Phthalate Exposure In Turkish Children Aged 8-9 Years In Konya

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Abstract. The present study aimed to investigate the concentrations of five phthalate metabolites in the urine samples from children aged 8-9 years from Konya in the central region of Turkey and to determine the effect of residence and gender on the concentrations of the metabolites. Children were selected by household sampling. Urine samples were collected in glass vials and were stored at −20 °C until analysis. Phthalate metabolites including mono-n-butyl phthalate (MnBP), monomethyl phthalate (MMP), monoethyl phthalate (MEP), monobenzyl phthalate (MzBP), mono-2-ethylhexyl phthalate (MEHP) were measured using LC–MS/MS. Enrolled sample was consisted of 587 children, 65.8% from urban area, 50.3% male. Measurements above the limit of quantification (LOQ) was 99.8% in MnBP, 97.6% in MzBP, 98.6% in MEP, 100% in MEHP and only 4.1 % in MMP. MnBP, MzBP, MEP, and MEHP was detected in urine with a median (25p-75p) concentration of of 106.9 (65.3-186.3), 10.5 (5.3—19.2), 26.8 (12.7-51.4), 20.9 (12.6-35.6) µg/g-creatinine, respectively. There was significant differences between rural and urban residence, whereas no gender difference was observed. Urinary concentration of MnBP, MzBP were found to be lower in urban areas, however, MEHP was higher. Findings indicated that phthalate exposure varied by types of phthalates, residence of children.

Keywords: Phthalates, metabolites, exposure, urine, children

1. Introduction

Children are exposed to a wide range of man-made environmental chemicals, including phthalates. The phthalates have the potential for harmful effects to child health including developmental and reproductive toxicity (Bergman *et al*. 2013; Braun *et al*. 2013; Wallner *et al*. 2016). Children might be exposed to phthalates easily by the routes of ingestion, inhalation, transdermal and transplacental ways. Measuring and following the concentrations of chemicals or their breakdown byproducts is of public health relevance (Angerer *et al*. 2006; Jones *et al*. 2015). Human biomonitoring (HBM) has been practiced for several years in several countries (Černá *et al*. 2015; CDC, 2015; Exley *et al*. 2015; Frederiksen *et al*. 2011; Haines *et al*. 2016; Kasper-Sonnenberg 2014; Rocha *et al*. 2017; Schwedler *et al*. 2017; Wang *et al*. 2015), however, there is no represensative published data in Turkey. This work is the first part of a larger study, CEKSA Biomonitoring Study that focuses on environmental toxicities from children aged 8-9 years in the central regions of Turkey (two regions of NUTS-II).

The present study aimed to investigate the concentrations of five phthalate metabolites in the urine samples from children aged 8-9 years from Konya in the central region of Turkey and to determine the effect of residence and gender on the concentrations of the metabolites.

2. Methods

Konya is the biggest city in the Central Anatolia Region of Turkey and is the seventh-most-crowded city in Turkey. As of 2014, Konya had a population of 1,174,536.

The survey was a representative cross-sectional study for rural and urban areas in Konya. Turkish Statistical Institute (TURKSTAT) calculated sample size and selected "primary sampling units (the blocks of 25 household lists)" by child population density. Selected household lists were checked for children aged 8-9 years by a field operation. After written informed consent was obtained from one parent, children were enrolled for the survey.

The study was approved by an ethical committee of the Faculty of Medicine Hacettepe University.

Urine samples from enrolled children were collected in glass vials in the morning and were stored and frozen at −20 °C until analysis.

Concentrations of urinary phthalate metabolites including mono-n-butyl phthalate (MnBP), monomethyl phthalate (MMP), monoethyl phthalate (MEP), monobenzyl phthalate (MzBP), mono-2-ethylhexyl phthalate (MEHP) were measured according to methods published previously (Koch *et al*., 2003; Kasper-Sonnenberg *et al*., 2012) using 1 mL aliquos. Briefly, after enzymatic hydrolysis of the conjugates with arylsulfatase-free ß-glucuronidase (from Escherichia coli K12), the samples were analysed with multidimensional liquid chromatography tandem mass spectrometry (LC/LC–MS/MS, Applied Biosystems; Foster City, CA. API-3200, AA14100B04).

All samples were analyzed in duplicate. The limit of detection (LOD) for MMP, MEP, MnBP, MBzP, and MEHP were 0.5, 0.4, 1.2, 0.4, and 0.4 µg/L, respectively. The limit of quantitation (LOQ) for MMP, MEP, MnBP, MBzP, and MEHP were 1.5, 1.2, 3.6, 1.2, and 1.2 µg/L, respectively. Internal quality control was performed by analysing control urine with a low and high concentration. The total mean recovery of each metabolite was between 90.6-108.1%.

Urine creatinine levels were analyzed using an kinetic colorimetric assay based on the Jaffé method (Taussky 1954).

Urinary phthalate metabolite concentrations are expressed either as a concentration (µg/L) or as a creatinine-corrected value (µg/g of creatinine).

The samples with phthalate metabolite concentrations higher than the LOQ by more than 75% were included in this study. Concentrations below the respective LOQ were set to LOQ/√2 for data treatment. Urinary phthalate metabolite concentrations were all log transformed because they were highly skewed to right side.

Geometric mean and 95% confidence intervals (95% CI) of unadjusted and creatinine-adjusted urinary phthalate concentrations were calculated. Correlation between the log transformed metabolites was evaluated with Pearson correlation coefficients. Student t-tests was carried out to check for differences in the geometric mean phthalate metabolite concentrations in residence (urban/rural) and gender (male/female). Data analysis was performed using SPSS, version 23.0.

3.Results

Enrolled sample consisted of 587 children, 65.8% from urban area, 50.3% male.

All analytes were detected in more than 97% of the study population except for MMP (4.1 % > LOD). Measurements above the limit of quantification (LOQ) was 99.8% in MnBP, 97.6% in MzBP, 98.6% in MEP, 100% in MEHP (Table 1). MnBP, MEP, MEHP, and MzBP, were detected in urine with GM [95 CI] concentrations of 93.6 [88.6-98.9], 23.1 [21.3-25.1], 19.1 [18.1-20.0] and 8.9 [8.2-9.5] µg/L, respectively. Urinary MnBP levels were between 200-299 µg/L in 7.4% of cases and more than 300 µg/L in 1.3% of cases.

Urinary concentration of MnBP, MzBP were found to be lower in urban areas, however, MEHP was higher in urban areas than rural areas (Table 2). Urinary MnBP levels were more than 200 µg/L in 9.3% in males and 8.1% in females.

No gender difference was found for any particular metabolite (Table 2). Urinary MBP levels were more than 200 µg/L in 4.7% for urban area and 18.2% for rural area.

Table 1. Urinary levels of phthalate metabolites in spot urine samples from Turkish children in Konya (n=587)

				Percentiles				
	%>LOQ	mean	GM [95 CI]	25	50	75	95	Range
Urinary phthalate metabolites (µg/L)								
MnBP	99.8	113.4	93.6 [88.6-98.9]	66.1	98.2	148.0	243.6	2.5-394.0
MEP	98.6	39.0	23.1 [21.3-25.1]	12.2	25.1	46.4	95.4	<LOQ-1600.0
MEHP	100	23.0	19.1 [18.1-20.0]	11.9	18.5	30.3	54.7	4.3-87.2
MBzP	97.6	12.9	8.9 [8.2-9.5]	5.1	8.8	16.9	36.3	<LOQ -118.0
MMP	4.1			<LOQ	<LOQ	<LOQ	<LOQ	<LOQ -27.5
Creatinine-corrected phthalate metabolites (µg/g-creatinine)								
MnBP		143.0	106.8 [100.6-113.9]	65.3	106.9	186.3	375.7	1.5-1322.1
MEP		51.8	26.4 [24.1-28.8]	12.7	26.8	51.4	165.0	0.6-2069.9
MEHP		30.5	21.8 [20.4-23.2]	12.6	20.9	35.6	78.7	2.8-290.8
MBzP		16.6	10.1 [9.4-11.0]	5.3	10.5	19.2	55.9	0.5-272.4

GM, geometric mean; CI, confidence interval; MMP, monomethyl phthalate; MEP, monoethyl phthalate; MnBP, mono-n-butyl phthalate; MEHP, mono (2-ethylhexyl) phthalate; MBzP, monobenzyl phthalate.

Table 2. Urinary levels of creatinine-corrected phthalate metabolites (geometric mean [%95 CI]) in spot urine samples from Turkish children in Konya (n=587) by region and gender.

	Urban	Rural		Male	Female	
	GMT [95% CI]	GMT [95% CI]	p	GMT [95% CI]	GMT [95% CI]	p
n	386	201		295	292	
MnBP	99.4 [91.5-108.0]	122.5 [11.3-134.9]	0.001	107.3 [98.0-117.5]	106.2 [97.0-116.4]	0.876
MEP	25.5 [22.8-28.6]	28.1 [24.4-32.4]	0.308	25.5 [22.5-29.0]	27.2 [24.0-30.9]	0.482
MEHP	23.1 [21.3-25.2]	19.3 [17.6-21.2]	0.001	21.1 [20.4-24.6]	22.4 [20.4-24.6]	0.949
MBzP	9.3 [8.3-10.3]	12.0 [10.7-13.3]	0.004	10.1 [9.0-11.3]	10.1 [9.1-11.3]	0.383

GMT, geometric mean titre; CI, confidence interval; MMP, monomethyl phthalate; MEP, monoethyl phthalate; MnBP, mono-n-butyl phthalate; MEHP, mono (2-ethylhexyl) phthalate; MBzP, monobenzyl phthalate.

There are correlations between the concentrations of the metabolites. MnBP was moderately correlated with MeHP ($p=0.454$, $p<0.001$), MEP ($p=0.452$, $p<0.001$), MBzP ($p=0.522$, $p<0.001$). There were also moderate correlation between MeHP and MBzP ($p=0.401$, $p<0.001$). Low-moderate correlations were present between MeHP and MEP ($p=0.358$, $p<0.001$), and between MEP and MBzP ($p=0.337$, $p<0.001$).

4. Discussion

We detected MnBP, MEP, MEHP and MBzP in > 97% of the samples demonstrating widespread exposure to these phthalates in children in Konya, Turkey. The median concentration of MnBP in the present survey was higher than those found in children in other countries (Rocha *et al*. 2017). MnBP was the highest and dominant compound among the studied metabolites whether the data was adjusted by creatinine or not. Similar to our study, China (Wang *et al*. 2015; Wu *et al*. 2017), Germany (Kasper-Sonnenberg *et al*. 2014) and Denmark (Frederiksen *et al*. 2011), Sweeden (Larsson *et al*. 2014) and Belgium (Geens *et al*. 2014) found MnBP as a dominant compound in these five metabolites. This might be attributed to the wide use of DBP. However, MEP was the highest metabolite in USA (CDC, 2015), Canada (Haines *et al*. 2016), Brazil (Rocha *et al*. 2017). Health based guidance values are available for MEP, MBzP, MnBP and for different combinations of DEHP metabolites (Aylward *et al*., 2009; Schulz *et al*., 2012b; Apel *et al*., 2016). As a limitation of study, one DEHP metabolite, MEHP metabolite was analyzed.

The detection rate of MMP was lower than those reported for other countries. This might be limited use of DMP.

For risk assessement, reference values, minimal risk levels (MRLs) and tolerable daily intakes (TDIs) and biomonitoring equivalents (BEs) for some contaminants were defined (Schulz *et al*., 2012; Apel *et al*., 2016). However, adverse health effects can not be excluded above these values. BE based on EFSA TDI for MnBP was calculated as 200 µg/L. In the present study, 8.7% of the children had concentrations of MnBP that exceeded health based guidance values, 200 µg/L, indicating reasons for concern. MnBP metabolites of children aged 6-11 years were higher than 200 µg/L in 12.2% in GerES IV (Schulz *et al*., 2012; Apel *et al*., 2016) and 4.2% in DEMOCOPHES (Schwedler *et al*., 2017). German Environmental Specimen Bank (ESB) samples from 1988 to 2015 showed high MnBP levels in 14% (Koch *et al*., 2016). however, these exceedances mainly occurred in urine samples collected before 2002.

Reference values (RV95) for metabolites of MnBP and MBzP phthalates in urine of children were reported to be 300 µg/L and 75 µg/L, respectively (Apel *et al*., 2016). Only, 1.3% of cases had MnBP levels higher than 300 µg/L and 0.3% had MzBP levels higher than 75 µg/L in the present study.

Among the metabolites analyzed, there was significant differences between rural and urban residence, whereas no gender difference was observed in the present study. In the literature, contradictory results have been reported regarding gender and regions. Living in the rural area in Sweeden was associated with significantly higher levels of MBzP, MnBP and MEP in children compared to living in the urban area (Larsson *et al* 2014), however, cases living in rural area in Spain had lower MEP levels (Cutanda *et al*. 2015) and MMP and MBP in urban area were significantly higher than those in the rural area in China (Wu *et al*. 2017). On the other hand, there was no regional difference in Korea (Ha *et al*. 2014). Gender specific differences were observed for MnBP and MBzP in USA (Silva *et al*., 2004) and in Denmark (Frederiksen 2013), for MBP in only univariate analysis in Korea (Ha *et al*., 2014) and for MEP in Brazil (Rocha *et al*. 2017). However, no gender difference was found in the German GerES IV study (Becker *et al*., 2009). Findings indicated that phthalate exposure varied by types of phthalates, residence of children, possibly affected by different lifestyles.

The observed significant positive correlations within samples between urinary metabolites in the present study have also previously been observed in a German study (Becker *et al*., 2009), Denmark study (Frederiksen *et al*., 2011) and Belgium (Geens, *et al*. 2014). They indicate that children exposed to high levels of one phthalate diester tend to be highly exposed to other phthalates as well, possibly due to simultaneous exposure to mixtures of phthalates present in consumer products.

As a conclusion, contact with phthalates is of considerable importance in our area and continuing biomonitoring surveillance is warranted.

Acknowledgments

This survey was funded by TÜBİTAK SBAG-107S407.

Conflict of interest: none declared.

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