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[원 저]

DEHP, DEP and DBP Exposure Analysis using Urinary Metabolites of Gyonggi Province University Students

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ABSTRACT

Objectives: Phthalates are used as plasticizers in polyvinyl chloride (PVC) plastics. As phthalate plasticizers are not chemically bound to the PVC, they can leach, migrate or evaporate into indoor air and atmosphere, foodstuffs, other materials, etc. Therefore, humans are exposed through ingestion, inhalation, and dermal exposure over their entire lifetime, including during intrauterine development. In particular, university students have a great number of opportunities to contact products including phthalates during campus life (food packaging, body care products, cosmetic, lotions, aftershave, perfume etc.). The purpose of this study was to examine levels of phthalate exposure as undergraduate students begin to use pharmaceuticals and personal care products including phthalates.

Methods: Phthalate metabolites, mono-ethyl phthalate (MEP), mono-n-butyl phthalate (MnBP), mono-isobutyl phthalate (MiBP), mono-2- ethylhexyl phthalate (MEHP), {(mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP}, and mono-(2-ethlyl-5-oxohexyl) phthalate (MEOHP} were examined. 80 urine samples collected from university students were analyzed using LC/MS/MS(API 4000, Applied Bioscience) with on-line enrichment and column-switching techniques. This study was carried out at Y university located in Gyonggi Province from 2008 to 2011.

Results: The detection limit of phthalate metabolites were 0.03 ng/mL for MEP, 0.11 ng/mL for MnBP, 0.08 ng/mL for MiBP, 0.93 ng/mL for MEHP, 0.19 ng/mL for MEOHP and 0.16ng/mL for MEHHP. MnBP showed the highest urinary levels (median: 31.6 ug/L, 24.8 ug/g creatinine (cr)). Concentrations were also high for MEHHP (median: 24.1 ug/L, 19.0 ug/g cr), followed by MEOHP (median: 22.8 ug/L, 17.9 ug/g cr). In individual cases, the maximum level reached up to 348 ug/L, and 291 ug/g cr, respectively. The urinary and creatinine adjusted levels of MEP were lower than those for DBP and DEHP metabolites, but were higher in 95th percentiles. As a result, the mean daily DEP intake value was 2.3 μg/kg bw/day, 3.5 μg/kg bw/day for DEHP and 4.9 μg/kg bw/day for DBP.

Conclusion: These students' phthalate exposure levels were below the international safe level set by the EU, but higher than the 2012 KFDA survey of the age group from 3 to 18.

Keywords: Phthalates, university student, daily intake, urine, metabolites

I. Introduction

Humans are exposed to various environmental contaminants, including phthalic acid diesters, commonly known as phthalates.[1)]

Phthalates are well known for their high production volume, a broad range of application, and ubiquity in environment. Because of their widespread use, all

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populations of people, domestic animals, and wildlife regularly encounter opportunities for exposure to phthalate.[2,3)]

In Korea, 4.0×10^5 tons of di-2- ethylhexyl phthalate (DEHP) was produced and 1.0×10^4 tons of di-n-butyl phthalate (DBP) was produced in 2002. In 2006, 4.4×10^5 tons of DEHP was produced and 1.0×10^5 tons is used and 7.7×10^3 tons of DBP was produced and 1.9×10^3 tons is used. The production of DEHP was increased whereas DBP was decreased.[4)]

Phthalate esters have adverse effects on liver, kidney, and the reproductive system, and act as weak endocrine disrupting agents.[5)] DEHP exerted toxic effects on the female and male reproductive system and interfered with the development of offspring.[6-8)] DBP has a lower toxic potency than DEHP with mainly developmental effects in rodents.[9)] Two studies provide preliminary evidence that the urinary concentrations of phthalate monoesters were associated with damage in human sperm and reduction in sperm motility as well as with shortened anogenital distance in male infants.[10,11)]

An assessment of the internal phthalate exposure is generally possible by measuring the amount of specific metabolites excreted via urine.[2,12,13)] For these biomonitering purpose specific metabolites of the phthalates, preferably in urine, can used. Since all phthalates are rapidly metabolized by cleavage of one or both of the two ester groups, metabolites like the primary monoesters are the obvious choice for analysis. However, even determination of those metabolites is susceptible to contamination, since they might be generated out of the parent diesters by various processes besides human metabolism, like chemical, enzymatical, microbiological, or photolytical hydrolysis.[12)]

At present a few studies review children and human exposure effects by various sources, but there are few studies for phthalate exposure of university students.

Since starting the university, undergraduate students take an interest in their physical appearance therefore they plentifully used body-care products (shampoo, body lotion, hair spray, wax and gel etc). Especially, female students frequently used to make-up products such as cosmetics, perfumes and lipsticks. And university students often eat fast-food that was put in plastic vessels and vinyl wraps. This reason include menu choices, cost, convenience, taste, socializing with friends, and a chance to get out.[14,15)]

The purpose of this study was to examine the level of phthalate exposure because the undergraduated students start to use the pharmaceuticals and personal care products (PPCP) including phthalates.

II. Materials and Method

1. Urine sample collection

The urine samples were independently collected in the university. More than eighty university students (19-27 years old) participated in this study and only 80 were sampled. They were not occupationally exposed to phthalates. They were living in the city of Seoul and the nearby suburban. A single spot urine sample was collected in 250 ml beaker, and then separated into two glass tube and stored at -20°C until analysis. Travel blanks were prepared and treated similarly.

2. Chemicals

Authentic standard substances (purity 99%), mono-ethyl phthalate, mono-n- butyl phthalate, mono-isobutyl phthalate, mono-2- ethylhexyl phthalate, mono-(2-ethlyl-5- oxohexyl) phthalate and mono-(2-ethyl-5-hydroxyhexyl) phthalate were obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA) as well as authentic isotopically labeled standard substances, mono-ethyl phthalate (MEP-1,2$^{13}C_2$, 99%), mono-n-butyl phthalate (MnBP-1,2$^{13}C_2$, 99%), mono-isobutyl phthalate (MiBP-1,2$^{13}C_2$, 99%), mono-2-ethylhexyl phthalate (MEHP -1,2$^{13}C_2$, 99%), mono-(2-ethlyl-5-oxohexyl) phthalate (MEOHP-1,2$^{13}C_2$, 99%) and mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP -1,2$^{13}C_2$, 99%).

Acetonitrile and water (>99.8%, LC/MS grade) were purchased from Burdick and Jackson (Honeywell international Inc, USA). Ammonium acetate (>97.0%) was purchased from sigma (Sigma-Aldrich Inc, USA). -glucuronidase enzyme solution (200units/ml, from *Escherichia coli* K12) was purchased from Roche Diagnostics (Mannheim, Germany).

3. Sample preparation

Each urine sample was thawed and vortex mixed, and 0.5 ml was transferred into a brown glass test

tube previously heated, cooled and acetone-washed. The sample was spiked with the labeled internal standard aqueous solution (20 μl, 1 μg/ml), ammonium acetate aqueous solution (100 μl, 1 M), and β-glucuronidase enzyme solution (10 μl, 200 units/ml). The sample was then incubated at 37°C for 2 hour in drying oven. The incubated sample was vortex mixed and then sonicated and the treated sample was mixed in 6:1 (sample : acetonitrile) and separated by centrifugation. Through a syringe filter (0.2 μm, nylon), the sample was transferred to a glass auto-sampler vial. A method blank and a spiked urine sample for reference were also analyzed in parallel with unknown samples in each batch.

4. LC-MS-MS analysis

Liquid chromatography was carried out on a Nanospace SI-2 HPLC apparatus with switching valve (auto sampler, quaternary pump, vacuum degasser). The HPLC equipped a pre-column (MF C8, 4.6×50 mm, 5 μm, Shiseido), trap-column (Cadenza C18, 2.0×30 mm, 5 μm, Imtakt) and analytical column (Cadenza C18, 2.0×75 mm, 3 μm, Imtakt). We used 0.1% formic acid (solvent A) and acetonitrile added 0.1% formic acid (solvent B) as a mobile phase with a constant flow rate (0.6 ml/min), and 20 μl of sample was injected into a HPLC column using an auto sampler.[32)]

For each analyte at least two specific parent daughter ion combinations were monitored with one combination being used for quantification and the other(s) for verification. Parameters of the HPLC-MS/MS methods for the analytes and its internal standards were used as Kho *et al.*.[32)]

5. Data analysis

1) Detection and measurement

Identification of the phthalate monoester was based on matching with the retention time of the corresponding labeled internal standard. Analytes were quantified using isotope dilution mass spectrometry. Two personal computer-based instrument software programs, Analyst® Software v1.5 (Applied Biosystem, U.S.), were used to acquire and process the data obtained. Integration, calibration, and quantification were carried out using these software programs.

2) Estimation of daily intake

The measured concentrations of phthalate metabolites in the urine samples were then translated into the daily intakes of the corresponding diesters. We assumed steady-state exposure, as in the previously published study.[16)] We employed the conversion model equation (1) as previously report.[16,17)] ME is the creatinine adjusted concentration of each phthalate monoester (mg/g cr), CE is the personal daily creatinine excretion rate normalized by individual body weight (g/kg/day), Fue is the molar fraction of the urinary excreted monoester related to the ingested diester, and MWd and MWm are the molecular weights of the phthalate diesters and monoesters:

$$\text{Daliy Intake} = \frac{\text{ME} \times \text{CE}}{\text{Fue} \times 1000} \times \frac{\text{MW}_\text{d}}{\text{MW}_\text{m}} \quad (1)$$

In the Equation. (1), CE is creatinine excretion and was set to be 18 mg/kg/day for women and 23 mg/kg/day for men.[17)] Fractional urinary excretion (Fue) values were provided by studies for the excretion of metabolites.[18,19)] The values were 0.13 for MEP, 0.13 for MEHP, 0.23 for MEHHP and 0.15 for MEOHP. For MBP we assumed the equal excretion factor as for MEP.

3) Statistical analysis

Considering the skewed distribution of the urinary phthalate monoesters, a natural log-transformation of data was used. The data were presented as range, median, 95th percentile, GM and GSD for the measurements and analyzed using the statistical software package Statistical Analysis System (SAS) version 8 (SAS Institute Inc., Cary, NC, USA). The Mann & Whitney U test (Wilcoxon rank-sum test) were applied to compare phthalate metabolite levels between men and women and one way ANOVA was applied to compare those among the age groups. A spreadsheet program (Excel 2003) was used for the table style database and for calculating some of the statistics.

III. Results

1. Metabolites in urine

The urinary concentrations and creatinine adjusted of the phthalate metabolites measured are given in Table 1 and 2. Among the phthalate metabolites measured in this study, MnBP level showed the highest urinary levels (median: 31.6 ug/L, 24.8 ug/g cr). The

Table 1. The concentration (ug/L) of phthalate metabolites measured in urine samples of university students

Phthalate	Metabolites	N	sex	GM	50P	95P	Range
		80	sum	16.2	12.5	188.0	0.7~537.0
DEP	MEP	40	male	10.4	9.9	53.3	0.7~122.0
		40	female	25.3	22.4	227.0	1.4~537.0
		80	sum	20.6	19.6	49.6	5.5~220.0
DEHP	MEHP	40	male	19.9	18.4	78.5	5.5~220.0
		40	female	21.2	20.8	40.4	9.0~67.6
		80	sum	24.1	25.5	67.8	1.8~348.0
	MEHHP	40	male	25.2	27.7	55.7	4.5~348.0
		40	female	23.1	23.2	67.8	1.8~83.3
		80	sum	22.8	23.2	67.5	2.0~291.0
	MEOHP	40	male	22.7	22.9	49.9	3.7~291.0
		40	female	22.8	23.8	67.5	2.0~79.6
		80	sum	31.6	35.2	84.5	4.5~204.0
DBP	MnBP	40	male	29.2	31.3	68.3	5.7~83.1
		40	female	34.2	36.0	115	4.5~204.0
		80	sum	8.4	8.9	22.4	1.4~44.5
	MiBP	40	male	8.1	8.1	22.2	1.4~44.5
		40	female	8.7	9.6	22.4	1.4~25.1

Urine samples were available for 80 university students. In all urine samples the DEP metabolite MEP, DEHP monoester MEHP and secondary metabolites MEOHP, MEHHP and DBP metabolites MnBP, MiBP were detectable respective LOQs. The detection limit of phthalate metabolites were 0.03 ng/mL for MEP, 0.11 ng/mL for MnBP, 0.08 ng/mL for MiBP, 0.93 ng/mL for MEHP, 0.19 ng/mL for MEOHP and 0.16 ng/mL for MEHHP, respectively.

Table 2. The creatinine adjusted concentration (ug/g) of phthalate metabolites measured in urine samples of university students

Phthalate	Metabolite	n	Detectable (%)	GM	GSD	50P	95P	Range
DEP	MEP	80	100	12.7	3.7	8.8	152.0	0.8-291.0
DEHP	MEHP	80	100	16.1	2.0	16.3	53.1	4.8-173.0
	MEHHP	80	100	19.0	1.8	20.0	48.4	4.1-138.0
	MEOHP	80	100	17.9	1.8	17.5	53.9	4.0-115.0
DBP	MnBP	80	100	24.8	1.8	24.9	53.1	2.5-118.0
	MiBP	80	100	6.6	1.8	6.6	17.4	1.5-27.6

concentrations were high for MEHHP (median : 24.1 ug/L, 19.0 ug/g cr), followed by MEOHP (median : 22.8 ug/L, 17.9 ug/g cr). In individual base, we observed maximum level reached up to 348.0 ug/L, 291.0 ug/g cr, respectively. The urinary and creatinine adjusted levels of MEP were lower than those of DBP and DEHP metabolites but were higher in 95th percentile.

We can compare the urinary phthalate metabolite levels determined in our samples with those determined in population studies conducted previously in Germany and the USA (Table 3). MEP value (median 12.5 ug/L) was lower compared with USA (median 181 and 188 ug/L) levels. MnBP (35.2 ug/L) and MiBP (8.9 ug/L) values were lower compared with Germany children (MnBP 57.4 ug/L, MiBP 31.9 ug/L) but higher slightly compared to the level of USA (MnBP 19.1~20.7, MiBP 2.4~4.9 ug/L). MEHP (19.6 ug/L), MEOHP (23.2 ug/L), MEHHP (25.5 ug/L) level of this study were higher compared

Table 3. Comparison between urinary excretion(ug/L) of the phthalate metabolites in the German and Korea generation population

Study	CDC NHANES (2005)		Wittassek *et al.* (2007)		CDC NHANES (2009)		This study	
Country	USA		Germany		USA		Korea	
Sampling year	2001-2002		2001/2003		2003-2004		2008	
N (age)	1683(>20)		120(20-29)		1534(>20)		80(19-27)	
	50P	95P	50P	95P	50P	95P	50P	95P
MEP	181	2720.0	-	-	188.0	2980.0	12.5	188.0
MnBP	19.1	95.4	57.4	338.0	20.7	108	35.2	84.5
MiBP	2.4	16.3	31.9	132.0	3.9	19.9	8.9	22.4
MEHP	4.1	39.5	5.0	28.6	1.7	29.5	19.6	49.6
MEOHP	12.2	115.0	13.4	42.3	12.4	139.0	23.2	67.5
MEHHP	17.7	175.0	14.6	58.6	18.4	225.0	25.5	67.8

Table 4. Daily intakes (μg/kg bw/day) of phthalate metabolites measured in urine samples of university students

Chemicals	Male (n=40)			Female (n=40)			Total (n=80)		
	GM	GSD	Range	GM	GSD	Range	GM	GSD	Range
MEP	1.5	2.8	0.2-30.7	3.5	3.8	0.6-46.2	2.3	3.5	0.2-46.2
MEHP	3.5	1.9	1.2-43.0	3.6	1.9	1.1-17.2	3.5	1.9	1.1-43.0
MEHHP	2.3	1.7	0.8-18.2	2.1	1.9	0.4-6.4	2.2	1.8	0.4-18.2
MEOHP	3.3	1.7	1.0-23.6	3.2	1.9	0.7-9.9	3.2	1.8	0.7-23.6
MnBP*	4.5	1.6	2.2-12.5	5.2	1.9	0.4-20.4	4.9	1.7	0.4-20.4
MiBP	1.3	1.8	0.3-6.1	1.3	1.7	0.4-3.5	1.3	1.8	0.3-6.1

* $P<0.05$ (one-way analysis of variance for daily intakes).

with USA (1.7~4.1, 12.2~12.4, 17.7~18.4 ug/L respectively) and Germany (5.0, 13.4, 14.6 ug/L).

2. Daily intake estimations

From the urinary phthalate metabolite levels we extrapolated daily intakes of the parent phthalates (DEP, DEHP. DBP), individually for each subject (Table 4). Overall, we found that DBP showed the highest daily intake values (mean : 1.3-4.9 μg/kg bw/day), follow by DEHP (mean : 2.2-3.5 μg/kg bw/day) and the daily intake of DEP was 2.3 μg/kg bw/day.

But DEP had about 2~8 times high daily intakes than those of DEHP and DBP in 95th percentile value. And we compared the daily intakes obtained with exposure limit values deduced by US EPA (EPA, 1990, 1991, 1993). The daily intakes of DEP, DBP were clearly lower than the RfD of US EPA (800 ug/kg bw/day, 100 ug/kg bw/day). The maximum intake value for DEHP of 43.0 ug/kg bw/day was 2 time higher than the RfD of US EPA and 2.5% (2 subjects out of 80) exceeded.

We compared the daily intakes of the different phthalate metabolites between the gender (Table 5). Female subjects had higher daily intakes for the DBP which can be calculated from the MnBP metabolites ($P<0.05$). The major routes of phthalate exposure are thought to be ingestion through dietary or dermal or respiratory sources.

In MEP, 22~22 age group had highest daily intake and 3 time higher at median than those of 25~27 age group. MnBP was 3~4 times higher than those of MiBP in all age groups. At median, 23~24 age group had highest daily intake in MEHHP and MEOHP and 21~22 age group was the highest in MEHP.

IV. Discussions

The concentrations were highest for MEHHP (median: 24.1 ug/L, 19.0 ug/g cr), followed by MEOHP (median: 22.8 ug/L, 17.9 ug/g cr). In individual cases we observed maximum levels reaching up to

Table 5. Daily intakes (μg/kg bw/day) of phthalate metabolites measured in urine samples of university students classified by age

phthalate	age	N	Daily intake (ug/kg bw/day)			
			GM(GSD)	Median	P95	Range
MEP	19-20	18	1.8(4.0)	1.5	27.7	0.2-40.0
	21-22	24	3.3(3.4)	3.6	20.7	0.7-30.7
	23-24	21	3.0(3.8)	2.8	40.7	0.5-46.2
	25-27	17	1.2(2.2)	1.1	4.1	0.3-4.3
MEHP	19-20	18	3.4(1.5)	3.7	6.3	1.5-6.5
	21-22	24	3.5(2.0)	4.2	10.1	1.2-13.8
	23-24	21	3.9(2.3)	3.1	17.2	1.1-43.0
	25-27	17	3.4(3.4)	3.3	8.7	1.5-10.8
MEHHP	19-20	18	2.1(1.9)	2.1	5.3	0.4-6.4
	21-22	24	2.2(1.9)	2.3	5.4	0.5-5.6
	23-24	21	2.4(1.6)	2.6	5.0	0.9-5.5
	25-27	17	2.2(2.0)	2.4	6.5	0.8-18.2
MEOHP	19-20	18	3.2(1.9)	3.1	7.9	0.6-9.9
	21-22	24	3.2(2.0)	3.2	8.7	0.8-9.4
	23-24	21	3.5(1.6)	3.6	7.1	1.6-7.4
	25-27	17	3.0(1.9)	3.0	9.2	1-23.6
MnBP*	19-20	18	4.4(1.7)	4.4	9.0	1.1-10.5
	21-22	24	4.8(1.9)	5.5	7.9	0.4-9.9
	23-24	21	6.4(1.6)	5.7	13.1	2.5-20.4
	25-27	17	4.0(1.5)	4.0	8.1	2.3-12.5
MiBP	19-20	18	1.2(1.9)	1.4	2.6	0.4-3.6
	21-22	24	1.4(1.8)	1.4	3.0	0.5-6.1
	23-24	21	1.4(1.7)	1.3	3.1	0.5-3.5
	25-27	17	1.1(1.8)	1.0	2.4	0.3-4.5

* $P<0.05$ (one-way analysis of variance for daily intakes).

348 ug/L, 291 ug/g, respectively, These observations are similar to those of Wittassek *et al*..[34] In our study, we found low amounts of MnBP and MiBP with a 15-fold lower level of MnBP and MiBP compared to the NHANES 2001-2002, 2003-2004 data. The difference of the results cause by different pattern and exposure pathways of DEP in the USA population. And the DBP metabolite levels in our study were higher than those of NHANES 2005, 2009, whereas were lower than those of Wittassek *et al*..[34] Apart from the result of Wittassek *et al*.,[34] the medians for the our data were higher than those of NHANES 2005, 2009, but were lower in 95th percentile. This may be caused by a different extent of exposure, but may also be due to different origin or age distribution of the study populations.

In generally female subjects had higher daily intakes for the DBP which can be deduced from the MnBP metabolites ($P<0.05$). These observations confirm the findings of Koch *et al*.[13] and Wittassek *et al*.,[34] who estimated elevated values of DBP for females as well. Women had higher daily intake values also DEP, but the difference was not significant. The major routes of phthalate exposure are thought to be ingestion through dietary sources, but recent evidence suggests that dermal absorption may also be a major route of exposure for people.[28,29]

Dermal absorption from phthalate -containing personal care products such as sunscreen, perfume, shampoo, and cosmetics may be an important route of exposure for university students. Several animal studies were reported that slow phthalate absorption and toxicity[30] but dermal absorption studies in humans are a few. Janjua and colleagues (2007) recently conducted a study of dermal absorption in healthy adults males and found a significant increase in MEP and MBP metabolites several hours after

Table 6. Daily intakes (μg/kg bw/day) of phthalates by the German and Korea university students deduced from urinary metabolite concentrations.

Study	Wittassek *et al*.[34]				This study	
Country	Germany				Korea	
Sampling year	1988-1993		2001/2003		2008	
n(age)	240(20-29)		119(20-29)		80(19-27)	
	50P	95P	50P	95P	50P	95P
DEP	-	-	-	-	1.6	25.8
DEHP	4.2[a]	12.6[a]	2.7[a]	6.4[a]	3.5[b]	10.5[b]
DBP	6.9	22.2	2.2	7.3	4.9	9.9

a Value based on MEHP, 5OH-MEHP, 5oxo-MEHP; metabolic conversion factor of Koch *et al*. (2005) applied.
b Value based on MEHP; metabolic conversion factor of Anderson *et al*. (2001) applied.

Table 7. Comparison of the daily intake value calculated in our study with those of Koch *et al*.(2000) and Wittassek *et al*.

Study	Koch *et al*.[17] (NHANES III, 1988-1994)		Wittassek *et al*.[34] Koch *et al*.[35]		This study	
n(age)	289(20-60)		239(2-14)		80(19-27)	
	50P	95P	50P	95P	50P	95P
DEP	12	110	-	-	1.6	25.8
DEHP	0.71[c]	3.6[c]	4.3[d]	15.2[d]	3.5[b]	10.5[b]
DBP	1.5[a]	7.2[a]	4.1	14.9	4.9	9.9

a Sum of DnBP and DiBP
b Value based on MEHP; metabolic conversion factor of Anderson *et al*. (2001) applied.
c Value based on MEHP; metabolic conversion factor of Peck and Albro (1982) applied.
d Value based on MEHP, 5OH-MEHP, 5oxo-MEHP; metabolic conversion factor of Koch *et al*. (2005) applied.

application of phthalate containing personal care products on the skin. These authors concluded that systemic uptake through skin is likely to be an important route of exposure for humans.[31]

The daily intake (ug/kg bw/day) of the DEP, DBP, DEHP are presented for different age groups in Table 6. Except for 25-27 age group, geometric means of all phthalate metabolites increase with increasing age. 21-22 and 23-24 groups were the highest daily intake in all phthalates.

We compared the daily intake of phthalates between the German and Korea university students (Table 6). The daily DEHP intakes estimated for 240 students (subset 1988-1993) in German were higher level than our data, whereas the value for 119 students (subset 2001, 2003) were somewhat lower. The estimated daily intakes for DBP were similar to those that compared our data with those of DEHP. These finding indicated that the Korean university students had higher DEHP and DBP exposure in comparison to the Germany. The differences in internal exposure between our study group and the German students are probably caused by different degrees of external exposure and also by differences in sampling location and time. The daily intake level of university students in our study differs from previously literatures for the children and adult by Koch *et al*.[17] and Wittassek *et al*.[34] (Table 7).

The 2011 survey of KFDA reported that phthalate exposure level of age group from 3 to 18, total 1,030 childrens were DEHP 2.75 ug/kg bw/day, DBP 1.22 ug/kg bw/day, respectively. The international safe level of phthalates from EU were DEHP 50 ug/kg bw/day, DBP 10 ug/kg bw/day. This study level were below the safe level.

The DEHP daily intakes of university students in this study was higher compared to the adult,[13] whereas lower than those of children,[34] Koch *et al*..[35] The daily DBP intake of university students were similar to children in median, but higher in 95th percentile. These finding indicated that children had the highest DEHP and DBP daily intakes,

follow by university students. And DEP was the highest in adult.

We quantified six monoesters, three DEHP metabolites, two DBP metabolites, and one DEP metabolite in undergraduate students urine sample. Incidentally, because of the problem of contamination and the high blank values, phthalate diesters determinations have only been applicable for determining phthalates in highly exposed subjects. Therefore monoesters of phthalates are generally more suitable as parameters for phthalate exposure, since they are primary metabolites of the diesters and specific to the respective parent phthalate diester.[12,20,21)]

Those monoesters, however, are also subject to contamination in the analytical and preanalytical phase as described above. During sample collection, transportation and the analytical sample preparation phthalate monoesters might be generated out of the ubiquitously present phthalate diesters through simple chemical, microbiological, or enzymatical ester cleavage and not human metabolism. Blount *et al.*,[12)] determined various monoester metabolites of the most applied phthalates.

MBP has a structural isomer, MiBP. MiBP and MBP were chromato- graphically inseparable in the present study. The complete chromatographic separation of MBP and MiBP can be achieved using a lower flow rate and a gentle slope gradient,[22)] or by using gas chromatography with a capillary column.[23)]

Achieving complete chromatographic separation of MBP and MiBP using these methods should be attempted in the future.

In the present study, we measured MEHP, MEHHP and MEOHP as an analytical standard substance. However, MEHP, the primary metabolite of DEHP, is not always our best choice as a biomarker for exposure to DEHP. Secondary metabolites of DEHP are also more important biomarkers for exposure to DEHP according to earlier studies.[24,25,26,27)]

These secondary metabolites of DEHP can be more suitable than MEHP because they are excreted in much higher concentrations in human urine (2.9 times higher), they show longer halflife times of elimination and they are not susceptible to any source of contamination.

We have to point out that this study does not represent the general university students in Korea because of the small sample size and limited geographic area.

V. Conclusion

We measured MEP, MnBP, MiBP, MEHP, MEOHP and MEHHP in urine of eighty university students (20-27 years old).

We found that MnBP the highest urinary levels (median: 31.6 ug/L, 24.8 ug/g cr), and the urinary levels of MiBP was clearly lower than those of other metabolites. The concentrations were high for MEHHP (median : 24.1 ug/L, 19.0 ug/g cr), followed by MEOHP (median : 22.8 ug/L, 17.9 ug/g cr). We observed maximum levels reached up to 348.0 ug/L, 291.0 ug/g cr, respectively. The urinary and creatinine adjusted level of MEP was lower than those of DBP and DEHP metabolites but the 95th percentile level of MEP was higher than DBP or DEHP.

Compared to the data of former studies, the daily DEHP and DBP intakes estimated for 240 students (subset 1988-1993) in German were higher compared to this data. And the daily DEHP and DBP intakes estimated for children in German were higher than those of university students in Korea, whereas the value for general population were somewhat lower. And compared to the data of adults, university students were higher than the adults. Female students had higher daily intakes for the DBP which can be calculated from the MnBP metabolites ($P<0.05$). This college student levels of phthalates were lower than the international safe level of EU, but somewhat higher than children of Korea.

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