Prenatal and Postnatal Exposure to Phthalate Esters and Asthma: A 9-Year Follow-Up Study of a Taiwanese Birth Cohort

Hsiu Ying Ku[1,2], Pen Hua Su[3], Hui Ju Wen[2], Hai Lun Sun[4], Chien Jen Wang[2], Hsiao Yen Chen[2], Jouni J. K. Jaakkola[5,6], Shu-Li Wang[1,2,7,8]*, TMICS Group[¶]

1 Graduate Institute of Life Science, National Defense Medical Center, Taipei, Taiwan, 2 Division of Environmental Health & Occupational Medicine, National Health Research Institutes, Miaoli, Taiwan, 3 Division of Genetics, Department of Pediatrics, Chung Shan Medical University Hospital, Taichung, Taiwan, 4 Department of Pediatrics, Chung Shan Medical University Hospital, Taichung, Taiwan, 5 Center for Environmental and Respiratory Health Research, University of Oulu, Oulu, Finland, 6 Medical Research Center, Oulu, Finland, 7 Department of Public Health, College of Public Health, China Medical University, Taichung, Taiwan, 8 School of Public Health, National Defense Medical Center, Taipei, Taiwan

¶ Membership of the Taiwan Mother and Infant Cohort Study (TMICS) Group is provided in the Acknowledgments.
* slwang@nhri.org.tw

Abstract

Previous studies have shown that phthalate exposure in childhood is associated with the development of respiratory problems. However, few studies have assessed the relative impact of prenatal and postnatal exposure to phthalates on the development of asthma later in childhood. Therefore, we assessed the impact of prenatal and postnatal phthalate exposure on the development of asthma and wheezing using a Taiwanese birth cohort. A total of 430 pregnant women were recruited, and 171 (39.8%) of them had their children followed when they were aged 2, 5, and 8 years. The International Study of Asthma and Allergies in Childhood questionnaire was used to assess asthma and wheezing symptoms and serum total immunoglobulin E levels were measured at 8 years of age. Urine samples were obtained from 136 women during their third trimester of pregnancy, 99 children at 2 years of age, and 110 children at 5 years. Four common phthalate monoester metabolites in maternal and children's urine were measured using liquid chromatography-electrospray ionization-tandem mass spectrometry. Maternal urinary mono-benzyl phthalate [MBzP] concentrations were associated with an increased occurrence of wheezing in boys at 8 years of age (odds ratio [OR] = 4.95 (95% CI 1.08–22.63)), for upper quintile compared to the others) after controlling for parental allergies and family members' smoking status. Urinary mono-2-ethylhexyl phthalate [MEHP] levels over the quintile at 2-year-old were associated with increased asthma occurrence (adjusted OR = 6.14 (1.17–32.13)) in boys. Similarly, the sum of di-2-ethyl-hexyl phthalate [DEHP] metabolites at 5 years was associated with asthma in boys (adjusted OR = 4.36 (1.01–18.86)). Urinary MEHP in maternal and 5-year-old children urine were significantly associated with increased IgE in allergic children at 8 years. Prenatal and postnatal exposure to phthalate was associated with the occurrence of asthma in children, particularly for boys.

Introduction

Over the past few decades, the prevalence of childhood asthma has been increasing throughout the world [1]. However, the prevalence of asthma has increased faster in Taiwan than in most countries, possibly due to increased exposure to environmental factors [1]. It has been hypothesized that the increase in asthma is attributable to exposure to endocrine disruptors that act as adjuvants to immunoglobulin E (IgE)-dependent mechanisms and allergen-specific T helper type 2 (Th2) immune responses [2,3].

Phthalate, an endocrine-disrupting chemical, is a widely used plasticizer that is added to consumer products, acts to soften plastics, and may augment allergic processes [3]. The most commonly used phthalates are di-2-ethyl-hexyl phthalate (DEHP) and benzyl butyl phthalate (BBzP), which is the most commonly found phthalate in indoor settings. Polyvinyl chloride (PVC) flooring is known to be a source for the 2 phthalates in indoor dust [4]. Some phthalates, including diethyl phthalate (DEP), are used as solvents in personal care products and are associated with decreased lung function in men [5]. Although the primary route of exposure to phthalates is through contaminated food, children have a higher total phthalate intake than adults, likely as a result of mouthing plastics and ingesting indoor dust [6]. The intensive use of plastic materials may be related to the increased exposure to phthalate esters observed in Taiwan, thereby increasing the risk of adverse effects of phthalates, particularly in pregnant women and children [7,8].

Experimental studies have proposed that DEHP may act as an adjuvant to promote allergic asthma. When DEHP and allergen exposure are combined, DEHP exposure has been found to increase levels of immunological and inflammatory markers, including total IgE, interleukin-4, interferon (IFN)-γ levels, and eosinophil counts [9]. A recent study also found that DEHP and BBzP may promote the Th2 response to increase allergies through the suppression of IFN-α/IFN-β expression and stimulation of T-cell responses [10]. Epidemiological studies have consistently shown that early phthalate exposure is associated with childhood asthma [11,12, 13]. A Norwegian study has observed that the risk of asthma in children is increased in the highest quartiles of mono-carboxyoctyl phthalate and mono-carboxynonyl phthalate exposure [12]. Whyatt *et al.* reported that prenatal exposure to BBzP and di-n-butyl phthalate (DnBP) may increase the risk of asthma among children at 5 to 11 years of age [13].

A nested case-control study demonstrated a relationship between phthalates in dust at home and asthma occurrence in children [14]. However, the effects of phthalate exposure during fetal and early childhood on the development of asthma later in life remain unknown. The immune system matures at 2 years of age; infants are particularly susceptible to environmental exposures during the fast developmental stage [15]. Therefore, our aim was to assess the association between prenatal and postnatal exposure to phthalates with the risk of asthma and wheezing using a 9-year birth cohort study.

Materials and Methods

Ethics statement

The study protocol was approved by the Institutional Review Board of the National Health Research Institutes, Taiwan. Prior to participating in the study, the pregnant women and/or children's caretakers provided written informed consent during three visits, having received detailed explanations of the benefits and risks.

Study population and data collection

This study was performed using data from an ongoing birth cohort study in central Taiwan and served as pilot for Taiwan Maternal and Infant Cohort Study. The study participants were
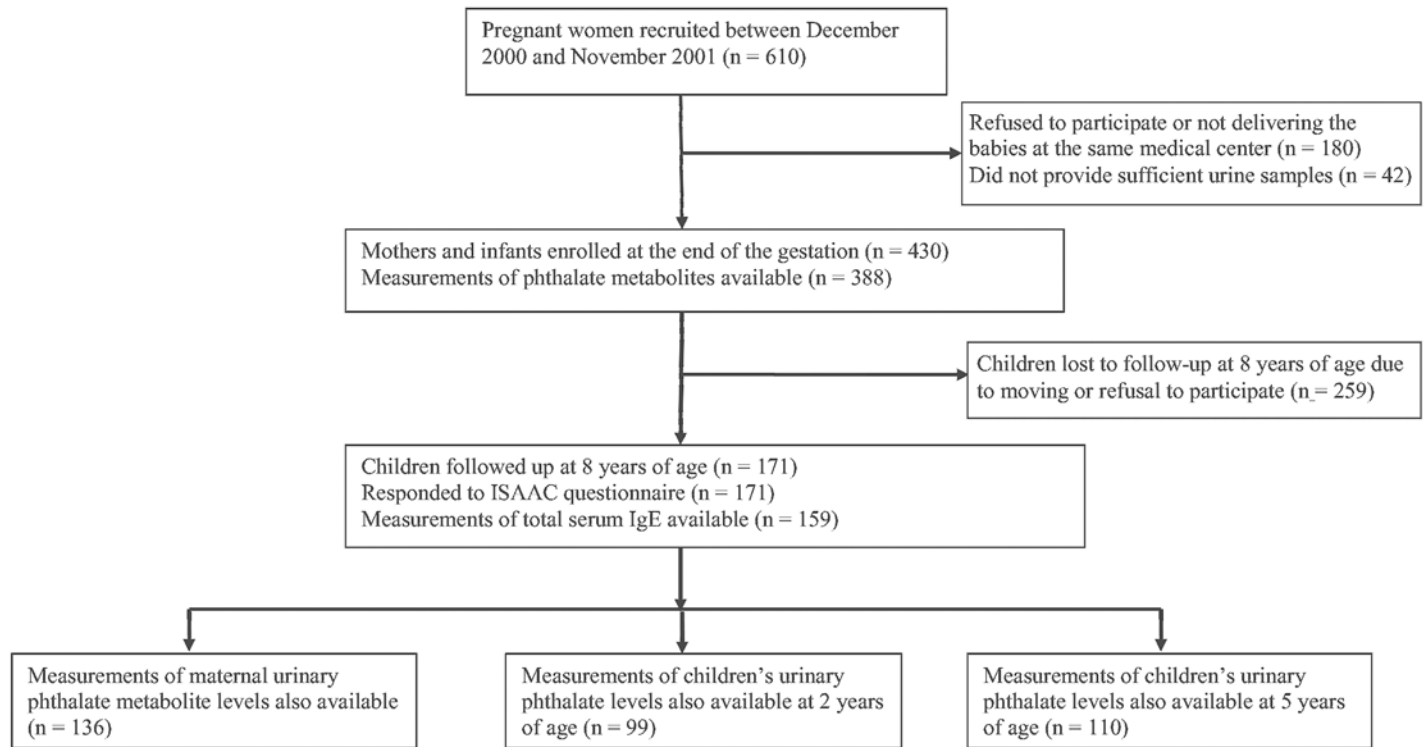
Fig 1. Flow chart of the recruitment process of pregnant women and follow up of their children. Pregnant women were invited to participate in the study. During the follow-up period, phthalate metabolites present in maternal and children's urine were measured. At 8 years of age, the presence of asthma and wheezing was assessed using the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire.

doi:10.1371/journal.pone.0123309.g001

pregnant women without clinical complications (eclampsia or preeclampsia), who delivered their babies in a local hospital between December 1, 2000 and November 30, 2001 (n = 610) [16,17]. We recruited 430 pregnant women and followed up their children at the ages of 2, 5, and 8 years in 2003, 2006, and 2009, respectively. We recruited the pregnant women during the third trimester of their pregnancies because pregnant women tended not to return for follow up when we attempted to recruit them during the first trimester. For example, they changed their childbirth hospital in the later stages of pregnancy to be closer to their parental homes. After receiving detailed explanations of the study and completing written informed consent forms, 430 women were invited to provide urine samples and information regarding their demographic and disease history (n = 388). A total of 171 (39.8%) children were evaluated at the age of 8 years to investigate for asthma symptoms; 259 children were lost to follow up because of moving or their parents' or caregivers' refusal to participate. At the follow-up visit, primary caretakers completed a questionnaire, and blood and urine samples were collected from the children. After excluding children that were lost to follow up, 136 maternal urine samples obtained during the third trimester of pregnancy, 99 urine samples obtained from children at 2 years of age, and 110 urine samples obtained from the children at 5 years of age were analyzed. A flow chart depicting the details of the study population is shown in Fig 1.

Disease definition and symptoms

The International Study of Asthma and Allergies in Childhood (ISAAC) is a standardized and validated method to investigate variations in childhood allergies [18]. We used the protocol of the ISAAC Chinese version to assess asthma in children at 8 years of age. A child was

considered to have asthma if the primary caretakers responded positively to the question "Has your child ever been diagnosed with asthma by a physician?" and was considered to have a history of wheezing if the primary caretaker responded positively to the question "Has your child ever had a chest wheeze when he/she did not have a flu?" Children were classified as having a parental history of asthma if at least one parent responded positively to the question "Have you ever been diagnosed with asthma by a physician?"

Analysis of total serum immunoglobulin E levels

To determine serum IgE levels in 8-year-old children, blood samples (0.5 mL) obtained via venipuncture were centrifuged and the sera stored at −20°C prior to analysis. Serum total IgE (tIgE) levels were measured using the ADVIA Centaur chemiluminescence immunoassay system (Siemens Healthcare Diagnostics; Deerfield, IL, USA). The assay range was approximately 1.5–3000 IU/mL.

Analysis of phthalate metabolites

Four phthalate monoester metabolites (mono-2-ethylhexyl phthalate [MEHP], mono-benzyl phthalate [MBzP], mono-butyl phthalate [MBP], and mono-ethyl phthalate [MEP]) that are representative of exposure to four commonly used phthalates (DEHP, BBzP, di-butyl phthalate [DBP], and DEP) were measured in maternal and children's urine samples (S1 Table). Details of the analysis and quality control procedures for the pregnant women's samples have been described previously [19]. Analysis of single urine samples from children aged 2, 5, and 8 years was performed according to a modified method previously described by Koch et al. [20]. In brief, 0.1 mL urine sample aliquots containing 1 M ammonium acetate (20 µL), β-glucuronidase (10 µL), and a mixture of isotopic phthalate metabolite standards were prepared. The samples were incubated at 37°C for 1.5 hours. Subsequent to hydrolysis, each sample was injected with 270 µL solvent (5% acetonitrile and 0.1% formic acid) in glass screw-cap vials and mixed for quantitative liquid chromatography-electrospray ionization-tandem mass spectrometry. Urinary creatinine levels were measured at the Kaohsiung Medical University Chung-Ho Memorial Hospital using a spectrophotometric method. Creatinine-corrected adjustment for each individual was used to examine associations between the compound exposure and asthma symptoms. If we used indirect adjustment by including urinary creatinine concentrations and uncorrected phthalate levels in multiple regression analyses, similar associations between the phthalate level and asthma status were found.

Statistical analysis

The differences between pregnant women lost to follow up and those included in the analysis were estimated using independent t-tests for continued variables and Chi-square tests for categorical variables. In our preliminary analyses, we applied exposure categories based on tertiles, quartile, and quintiles of biomarker concentrations. We found that increased risk was first observed when exposure levels achieved the highest quintile. We believe that this may have resulted from lower levels of MBzP and MEP in our subjects relative to those of other studies [21,22]. Therefore, we divided the participants into 2 groups: the 'highest quintile' group, which consisted of the participants within the 80th to 100th percentiles, and the reference group, which consisted of the participants that were below the 80th percentile. The adjusted odds ratio (aOR) for asthma or wheezing was calculated using logistic regression models adjusted for parental allergies and family members' smoking status. We defined parental allergies as: at least one parent having been diagnosed with any of asthma, dermatitis, rhinitis, or conjunctivitis. Multiple linear regression analyses were performed to examine the association

between different phthalate metabolites and total IgE levels, after controlling for sex and parental allergies. Measured phthalate metabolite and total IgE levels were transformed by taking the base 10 log to fit normal distributions. The creatinine-corrected adjusted urinary concentrations of MEHP, mono-2-ethyl-5-oxohexyl phthalate, and mono-2-ethyl-5-hydroxyhexyl phthalate were combined to assess DEHP exposure (ΣDEHP). Statistical analyses were carried out using SPSS 17.0 software (Chicago, IL, USA). Hsiu-Ying Ku, Hui-Ju Wen, and Shu-Li Wang had full access to all data in this study and took responsibility for the integrity of the data and accuracy of the data analysis.

Results

Characteristics of the study population

Demographic characteristics of pregnant women with children who were followed up successfully at 8 years (n = 171) and those lost to follow up (n = 217) are shown in Table 1. Pregnant women who were followed up tended to be educated to a higher level (i.e., ≥university) relative to those lost to follow up. The distribution of phthalate metabolite levels was skewed to the right (Fig 2). We found that pregnant women who were lost to follow up tended to exhibit higher geometric mean concentrations on four phthalate metabolites, with the exception of MEP concentration.

Childhood asthma risk

At 8 years of age, 32 of the 171 participating children (19%) showed a history of wheezing (24% of boys, 14% of girls; S2 Table). Boys were more likely to have allergic asthma relative to girls (19% and 10%, respectively). In order to explore potential confounding for associations between phthalate exposure and asthma, we examined differences in the distributions of possible confounders among children with and without asthma using Chi-square or Fisher's exact tests (Table 2). There was a borderline significant difference in family members' smoking status between asthmatic and non-asthmatic groups. In addition, based on current knowledge, gender and parental history of asthma are important predictors for asthma in children. Therefore, model covariates included parental allergies and family members' smoking status for sex-stratified analyses. MEHP levels appeared to be higher in our children as compared to Danish ones (S3 Table).

Phthalates and asthma

The association between different phthalate metabolites and asthma or wheezing in children is shown in Table 3. An overview of the results indicated positive associations between maternal phthalate metabolite concentrations and ever wheezing in children of both sexes, with the exception of MBP. In boys, there was a significant association between the prevalence of ever wheezing and a maternal MBzP concentration greater than the 80th percentile (aOR = 4.95; 95% CI: 1.08–22.63), compared with the reference group with MBzP levels less than or equal to the 80th percentile, after adjusting for maternal and paternal allergies and family members' smoking status. In addition, there was a positive association of borderline significance between the prevalence of ever wheezing and maternal ΣDEHP metabolite concentrations in boys (aOR = 4.12; 95% CI: 0.87–19.61). In boys, the upper quintile of 2-year-old MEHP concentrations had a significant odds ratio for asthma at 8 years (aOR = 6.14; 95% CI: 1.17–32.13) and similar association patterns were found in 5-year-old MEHP. Boys in the upper quintile concentration of 5-year-old ΣDEHP were at an approximately four fold increased risk of asthma at 8 years of age (aOR = 4.36; 95% CI: 1.01–18.86), relative to the reference group. Similarly, we observed significantly increased odds ratios for asthma in the upper quintile of 5-year-old MEP

Table 1. Demographics and maternal urinary phthalate metabolite concentrations (μg/g creatinine) in pregnant women with and without children who were followed up for asthma at 8 years of age.

Variables	Pregnant women with children lost to follow up (n = 217)	Pregnant women with children followed up (n = 171)
Mean age at delivery (y)	28.44 ± 4.29	29.10 ± 4.00
Age at delivery (y)		
≤35	205 (94.5%)	160 (93.6%)
>35	12 (5.5%)	11 (6.4%)
Maternal education		
≤High school	112 (51.6%)	70 (40.9%)
Junior college	78 (35.9%)	75 (43.9%)
≥University	27 (12.4%)	26 (15.2%)
Paternal education		
≤High school	105 (48.4%)	67 (39.2%)
Junior college	83 (38.2%)	76 (44.4%)
≥University	29 (13.4%)	28 (16.4%)
Family income per year (US Dollars)		
≤$20,000	96 (44.2%)	67 (39.2%)
$20,000–50,000	87 (40.1%)	76 (44.4%)
>$50,000	34 (15.7%)	28 (16.4%)
Smoking during pregnancy		
Yes	5 (3.5%)	0 (0%)
No	212 (96.5%)	171 (100%)
Passive smoking prior to pregnancy		
Yes	93 (42.9%)	81 (47.4%)
No	124 (57.1%)	90 (52.6%)
MEHP[a,b] (μg/g creatinine)	20.11 (17.83–22.70)	16.90 (14.49–19.72)
ΣDEHP[a,b] (μg/g creatinine)	54.24 (48.28–60.93)	50.22(42.22–59.72)
MBzP[a,b] (μg/g creatinine)*	18.76 (17.08–20.61)	15.48 (13.59–17.63)
MBP[a,b] (μg/g creatinine)	74.86 (66.73–83.97)	66.14 (56.06–78.03)
MEP[a,b] (μg/g creatinine)	61.37 (52.62–71.57)	65.15 (58.52–72.53)

Data are presented as number (%) or mean.

ΣDEHP, sum of metabolites of di-2-ethylhexyl phthalate; MEHP, mono-2-ethylhexyl phthalate; MBzP, mono-benzyl phthalate; MBP, mono-butyl-phthalate; MEP, mono-ethyl phthalate.

[a]Phthalate metabolite data available for n = 252 pregnant women with children lost to follow up and n = 136 pregnant women with followed-up children.

[b]Data are presented as geometric mean (95% confidence interval).

*Indicates a significant ($p < 0.05$) difference between lost to follow up and followed up participants by Mann-Whitney U test.

doi:10.1371/journal.pone.0123309.t001

concentration in boys (aOR = 8.92; 95% CI: 1.87–42.54). There was also a borderline significant positive association between the prevalence of ever wheezing and 5-year-old MEP (aOR = 4.22; 95% CI: 0.87–20.51). In girls, no significant association was observed between phthalate metabolite concentrations and risk of asthma or wheezing.
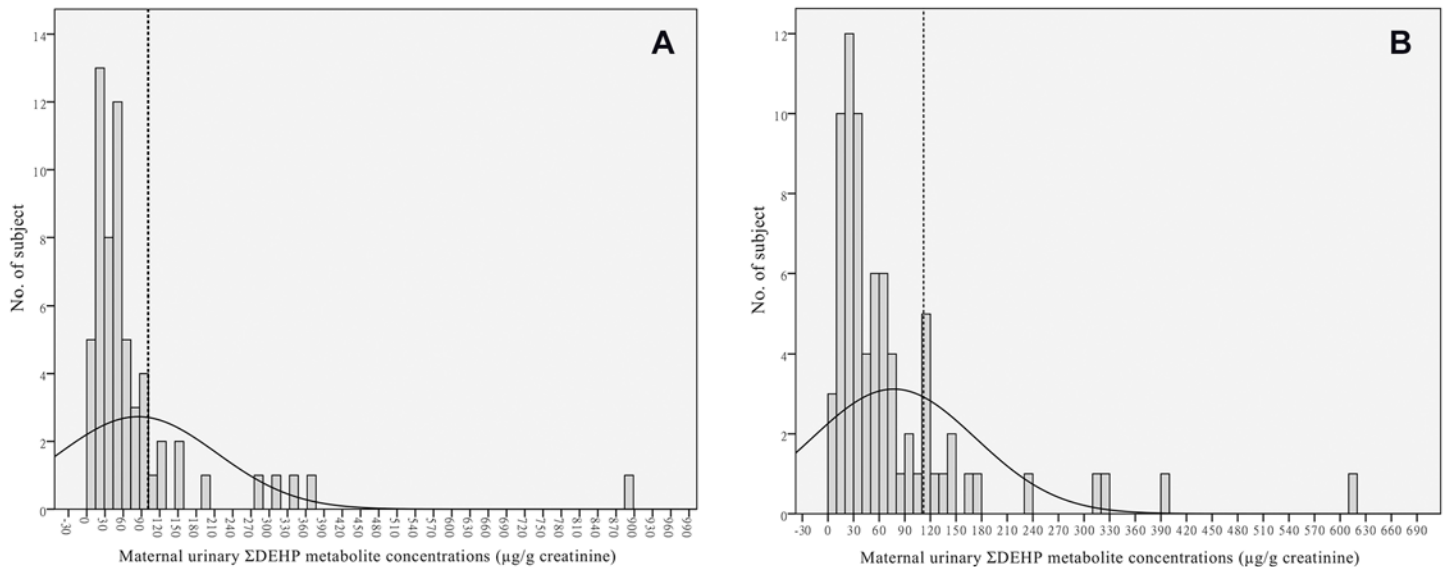
Fig 2. Distribution of urinary phthalate metabolite concentrations (µg/g creatinine) in pregnant women. The sum of di-2-ethylhexyl phthalate (ΣDEHP) metabolite concentrations was skewed to the right. Subjects in the highest quintile (80th to 100th percentile) showed the strongest determinant of the outcomes of interest. (A). The cut-point for the highest quintile of maternal ΣDEHP metabolite concentration was 101 µg ΣDEHP/g creatinine in boys. (B). The cutoff was 112 µg ΣDEHP/g creatinine in girls.

doi:10.1371/journal.pone.0123309.g002

Phthalate metabolites and total immunoglobulin E levels

Log_{10}-transformed concentrations of 8-year-old serum total IgE and creatinine-corrected urine phthalate metabolite were applied for further analysis. The unstandardized regression coefficients (B) for tIgE at 8 years of age, according to urine phthalate metabolite concentrations in pregnant women and children aged 2 and 5 years are shown in Table 4. A significant positive association was observed between maternal MBzP concentrations and tIgE (per log-unit: B = 0.39; p = 0.03) in 8-year-old children after adjusting for gender and paternal allergies. In allergic children, higher MEHP concentrations in pregnant women and 5-year-old children were positively correlated with tIgE levels at 8 years of age (per log-unit: B = 0.50, p <0.05 and B = 0.36, p <0.05, respectively). A significant positive correlation between MBzP levels at 2 years of age and tIge in allergic children (per log-unit: B = 0.04, p = 0.02) was also observed. However, no association was observed in non-allergic children.

Discussion

To our knowledge, this is the first study to assess the effects of both prenatal and postnatal exposure to phthalates on the risk for childhood asthma. In this study, higher maternal MBzP levels were associated with an approximately five-fold increase in the odds of wheezing in boys and substantial increases in tIgE levels in 8-year-old children of both sexes. In addition, we found that increased maternal ΣDEHP metabolite concentrations were associated with increased risk of wheezing in boys, although the association only had borderline statistical significance. Maternal MEHP levels were positively associated with increased tIgE levels in allergic children. This indicates that IgE tends to increase with increasing DEHP and BBzP metabolite concentrations in these allergic children.

Wheezing is one of the earliest symptoms of asthma, and in young children, it results from narrowing of the airways of the lungs as a result of inflammation [23]. Children who experience persistent wheezing tend to have higher asthma rates later in life [24]. Jaakkola et al. reported

Table 2. Characteristics of children at 8 years of age classified by asthma status (n = 171).

Variables	Children without asthma n (%)	Children with asthma n (%)	p-value[a]
Gender			0.09
Girls	82 (55.8%)	9 (37.5%)	
Boys	65 (44.2%)	15 (62.5%)	
Maternal education			0.56
≤High school	51 (35.2%)	10 (41.7%)	
Junior college	55 (37.9%)	10 (41.7%)	
≥University	39 (26.9%)	4 (16.7%)	
Maternal allergies			0.30
No	90 (61.2%)	12 (50.0%)	
Yes	57 (38.8%)	12 (50.0%)	
Paternal allergies			0.79
No	119 (81.0%)	19 (79.2%)	
Yes	28 (19.0%)	5 (20.8%)	
Parental allergies			0.46
No	73 (49.7%)	10 (41.7%)	
Yes	74 (50.3%)	14 (58.3%)	
Family income per year (US Dollars)			0.62
<US$20,000	49 (36.0%)	6 (31.6%)	
$20,000–50,000	49 (36.0%)	9 (47.4%)	
>$50,000	38 (27.9%)	4 (21.1%)	
Family members' moking status			0.06
No	79 (53.7%)	8 (33.3%)	
Yes	68 (46.3%)	16 (66.7%)	
Breastfeeding			0.67
≤3 months	116 (78.9%)	18 (75.0%)	
>3 months	31 (21.1%)	6 (25.0%)	
Water leakage at home (days/year)			0.49
0	130 (89.0%)	20 (83.3%)	
≥1	16 (11.0%)	4 (16.7%)	
Cockroaches observed at home (cockroaches/month)			0.96
0–2	57 (39.6%)	9 (39.1%)	
≥3	87 (60.4%)	14 (60.9%)	
Number of walls with mold at home			0.46
0	66 (45.2%)	10 (41.7%)	
≥1	80 (54.8%)	14 (58.3%)	
Serum total IgE levels (IU/mL)	74.8 ± 22.74	132.5 ± 90.58	0.27[b]

Data are presented as number (%) or median ± standard error.

IgE, immunoglobulin E.

[a] p values for differences between groups were estimated using Chi-square (Fisher's exact) test.

[b] p values for the difference between non-asthmatic children and asthmatic children using a Mann-Whitney U test.

doi:10.1371/journal.pone.0123309.t002

that exposure to plastic wall material was associated with a three fold increase in risk of persistent wheezing in children [25]. A recent study found that maternal urinary MBzP concentration was associated with history of asthma-like symptoms and current asthma [13]. Similarly, Kolarik et al. showed that DEHP in indoor dust was correlated with wheezing in children in

Table 3. Adjusted odds ratios for wheezing and asthma occurrence at 8 years for the upper quintile in urinary phthalate metabolite concentrations *in utero* and at 2 and 5 years of age.

Phthalate metabolite upper quintile	Boys					Girls					Total			
	Wheezing		Asthma		Upper quintile	Wheezing		Asthma		Upper quintile	Wheezing		Asthma	
	aOR	95% CI	aOR	95% CI		aOR	95% CI	aOR	95% CI		aOR	95% CI	aOR	95% CI
A. Urinary metabolites during the 3rd trimester														
					n = 62					n = 74				
														n = 136
MEHP	2.38	(0.48–11.84)	0.93	(0.16–5.19)	>33.5	1.09	(0.11–10.80)	1.04	(0.10–10.43)	>38.5	1.68	(0.47–6.02)	0.96	(0.24–3.81)
ΣDEHP	4.12#	(0.87–19.61)	0.77	(0.14–4.28)	>101.3	2.80	(0.42–18.69)	0.74	(0.07–7.39)	>112.7	3.12#	(0.98–9.98)	0.81	(0.21–3.14)
MBzP	4.95*	(1.08–22.63)	0.83	(0.15–4.66)	>23.9	1.00	(0.10–9.98)	1.00	(0.10–10.08)	>31.9	1.71	(0.46–6.38)	0.68	(0.14–3.38)
MEP	1.17	(0.20–6.80)	0.88	(0.16–4.80)	>118.6	2.94	(0.44–19.70)	-	-	>121.2	1.54	(0.44–5.40)	0.48	(0.10–2.30)
MBP	0.35	(0.04–3.17)	-	-	>142.0	0.99	(0.10–9.68)	0.72	(0.07–7.12)	>180.8	0.66	(0.14–3.22)	0.23	(0.03–1.83)
B. Urinary metabolites at 2 years														
					n = 50					n = 49				
														n = 99
MEHP	2.39	(0.47–12.23)	6.14*	(1.17–32.13)	>31.8	1.05	(0.10–11.41)	1.46	(0.10–21.47)	>43.2	1.74	(0.46–6.58)	2.94	(0.70–12.26)
ΣDEHP	1.03	(0.17–6.23)	3.78	(0.64–22.30)	>322.9	0.99	(0.09–10.33)	-	-	>323.7	1.00	(0.24–4.13)	1.68	(0.38–7.48)
MBzP	1.47	(0.24–9.10)	1.48	(0.24–9.34)	>27.3	-	-	-	-	>19.9	0.57	(0.11–2.96)	0.71	(0.13–3.77)
MEP	-	-	-	-	>54.3	-	-	-	-	>83.0	0.27	(0.03–2.32)	-	-
MBP	0.40	(0.04–3.75)	1.33	(0.22–8.06)	>283.4	1.13	(0.11–11.70)	-	-	>276.6	0.59	(0.12–2.93)	0.75	(0.14–3.96)
C. Urinary metabolites at 5 years														
					n = 55					n = 55				
														n = 110
MEHP	0.86	(0.15–4.88)	2.61	(0.60–11.37)	>25.2	-	-	-	-	>25.1	0.59	(0.12–2.91)	1.27	(0.36–4.49)
ΣDEHP	1.70	(0.35–8.24)	4.36*	(1.01–18.86)	>341.8	1.40	(0.12–15.98)	0.96	(0.09–9.98)	>284.7	1.59	(0.43–5.86)	3.48*	(1.10–10.99)
MBzP	-	-	0.91	(0.16–5.25)	>37.8	-	-	-	-	>29.2	-	-	0.43	(0.09–2.14)
MEP	4.22#	(0.87–20.51)	8.92*	(1.87–42.54)	>56.9	1.74	(0.14–21.20)	-	-	>51.5	2.85	(0.81–10.04)	2.74#	(0.85–8.90)
MBP	0.41	(0.05–3.74)	1.50	(0.32–7.04)	>201.5	-	-	0.76	(0.07–8.55)	>140.5	0.23	(0.03–1.90)	0.66	(0.17–2.64)

Data are adjusted for parental allergies and family members' smoking status.

Urinary metabolites are measured as µg metabolite/g creatinine.

[a]OR: adjusted odds ratio; CI: confidence interval; MEHP: mono-2-ethylhexyl phthalate; ΣDEHP: sum of metabolites of di-2-ethylhexyl phthalate; MBzP: mono-benzyl phthalate; MEP: mono-ethyl phthalate; MBP: mono-butyl-phthalate.

* Indicates a significant ($p < 0.05$) finding;

# indicates a borderline significant ($p < 0.10$) finding.

doi:10.1371/journal.pone.0123309.t003

Table 4. Association between maternal and children's log-transformed urinary phthalate metabolite concentrations (μg/g creatinine) and log-transformed total serum immunoglobulin E levels (IU/ml) in 8-year-old children.

Phthalate metabolite	Allergic children[a]	Non-allergic children	All children
A. Urinary metabolites during the 3rd trimester			
	n = 72	n = 58	n = 130
MEHP	**0.50 (0.02)***	0.24 (0.26)	0.38 (0.16)
ΣDEHP	0.20 (0.32)	0.12 (0.51)	0.03 (0.89)
MBzP	0.20 (0.94)	0.22 (0.39)	**0.39 (0.03)***
MEP	0.11 (0.55)	0.43 (0.09)	0.10 (0.58)
MBP	0.20 (0.34)	0.23 (0.20)	0.06 (0.72)
B. Urinary metabolites at 2 years			
	n = 56	n = 36	n = 92
MEHP	0.07 (0.77)	0.36 (0.18)	0.08 (0.66)
ΣDEHP	0.28 (0.36)	0.23 (0.36)	0.01 (097)
MBzP	**0.40 (0.02)***	0.16 (0.32)	0.20 (0.11)
MEP	−0.29 (0.15)	−0.20 (0.29)	−0.23 (0.10)
MBP	−0.27 (0.37)	0.21 (0.41)	0.04 (0.85)
C. Urinary metabolites at 5 years			
	n = 63	n = 40	n = 103
MEHP	**0.36 (0.04)***	−0.22 (0.20)	0.10 (0.43)
ΣDEHP	0.29 (0.16)	−0.14 (0.53)	0.14 (0.35)
MBzP	−0.03 (0.90)	0.09 (0.65)	0.03 (0.85)
MEP	−0.14 (0.43)	0.18 (0.30)	0.01 (0.97)
MBP	0.21 (0.29)	0.18 (0.51)	0.22 (0.17)

Data are presented as unstandardized regression coefficient (B) and *p*-value.

Associations were adjusted for gender and parental allergies.

MEHP: mono-2-ethylhexyl phthalate; ΣDEHP: sum of metabolites of di-2-ethylhexyl phthalate; MBzP: mono-benzyl phthalate; MEP: mono-ethyl phthalate; MBP: mono-butyl-phthalate.

[a]Allergic children included children with asthma, dermatitis, rhinitis, and conjunctivitis.

*Indicates a significant (*p* <0.05) association, estimated using linear regression, between log-transformed phthalate metabolite concentrations and log-transformed serum immunoglobulin E concentrations.

doi:10.1371/journal.pone.0123309.t004

Bulgaria [26]. Furthermore, Just *et al.* reported a positive association between current MBzP concentrations and airway inflammation, particularly in children who wheezed [27].

Boys in the upper quintile of DEHP metabolite and MEP concentrations at 2 and 5 years of age were more likely to experience asthma during the follow-up period. These results are consistent with those of a previous case-control study of children in which risk of asthma was significantly associated with DEHP concentrations [14]. In addition, current DEP exposure was positively associated with airway inflammation in children and decreased forced expiratory volume in 1 second in adults [5,27]. However, DEP and BBzP air samples were shown to be significantly correlated with urinary metabolite concentrations, suggesting that inhalation of phthalates may be an important pathway to the development of allergic asthma [28].

Our data showed positive correlations between urinary DEHP metabolites at different ages and tIgE levels in allergic children at 8 years of age. In addition, urinary MBzP concentrations at 2 years of age were positively associated with tIgE levels in 8-year-old allergic children. A strong positive association has previously been reported to exist between airway reactivity and DEHP levels in combination with allergen exposure and tIgE levels [9]. In addition, an association between MBzP and tIgE levels in 2-year-old boys has been observed in Taiwan [29].

Allergic asthma is thought to be a chronic inflammatory disease that is mainly driven by an allergen-specific Th2 immune response [30]. Therefore, it has been hypothesized that certain phthalates may be a trigger for asthma-related airway inflammation and an increase in Th2 immunoglobulins including IgE [30]. Our findings show that DEHP and MBzP metabolites might trigger increased tIgE levels in children with allergic diseases. Therefore, it appears that the allergy status of children makes them vulnerable to the effects of phthalate exposure.

There is limited knowledge regarding the mechanism through which phthalate exposure *in utero* promotes allergies. DEHP can cross the placenta and potentially result in abnormal fetal development [31]. We have previously shown a statistically significant association between maternal DEHP metabolite concentrations and cord blood concentrations of sex hormones [19]. In addition, prenatal BBzP exposure may predispose children to allergic sensitization, perhaps through the disruption of maternal estrogen during pregnancy [32]. Sex hormones during pregnancy influence the risk of allergic diseases in children [33]. Therefore, *in utero* phthalate exposure could disrupt maternal sex hormone concentrations and affect the development of the immune system in children. Moreover, *in utero* exposure to BBzP may modify the expression of genes related to immunity, including cluster of differentiation (CD) 24, CD5, cathepsin E, protein tyrosine phosphatase receptor type C, and secretory leukocyte protease inhibitor, through epigenetic effects [34]. Besides, MEHP may trigger release of TNF-α and initiate a possible differentiation towards anti-inflammatory macrophages [35].

We report the results of a long-term cohort study in which maternal exposure levels were determined prior to the diagnosis of the children's allergic disease. In addition, we studied the role of tIgE levels as a marker of allergy and assessed its association with different phthalate metabolites. A limitation of this study was the relatively small sample size, which may have resulted in insufficient statistical power to conduct further stratification analyses. However, despite the sample size, significant associations between phthalate metabolites and asthma incidence in children were observed. A second limitation of the study was that maternal urine samples were collected during the third trimester of pregnancy. It has been reported that the relatively high temporal variability in DEHP metabolites in pregnant women result in non-differential exposure misclassification of DEHP; therefore, bias has a minimal effect [36]. A third limitation was the high percentage (60%) of participants lost to follow up at 8 years of age. However, a higher proportion of pregnant women who returned for follow up (15%) were educated to higher levels relative to those lost to follow up (12%). Our results also showed that geometric mean values of phthalate metabolites (except for MEP) were usually lower in the follow-up group. In addition, we used lifetime prevalence of physician-diagnosed asthma in the ISAAC questionnaire for respondents but did not record the onset of asthma that is generally diagnosed after 5 years of age. However, non-random misclassification of the exposure was unlikely, as asthmatic and non-asthmatic people are equally likely to be misclassified according to exposure. Together with previous findings that pregnant women's exposure to phthalates has been reported to associate with asthma symptoms in their children. Therefore, it is not likely that our conclusion was biased. Future study is directed to record time of the disease onset in consecutive follow-ups.

Concerning the prevalence of asthma and wheezing, asthma rate (14%) in the present study was similar to that found in an earlier similar survey conducted in the same area (15%) [37]. However, the prevalence of asthma was higher than the prevalence reported in nationwide study on school-age children in Taiwan (8.8%) [38]. The reason for this discrepancy likely lies in the fact that the participants in our study were living in urban areas where prevalence of asthma tended to be higher than in rural areas. As compared to the international data, the prevalence of wheezing in our study (19%) was similar to that reported in international data (16–29%) [39] However, asthma prevalence among boys in our study (19%) appeared to be

higher than that reported in international data (8–12%) [1]. It is relatively difficult to distinguished between cold and wheezing symptoms for parents and primary caretakers in Taiwan with high incidence of flu and cold. Thus, the prevalence of wheezing could have been underestimated in our study.

Conclusions

We provide new evidence that early exposure to certain phthalates may associate with allergic sensitization and play a role in the development of asthma during the first 8 years of life. This observation raises substantial public health concerns given the widespread use of phthalate esters and increasing prevalence of asthma in children.

Supporting Information

S1 Table. Pearson's correlation between log-transformed phthalate metabolite concentrations in urine samples of mothers and children aged 2, 5, and 8 years.
(DOC)

S2 Table. Demographic characteristics and the prevalence of allergic diseases in 8-year-old children.
(DOC)

S3 Table. Pregnant women and children's geometric means of phthalate metabolites in different studies.
(DOC)

Acknowledgments

Author Contributions

References

1. Pearce N, Ait-Khaled N, Beasley R, Mallol J, Keil U, Mitchell E, et al. Worldwide trends in the prevalence of asthma symptoms: phase III of the International Study of Asthma and Allergies in Childhood (ISAAC). Thorax. 2007; 62: 758–766. PMID: 17504817

2. Delacourt C. Role of IgE-dependent mechanisms in infantile asthma. Revue Francaise D Allergologie Et D Immunologie Clinique. 2002; 42: 267–270.

3. Chalubinski M, Kowalski ML. Endocrine disrupters—potential modulators of the immune system and allergic response. Allergy. 2006; 61: 1326–1335. PMID: 17002710

4. Carlstedt F, Jonsson BA, Bornehag CG. PVC flooring is related to human uptake of phthalates in infants. Indoor Air. 2013; 23: 32–39. doi: 10.1111/j.1600-0668.2012.00788.x PMID: 22563949

5. Hoppin JA, Ulmer R, London SJ. Phthalate exposure and pulmonary function. Environ Health Perspect. 2004; 112: 571–574. PMID: 15064163

6. Heudorf U, Mersch-Sundermann V, Angerer E. Phthalates: Toxicology and exposure. Int J Hyg Environ Health. 2007; 210: 623–634. PMID: 17889607

7. Lin S, Ku HY, Su PH, Chen JW, Huang PC, Angerer J, et al. Phthalate exposure in pregnant women and their children in central Taiwan. Chemosphere. 2011; 82: 947–955. doi: 10.1016/j.chemosphere.2010.10.073 PMID: 21075419

8. Wu MT, Wu CF, Wu JR, Chen BH, Chen EK, Chao MC, et al. The public health threat of phthalate-tainted foodstuffs in Taiwan: the policies the government implemented and the lessons we learned. Environ Int. 2012; 44: 75–79. doi: 10.1016/j.envint.2012.01.014 PMID: 22361240

9. Guo J, Han B, Qin L, Li B, You H, Yang J, et al. Pulmonary toxicity and adjuvant effect of di-(2-exylhexyl) phthalate in ovalbumin-immunized BALB/c mice. PLOS One. 2012; 7(6): e39008 doi: 10.1371/journal.pone.0039008 PMID: 22701742

10. Kuo CH, Hsieh CC, Kuo HF, Huang MY, Yang SN, Chen LC, et al. Phthalates suppress type I interferon in human plasmacytoid dendritic cells via epigenetic regulation. Allergy. 2013; 68: 870–879. doi: 10.1111/all.12162 PMID: 23738920

11. Larsson M, Hagerhed-Engman L, Kolarik B, James P, Lundin F, Janson S, et al. PVC—as flooring material—and its association with incident asthma in a Swedish child cohort study. Indoor Air. 2010; 20: 494–501. doi: 10.1111/j.1600-0668.2010.00671.x PMID: 21070375

12. Bertelsen RJ, Carlsen KC, Calafat AM, Hoppin JA, Haland G, Mowinckel P, et al. Urinary biomarkers for phthalates associated with asthma in Norwegian children. Environ Health Perspect. 2013; 121: 251–256. doi: 10.1289/ehp.1205256 PMID: 23164678

13. Whyatt RM, Perzanowski MS, Just AC, Rundle AG, Donohue KM, Calafat AM, et al. Asthma in inner-city children at 5–11 years of age and prenatal exposure to phthalates: the Columbia Center for Children's Environmental Health Cohort. Environ Health Perspect. 2014; 122: 1141–1146. doi: 10.1289/ehp.1307670 PMID: 25230320

14. Bornehag CG, Sundell J, Weschler CJ, Sigsgaard T, Lundgren B, Hasselgren M, et al. The association between asthma and allergic symptoms in children and phthalates in house dust: a nested case-control study. Environ Health Perspect. 2004; 112: 1393–1397. PMID: 15471731

15. Dietert RR, Zelikoff JT. Early-life environment, developmental immunotoxicology, and the risk of pediatric allergic disease including asthma. Birth Defects Res B Dev Reprod Toxicol. 2008; 83: 547–560. doi: 10.1002/bdrb.20170 PMID: 19085948

16. Su PH, Huang PC, Lin CY, Ying TH, Chen JY, Wang SL. The effect of in utero exposure to dioxins and polychlorinated biphenyls on reproductive development in eight year-old children. Environ Int. 2012; 39: 181–187. doi: 10.1016/j.envint.2011.09.009 PMID: 22208758

17. Wang SL, Lin CY, Guo YLL, Lin LY, Chou WL, Chang L W. Infant exposure to polychlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls (PCDD/Fs, PCBs)—correlation between prenatal and postnatal exposure. Chemosphere. 2004; 54: 1459–1473. PMID: 14659948

18. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. Eur Respir J. 1995; 8: 483–491. PMID: 7789502

19. Lin LC, Wang SL, Chang YC, Huang PC, Cheng JT, Su PH, et al. Associations between maternal phthalate exposure and cord sex hormones in human infants. Chemosphere. 2011; 83: 1192–1199. doi: 10.1016/j.chemosphere.2010.12.079 PMID: 21272909

20. Koch HM, Gonzalez-Reche LM, Angerer J. On-line clean-up by multidimensional liquid chromatography-electrospray ionization tandem mass spectrometry for high throughput quantification of primary and secondary phthalate metabolites in human urine. J Chromatogr B Analyt Technol Biomed Life Sci. 2003; 784: 169–182. PMID: 12504195

21. Boas M, Frederiksen H, Feldt-Rasmussen U, Skakkebaek NE, Hegedus L, Hilsted L, et al. Childhood exposure to phthalates: associations with thyroid function, insulin-like growth factor I, and growth. Environ Health Perspect. 2010; 118: 1458–1464. doi: 10.1289/ehp.0901331 PMID: 20621847

22. Woodruff TJ, Zota AR, Schwartz JM. Environmental chemicals in pregnant women in the United States: NHANES 2003–2004. Environ Health Perspect. 2011; 119: 878–885. doi: 10.1289/ehp.1002727 PMID: 21233055

23. Krawiec ME, Westcott JY, Chu HW, Balzar S, Trudeau JB, Schwartz LB, et al. Persistent wheezing in very young children is associated with lower respiratory inflammation. Am J Respir Crit Care Med. 2001; 163: 1338–1343. PMID: 11371398

24. Guilbert TW, Morgan WJ, Zeiger RS, Bacharier LB, Boehmer SJ, Krawiec M, et al. Atopic characteristics of children with recurrent wheezing at high risk for the development of childhood asthma. J Allergy Clin Immunol. 2004; 114: 1282–1287. PMID: 15577824

25. Jaakkola JJ, Verkasalo PK, Jaakkola N. Plastic wall materials in the home and respiratory health in young children. Am J Public Health. 2000; 90: 797–799. PMID: 10800434

26. Kolarik B, Naydenov K, Larsson M, Bornehag CG, Sundell J. The association between phthalates in dust and allergic diseases among Bulgarian children. Environ Health Perspect. 2008; 116: 98–103. doi: 10.1289/ehp.10498 PMID: 18197306

27. Just AC, Whyatt RM, Miller RL, Rundle AG, Chen Q, Calafat AM, et al. Children's urinary phthalate metabolites and fractional exhaled nitric oxide in an urban cohort. Am J Respir Crit Care Med. 2012; 186: 830–837. doi: 10.1164/rccm.201203-0398OC PMID: 22923660

28. Adibi JJ, Perera FP, Jedrychowski W, Camann DE, Barr D, Jacek R, et al. Prenatal exposures to phthalates among women in New York City and Krakow, Poland. Environ Health Perspect. 2003; 111: 1719–1722. PMID: 14594621

29. Wang IJ, Lin CC, Lin YJ, Hsieh WS, Chen PC. Early life phthalate exposure and atopic disorders in children: A prospective birth cohort study. Environ Int. 2013; 62C: 48–54.

30. Rosenwasser LJ. Mechanisms of IgE Inflammation. Current Allergy and Asthma Reports. 2011; 11: 178–183. doi: 10.1007/s11882-011-0179-6 PMID: 21302007

31. Li LX, Chen L, Meng XZ, Chen BH, Chen SQ, Zhao Y, et al. Exposure levels of environmental endocrine disruptors in mother-newborn pairs in China and their placental transfer characteristics. PLoS One. 2013; 8(5): e62526 doi: 10.1371/journal.pone.0062526 PMID: 23667484

32. Marsee K, Woodruff TJ, Axelrad DA, Calafat AM, Swan SH. Estimated daily phthalate exposures in a population of mothers of male infants exhibiting reduced anogenital distance. Environ Health Perspect. 2006; 114: 805–809. PMID: 16759976

33. Osman MF, Black C, Jick S, Hannaford P. Previous maternal oral contraception and the risk among subsequent offspring of asthma diagnosis in early childhood. Paediatr Perinat Epidemiol. 2009; 23: 567–573. doi: 10.1111/j.1365-3016.2009.01064.x PMID: 19840293

34. Moral R, Santucci-Pereira J, Wang R, Russo IH, Lamartiniere CA, Russo J. In utero exposure to butyl benzyl phthalate induces modifications in the morphology and the gene expression profile of the mammary gland: an experimental study in rats. Environ Health. 2011; 10: e5.

35. Bølling AK, Ovrevik J, Samuelsen JT, Holme JA, Rakkestad KE, Mathisen GH, et al. Mono-2-ethylhexylphthalate (MEHP) induces TNF-α release and macrophage differentiation through different signalling pathways in RAW264.7 cells. Toxicol Lett. 2012; 209: 43–50. doi: 10.1016/j.toxlet.2011.11.016 PMID: 22143055

36. Braun JM, Smith KW, Williams PL, Calafat AM, Berry K, Ehrlich S, et al. Variability of urinary phthalate metabolite and bisphenol A concentrations before and during pregnancy. Environ Health Perspect. 2012; 120: 739–745. doi: 10.1289/ehp.1104139 PMID: 22262702

37. Chiang LC, Chen YH, Hsueh KC, Huang JL. Prevalence and severity of symptoms of asthma, allergic rhinitis, and eczema in 10- to 15-year-old schoolchildren in central Taiwan. Asian Pac J Allergy Immunol. 2007; 25: 1–5. PMID: 17891915

38. Chen YC, Tu YK, Huang KC, Chen PC, Chu DC, Lee YL. Pathway from central obesity to childhood asthma. Physical fitness and sedentary time are leading factors. Am J Respir Crit Care Med. 2014; 189: 1194–1203. doi: 10.1164/rccm.201401-0097OC PMID: 24669757

39. Kuehni CE, Davis A, Brooke AM, Silverman M. Are all wheezing disorders in very young (preschool) children increasing in prevalence? Lancet. 2001; 357: 1821–1825. PMID: 11410189