



Urinary phthalate metabolites in relation to childhood asthmatic and allergic symptoms in Shanghai

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ABSTRACT

Background: Few studies can be found on phthalate exposure in relation to childhood asthma and allergic symptoms from Mainland China, where a persistent increase in prevalence of childhood asthma and allergic disease has been observed.

Objectives: This study aimed to assess the exposure levels to phthalates and its relationship with asthmatic and allergic symptoms among children in Shanghai, which has the highest prevalence of childhood asthma in Mainland China.

Methods: A follow-up study (2013–2014) of 434 children aged 5–10 years was conducted, based on the China, Children, Homes, Health (CCHH) study (2011–2012) in Shanghai, China. Information on asthmatic and allergic symptoms (wheeze, rhinitis, and eczema) were collected using validated questionnaires. Ten phthalate metabolites in morning urine samples were analyzed by high-performance liquid chromatography with triple quadrupole tandem mass spectrometry (HPLC-MS/MS). Multivariable logistic regression was used to estimate the associations between symptoms and urinary phthalate metabolites controlling for demographics, family history of allergic diseases and other covariates.

Results: Nine out of 10 phthalate metabolites were detected in all subjects (average detection rate of 93.2%). By multivariable logistic regression analyses, the 4th quartile of Mono-*n*-butyl phthalate (MnBP) (reference: 1st quartile) had adjusted prevalence odds ratios (aPOR_s) and 95% confidence intervals (95%CI_s) of 2.27(1.06–4.88), 2.14(1.02–4.46) and 2.98(1.19–7.50) for wheeze, rhinitis and eczema, respectively, while those of Mono-isobutyl phthalate (MiBP) were 2.23(1.08–4.62) and 2.96(1.02–8.60) for rhinitis and eczema, respectively. The highest quartile of mono-2-ethyl-5-hydroxyhexyl phthalate(MEHHP) and mono-2-ethyl-5-oxohexyl phthalate(MEOHP) had aPOR_s and 95%CI_s of 3.10(1.10–8.74) and 2.63(1.02–6.80) for eczema, respectively. By summing up the 4 low molecular weight metabolites (Σ_4 LMWP) and all 9 metabolites (Σ_9 Total), the highest quartiles of Σ_4 LMWP and Σ_9 Total were significantly associated with all symptoms. In most of the above associations, a significantly increasing trend from the 1st to the 4th quartile was observed. Subjects with 2 or 3 concomitant symptoms (reference: no symptoms) had significant positive associations with a higher level (the 4th quartile) of phthalate metabolites.

Abbreviations: MMP, Mono-methyl phthalate; MEP, Mono-ethyl phthalate; MiBP, Mono-isobutyl phthalate; MnBP, Mono-*n*-butyl phthalate; MBzP, Mono-benzyl phthalate; MCHP, Mono-cyclohexyl phthalate; MEHP, Mono-2-ethylhexyl phthalate; MECPP, Mono-2-ethyl-5-carboxypentyl phthalate; MEHHP, Mono-2-ethyl-5-hydroxyhexyl phthalate; MEOHP, Mono-2-ethyl-5-oxohexyl phthalate; DMP, Dimethyl phthalate; DEP, Diethyl phthalate; DiBP, Di-isobutyl phthalate; DnBP, Di-*n*-butyl phthalate; BBzP, Butyl benzyl phthalate; DCHP, Dicyclohexyl phthalate; DEHP, Di-2-ethylhexyl phthalate; Σ_4 DEHP, Sum of MEHP, MEHHP, MEOHP and MECPP; Σ_4 LMWP, Sum of low-molecular-weight phthalates (MMP, MEP, MiBP and MnBP); Σ_5 HMWP, Sum of high-molecular weight phthalates (MEHP, MEHHP, MEOHP, MECPP and MBzP); Σ_9 Total, Sum of all 9 phthalate metabolites; PAEs, Phthalate esters; HPLC-MS/MS, High performance liquid chromatography with triple quadrupole tandem mass spectrometry; LOD, Limit of detection; CI_s, Confidence intervals

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Conclusions: Low MW metabolites such as MnBP and MiBP, high MW DEHP and the total amount of phthalate metabolites might have adverse health effects on asthma and allergic symptoms in Chinese children.

1. Introduction

The prevalence of asthma and allergic diseases has been rapidly increasing among young children in China (Huang et al., 2015; Pearce et al., 2007; Yangzong et al., 2012; Zhang et al., 2013). Among other cities, Shanghai has been ranked in top place in the prevalence of childhood asthma within the last three decades. In 1990, the prevalence of diagnosed asthma was 1.50% among children aged 0–14 years (yrs) in Shanghai. In 2000, it increased to 3.34%, and in 2010, it increased to 5.73% (Chen, 2004; Chen, 2013). Among others, preschool children aged 3–6 yrs had the highest prevalence of diagnosed asthma in Shanghai.

Exposure to environmental risk factors is considered as a predominant contributor to the increase in childhood asthma prevalence and morbidity (Burbank et al., 2017; Deng et al., 2016; Mendell, 2007). Phthalate esters (PAEs), a family of synthetic chemicals widely used in commercial products as plasticizers, have been implicated as one of the potential chemicals affecting asthma and allergic disorders in children (Jaakkola and Knight, 2008). Although exposure to PAEs can occur through different pathways including ingestion, inhalation and skin contact (Braun et al., 2013; Wittassek et al., 2011), measuring the urinary levels of PAE metabolites was considered as a feasible and objective method in evaluating the total exposure levels to PAEs (Guo et al., 2011).

Based on the measurements of urinary levels, positive reports on the associations between PAE metabolites and childhood asthmatic and allergic disorders or symptoms have been published in recent years, in Norway (Bertelsen et al., 2013), Spain (Gascon et al., 2015), Belgium (Franken et al., 2017), Taiwan (Hsu et al., 2012; Ku et al., 2015; Wang et al., 2014) and America (Whyatt et al., 2014). On the other hand, negative or non-significant results have also been reported on the associations between urinary metabolites of PAEs and childhood asthma and allergic sensitization, including studies of children aged 3–5 yrs in Denmark (Beko et al., 2015; Callesen et al., 2014a) and children aged 6–17 yrs in America (Hoppin et al., 2004).

To our knowledge, no data have been reported on the urinary phthalate exposure in relation to childhood asthmatic and allergic symptoms in Mainland China. On the other hand, some recent studies reported certain phthalate metabolites, including Mono-*n*-butyl phthalate (MnBP) (95.7 µg/L vs. 76.9 µg/L) and Mono-2-ethylhexyl phthalate (MEHP) (12.3 µg/L vs. 2.7 µg/L) (Wu et al., 2017), Mono-methyl phthalate (MMP) (7.9 µg/L vs. 4.9 µg/L) (Wang et al., 2015) was at higher urinary levels in Chinese children (aged 5–7 yrs and 8–11 yrs) than those in Sweden (aged 6–11 yrs) (Larsson et al., 2014) and Germany (aged 5–9 yrs) (Kasper-Sonnenberg et al., 2012). Furthermore, compared with children living in rural areas, urban children had higher levels (Wu et al., 2017).

The objectives of the current study were first to quantify and assess the urine phthalate exposure levels in children who lived in Shanghai. The second aim was to analyze the associations between asthma and allergic symptoms and phthalate metabolite exposure levels. This study will contribute by filling the knowledge gap regarding phthalate exposure and health effects on asthma and allergic diseases in Chinese children.

2. Material and methods

2.1. Study design and participants

This cross-sectional study collected health information via a

questionnaire survey and analyzed PAE metabolite levels in morning urine samples. The questionnaire survey was a 2-year follow-up survey (2013–2014) based on the baseline questionnaire used in Shanghai ($N = 16,948$) in 2011–2012 as part of the China, Children, Homes, Health (CCHH) project in China (Hu et al., 2014; Huang et al., 2016). In the follow-up survey, those who never moved to another home and neither had their homes decorated nor renovated (neither the home interior surface including wall/floor material was renovated/repainted nor new large furniture was added) since the baseline survey were invited to participate. After obtaining written informed consent from parents, a questionnaire on asthma and allergic diseases or symptoms was administered to parents and children's morning urine samples were collected. A total of 454 children were invited and 434 children (95.6%) finally participated in the questionnaire survey or contributed urine samples. As in the baseline survey, the children in the follow-up study were from 6 administrative districts in Shanghai – 4 urban districts (Yang-Pu district, Hong-kou district, Jing-An district, and Zha-Bei district) and 2 suburban ones (Feng-Xian district and Bao-Shan district) (Hu et al., 2014; Huang et al., 2016). There was a smaller population and less traffic in suburban areas than in urban areas, but no differences in nationality or life habits. The average annual gross domestic product of these two suburban areas (77 billion RMB) was lower than that in the urban areas (85 billion RMB) in 2013. The study was approved by the Ethical Committee of the School of Public Health, Fudan University (International Registered Number: IRB00002408 & FWA 00002399).

2.2. Questionnaire

The questions on children's health were essentially the same as in the baseline survey and were from the core questionnaire of the International Study of Asthma and Allergies in Childhood (ISAAC). Three symptoms were mainly assessed, “Have you ever had wheezing during the past 12 months?”, “Have you ever had sneezing, runny or stuffy nose without cold during the past 12 months?” and “Have you ever had eczema during the past 12 months?” They were termed as ‘current wheezing,’ ‘current rhinitis’ and ‘current eczema’ in this study. In addition to each individual symptom of interest, the number of concomitant symptoms were calculated (none, 1 symptom only, and ≥ 2 symptoms) and applied to classify children into groups with different number of concomitant symptoms.

Demographic and socio-demographic information was collected including gender, age, body mass index (BMI), family history of asthma and allergic diseases, area of residence (urban or suburban district), maternal education, breastfeeding time and annual household income. To the above questions, the answer ‘yes’ was considered to be positive and the answer ‘no’ was considered negative. The answer ‘I don't know’ was considered as missing data.

Information on children's home environment was collected both in the questionnaire and by on-site inspection. The questions were developed based on the previous questionnaire used in the “Dampness in Buildings and Health” study conducted in Sweden (Bornehag et al., 2004) and the ALLHOME study conducted in Bulgaria (Naydenov et al., 2008), and was adapted to the local Chinese setting (Zhang et al., 2013). Information on building characteristics, indoor environment and lifestyles was collected, as well as tobacco smoking at home (yes/no), furry pets inside the home (yes/no), floor materials in children's bedrooms (wood/parquet or others), wall materials in children's bedrooms (paint/wallpaper or tiles/lime), and visible moisture in the house (yes/no).

2.3. Urine sample collection and analyses

A total of 434 morning urine samples (each around 50 mL) were successfully collected with the help of parents. First, they were collected into clean glass containers and then stored at 4 °C in a refrigerator until transferred into an amber glass bottle within 6 h. All the glass containers were prewashed with acetonitrile and methanol. Internal standards were added and samples were treated with glucuronidase before being transferred and frozen at −40 °C in 2 mL polypropylene tubes until analysis.

Ten phthalate metabolites including MMP, Mono-ethyl phthalate (MEP), Mono-isobutyl phthalate (MiBP), MnBP, Mono-benzyl phthalate (MBzP), Mono-cyclohexyl phthalate (MCHP), MEHP, Mono-2-ethyl-5-carboxypentyl phthalate (MECPP), Mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP) and Mono-2-ethyl-5-oxohexyl phthalate (MEOHP) were analyzed in this study. The full list of 10 metabolites and their parent phthalates are shown in Table S1. Creatinine concentrations were analyzed to correct for urine dilution (Wang et al., 2015). Creatinine was determined using a Roche Cobas C501 biochemical analyzer (Roche Diagnostics, Basel, Switzerland). Ten metabolites and corresponding isotopically labelled internal standards ($^{13}\text{C}_4\text{MMP}$, $^{13}\text{C}_4\text{MEP}$, $^{13}\text{C}_4\text{MiBP}$, $^{13}\text{C}_4\text{MnBP}$, $^{13}\text{C}_4\text{MBzP}$, $^{13}\text{C}_4\text{MCHP}$, $^{13}\text{C}_4\text{MEHP}$, $^{13}\text{C}_4\text{MECPP}$, $^{13}\text{C}_4\text{MEHHP}$, and $^{13}\text{C}_4\text{MEOHP}$) were purchased from Cambridge Isotope Laboratories (Andover, MA, USA).

2.3.1. Sample glucuronidase pretreatment

A previously published method (Liu et al., 2012) was used as a reference method to analyze the concentrations of urinary phthalate metabolites. An aliquot (1.0 mL) of urine was spiked with 20 μL of stock solution containing 500 $\mu\text{g/L}$ of 10 mixed internal standard and then acidified with 1 mL sodium acetic acid (pH 6.5). The mixture was incubated with *Escherichia coli* β -glucuronidase (Sigma-Aldrich, St. Louis, MO, USA) enzymes at 37 °C overnight on a shaking water bath for deconjugation. After enzymatic deconjugation, solid-phase extraction (SPE) was applied to separate the phthalate metabolites from other urinary components. The separation was performed by loading the samples into Oasis HLB column (Waters, USA) which had been previously activated with 2 mL acetonitrile and 3 mL of aqueous sodium phosphate (pH 2.0). After neutral or basic compounds were washed with 2 mL methanoic acid (0.1 mol/L) and 1 mL pure water (high-performance liquid chromatography [HPLC] level, CNW, Germany), the SPE material was vacuumed for 30 min. The target phthalate metabolites were eluted with 2 mL acetonitrile and 2 mL ethyl acetate. The eluate was concentrated to dryness with weak nitrogen in a 37 °C water bath and then dissolved to 200 μL with aqueous 50% (v/v) acetonitrile.

2.3.2. Instrumental analysis

HPLC coupled with triple quadrupole tandem mass spectrometry (HPLC-MS/MS, Waters Corp., USA) was applied to measure the target compounds. Five microliters of pretreated samples were injected onto a Hypersil Gold Phenyl C18 column (1.9 μm , 100 mm $\times$ 2.1 mm; Thermo Fisher, USA). The following solvents were used for liquid chromatographic separation: 0.1% acetic acid in water (A) and 0.1% acetic acid in acetonitrile (B). Solvent programming involved 0–7.8 min, 20–50% B; 7.8–9.0 min, 50–100% B; 10.5–10.51 min, 100–20% B. The total programming time was 12 min. The column temperature was set to 30 °C.

Target compounds were analyzed under negative ion mode. Nitrogen was used as the desolvation gas. The spray voltage was set to 2.2 kV, and the capillary temperature was set to 350 °C. Multiple reaction monitoring (MRM) mode was used. More specific settings of the instrumental analyses are listed in Table S2.

Laboratory blank samples were prepared by mixing urine samples obtained from eight anonymous healthy donors (total volume 1 L). After adding the activated carbon powder in a ratio of 1% to the mixed urine, the mixture was stirred at 4 °C overnight, and then filtered to

remove the activated carbon to obtain the blank samples.

2.3.3. Quality assurance

For each batch of 24 samples, 3 procedural blank samples and 6 matrix spiked samples at two different concentrations (10 ng/mL and 50 ng/mL) were processed. Substitution of certain materials that might contain phthalates were made to eliminate phthalate contamination from the laboratory environment. If a blank had > 5 ng/mL of any phthalate metabolite, all the analytical results for that day were rejected until the source of contamination was identified and eliminated. The laboratory provided values for the phthalate congeners measured in three blank cartridges analyzed along with morning urine samples. MiBP, MnBP, MECPP, MEHP, and MBzP were detected in procedural blanks, and the average blank values for these metabolites were 1.51, 1.22, 2.03, 1.03, and 0.19 ng/mL, respectively. The final concentrations of these compounds in samples were determined as their measured levels minus the average blank values. The limit of detection (LOD) was determined at the point when the measured levels were at least 3 times higher than the average signal levels of all blank samples (Wang et al., 2015). The SPE recoveries and relative standard deviations of target phthalate metabolites ranged from 72.7% to 122.5% and from 1.5% to 9.1% at 10 ng/mL, and from 72.7% to 102.1% and from 2.2% to 16.0% at 50 ng/mL, respectively. The mixture of isotopically-labelled internal standards ($^{13}\text{C}_4\text{MMP}$, $^{13}\text{C}_4\text{MEP}$, $^{13}\text{C}_4\text{MiBP}$, $^{13}\text{C}_4\text{MnBP}$, $^{13}\text{C}_4\text{MECPP}$, $^{13}\text{C}_4\text{MEHHP}$, $^{13}\text{C}_4\text{MEOHP}$, $^{13}\text{C}_4\text{MBzP}$, $^{13}\text{C}_4\text{MEHP}$, and $^{13}\text{C}_4\text{MCHP}$; Cambridge Isotope Laboratories) were added to each sample. The LOD of phthalate metabolites ranged from 0.03 to 0.3 ng/mL. A new standard curve was made by 10 levels of standard samples varying from 0.2 to 500 ng/mL for each batch of measured samples. The R^2 value of the linear regression for each calibration curve was above 0.990. Other information regarding the quality control during the instrumental analyses are presented in Table S3.

2.4. Statistical analyses

Among the 10 metabolites analyzed, 9 metabolites (MMP, MEP, MiBP, MnBP, MBzP, MEHP, MEOHP, MECPP, and MEHHP) had average high detection rates of 93.2% except for MBzP and MCHP with 51.2% and 8.8%, respectively. MCHP was removed from the analyses due to lack of enough representative samples. Among the 9 metabolites, samples below the LOD were allocated half of the LOD value for subsequent calculations (Beko et al., 2015; Liu et al., 2012). We used a previously published method to create four groups of phthalate metabolites (Liao et al., 2018; Wang et al., 2013): 1) $\Sigma_4\text{DEHP}$ (sum of MEHP, MEHHP, MEOHP, and MECPP, all metabolites of DEHP), 2) $\Sigma_4\text{LMWP}$ (sum of MMP, MEP, MiBP, and MnBP, all with molecular weight [MW] < 250 Da), 3) $\Sigma_5\text{HMWP}$ (sum of MEHP, MEHHP, MEOHP, MECPP, and MBzP, all with MW $\geq$ 250 Da), and 4) $\Sigma_9\text{Total}$ (sum of all 9 individual phthalate metabolites). The group concentrations were calculated by converting the concentration ($\mu\text{g/L}$) of each member metabolite to molar concentrations, then multiplied by the MW of a certain representative metabolite and summing them. The MWs of representative metabolites were 278 Da, 222 Da, 278 Da, and 278 Da for the 4 groups, respectively (Liao et al., 2018; Wang et al., 2013). The levels of phthalate metabolites were presented with and without the creatinine correction. The creatinine corrected levels of phthalate metabolites were calculated as $\text{Pc} = \text{P}/\text{Cr}$, where Pc is the creatinine-corrected phthalate metabolite concentration ($\mu\text{g/g Cr}$), P is the measured phthalate metabolite concentration, and Cr is the creatinine concentration of the individual urine sample (Meeker et al., 2009).

Using the Kolmogorov-Smirnov test, the concentrations of phthalate metabolites were found to follow a normal distribution after natural logarithmic (ln) transformation. First, the measurements of 9 phthalate metabolites were described and summarized for all subjects as the geometric mean (GM), Min-Max, and percentiles.

Second, the levels of phthalate metabolites were compared among

subjects with or without each of the symptoms (by students' *t*-test) and between groups with different numbers of concomitant symptoms (by one-way analysis of variance). Dunnett's test was further used to compare any two groups when statistical difference was found in the one-way analysis of variance. Similarly, the levels of phthalate metabolites were compared between different demographic and home environmental factors. Spearman's rank correlation coefficients were applied to analyze the correlations between any two metabolites out of the total 9 phthalate metabolites.

Third, we used a published method (covariate-adjusted standardization) to adjust for creatinine when investigating the associations between urinary phthalate metabolites and asthmatic and allergic symptoms (O'Brien et al., 2016). The covariate-adjusted standardization was calculated as $C_{ratio} = P/(Cr/\hat{Cr})$, where C_{ratio} was the creatinine-corrected phthalate metabolite concentration ($\mu\text{g/L}$), P was the measured phthalate metabolite concentration, and Cr and $\hat{Cr}$ denoted the observed and fitted creatinine concentrations, respectively. The fitted creatinine concentration was calculated as $\hat{Cr} = \ln(Cr)$ (O'Brien et al., 2016). The associations were estimated by the prevalence odds ratios (PORs) and 95% confidence intervals (CIs) and presented as both the crude and adjusted prevalence ORs in multivariable logistic regression models. By comparing with the prevalence of symptoms in the lowest quartile of creatinine-corrected phthalate metabolites (by covariate-adjusted standardization), the PORs were calculated in each of the other 3 higher quartiles of phthalate metabolites. Potential confounders were selected from those known or suspected of being associated with asthma and allergic symptoms and phthalates (Bertelsen et al., 2013; Callesen et al., 2014b; Whyatt et al., 2014). Variables were retained in the models if they were significantly associated with the outcome (symptoms) and/or their removal changed the coefficient of the exposure (PAE metabolites) to the outcome relationship by > 10% (Whyatt et al., 2014). The adjusted model covariates included age (5~ < 7.5 vs. ≥ 7.5 –10 yrs), gender (boys vs. girls), BMI (< 16.1 vs. ≥ 16.1 kg/m²), maternal education (< high school vs. $\geq$ high school), breastfeeding time (< 6 vs. ≥ 6 months), family smoking exposure (yes vs. no), history of parental asthma (yes vs. no), residential area (urban vs. suburban), annual household income ($\leq 200,000$ RMB vs. > 200,000 RMB), wall materials in children's bedrooms (paint/wallpaper vs. tiles/lime) and floor materials in children's bedrooms (wood/parquet vs. others). The trend test of PORs was also performed by adding the ordinal quartiles of phthalate metabolites as continuous variables in the multivariable logistic regression models controlling for the same covariates. The interaction effects of sex strata and phthalate metabolites on the association between urinary PAE metabolites and asthmatic and allergic symptoms were also analyzed.

In all analyses, two-sided *p*-values equal to or < 0.05 were considered to be statistically significant. All statistical analyses were performed using SPSS version 20.0 (IBM Corp., Chicago, USA).

3. Results

3.1. Participant characteristics

In total, 419 out of the 434 participating children completed both the questionnaire and measurements of 9 urinary metabolites. Among them, 215 were boys (51.3%) and 204 were girls (48.7%) (Table 1). Most children (73%) were aged 5.0 to 7.5 yrs (min-max: 5–10 yrs). The prevalences of wheeze, rhinitis, and eczema within the preceding 12 months were 35.1%, 54.2% and 19.1%, respectively. Compared with the data obtained from the same subjects 2 years prior (the baseline survey), the prevalence of rhinitis and eczema symptoms increased while the prevalence of wheeze decreased slightly. In the baseline survey, the 419 children had a relatively higher prevalence of wheeze ($P < 0.001$), rhinitis ($P < 0.001$) and eczema ($P < 0.001$), compared with all subjects of the same age ($n = 15,026$; 88.7% of the total 16,948 subjects).

Table 1

Prevalence of wheeze, rhinitis and eczema symptoms in the preceding 12 months in the follow-up study and baseline survey as well as the distribution of the different characteristics ($N = 419$).

	Total <i>n</i> (%)	Number and prevalence of symptoms [<i>n</i> (%)] in the preceding 12 months		
		Wheeze	Rhinitis	Eczema
Subjects in this study (2013–2014)	419	147(35.1)	227(54.2)**	80(19.1)
Number of same subjects in the baseline survey (2011–2012)	419	161(38.4)**	58(13.8)	61(14.6)
Subjects in the baseline survey at the same age level (2011–2012)	15,026	3229(21.5)	1803(12.0)	1923(12.8)
Gender				
Male	215(51.3)	79(36.7)	120(55.8)	40(18.6)
Female	204(48.7)	68(33.3)	107(52.5)	40(19.6)
Age (years)				
5~ < 7.5	306(73.0)	117(38.2)*	168(54.9)	63(20.6)
≥ 7.5 –10	113(27.0)	30(26.5)	59(52.2)	17(15.0)
BMI (kg/m ²)				
< mean (16.1)	247(58.9)	73(29.6)	117(47.4)	44(17.8)
$\geq$ mean (16.1)	172(41.1)	59(34.3)	91(52.9)	31(18.0)
Breastfeeding time				
> 6 months	176(42.0)	57(32.3)	92(52.3)	31(17.6)
≤ 6 months	243(58.0)	90(37.0)	135(55.6)	49(20.2)
Maternal education				
< High school	88(21.0)	28(31.8)	40(45.5)	7(8.0)
$\geq$ High school	331(79.0)	111(33.5)	175(52.9)	68(20.5)*
Residence area				
Urban	242(57.8)	85(35.1)	138(57.0)*	54(22.3)*
Suburban	177(42.2)	62(35.0)	89(50.3)	26(14.7)
Annual household income				
$\leq 200,000$ RMB	261(52.3)	80(30.7)	120(46.0)	42(16.1)
> 200,000 RMB	158(37.7)	54(34.2)	82(51.9)	31(19.6)
Family smoking exposure				
Yes	225(53.7)	81(36.0)	119(52.9)*	49(21.8)*
No	194(46.3)	54(27.8)	82(42.3)	23(11.9)
History of parental asthma				
Yes	37(8.8)	19(51.4)*	26(70.3)*	17(45.9)*
No	382(91.2)	125(32.7)	198(51.8)	62(16.2)
Wall materials in children's bedrooms				
Paint/wallpaper	376(89.7)	127(33.8)	204(54.3)	73(19.4)
Tiles/lime	43(10.3)	12(27.9)	13(30.2)	5(11.6)
Floor materials in children's bedrooms				
Wood/parquet	353(84.2)	118(33.4)	184(52.1)	70(19.8)
Others	66(15.8)	24(36.4)	33(50.0)	8(12.1)

* $P < 0.05$ refers to the statistically significant differences in prevalence in paired subgroups by demographic or personal characteristics.

** $P < 0.001$ refers to the statistically significant differences in prevalence between baseline and follow-up studies.

In the present study, boys had a higher prevalence of wheeze (36.7% vs. 33.3%, $P = 0.465$) and allergic rhinitis symptoms in the preceding 12 months (55.8% vs. 52.5%, $P = 0.49$) than girls. Children with maternal education $\geq$ high school (for eczema, $P < 0.05$), living in urban areas (for rhinitis and eczema, $P < 0.05$), with family smoking exposure (for rhinitis and eczema, $P < 0.05$) and history of parental asthma showed significantly higher prevalence of allergic symptoms ($P < 0.05$). However, we did not observe statistical significance between BMI, breastfeeding time, annual household income, wall materials in children's bedrooms, floor materials in children's bedrooms and prevalence of wheeze, rhinitis and eczema ($P > 0.05$). More detailed information on the prevalence distribution is presented in Table 1.

Table 2

Summary of the urinary levels of phthalate metabolites in children (with and without creatinine adjustment).

	GM	Min	Percentile				Max	LOD (ng/mL)	Detectable sample No. (%)
			P ₂₅	P ₅₀	P ₇₅	P ₉₅			
PAEs metabolites (ng/mL)/(μg/g) ¹⁾									
MMP	6.9	< LOD	6.8	11.4	17.8	51.9	265.2	0.03	391(90.1)
Cr-adjusted	11.2	< LOD	10.7	18.1	30.9	83.8	733.2		
MEP	11.2	< LOD	5.6	10.1	23.5	98.1	679.7	0.10	425(97.9)
Cr-adjusted	18.3	< LOD	9.2	17.7	34.1	158.2	708.0		
MiBP	47.8	2.3	25.9	46.2	89.2	282.1	1166.4	0.05	434(100.0)
Cr-adjusted	77.9	3.7	39.8	79.5	144.0	411.9	3256.0		
MnBP	65.4	3.0	32.7	58.1	133.1	447.8	1907.8	0.30	434(100.0)
Cr-adjusted	106.5	7.2	49.3	101.4	205.7	701.3	5487.0		
MECPP	39.9	3.2	22.6	39.0	69.0	168.3	1955.2	0.03	434(100.0)
Cr-adjusted	64.9	11.1	40.3	60.3	96.4	259.3	2227.0		
MEHHP	26.3	1.4	14.4	24.7	47.5	120.5	1715.3	0.10	434(100.0)
Cr-adjusted	42.8	3.9	24.7	41.1	69.5	175.5	1421.0		
MEOHP	14.9	0.2	8.2	14.5	26.8	69.8	794.1	0.04	434(100.0)
Cr-adjusted	24.3	0.1	14.9	22.8	38.4	109.9	792.0		
MEHP	4.7	0.2	2.7	4.5	8.1	22.0	372.9	0.05	434(100.0)
Cr-adjusted	7.7	0.8	4.2	7.1	12.4	42.8	894.0		
MBzP	0.3	< LOD	< LOD	0.2	0.8	4.4	72.9	0.20	222(51.2)
Cr-adjusted	0.5	< LOD	< LOD	0.4	1.3	7.8	61.0		
Four groups of phthalate metabolites									
Σ ₄ DEHP	81.7	5.7	47.8	78.7	137.7	331.9	4515.7	—	—
Cr-adjusted	132.3	20.5	79.1	124.5	195.9	563.1	5001.8	—	—
Σ ₄ LMWP	160.6	11.1	84.8	147.9	283.0	879.4	2424.4	—	—
Cr-adjusted	260.5	37.7	130.7	241.8	491.0	1254.4	8209.6	—	—
Σ ₅ HMWP	82.6	5.8	48.0	79.6	139.6	341.3	4517.0	—	—
Cr-adjusted	133.8	20.6	79.7	127.4	196.3	565.8	5068.0	—	—
Σ ₉ Total	301.2	19.7	170.3	279.5	484.6	1411.9	5111.1	—	—
Cr-adjusted	488.8	71.5	273.6	456.3	802.6	2170.0	15,348.5	—	—

LOD: limit of detection; Cr: creatinine.

^a The unit of ng/mL is for the concentration levels without creatinine adjustment while μg/g was for the concentration levels with creatinine adjustment.

3.2. Urinary phthalate metabolites

Of the 9 metabolites, 8 compounds (except MBzP detected in 51.2%) were detected in > 90% of samples (Table 2). Among them, MnBP, MiBP, MEHHP, MECPP, MEOHP, and MEHP were detected in 100% of samples. The generally high detection rate indicated the potentially wide exposure of children to these compounds.

Among the 9 phthalate metabolites, MnBP had the highest average level (GM 106.5 μg/g) followed by MiBP (GM 77.9 μg/g) and 3 secondary metabolites of DEHP – MECPP (64.9 μg/g), MEHHP (42.8 μg/g) and MEOHP (24.3 μg/g). MEP (18.3 μg/g), MMP (11.3 μg/g), MEHP (7.7 μg/g), and MBzP (0.5 μg/g) were at relatively lower levels. The total levels of phthalate metabolites (9 compounds) were on average 722.5 μg/g. Spearman's rank correlation analyses revealed a high correlation between MnBP and MiBP (correlation coefficient 0.79, $P < 0.001$) and the four metabolites of DEHP among each other (MEHP, MECPP, MEOHP, and MEHHP, correlation coefficient > 0.75, $P < 0.001$). Detailed information on the correlation analyses is shown in Table S4 (Supplementary material).

Certain demographic and home environmental factors were univariately associated with phthalate metabolite concentrations (Table S5). Children with < 6 months of breastfeeding had significantly higher levels of MiBP, MEHHP, MEOHP, MBzP, Σ₄DEHP, and Σ₅HMWP than those breastfed for 6 months or more. Higher levels of MiBP and MBzP were found in children living in urban areas and with maternal education equal to or higher than high school level. We did not find statistical significance in other metabolites among different subgroups (Table S5).

3.3. Associations between phthalate metabolites and symptoms

By univariate comparisons (Table 3), the urinary levels of phthalate

metabolites (GM) including MiBP, MnBP, MBzP, Σ₄LMWP and Σ₉Total were statistically higher in children with positive reports of symptoms of wheeze, rhinitis or eczema within the preceding 12 months. The 4 metabolites of DEHP were statistically higher in children with wheeze or eczema, but not in those with rhinitis. Compared with children with no symptoms or one symptom only, children with two or more symptoms had generally higher levels of phthalate metabolites for all compounds ($P < 0.05$).

By multivariate logistic regression analyses (Table 4), compared with the 1st quartile, the 4th quartile of MnBP, Σ₄LMWP and Σ₉Total were associated with wheezing with aPOR_s (95% CIs) of 2.27 (1.06–4.88), 2.15 (1.01–4.62), and 2.25 (1.05–4.81), respectively. The highest quartiles of MiBP, MnBP, MBzP, Σ₄LMWP, and Σ₉Total were associated with rhinitis symptoms with aPOR_s (95% CIs) of 2.23 (1.08–4.62), 2.14 (1.02–4.46), 2.46 (1.17–5.14), 2.28 (1.09–4.76), and 3.19 (1.49–6.82), respectively. The highest interquartile range (IQR) of MiBP, MnBP, MEHHP, MEOHP, Σ₄LMWP, and Σ₉Total was associated with eczema with aPOR_s (95% CIs) of 2.96 (1.02–8.60), 2.98 (1.19–7.50), 3.10 (1.10–8.74), 2.63 (1.02–6.80), 4.30 (1.42–13.01), and 4.66 (1.52–14.30), respectively.

The trend analyses showed that each increase in IQR of MiBP and MnBP was associated with an increased prevalence of current rhinitis ($P_{\text{trend}} = 0.03, 0.04$) and eczema ($P_{\text{trend}} = 0.01, 0.01$), respectively. We also observed a significant trend in the associations between MEHHP, MEOHP and eczema among children ($P_{\text{trend}} = 0.02$ and 0.04), respectively. The trend for associations between Σ₉Total and current rhinitis and eczema was significant (both $P_{\text{trend}} \leq 0.01$) (Table 4). There was no statistical significance regarding the modification by sex for all phthalate metabolites on any symptom in children (all $P_{\text{interaction}} > 0.05$) (Table 4).

By grouping the subjects into 0, 1 and equal to 2 or > 2 concomitant symptoms, significant positive associations were found between the 4th

Table 3

The average levels (GM, creatinine-adjusted) of the urinary phthalate metabolites in subgroups with and without each symptom or with none, one and two/three symptoms.

Metabolites	Wheeze		Rhinitis		Eczema		Any symptoms		
	Yes	No	Yes	No	Yes	No	None	One	Two/three
MMP	13.6	10.0	11.5	10.7	13.7	10.6	11.1	9.3	13.6
MEP	20.7	16.6	17.9	18.0	19.9	17.6	17.8	16.0	20.5*
MiBP	89.6*	72.6	87.6*	68.5	99.5**	74.1	72.2	69.4	97.6**
MnBP	120.8*	99.9	115.3*	97.7	145.5**	99.7	100.2	94.4	131.6**
MEHHP	48.0*	40.0	44.9	40.1	50.3*	41.1	39.9	39.8	49.5
MECPP	72.5*	60.8	67.7	61.3	71.9	63.2	60.5	61.8	73.3
MEOHP	28.1*	22.3	25.9	22.3	28.8*	23.2	21.9	22.7	28.8*
MEHP	8.40	7.20	8.10	7.10	8.8*	7.3	7.1	7.1	8.7
MBzP	0.60*	0.50	0.60*	0.40	0.8**	0.5	0.5	0.4	0.8***
Σ ₄ DEHP	159.6*	133.6	149.6	134.1	162.9	138	133.1	134.5	163.1*
Σ ₄ LMWP	287.7*	233.0	273.3*	227.2	320.1**	237.6	235	220.5	311.7**
Σ ₅ HMWP	161.1*	134.9	150.9	135.6	165.1	139.2	134.6	135.4	164.9*
Σ ₉ Total	481.5*	392.9	455.6*	386.2	518.8**	403	389.8	387	508.1**

GM: geometric mean;

The numbers with * represent the significant differences with or without the symptoms (*t*-test), or between the subgroup with one symptom, two or three concomitant symptoms (Dunnett's test).

* *P* < 0.5.

** *P* < 0.01.

*** *P* < 0.001.

quartile of PAE metabolites (compared with the 1st quartile) and 2 or > 2 concomitant symptoms (Fig. 1) for MiBP, MnBP, MEHHP, MEOHP, MBzP, Σ₄LMWP and Σ₉Total.

4. Discussion

In this study, low-MW phthalate metabolites such as MnBP and MiBP found at higher levels in children's urine samples and were more pronouncedly associated with an increased risk of asthmatic and allergic symptoms, compared with other metabolites. High MW phthalate metabolites such as MEHHP and MEOHP (metabolites of DEHP) were associated with an increased risk of allergic rhinitis and eczema. The sum of low-MW metabolites (Σ₄LMWP) and the total level of 9 metabolites (Σ₉Total) showed significant associations with all symptoms in children.

Research has been conducted on phthalate exposure and childhood asthma and allergy in different countries, but the conclusions have not been consistent (Callesen et al., 2014a; Franken et al., 2017; Wang et al., 2014). Among children aged 3–8 years in Sweden, house dust concentrations of DEHP were associated with physician-confirmed asthma (Bornehag et al., 2004). Similar results about positive associations between house dust DEHP concentrations and childhood asthma were reported in Bulgaria (Kolarik et al., 2008) and Japan (Ait et al., 2014). In addition, a cross-sectional study conducted in Norway showed that higher concentrations of urinary mono(carboxyoctyl) phthalate (MCOP) and mono(carboxynonyl) phthalate (MCNP) were associated with current asthma in 10-year-old children (Bertelsen et al., 2013). Hsu et al. (2012) found that higher urinary MBzP levels were associated with an increased risk of diagnosed asthma, and MEHP was associated with allergic rhinitis. On the other hand, two birth cohort studies from Spain (Gascon et al., 2015) and Taiwan (Wang et al., 2014) reported that maternal exposure to DEHP was not associated with childhood eczema. Callesen et al. (2014a) found no positive associations between the urinary concentration of any phthalate metabolite and either asthma or rhinitis; only a weak positive association was found between a metabolite of DEP and atopic dermatitis in young children.

The present study was conducted in Shanghai which has the highest prevalence of childhood asthma in Mainland China (Chen, 2013). The urinary levels of phthalate metabolites were similar to the reported levels in previous studies among children, in Shanghai and areas close

to Shanghai (Wang et al., 2015; Wang et al., 2013). Together with another study in the Chinese general population (Guo et al., 2011), all these studies showed that there were higher urinary levels of MiBP and MnBP (Wang et al., 2015; Wang et al., 2013) in the Chinese population. Other studies also showed that MnBP and MiBP were the dominant phthalate metabolites in Chinese and German children (Kasper-Sonnenberg et al., 2012; Koch et al., 2011; Wang et al., 2015; Wang et al., 2013), which were consistent with our findings. This exposure pattern was consistent with the situation that China is one of the largest producers and consumers of Di-*n*-butyl phthalate (DBP) and Di-isobutyl phthalate (DiBP) in the world (Guo et al., 2011). Some other factors related with the higher exposure levels of MiBP in our study included shorter time of breastfeeding among children (e.g. less than six months), children with maternal education over high school and living in urban districts, compared to suburban/rural areas (Table S5).

In the present study, urinary MnBP and MiBP, representative of low-MW phthalates, were significantly related to asthmatic and allergic symptoms, according to parents' reports in the questionnaire. Another low-MW metabolite, MEP, was significantly higher in the subgroup having two or three allergic symptoms than that with none or one symptom in this study. Low-MW phthalates function as plasticizers, fixatives or solvents in personal care products, but may also be used in plastic containers in small quantities (Overgaard et al., 2017). Liao et al. (2018) found a significant association between higher urinary level of MiBP and window frame plastic material compared to wood or aluminum material in children's bedrooms. Evidence has shown that use or exposure to personal care products can significantly increase the level of DEP (Koch et al., 2013). Since DEP is added to cosmetic products, the frequent use of these cosmetics will lead to a higher internal burden due to absorption through the skin. On the other hand, inhalation may also be an important exposure pathway to low-MW metabolites. According to Adibi et al. (2003), significant correlation was found between personal levels of airborne DEP, DBP and concentrations of corresponding urinary metabolites.

In addition to the low-MW metabolites, high-MW metabolites, such as DEHP, were also reported to be associated with asthma and allergic disease in previously published studies (Jaakkola and Knight, 2008; Kimber and Dearman, 2010). In our study, the average level of the sum of the four metabolites (Σ₄DEHP) was 132.3 μg/g, which was consistent with the reported value (137 μg/g) in a previous study (Wang et al.,

Table 4

The crude and adjusted prevalence ORs of asthma and allergic symptoms per interquartile range (IQR) of urinary phthalate metabolites (creatinine-adjusted) as well as the trend analyses of associations ^a.

Metabolites	IQR levels	Wheeze		Rhinitis		Eczema	
		cPOR (95%CI)	aPOR (95%CI)	cPOR (95%CI)	aPOR (95%CI)	cPOR (95%CI)	aPOR (95%CI)
MMP	1st	1.0	1.0	1.0	1.0	1.0	1.0
	2nd	0.92 (0.51, 1.66)	0.88 (0.43, 1.87)	1.52 (0.86, 2.68)	2.11 (1.02, 4.37)*	1.31 (0.65, 2.64)	1.24 (0.50, 3.03)
	3rd	1.04 (0.58, 1.87)	1.07 (0.52, 2.22)	1.06 (0.61, 1.87)	1.75 (0.85, 3.62)	1.19 (0.59, 2.44)	0.99 (0.39, 2.54)
	4th	0.83 (0.44, 1.54)	1.01 (0.50, 2.06)	0.87 (0.48, 1.58)	1.31 (0.65, 2.63)	0.90 (0.41, 1.95)	1.03 (0.42, 2.54)
	P-trend	–	0.85	–	0.64	–	0.92
	P for interaction ^b	–	0.09	–	0.07	–	0.38
MEP	1st	1.0	1.0	1.0	1.0	1.0	1.0
	2nd	0.99 (0.54, 1.81)	0.77 (0.35, 1.67)	0.88 (0.51, 1.53)	0.89 (0.43, 1.85)	1.33 (0.61, 2.90)	0.72 (0.25, 2.07)
	3rd	1.55 (0.86, 2.78)	1.38 (0.65, 2.94)	1.37 (0.78, 2.40)	1.63 (0.78, 3.41)	2.24 (1.06, 4.68)*	1.77 (0.68, 4.64)
	4th	1.56 (0.86, 2.83)	1.11 (0.51, 2.39)	1.00 (0.57, 1.77)	1.06 (0.51, 2.22)	1.99 (0.94, 4.26)	1.75 (0.66, 4.62)
	P-trend	–	0.40	–	0.48	–	0.07
	P for interaction ^b	–	0.08	–	0.08	–	0.72
MiBP	1st	1.0	1.0	1.0	1.0	1.0	1.0
	2nd	1.30 (0.73, 2.34)	0.99 (0.45, 2.15)	1.21 (0.70, 2.11)	1.15 (0.54, 2.43)	1.50 (0.68, 3.30)	1.01 (0.31, 3.36)
	3rd	1.02 (0.56, 1.87)	0.87 (0.41, 1.85)	0.95 (0.55, 1.66)	1.07 (0.52, 2.20)	2.33 (1.09, 4.95)*	3.70 (1.31, 10.47)*
	4th	1.63 (0.91, 2.95)	1.45 (0.70, 3.04)	1.86 (1.04, 3.29)*	2.23 (1.08, 4.62)*	2.17 (1.01, 4.66)*	2.96 (1.02, 8.60)*
	P-trend	–	0.36	–	0.03*	–	0.01*
	P for interaction ^b	–	0.27	–	0.09	–	0.80
MnBP	1st	1.0	1.0	1.0	1.0	1.0	1.0
	2nd	1.25 (0.70, 2.24)	1.61 (0.74, 3.52)	1.11 (0.63, 1.93)	1.12 (0.54, 2.35)	1.23 (0.57, 2.65)	0.61 (0.21, 1.76)
	3rd	0.77 (0.42, 1.41)	0.82 (0.37, 1.85)	1.12 (0.64, 1.95)	1.17 (0.56, 2.42)	1.25 (0.58, 2.67)	0.58 (0.20, 1.68)
	4th	1.62 (0.90, 2.92)	2.27 (1.06, 4.88)*	1.53 (0.86, 2.71)	2.14 (1.02, 4.46)*	2.60 (1.27, 5.33)*	2.98 (1.19, 7.50)*
	P-trend	–	0.12	–	0.04*	–	0.01*
	P for interaction ^b	–	0.13	–	0.06	–	0.62
MEHHP	1st	1.0	1.0	1.0	1.0	1.0	1.0
	2nd	1.53 (0.85, 2.73)	1.32 (0.61, 2.85)	1.24 (0.72, 2.16)	1.28 (0.61, 2.65)	2.50 (1.12, 5.59)*	2.38 (0.79, 7.19)
	3rd	1.20 (0.66, 2.18)	1.34 (0.65, 2.76)	0.70 (0.40, 1.23)	0.88 (0.44, 1.76)	3.00 (1.36, 6.68)*	3.89 (1.38, 10.98)*
	4th	1.47 (0.80, 2.67)	1.49 (0.73, 3.05)	1.19 (0.67, 2.10)	1.42 (0.72, 2.81)	2.63 (1.16, 5.97)*	3.10 (1.10, 8.74)*
	P-trend	–	0.30	–	0.47	–	0.02*
	P for interaction ^b	–	0.71	–	0.10	–	0.51
MECPP	1st	1.0	1.0	1.0	1.0	1.0	1.0
	2nd	1.24 (0.69, 2.25)	1.22 (0.55, 2.72)	0.99 (0.57, 1.73)	0.82 (0.39, 1.72)	1.69 (0.78, 3.64)	1.24 (0.42, 3.64)
	3rd	1.37 (0.75, 2.48)	1.48 (0.70, 3.15)	0.76 (0.43, 1.33)	0.72 (0.35, 1.47)	2.28 (1.07, 4.84)*	2.60 (0.99, 6.83)
	4th	1.47 (0.81, 2.66)	1.60 (0.77, 3.32)	1.13 (0.64, 2.00)	1.21 (0.61, 2.42)	1.97 (0.92, 4.23)	2.04 (0.78, 5.33)
	P-trend	–	0.18	–	0.58	–	0.07
	P for interaction ^b	–	0.93	–	0.78	–	0.48
MEOHP	1st	1.0	1.0	1.0	1.0	1.0	1.0
	2nd	1.10 (0.61, 1.98)	1.02 (0.47, 2.24)	1.37 (0.79, 2.39)	1.36 (0.65, 2.84)	2.01 (0.93, 4.36)	1.69 (0.60, 4.75)
	3rd	1.21 (0.67, 2.17)	1.34 (0.64, 2.80)	0.81 (0.47, 1.42)	0.84 (0.42, 1.71)	1.89 (0.87, 4.14)	1.95 (0.72, 5.25)
	4th	1.52 (0.84, 2.75)	1.54 (0.75, 3.15)	1.18 (0.67, 2.09)	1.39 (0.69, 2.76)	2.61 (1.21, 5.63)*	2.63 (1.02, 6.80)*
	P-trend	–	0.17	–	0.56	–	0.04*
	P for interaction ^b	–	0.75	–	0.07	–	0.46

(continued on next page)

Table 4 (continued)

Metabolites	IQR levels	Wheeze		Rhinitis		Eczema	
		cPOR (95%CI)	aPOR (95%CI)	cPOR (95%CI)	aPOR (95%CI)	cPOR (95%CI)	aPOR (95%CI)
MEHP	1st	1.0	1.0	1.0	1.0	1.0	1.0
	2nd	0.71 (0.40, 1.27)	0.93 (0.44, 1.98)	1.02 (0.59, 1.77)	1.21 (0.59, 2.50)	1.65 (0.78, 3.53)	2.23 (0.78, 6.32)
	3rd	0.64 (0.35, 1.17)	0.89 (0.43, 1.86)	0.94 (0.54, 1.67)	1.19 (0.59, 2.41)	1.82 (0.85, 3.92)	2.48 (0.90, 6.82)
	4th	1.11 (0.61, 2.01)	1.45 (0.71, 2.99)	1.07 (0.59, 1.92)	1.28 (0.63, 2.59)	2.08 (0.95, 4.53)	2.55 (0.93, 6.96)
	P-trend	–	0.31	–	0.52	–	0.08
	P for interaction ^b	–	0.67	–	0.08	–	0.06
MBzP	1st	1.0	1.0	1.0	1.0	1.0	1.0
	2nd	0.94 (0.50, 1.76)	1.29 (0.60, 2.79)	1.18 (0.66, 2.14)	1.89 (0.91, 3.95)	0.86 (0.37, 1.99)	0.72 (0.26, 2.01)
	3rd	1.30 (0.71, 2.35)	1.10 (0.52, 2.33)	1.57 (0.89, 2.77)	1.88 (0.93, 3.82)	1.74 (0.84, 3.59)	1.12 (0.44, 2.86)
	4th	1.38 (0.75, 2.55)	1.75 (0.82, 3.71)	1.59 (0.88, 2.87)	2.46 (1.17, 5.14)*	2.15 (1.03, 4.57)*	1.81 (0.72, 4.57)
	P-trend	–	0.20	–	0.03*	–	0.11
	P for interaction ^b	–	0.47	–	0.43	–	0.26
Σ ₄ DEHP	1st	1.0	1.0	1.0	1.0	1.0	1.0
	2nd	1.08 (0.60, 1.95)	0.98 (0.45, 2.16)	1.19 (0.68, 2.08)	0.92 (0.44, 1.92)	1.99 (0.92, 4.31)	1.62 (0.56, 4.68)
	3rd	1.02 (0.57, 1.85)	1.20 (0.58, 2.48)	0.75 (0.43, 1.31)	0.91 (0.46, 1.84)	2.20 (1.02, 4.74)*	2.79 (1.06, 7.36)*
	4th	1.42 (0.78, 2.58)	1.45 (0.72, 2.93)	1.15 (0.65, 2.05)	1.26 (0.64, 2.50)	2.18 (0.99, 4.77)	2.14 (0.82, 5.59)
	P-trend	–	0.24	–	0.49	–	0.08
	P for interaction ^b	–	0.80	–	0.07	–	0.39
Σ ₄ LWMP	1st	1.0	1.0	1.0	1.0	1.0	1.0
	2nd	1.18 (0.65, 2.12)	1.49 (0.68, 3.29)	1.19 (0.68, 2.08)	1.25 (0.59, 2.64)	3.22 (1.41, 7.37)*	2.09 (0.66, 6.57)
	3rd	0.84 (0.46, 1.52)	0.93 (0.43, 2.02)	0.99 (0.58, 1.73)	1.21 (0.60, 2.46)	2.70 (1.17, 6.21)*	2.62 (0.86, 7.99)
	4th	1.68 (0.94, 3.03)	2.15 (1.01, 4.62)*	1.72 (1.02, 3.03)*	2.28 (1.09, 4.76)*	3.14 (1.36, 7.26)*	4.30 (1.42, 13.01)*
	P-trend	–	0.13	–	0.04*	–	0.01*
	P for interaction ^b	–	0.06	–	0.32	–	0.93
Σ ₅ HWMP	1st	1.0	1.0	1.0	1.0	1.0	1.0
	2nd	1.11 (0.62, 1.99)	1.07 (0.49, 2.34)	1.30 (0.74, 2.27)	1.04 (0.49, 2.17)	2.28 (1.05, 4.91)*	1.79 (0.63, 5.08)
	3rd	0.90 (0.50, 1.64)	1.13 (0.54, 2.34)	0.65 (0.37, 1.14)	0.83 (0.41, 1.67)	1.96 (0.91, 4.29)	2.64 (0.99, 7.03)
	4th	1.37 (0.76, 2.48)	1.45 (0.72, 2.93)	1.12 (0.63, 1.99)	1.26 (0.64, 2.50)	2.18 (0.99, 4.79)	2.15 (0.83, 5.60)
	P-trend	–	0.30	–	0.62	–	0.11
	P for interaction ^b	–	0.78	–	0.06	–	0.38
Σ ₉ Total	1st	1.0	1.0	1.0	1.0	1.0	1.0
	2nd	1.11 (0.62, 1.99)	1.40 (0.63, 3.11)	1.32 (0.75, 2.30)	1.43 (0.67, 3.06)	1.77 (0.79, 3.98)	2.05 (0.62, 6.73)
	3rd	0.74 (0.40, 1.35)	0.70 (0.32, 1.54)	1.03 (0.59, 1.78)	1.31 (0.63, 2.70)	2.44 (1.12, 5.32)*	3.19 (1.05, 9.70)*
	4th	1.68 (0.93, 3.01)	2.25 (1.05, 4.81)*	2.14 (1.19, 3.83)*	3.19 (1.49, 6.82)*	2.81 (1.28, 6.16)*	4.66 (1.52, 14.30)*
	P-trend	–	0.12	–	0.01*	–	0.00**
	P for interaction ^b	–	0.16	–	0.42	–	0.72

Abbrev: cPOR: crude prevalence odds ratio.

Trend analyses were performed by adding the ordinal quartiles of PAE metabolites as continuous variables in the same multivariable regression analyses adjusted for the same covariates.

^a The model adjusted for gender, age, BMI, breastfeeding time, family smoking exposure, residence area, maternal education, annual household income, history of parental asthma, wall materials in children's bedrooms and floor materials in children's bedrooms.

^b P for interaction was separately estimated by adding an interaction term of gender strata and phthalate metabolites into the model adjusted for the same variables as above.^a

* $P < 0.05$.

** $P < 0.01$.

2015). Higher concentrations of MEOHP and MEHP were detected as the dominant metabolites of DEHP in this study. These two compounds were positively associated with eczema symptoms in children in Shanghai which was consistent with other Asian studies. A matched case-control study conducted in Korea showed that DEHP increased the

risk of atopic dermatitis in 3-year-old children (Choi et al., 2014). A 10-year cohort study showed that the effect of early life phthalate exposure on IgE levels could potentially cause atopic dermatitis in children (Shu et al., 2014).

Although phthalate metabolites had the same core chemical

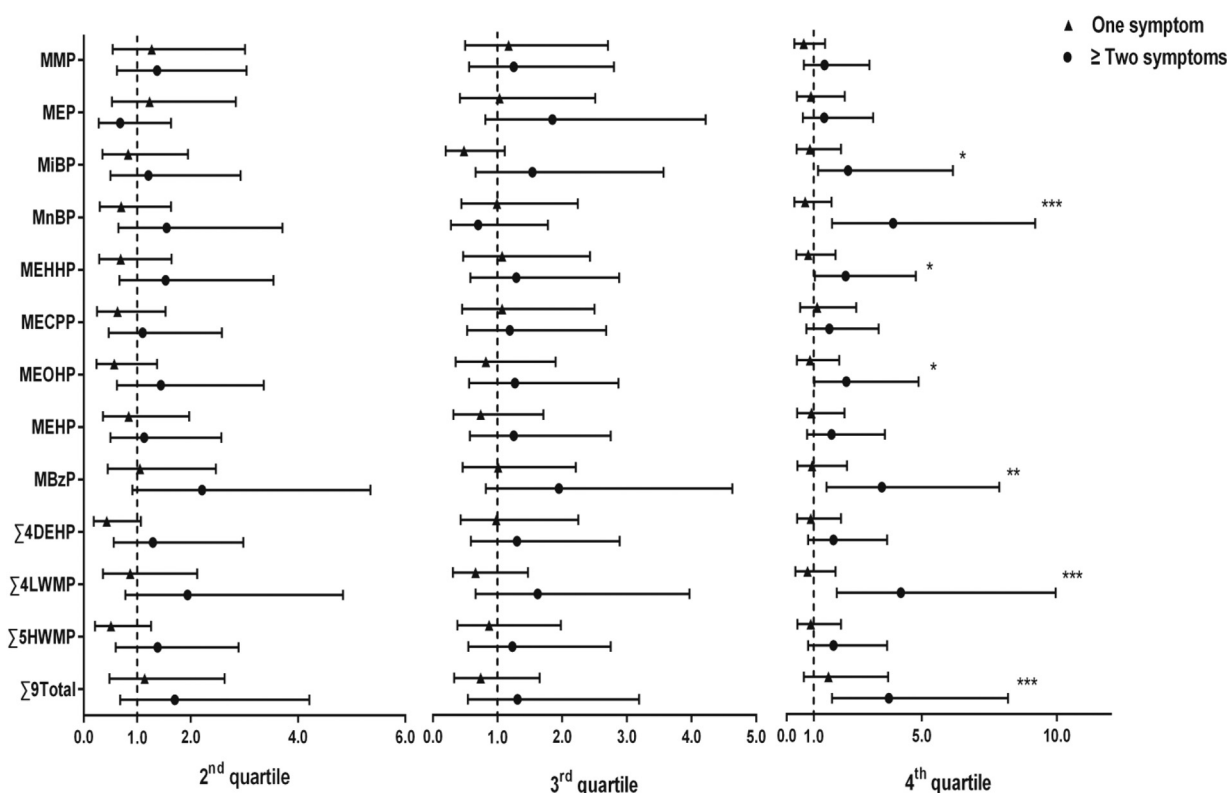


Fig. 1. Associations (aPORs and 95% CIs) of one symptom, and equal to 2 or > 2 concomitant symptoms with PAE metabolites (creatinine-adjusted) in the 2nd, 3rd and 4th quartile, compared with the 1st quartile ^a.

^a The model was adjusted for gender, age, BMI, breastfeeding time, family smoking exposure, residence area, maternal education, annual household income, history of parental asthma, wall materials in children's bedrooms and floor materials in children's bedrooms. *P < 0.05, **P < 0.01, ***P < 0.001.

structure, there was no high correlation with each other except for those from the same parent compounds (Table S4). By summing up Σ₄DEHP, Σ₄LMWP, Σ₅HMWP and Σ₉Total, it was found that Σ₄LMWP and Σ₉Total metabolites were positively associated with asthma and allergic symptoms, consistent with the significant results in respect of the individual corresponding compounds.

Animal experimental studies have indicated that PAEs can act as adjuvants to IgE-dependent mechanisms and allergen-specific T helper type 2 (Th2) immune responses to increase the prevalence of asthma (Guo et al., 2012; You et al., 2016). Nishioka et al. (2012) found that phthalates can affect the immune system by increasing the production of inflammatory cytokines, including tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), IL-18, and IL-6. In addition, DBP also showed strong adjuvant effects for the hapten fluorescein isothiocyanate in contact hypersensitivity models in a toxicological study (Matsuda et al., 2010). Shigeno et al. (2009) further suggested that DBP exposure might potentially modify the mouse immune system, resulting in Th2 cell-mediated allergic skin inflammation.

DEHP is a common contaminant of the human diet, which can be easily released into the surrounding environment from hydrolysis products and affect the bronchial contracting receptors, thereby generating a hyper-responsive condition in the lungs (Oie et al., 1997). Much of the mechanistic work on phthalate and allergy has focused on DEHP and its ability to modulate responses to allergens (Kimber and Dearman, 2010). Evidence has shown that DEHP has an adjuvant effect on the immune response to allergens in human lung epithelial cells (Guo et al., 2012; Nishioka et al., 2012). Miller and Marty (2010) found that DEHP altered airway cell differentiation and surfactant protein production in the lungs. Previous animal studies as well as in vitro studies have shown that DEHP has an adjuvant effect on the immunological response (Koike et al., 2009; Yanagisawa et al., 2008). Vetrano et al. (2010) showed that an alternation of inflammatory reactions including inactivation of the

peroxisome proliferator-activated receptor-gamma (PPAR-γ) or enhancement of dendritic cell differentiation might increase susceptibility to atopic disorders.

This is the first cross-sectional study to investigate the relationship between urinary phthalate metabolites and asthmatic and allergic symptoms in children in Mainland China. In this study, we used first-morning urine instead of a spot sample, which may strengthen the predictive ability of one measurement for long-term exposure compared to a pure spot sample. The use of the validated questionnaires could ensure the results were obtained with convincing statistical power. The significant findings of trend analyses by quartiles as well as the associations with 2 or > 2 concomitant symptoms indicated that there might be a dose-effect relationship between LMWPs (such as MnBP and MiBP), oxidative metabolites of DEHP (MEHHP and MEOHP) and MBzP and the risk of asthmatic and allergic symptoms.

However, potential limitations in this study should also be noted. First, the subjects in this study were not randomly selected in the same age level of children in Shanghai, since the prevalence of asthmatic and allergic symptoms (38.4% vs. 21.5%, 13.8% vs. 12.0%, and 14.6% vs. 12.8%) in these subjects were higher than the average prevalence in the source population (Table 1). This might be due to the fact that parents who had children with asthmatic or allergic symptoms were more likely to be interested in having further details and agreed to participate in the follow-up study. Second, self-reported symptoms, not by clinical diagnosis, might introduce information bias regarding the health of children. However, the aim of this study was to analyze the symptoms in relation to urinary metabolite levels which was hardly biased by the parents' own reports. Thirdly, the substitution value by half of LOD for those below LOD, especially MBzP, could bias the results (Helsel, 2006) and needs to be interpreted with particular caution. Also, since the metabolites of phthalates have short half-lives, the one-time urine sample was a limited representative of the long-term exposure. Long-

term sampling by multiple measurements could better describe the true exposure levels in children. However, this had a low feasibility with further low rate of parental consent. Finally, non-causal associations (e.g. due to residual confounding by some factors associated with asthma and allergic symptoms and phthalates) cannot be ruled out. For all of these reasons, the findings should be interpreted with caution before replication in other cohorts.

5. Conclusions

Both low-MW phthalate metabolites such as MiBP, MnBP and high-MW phthalate metabolites such as DEHP as well as the total phthalate levels (Σ_9 Total) in urine were associated with childhood asthmatic and allergic symptoms in Shanghai. Exposure to phthalates might have adverse health effects on asthma and allergic symptoms in Chinese children. Further studies are needed to confirm and explore our findings.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2018.08.043>.

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Supporting information

Urinary phthalate metabolites in relation to childhood asthmatic and allergic symptoms in Shanghai

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Table S1 List of phthalate esters (PAEs) and corresponding monoester metabolites

No.	Metabolites	Abbrev.	MW	PAEs	Abbrev.	MW
1	Mono-methyl phthalate	MMP	180	Dimethyl phthalate	DMP	193
2	Mono-ethyl phthalate	MEP	194	Diethyl phthalate	DEP	222
3	Mono-isobutyl phthalate	MiBP	222	Di-isobutyl phthalate	DiBP	278
4	Mono-n-butyl phthalate	MnBP	222	Di-n-butyl phthalate	DnBP	278
5	Mono-benzyl phthalate	MBzP	256	Butyl benzyl phthalate	BBzP	312
6	Mono-cyclohexyl phthalate	MCHP	248	Dicyclohexyl phthalate	DCHP	330
7	Mono-2-ethylhexyl phthalate	MEHP	278	Di-2-ethylhexyl phthalate	DEHP	390
8	Mono-2-ethyl-5-carboxypentyl phthalate	MECPP	308	Di-2-ethylhexyl phthalate	DEHP	390
9	Mono-2-ethyl-5-hydroxyhexyl phthalate	MEHHP	294	Di-2-ethylhexyl phthalate	DEHP	390
10	Mono-2-ethyl-5-oxohexyl phthalate	MEOHP	292	Di-2-ethylhexyl phthalate	DEHP	390

MW: molecular weight

Abbrev. : abbreviation

Table S2. Settings for multiple reaction monitoring (MRM) mode in the instrumental analyses

Compounds	Precursor ion(V)	Cone Voltage(V)	Product ion(V)	Collision energy(V)
MMP	179	51	107	12
MEP	193	54	121	13
MiBP	221	61	77	16
MnBP	221	61	77	16
MECPP	221	61	77	16
MEHHP	293	80	121	18
MEOHP	291	80	121	21
MEHP	277	74	134	17
MBzP	255	70	107	18
MCHP	247	68	97	17

Table S3. Data on the quality assurance/quality control in the instrumental analyses

Compounds	Retention time	Regression equation	Linear	Spiked(10ng/ml)		Spiked(50ng/ml)	
			regression coefficient	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
MMP	2.42	$Y=0.00981653+0.0434931x$	0.9954	122.5	1.5	102.1	16.0
MEP	3.45	$Y=0.00174556+0.0124419x$	0.9940	97.7	6.3	98.4	5.0
MiBP	6.05	$Y=0.0216629+0.0190406x$	0.9999	94.3	9.1	94.4	6.4
MnBP	6.24	$Y=0.0086677+0.0150875x$	0.9995	107.0	6.2	100.4	2.2
MECPP	6.09	$Y=0.00068137+0.0160076x$	0.9921	72.7	7.6	72.7	5.2
MEHHP	6.18	$Y=0.00591744+0.012086x$	0.9989	103.4	7.3	111.9	10.9
MEOHP	6.53	$Y=-0.00174496+0.0187985x$	0.9951	97.6	7.4	101.6	6.3
MEHP	10.01	$Y=0.030581+0.0187102x$	0.9971	99.2	2.6	106.8	6.7
MBzP	7.03	$Y=0.0031436+0.00135945x$	0.9968	97.6	7.4	101.7	5.5
MCHP	7.32	$Y=-0.0335918+0.0302792x$	0.9999	98.8	4.9	106.8	2.3

Table S4. Spearman’s rank correlation coefficients among urinary phthalate metabolites (N=434)

	MEP	MiBP	MnBP	MEHHP	MECPP	MEOHP	MEHP	MBzP	Σ ₄ DEHP	Σ ₄ LMWP	Σ ₅ HMWP	Σ ₉ Total
MMP	0.42	0.46	0.47	0.40	0.40	0.41	0.40	0.34	0.42	0.57	0.42	0.56
MEP	—	0.47	0.49	0.32	0.32	0.33	0.31	0.34	0.33	0.62*	0.34	0.58
MiBP		—	0.79*	0.56	0.46	0.56	0.49	0.45	0.53	0.89*	0.54	0.85*
MnBP			—	0.50	0.43	0.51	0.46	0.40	0.49	0.94*	0.49	0.87*
MEHHP				—	0.91*	0.96*	0.79*	0.38	0.97*	0.54	0.97*	0.76*
MECPP					—	0.91*	0.76*	0.34	0.98*	0.47	0.97*	0.71*
MEOHP						—	0.77*	0.37	0.97*	0.55	0.96*	0.77*
MEHP							—	0.36	0.82*	0.49	0.82*	0.67*
MBzP								—	0.38	0.46	0.40	0.46
Σ ₄ DEHP									—	0.53	0.99*	0.77*
Σ ₄ LMWP										—	0.53	0.93*
Σ ₅ HMWP											—	0.77*

*All correlations were significant with p values <0.001.

Table S6. Urinary phthalate metabolite concentrations (GM in µg/g) in this study compared with other studies in children

Region	Reference	Study	Age	Phthalate metabolites								
		Year	(N)	MMP	MEP	MiBP	MnBP	MEHHP	MECPP	MEOHP	MEHP	ME
Germany	Kasper-Sonnenberg et al., (2012)	2007 ~2009	5-9y (104)	4.9	39.1	66.2	48.1	29.3	—	26.2	3.9	12
Taiwan	Chen et al., (2017)	2014	3-11y (181)	7	3.4	13.7	27.6	22.5	—	15.2	6	0.
USA	CDC (2015)	2015	6-11y (395)	2.3	23.4	8.3	11.1	10.5	—	6.96	1.4	8.
Sweden	Larsson et al., (2014)	—	6-11y (97)	—	28.8	—	76.9	24.6	—	15.7	2.7	19
China	Wang et al., (2015)	2012	8-11y (782)	16.5	45.4	79.7	94.4	24.3	42.3	36.4	11.4	0.5
This study	—	2013 ~2014	5-10y (419)	11.2	18.3	77.9	106.5	64.9	42.8	24.3	7.7	0.

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