exposure assessment of DEHP via inhalation when people mainly exposed to DEHP formed particulate matters in industrial field or somewhere.

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Ⅶ. Supplementary information

Dose reconstruction from urinary biomarkers in a human panel exposed to bis (2-ethylhexyl) phthalate via inhalation

Table S1. Intra and inter-day precision and accuracy of DEHP-d_4 and their four metabolites for method validation (*n*=9)

Table S2. Precision and accuracy of DEHP-d_4 and their four metabolites for QA/QC during sample analysis

Table S1. Intra and inter-day precision and accuracy of DEHP-d_4 and their four metabolites for method validation (n=9)

Metrix	Analyte	Nominal Concentration (ng/mL)	Precision (%)		Accuracy (%)
			Intra	Inter	
Air	DEHP-d_4	100	4.1	4.0	98.5
		200	3.2	3.5	103.2
		1600	2.8	2.8	100.0
Urine	MEHP-d_4	1	18.4	20.6	63.0
		10	11.5	16.8	67.5
	MEHHP-d_4	1	3.7	4.1	98.8
		10	2.9	3.2	99.7
	MEOHP-d_4	1	4.2	4.1	93.8
		10	3.0	3.3	98.6
	5cxMEPP-d_4	1	3.1	3.1	101.7
		10	2.5	2.3	103.3

Table S2. Precision and accuracy of DEHP-d_4 and their four metabolites for QA/QC during sample analysis

Metrix	Analyte	N	Nominal Concentration (ng/mL)	Precision (%)	Accuracy (%)
Air	DEHP-d_4	10	200	8.7	97.1
Urine	MEHP-d_4	30	1	28.9	76.1
		30	10	27.4	80.9
	MEHHP-d_4	30	1	17.2	104.3
		30	10	9.7	99.2
	MEOHP-d_4	30	1	10.6	94.1
		30	10	9.8	101.8
	5cxMEPP-d_4	30	1	10.0	98.5
		30	10	10.5	105.0

국문초록

요 중 바이오마커를 이용한 DEHP의 인체 흡입 노출량 추정

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일반 인구집단에서 DEHP 주로 식음료, 먼지 등의 섭취가 주요 노출경로로 알려져 있지만, DEHP가 함유된 생활화학용품을 사용하는 경우 흡입이나 피부접촉을 통한 노출이 일어난다고 보고되고 있다. 그럼에도 불구하고 아직까지 DEHP에 흡입 노출을 평가할 수 있는 연구는 이루어져 있지 않다. 따라서 본 연구의 목적은 1) 노출모델을 이용하여 DEHP 흡입 노출량을 추정하고, 2) DEHP 흡입노출 시 소변 중 배출율을 산출하여, 이를 구강섭취 노출로 산출된 타 문헌의 소변 중 배출율

을 비교하는 것이다. 본 연구는 한국 성인남성 7명을 대상으로 압축식 스프레이를 이용하여 DEHP-d_4 용액을 분사하여 흡입노출 시켰고, 피험자의 호흡 반구에서 채취한 공기 시료와 노출 후 48시간 동안 배출되는 모든 소변을 채취하여 DEHP와 그 대사산물을 분석하였다. 공기 중 DEHP-d_4의 시간가중평균 농도는 1.73 ± 0.42 mg/m^3 이었고, 노출 후 48시간 동안 소변을 통하여 배설되는 대사산물의 누적 배출량은 MEHHP-d_4 0.8 ± 0.5 μg, MEOHP-d_4 1.6 ± 0.4 μg 그리고 5cxMEPP-d_4 3.3 ± 0.9 μg 이었다. 공기 시료의 농도를 이용한 노출모델을 통하여 추정된 노출량은 평균 70.8 μg 이었고, 이와 소변으로 배출된 대사산물의 양을 이용하여 산출한 소변 중 배출율을 계산하였으며 MEHHP-d_4 1.54 ± 0.74 %, MEOHP-d_4 3.26 ± 1.49 % 그리고 5cxMEPP-d_4 4.62 ± 3.42 % 이었다. 소변 중 대사산물의 경우, 구강섭취 노출되었던 타 연구에서 배출된 대사산물의 종류와 동일하였으나, 단회의 스프레이를 통한 흡입 노출되었던 본 연구에서는 배출되는 수준이 보다 약 2 - 20 배 가량 낮았고 배출되는 비율이

달랐다. 본 연구의 결과는 산업현장 같은 DEHP 흡입노출이 주로 발생하는 경우, DEHP 흡입 노출평가를 위하여 제한적으로 사용될 수 있는 기초데이터로 활용될 수 있을 것으로 여겨진다.

주요어: Bis (2-ethylhexyl) phthalate, 디에틸헥실프탈레이트, 노출모델, 생체모델, 노출량, 소변 중 배출율

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