



Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

journal homepage: www.elsevier.com/locate/ijheh



Phthalate metabolites in urine and asthma, allergic rhinoconjunctivitis and atopic dermatitis in preschool children

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ARTICLE INFO

Article history:

Received 7 October 2011

Received in revised form

27 November 2013

Accepted 5 December 2013

Keywords:

Allergic diseases

Clinical examination

Eczema

Exposure pathways

Wheeze

ABSTRACT

Phthalate esters are among the most ubiquitous of indoor pollutants and have been associated with various adverse health effects. In the present study we assessed the cross-sectional association between eight different phthalate metabolites in urine and allergic disease in young children. As part of the Danish Indoor Environment and Children's Health study, urine samples were collected from 440 children aged 3–5 years, of whom 222 were healthy controls, 68 were clinically diagnosed with asthma, 76 with rhinoconjunctivitis and 81 with atopic dermatitis (disease subgroups are not mutually exclusive; some children had more than one disease). There were no statistically significant differences in the urine concentrations of phthalate metabolites between cases and healthy controls with the exception of MnBP and MECP, which were *higher* in healthy controls compared with the asthma case group. In the crude analysis MnBP and MiBP were *negatively* associated with asthma. In the analysis adjusted for multiple factors, only a weak positive association between MEP in urine and atopic dermatitis was found; there were no *positive* associations between any phthalate metabolites in urine and either asthma or rhinoconjunctivitis. These findings appear to contradict earlier studies. Differences may be due to higher exposures to certain phthalates (e.g., BBzP) via non-dietary pathways in earlier studies, phthalates serving as surrogates for an agent associated with asthma (e.g., PVC flooring) in previous studies but not the present study or altered cleaning habits and the use of “allergy friendly” products by parents of children with allergic disease in the current study in contrast to studies conducted earlier.

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Introduction

An increased awareness of the potential impact of indoor exposures on allergic diseases has resulted in considerable research in this area over the past quarter century (Bornehag and Nanberg, 2010; Kimber and Dearman, 2010; Nielsen et al., 2007; Peat et al., 1998; Platt et al., 1989). Phthalate esters are commonly used as plasticizers and they are among the most frequently encountered indoor pollutants (Rudel et al., 2003). There has been growing evidence that certain phthalate esters can function as endocrine

disruptors. For example, recent studies have found associations between phthalate esters and adverse effects on genital development, semen quality, children's neurodevelopment, thyroid function, onset of puberty in females, children's mental, psychomotor and behavioral development and possibly respiratory problems (e.g. Boas et al., 2010; Bornehag and Nanberg, 2010; Frederiksen et al., 2012; Jurewicz and Hanke, 2011; Kimber and Dearman, 2010; Meeker et al., 2009; Whyatt et al., 2012; Wigle et al., 2008). There is somewhat less evidence for links between phthalate esters and allergic diseases. Several epidemiological studies have reported associations between certain phthalate esters in dust or PVC materials in homes and allergic diseases in children (Bornehag et al., 2004a; Hsu et al., 2012; Jaakkola et al., 1999, 2000, 2004; Kolarik et al., 2008; Larsson et al., 2010; Oie et al., 1999). However, Kimber and Dearman (2010) have questioned whether phthalates themselves are the causative agents in these studies and, more broadly, Nielsen et al. (2007) have questioned the role of indoor chemicals in

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the promotion of allergic diseases. Although phthalate metabolites in urine have been associated with various adverse health outcomes, studies that have examined potential associations between phthalate metabolites in urine and allergic diseases are limited. Just et al. (2012) found that prenatal exposure to BBzP, based on measurements of MBzP in spot urine samples during the third trimester of pregnancy, may influence the child's risk of developing eczema in early childhood. Hsu et al. (2012) found higher MBzP levels in asthmatics and an increased risk of diagnosed asthma with higher quartiles. MEHP levels were associated with the severity of allergic rhinitis. Ferguson et al. (2011) reported elevation of C-reactive protein, a serum marker of inflammation, with interquartile range increases in urinary MBzP.

The Danish Indoor Environment and Children's Health (IECH) study is an investigation of potential associations between different indoor environmental factors and children's health, with a focus on asthma, allergic rhinoconjunctivitis and atopic dermatitis (Clausen et al., 2012). As part of the IECH study, we have measured the mass fractions of five selected phthalates (diethyl phthalate (DEP), di(isobutyl) phthalate (DiBP), di(*n*-butyl) phthalate (DnBP), benzylbutyl phthalate (BBzP) and di(2-ethylhexyl) phthalate (DEHP)) in dust samples collected from the children's bedrooms and day-care centers (Langer et al., 2010). The five simple monoesters of these phthalates (MEP, MiBP, MnBP, MBzP, MEHP) and three of the oxidized metabolites of DEHP (MEHHP, MEOHP, MECPP) were measured in urine samples collected from the children (Langer et al., 2014). Callesen et al. (2014) examined the potential association between phthalate esters in the dust and allergic diseases. The only significant association was between DEHP and parent-reported current wheeze. The objective of the present paper is to assess potential associations between the *phthalate metabolites in urine* samples from children aged 3–5 years and asthma, allergic rhino-conjunctivitis and atopic dermatitis.

Material and methods

Ethics statement

The study was approved by The Regional Scientific Ethical Committee for Southern Denmark (Case # S-20070108).

First phase of study, selection of subjects and clinical examination

The first phase of the study included a written questionnaire (WQ) with 116 questions regarding characteristics of the building, indoor environment, family habits and occupant health with a focus on allergic diseases (asthma, rhinoconjunctivitis and eczema). The questionnaire resembled the one that was used in the original DBH study in Sweden (Bornehag et al., 2004b). The questions related to the child's health were similar to those used in the ISAAC study (Asher et al., 1995). The questionnaire was mailed to 17,486 families living in Funen with a child aged 1–5 years. The response rate was 63% corresponding to 11,084 families. Based on power calculations, a primary case–control group of 500 subjects was established among questionnaire responders aged 3–5 years from the municipality of Odense ($n=2835$). The control group was a sample of 300 randomly selected children (random controls). The selection criteria for the 200 children in the case group was at least two parentally reported disease/symptoms regarding asthma, allergic rhinoconjunctivitis or eczema/atopic dermatitis on the WQ (Clausen et al., 2012).

All children underwent a clinical examination coupled with a structured interview (CEI) by the same experienced medical doctor. Weight and height were recorded and an extensive questionnaire-based interview, including medical history, time and onset of

symptoms, trigger factors, allergic heredity and exposure to pets and tobacco smoke, was conducted. The medical history was supplemented with the use of a video exemplifying asthma in 3–5 year olds and a demonstration of sounds indicative of asthma. The diagnostic criteria that had to be met for a child to be labeled with one of the allergic diseases during the clinical examination (asthma, rhinoconjunctivitis and atopic dermatitis) are described in detail in Callesen et al. (2014). Children with clinically confirmed asthma, allergic rhinoconjunctivitis or atopic dermatitis were subsequently excluded from the *random control group* (and admitted in the disease-specific case groups, referred to as DS cases in what follows), leaving 243 children in the *healthy control group*. Additionally, doctor-diagnosed allergic diseases and “wheeze ever”, as reported by the parents using the written questionnaires, were used to obtain four new DS case groups.

Urine collection and chemical analyses

A phthalate-free urine collection vessel was packed at the sterile center of the Odense University Hospital and mailed to each of the families. It included instructions on sampling and handling. First morning urine sample was collected and stored at 5 °C until the family attended the clinical examination later that same day, at which point the samples were stored at –23 °C. Samples were collected between August 2008 and April 2009.

The targeted monophthalates were: mono-ethyl phthalate (MEP, metabolite of DEP), mono-*n*-butyl phthalate (MnBP, metabolite of DnBP), mono-isobutyl phthalate (MiBP, metabolite of DiBP), mono-benzyl phthalate (MBzP, metabolite of BBzP) and mono-2-ethylhexyl phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP) (all metabolites of DEHP). The procedure and results of the chemical analyses are described in detail in Langer et al. (2014). In brief, the urine samples were thawed and 250 µl aliquots were used for analysis. $^{13}\text{C}_4$ -labeled MEP and MEHP were added to each aliquot. The native phthalate metabolites present in the children's urine were deconjugated from their glucuronated form by incubation with β -glucuronidase for 2 h at 37 °C. After cooling to room temperature, the enzyme reaction was quenched by the addition of 125 µl of formic acid and diluted with 1 ml of 5% acetonitrile. The analytes in the enzyme treated children's urine samples, calibration standards and blanks were separated from their urinary matrix using solid phase extraction with preconditioned (2 ml methanol followed by 2 ml water) C18 Isolute SPE columns. After rinsing with 2 ml water, the monophthalates were eluted by the addition of 0.5 ml 1% formic acid in acetonitrile. The eluents were divided in three vials each and analyzed by liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS). The system used was a Waters 2695 LC unit connected to a Waters Quattro Ultima operated in the negative ESI-MS/MS mode by MassLynx 4.1 (all from Waters Corp., USA). The separation was performed using a 3 µm particle size phenyl column (150 mm × 2.1 mm; Fortis Technologies, UK). The analytes were detected under MRM-conditions optimized using direct infusion of reference substances. The collected MRM-traces for all analyzed ions were integrated and quantified using QuanLynx 4.1. All automatic integrations were manually inspected for all samples and standards, and corrected, if necessary. Glass vials and polypropylene microcentrifuge tubes were used during the analyses. Randomly selected samples were analyzed as duplicates or triplicates. For reasons outlined in Langer et al. (2014), we did not adjust our values using the creatinine correction approach. Concentrations below the limit of detection (LOD) were set to one-half of the LOD for subsequent calculations.

Table 1Prevalence of allergic diseases in the “primary case–control” group, limited to those children that delivered a urine sample ($n = 171 + 269$ out of $200 + 300$, respectively).

	Primary (random) controls ($n = 269$) ^a	Primary cases ($n = 171$) ^a
	<i>n</i> (%)	<i>n</i> (%)
Clinical examination coupled with a structured interview (CEI)		
Asthma	13 (4.8)	55 (32.2)
Rhinoconjunctivitis	15 (5.6)	61 (35.7)
Atopic dermatitis	31 (11.5)	50 (29.2)
Written questionnaire (WQ)		
Wheeze ever	78 (30.0)	151 (88.3)
Doctor diagnosed asthma	28 (10.4)	89 (52)
Doctor diagnosed rhinoconjunctivitis	5 (1.9)	47 (27.5)
Doctor diagnosed atopic dermatitis	24 (8.9)	60 (35.1)

^a 269 of the 300 primary random controls and 171 of the 200 primary cases delivered a urine sample. See section “First phase of study, selection of subjects and clinical examination” for details on the primary case–control group.

Statistical method

The number of children in the analyses was reduced by including only those that delivered a urine sample. Children with asthma, allergic rhinoconjunctivitis or atopic dermatitis based on the CEI (DS cases) were compared to the healthy control group subjects to investigate potential associations between the health outcomes and phthalate metabolite levels. In these analyses, children in the original case group ($n = 200$) without clinically confirmed allergic disease were excluded.

In a similar approach, DS case groups based on the written questionnaire were compared to a unique control group obtained for each endpoint by excluding the children with the particular parent-reported disease/symptom from the healthy control group. The analyses approach used to compare these groups with the healthy controls were identical to the analyses used for the DS case groups determined from the CEI.

For the purpose of correcting for social class, the participants were divided into two groups, following the education and employment status criteria suggested by Hansen (1986) – Group I (higher social class): Hansen’s classes 1–3 and Group II (lower social class): Hansen’s classes 4–5.

For comparisons of medians for healthy controls and the various DS case groups as well as for various factors related to allergic diseases the Mann–Whitney *U* test was used. Multiple

logistic regressions were performed after factoring the phthalate metabolites into quartiles. Both crude and adjusted analyses were performed. Stepwise backward and forward regression analyses identified the significant variables for which the final adjustment was made. These included breastfeeding less than 3 months, family members smoking inside the home, single allergic predisposition, and sex group. We also adjusted for social class, although this was not a significant variable. The limit for statistical significance was set to $p < 0.05$. STATA software, release 11.2 (StataCorp LP, College Station, TX, USA), was used for statistical analysis.

Results

Concentrations of phthalate metabolites

Of the 500 children in the primary case-control group, 20 dropped out prior to the clinical examination; of the 480 remaining children, 92% ($n = 441$) delivered a urine sample. Valid data for the present analyses (data on both urinary metabolites and health status) was available for 440 children. The prevalence of allergic diseases among the 440 subjects is reported in Table 1. The concentrations of urinary phthalate metabolites for healthy controls and for the various DS case groups determined by CEI are reported in Table 2. Among healthy controls and the different case groups,

Table 2

Concentrations of urinary phthalate metabolites (ng/ml) in the healthy control group and the three DS-case groups determined by clinical examination coupled with a structured interview (CEI).

Phthalate metabolites	<LOD, <i>n</i> (%)	Median	GM (95% CI)	Min	Max	95%	<LOD, <i>n</i> (%)	Median	GM (95% CI)	Min	Max	95%
Healthy controls, <i>n</i> = 222^a							Asthma, <i>n</i> = 68^a					
MEP	8 (3.6)	16.0	15.8 (13.5–18.5)	0.47	888.5	111.6	3 (4.4)	17.0	14.5 (10.6–19.8)	0.47	258.7	124.9
MnBP	0	84.7	82.2 (74.0–91.2)	8.4	4007	256.8	0	66.7	60.6 (50.1–73.4)	6.2	228.3	200
MiBP	0	74.2	70.2 (63.6–77.4)	6.5	1418	206.7	0	59.5	58.1 (47.6–70.8)	6.6	594.1	207
MBzP	1 (0.5)	13.7	13.7 (12.0–15.7)	0.6	597	71.4	3 (4.4)	10.4	11.2 (8.5–14.8)	0.6	105.3	65.1
MEHP	4 (1.8)	5.2	4.8 (4.3–5.3)	0.14	529.9	13.7	1 (1.5)	4.0	4.1 (3.4–5.0)	0.14	22.2	12.4
MEHHP	0	33.5	34.1 (30.6–37.9)	2.5	2580	118.1	0	32.0	27.7 (21.8–35.2)	0.53	197.9	92.1
MEOHP	29 (13.1)	19.2	17.1 (15.0–19.5)	2.9	2092	71.3	11 (16)	16.3	14.5 (11.5–18.2)	2.9	142	55.3
MECPP	0	37.0	38.8 (35.0–43.0)	5.0	5541	135.8	0	32.3	29.9 (24.9–35.8)	3.2	227.1	101.4
Rhinoconjunctivitis, <i>n</i> = 76^a							Atopic dermatitis, <i>n</i> = 81^a					
MEP	2 (2.6)	17.3	17.1 (13.3–22.0)	0.47	193.3	93.7	1 (1.2)	18.0	19.8 (15.6–25.1)	0.47	258.7	90.1
MnBP	0	79.2	79 (68.0–91.7)	9.5	395.9	210.2	0	87.9	80.0 (68.0–94.1)	9.5	422.9	235
MiBP	0	63.7	67.8 (57.4–80.0)	14.7	331.4	226.8	0	65.4	67.4 (56.9–79.9)	11.9	562.1	239.2
MBzP	0	12.2	14.1 (11.4–17.5)	1.5	131	82.9	4 (4.9)	13.3	13.1 (10.1–16.9)	0.61	153.3	77.8
MEHP	2 (2.6)	4.2	4.1 (3.4–5.0)	0.14	22.2	13.2	4 (4.9)	4.6	4.3 (3.4–5.4)	0.14	60.9	13.2
MEHHP	0	34.3	31.7 (26.4–38.2)	0.53	197.9	105	0	34.1	30.0 (24.3–37.0)	0.64	385.5	102.3
MEOHP	8 (10.5)	16.3	15.8 (13.1–19.1)	2.9	142	62.8	16 (19.8)	16.9	14.9 (11.9–18.7)	2.87	260.8	62.8
MECPP	0	31.6	33.7 (29.3–39.1)	6.5	227.1	103.7	0	33.6	34.7 (28.8–41.7)	3.5	645.5	103.7

<LOD, the number of samples below limit of detection.

^a Urine was not delivered by $n = 21$ among healthy controls, $n = 6$ among children with asthma, $n = 7$ among children with rhinoconjunctivitis and $n = 9$ among children with atopic dermatitis.

* $p < 0.05$ using Mann–Whitney *U* rank sum test (compared to healthy controls).

Table 3

Median urinary phthalate metabolite concentrations and population characteristics.

	MEP	MnBP	MiBP	MBzP	MEHP	MEHHP	MEOHP	MECPP
Sex group								
Girl, N = 200	17.8	85.3	75.4	13.8	4.9	33.3	17.4	33.8
Boy, N = 240	15.5	78.8	69.2	12.4	4.7	33.4	18.0	36.1
Breastfed >3 months								
Yes, N = 327	16.4	84.8	74.5	13.7	4.8	33.8	17.4	35.6
No, N = 109	16.7	69.8	68.8	10.8	4.6	32.4	18.9	33.8
Antibiotics								
Yes, N = 381	16.7	78.6	70.2	12.8	4.7	32.8	17.6	34.2
No, N = 60	15.2	95.8	74.4	19.6	4.8	36.1	17.4	38.7
Single allergic predisposition								
Yes, N = 265	15.7	78.3	70.9	13.0	4.7	33.9	17.9	35.9
No, N = 175	17.4	84.6	73.8	13.8	4.9	31.3	17.5	33.5
Visible mold								
Yes, N = 135	17.8	88.1	73.8	15.1	4.9	34.9	20.0	40.2
No, N = 281	16.0	77.2	72.2	12.2	4.7	32.4	17.0	33.4
Visible moisture								
Yes, N = 121	14.6	89.8	70.2	14.7	4.8	30.7	17.4	34.1
No, N = 318	16.7	77.7	74.1	12.8	4.9	33.4	17.6	34.7
Condensation >5 cm on the window pane in winter								
Yes, N = 66	14.1	85.2	79.2	12.7	4.4	28.7	16.0	32.0
No, N = 354	16.8	79.8	72.0	13.5	4.9	40.0	18.5	36.4
Cat ^a								
Yes, N = 81	17.7	93.1	81.4	17.7[*]	4.9	36.4	19.9	38.0
No, N = 359	16.0	78.9	69.4	12.3	4.7	32.8	17.2	33.8
Dog ^a								
Yes, N = 49	19.5	93.9	87.6	16.6	5.2	31.0	17.8	40.5
No, N = 391	15.5	79.3	70.8	12.9	4.7	33.4	17.6	34.2
Pet avoidance								
Yes, N = 132	15.0	83.8	74.2	11.4	4.6	35.0	16.9	36.5
No, N = 301	17.3	80.1	72.0	14.0	4.9	32.8	18.5	34.5
Changed cleaning habits								
Yes, N = 53	18.0	80.1	68.4	11.8	4.7	30.7	17.0	33.3
No, N = 380	16.5	80.1	72.7	13.4	4.7	33.7	18.1	35.0
Smoke inside								
Yes, N = 54	16.1	92.2	61.6	13.6	4.1	33.0	15.5	33.6
No, N = 386	16.5	79.5	74.4	13.0	4.9	33.4	17.9	34.7
Social class								
I (high), N = 367	16.4	78.4	72.2	12.0[*]	4.6[*]	32.4	17.3[*]	34.0
II (low), N = 54	21.4	98.6	67.3	17.3	5.4	36.1	22.3	42.0

^a Current ownership at the time of filling the questionnaire.^{*} $p < 0.05$ using Mann–Whitney U rank sum test.

MEOHP was below the LOD in between ~10 and 20% of the urine samples. Less than ~5% of the other metabolite levels were below the LOD.

It is apparent from Table 2 that there is little difference in the concentrations of phthalate metabolites between the healthy controls and the groups clinically diagnosed with asthma, rhinoconjunctivitis or atopic dermatitis. The only statistically significant differences were between concentrations of MnBP and MECPP measured in the urine of the healthy controls and the asthma cases. However, *higher* levels of these phthalate metabolites were measured in the healthy control group compared to the asthma group.

Median metabolite concentrations among the factors that may impact allergic diseases are shown in Table 3. A significant association was indicated between higher concentrations of MBzP and (i) being in a lower social class and (ii) having a cat inside the home. A similar association was also found between higher concentrations of MEHP and MEOHP (two DEHP metabolites) and being in a lower social class. For children with an allergic predisposition, the concentrations of MEP, MnBP and MiBP tended to be lower than for children without such a predisposition.

Association between phthalate metabolites and allergic diseases

More detailed results regarding phthalate metabolites in urine and clinically diagnosed asthma, rhinoconjunctivitis and atopic dermatitis are presented in Table 4. We found negative associations between MnBP and MiBP and asthma in the crude analyses, but the associations disappeared in the adjusted analyses. In the adjusted analyses, MEP was correlated with atopic dermatitis (4th quartile only) and MECPP was associated with rhinoconjunctivitis (2nd quartile only).

None of the doctor diagnosed allergic diseases or “wheeze ever”, as reported by WQ, were associated with any of the phthalate metabolites. Instead, there was a tendency for slightly lower median concentrations of phthalate metabolites in the case groups compared to the respective controls based on the WQ (see Tables S1–S4 in the Supporting Information).

Discussion

The differences in the levels of phthalate metabolites between cases diagnosed at the clinical examination that included a

Table 4

Associations between phthalate metabolites in urine and asthma, rhinoconjunctivitis and atopic dermatitis diagnosed by CEI. Crude and adjusted Odds Ratios and 95% confidence intervals are shown.

Phthalate	Quartile	Asthma crude (n = 68/222) ^a		Asthma adjusted (n = 60/216) ^a		Rhinoconjunctivitis crude (n = 76/222) ^a		Rhinoconjunctivitis adjusted (n = 71/216) ^a		Atopic dermatitis crude (n = 81/222) ^a		Atopic dermatitis adjusted (n = 76/216) ^a	
		OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
MEP	1	1		1		1		1		1		1	
	2	0.57	0.26–1.25	0.61	0.27–1.39	1.31	0.54–2.41	1.23	0.55–2.77	1.11	0.39–1.69	1.13	0.52–2.49
	3	0.80	0.39–1.66	0.91	0.41–2.00	1.06	0.50–2.26	1.08	0.48–2.44	0.81	0.37–1.75	0.89	0.39–2.01
	4	0.87	0.42–1.80	1.07	0.49–2.34	1.35	0.68–2.70	1.60	0.75–3.41	1.88	0.98–3.66	2.27	1.12–4.62
MnBP	1	1		1		1		1		1		1	
	2	0.58	0.28–1.19	0.68	0.31–1.49	1.58	0.76–3.26	1.80	0.82–3.96	0.81	0.39–1.69	0.71	0.39–1.87
	3	0.58	0.28–1.19	0.77	0.35–1.69	0.91	0.42–1.97	0.95	0.42–2.18	0.95	0.46–1.95	0.97	0.47–2.19
	4	0.42	0.19–0.90	0.60	0.26–1.36	1.16	0.58–2.34	1.36	0.64–2.89	1.10	0.57–2.11	0.62	0.60–2.39
MiBP	1	1		1		1		1		1		1	
	2	0.43	0.20–0.91	0.49	0.22–1.09	1.05	0.51–2.16	1.18	0.54–2.55	1.00	0.50–2.02	1.11	0.53–2.34
	3	0.55	0.27–1.21	0.91	0.41–1.99	0.68	0.31–1.45	0.89	0.39–2.02	0.76	0.37–1.57	0.88	0.41–1.91
	4	0.47	0.23–0.99	0.61	0.27–1.34	0.99	0.50–1.94	1.07	0.52–2.22	0.90	0.47–1.75	0.97	0.48–1.94
MBzP	1	1		1		1		1		1		1	
	2	1.20	0.59–2.47	1.18	0.55–2.55	1.38	0.67–2.84	1.48	0.68–3.23	1.09	0.53–2.23	1.23	0.58–2.63
	3	0.49	0.21–1.13	0.63	0.26–1.53	0.84	0.39–1.82	0.89	0.39–2.01	0.69	0.32–1.47	0.69	0.31–1.55
	4	1.07	0.52–2.22	1.11	0.51–2.44	1.08	0.54–2.16	1.18	0.56–2.48	1.34	0.70–2.57	1.43	0.72–2.88
MEHP	1	1		1		1		1		1		1	
	2	0.66	0.32–1.36	0.64	0.31–1.44	1.34	0.67–2.68	1.54	0.72–3.30	1.13	0.56–2.29	1.21	0.58–2.55
	3	0.48	0.22–1.02	0.53	0.24–1.21	0.53	0.24–1.16	0.57	0.25–1.31	0.81	0.39–1.69	0.91	0.42–1.96
	4	0.57	0.28–1.20	0.61	0.28–1.34	0.74	0.37–1.48	0.71	0.34–1.47	0.96	0.50–1.87	0.93	0.46–1.87
MEHHP	1	1		1		1		1		1		1	
	2	0.79	0.38–1.67	1.17	0.52–2.65	0.91	0.43–1.92	1.00	0.44–2.24	0.58	0.28–1.20	0.58	0.27–1.27
	3	0.98	0.48–2.03	1.14	0.52–2.51	1.45	0.71–2.94	1.43	0.66–3.11	1.00	0.50–1.98	0.99	0.48–2.05
	4	0.68	0.32–1.48	0.84	0.37–1.93	0.76	0.37–1.54	0.70	0.33–1.50	0.72	0.37–1.39	0.68	0.34–1.36
MEOHP	1	1		1		1		1		1		1	
	2	0.80	0.39–1.66	1.03	0.46–2.30	1.46	0.71–3.01	1.41	0.64–3.10	1.13	0.56–2.29	1.06	0.50–2.25
	3	0.98	0.48–2.00	1.04	0.48–2.26	1.21	0.58–2.52	1.10	0.50–2.44	0.93	0.46–1.92	0.83	0.39–1.79
	4	0.48	0.21–1.08	0.55	0.23–1.31	0.77	0.37–1.58	0.65	0.30–1.41	0.87	0.45–1.71	0.79	0.39–1.60
MECPP	1	1		1		1		1		1		1	
	2	0.86	0.42–1.76	1.50	0.66–3.41	1.85	0.91–3.76	2.41	1.07–5.42	0.67	0.33–1.38	0.76	0.35–1.66
	3	0.75	0.36–1.55	0.84	0.38–1.88	0.77	0.35–1.66	0.77	0.33–1.79	0.79	0.39–1.59	0.72	0.34–1.52
	4	0.45	0.20–1.01	0.59	0.25–1.41	0.88	0.43–1.79	0.89	0.41–1.91	0.79	0.41–1.51	0.78	0.39–1.56

^a n = DS-cases/healthy controls. The number of subjects is lower in the adjusted analyses due to missing values in the adjusting variables. The regression model initially included all variables listed in Table 3. Stepwise backward and forward regression analyses identified the significant variables for which the final adjustment was made.

structured interview (CEI) and healthy controls were relatively small. Statistically significant differences were observed only for MnBP and MECPP, which were *higher* in healthy controls compared with the asthma case group. We found a *negative* association between levels of MnBP and MiBP in urine and asthma in the crude analysis. In the analysis adjusted for multiple factors, only a weak positive association between MEP in urine and atopic dermatitis was found; we found no *positive* associations between any phthalate metabolites in urine and either asthma or rhinoconjunctivitis. We also found no association between the levels of phthalate metabolites in urine and responses on the written questionnaire to questions addressing doctor diagnosed allergic diseases/“wheeze ever”. We based our diagnoses of allergic diseases on generally accepted criteria (Bateman et al., 2008; Bousquet et al., 2008; Kjaer et al., 2008; Niggemann et al., 2006; Pekkanen and Pearce, 1999; Soto-Martinez et al., 2009) including a clinical examination coupled with a structured interview (each by the same experienced doctor), conducted in both the random control group (n = 300) and the criteria selected “case” group (n = 200). Furthermore, the urine samples were collected over a time span of more than half a year (summer through winter), during which period the availability of fresh foods varied. Given that diet has a large impact on exposure to certain phthalates (Bosgra et al., 2005; Fromme et al., 2007b; Koch et al., 2013; Wittassek et al., 2011; Wormuth et al., 2006), we

believe that the extended collection period results in diminished risk for collection bias regarding contributions from diet.

In the “Dampness in Buildings and Health” study conducted among children in Värmland, Sweden, the level of DEHP measured in dust samples from the children’s bedrooms was statistically larger for cases compared with controls (966 µg/g asthma vs. 741 µg/g controls; *p* = 0.022; Bornehag et al., 2004a). Additionally, a dose-response relationship was found between DEHP and asthma. Among the children in our study no statistically significant difference in DEHP levels in dust between asthma cases and healthy controls was found (bedroom samples: 242 µg/g asthma vs. 197 µg/g controls; daycare samples: 421 µg/g asthma vs. 485 µg/g controls; weighted for the two environments: 300 µg/g asthma vs. 285 µg/g controls; Callesen et al., 2014). Hence, among the Odense children there was no association between clinically diagnosed asthma and either DEHP in dust samples from their environment or DEHP metabolites in their urine. However, children in the Värmland study had higher levels of DEHP in bedroom dust (median 770 µg/g; Bornehag et al., 2005a) compared with the study children from Odense (median 210 µg/g; Langer et al., 2010). Yet a factor of 3.7 in median mass fractions is not exceptionally large given the wide range of levels typically observed for organic compounds present in dust (Weschler and Nazaroff, 2008). Furthermore, dust samples from the daycare centers attended by the Odense

children had a median mass fraction of 500 µg/g – not that different from the bedroom values in Värmland (dust samples were not collected from daycare centers in Värmland). An alternative explanation for this observed difference is that DEHP was a surrogate for another agent responsible for asthma in the Värmland study, but was not a surrogate for this agent in the Odense study. For example, in Värmland 187 out of 346 homes had PVC flooring in the children's bedrooms, and DEHP in dust was significantly higher in bedrooms with PVC flooring compared with other flooring materials ($p = 0.001$; [Bornehag et al., 2005a](#)). In contrast, in Odense only 4 out of 500 homes had PVC flooring in the child's bedroom, precluding any association between DEHP in dust and PVC flooring. In the original phase of the Värmland study, there was an association between PVC flooring and case status ([Bornehag et al., 2004a](#)). In a five-year follow-up study of the Värmland children, the incidence of asthma among the children was again associated with PVC flooring in the child's bedroom (AOR: 1.52; 95% CI: 0.99–2.35; [Larsson et al., 2010](#)). If asthma is associated with PVC flooring, or the combination of water leaks and PVC flooring ([Bornehag et al., 2005b](#)), then an association between DEHP and asthma would be observed in the Värmland study but not the Odense study.

In the Värmland study [Bornehag et al. \(2004a\)](#) found an association between BBzP levels in settled dust and atopic dermatitis, while in a New York City study the investigators ([Just et al., 2012](#)) found an association between prenatal exposure to BBzP, as determined by the level of MBzP in the mother's urine, and eczema. In the Odense study we found no association between BBzP levels in settled dust ([Callesen et al., 2014](#)) or MBzP in a child's urine and any of the other allergic diseases. The explanation could be that BBzP in settled dust is a surrogate for another agent in Värmland but not in Odense, as speculated above for DEHP. It could also be due to different levels of BBzP in settled dust in the two studies. The median concentration of BBzP in settled dust collected from children's bedrooms was more than 35 times larger in Värmland than in Odense (135 µg/g in the former ([Bornehag et al., 2004a](#)) and 3.7 µg/g in the latter ([Langer et al., 2010](#))). This, in turn, likely reflects the much greater use of PVC flooring in Värmland homes compared with Odense homes (see above), coupled with the likely more common use of BBzP as a plasticizer in PVC flooring at the time of the Swedish study. Indeed, a recent study from Sweden reported significantly higher urine levels of MBzP in infants with PVC flooring in their bedrooms ([Carlstedt et al., 2013](#)). In the study by [Just et al. \(2012\)](#), the geometric mean concentration of MBzP in the mother's urine (13.6 ng/g) was similar to that in the Odense children's urine (13.3 ng/ml; [Langer et al., 2014](#)). However, exposure to BBzP as a developing fetus may have a different impact than exposure to BBzP as a 3–5 year old.

In a study analogous to the present study, [Hsu et al. \(2012\)](#) collected urine samples from 101 children in the greater Tainan metropolitan area of southern Taiwan. The investigators found an association between urinary concentrations of MBzP and asthma. However, results from this Taiwanese study should be interpreted with caution given the number of cases ($n = 59$) and controls ($n = 42$) coupled with relatively few cases of asthma ($n = 9$). Recently [Whyatt et al. \(2013\)](#) have reported that the combination of prenatal MBzP in mother's urine and postnatal BPA in the child's urine enhanced the likelihood of asthma and any report of wheeze in the child between 5 and 11 years of age.

In a recent paper ([Bekö et al., 2013](#)) we used the dust concentrations of a given phthalate measured in a child's bedroom and daycare center to estimate its gas phase concentrations in these environments and proceeded to estimate, for each child, what fraction of her/his total phthalate exposure, as determined from metabolites in urine, was a consequence of indoor exposures. The indoor exposure pathways considered were dust ingestion, inhalation and direct dermal absorption from air. Our findings,

while specific for the Odense study, were consistent with earlier apportionment of phthalate intake via different pathways ([Guo and Kannan, 2011](#); [Koch et al., 2013](#); [Wormuth et al., 2006](#)). For both DEHP and BBzP, indoor pathways contributed less than 10% to total intake, with the bulk of the remainder presumably coming from diet. For DEP, DnBP and DiBP, indoor pathways were responsible for a meaningful fraction of total intake, with inhalation and dermal absorption contributing substantially to indoor intake. While the metabolites in urine provide information regarding the total intake of each phthalate for each child, these measurements provide no information regarding the different exposure pathways responsible for these intakes ([Koch et al., 2013](#); [Wittassek et al., 2011](#)). Yet the impact of a phthalate on a particular health endpoint may be somewhat dependent on the route of exposure. For example, while diet may make the largest contribution to the total intake of DEHP and BBzP, it may be that inhalation intake plays a larger role than diet in the case of asthma and dermal intake plays a larger role than diet in the case of eczema. There are differences in the "tissue insult" that occurs when phthalates are ingested versus when they are inhaled or dermally absorbed. When ingested these chemicals pass through the gastrointestinal tract and liver before they enter the blood, encountering enzymes that metabolize them relatively quickly ([Kluwe, 1982](#)). When phthalates are inhaled or dermally absorbed, they enter the blood without first passing through the gastrointestinal tract. Inhalation results in exposure of respiratory and lung tissue ([Roth et al., 1988](#)), while dermal absorption exposes the stratum corneum and viable epidermis. There are enzymes in the skin that can metabolize phthalate esters ([Beydon et al., 2010](#)), but the ratio of hydrolyzed to oxidized metabolites may differ for the dermal pathway versus the ingestion pathway. In summary, the urine metabolites are a measure of total intake for each of the phthalate esters targeted in this study, while one pathway may be more important than another in the development of allergic disease. It could be difficult to detect evidence of such a role for a given pathway (e.g., inhalation) if another pathway (e.g., ingestion) dominates the levels found in the urine.

The median concentrations of phthalate metabolites in urine were higher in children with pets than in those without pets. The phthalate esters targeted in the present study sorb to settled dust and indoor surfaces ([Weschler and Nazaroff, 2008](#)). Elevated levels of flame retardants have been reported on the fur of house cats ([Dye et al., 2007](#)), and for similar reasons, elevated levels of phthalates are anticipated on the fur of pets. Children petting their cats, followed by hand-to-mouth activity, could contribute to the higher urinary concentrations of phthalate metabolites among pet owners.

The concentrations of BBzP and DEHP metabolites tended to be higher in the urine samples of children from lower social class families. This may reflect a higher percentage of packaged foods in the diets of lower social class families. Packaged foods tend to contain more phthalates than "fresh-foods" ([Rudel et al., 2011](#)). Additionally, household products (e.g., shower curtains, rain jackets, boots, upholstery) made with phthalate-plasticized PVC tend to be less expensive than analogous products made with alternative materials.

A tendency towards lower urinary concentrations of MEP, MnBP and MiBP was observed in children with allergic predisposition. DEP, the parent of MEP, is a common constituent of personal care products; to a lesser extent the same is true for DnBP and DiBP, the respective parents of MnBP and MiBP ([Dodson et al., 2012](#); [Koniecki et al., 2011](#); [Romero-Franco et al., 2011](#)). In families where one or both parents suffer from an allergic disease, avoidance behavior may result in less exposure to personal care products containing DEP, DnBP and DiBP. Certain waxes, polishes and PVC products also contain DnBP and DiBP ([Dodson et al., 2012](#)). Indoor exposure pathways are anticipated to contribute substantially to the urinary levels of these phthalates ([Bekö et al., 2013](#); [Guo and Kannan, 2011](#);

Koch et al., 2013; Wormuth et al., 2006). Families with a history of allergic disease may vacuum and vent their homes more frequently (Callesen et al., 2014), leading to decreased levels of DnBP and DiBP in air and dust and decreased uptake via indoor pathways. Consistent with this speculation, the median levels of DnBP and DiBP in bedroom dust samples were lower for the children with allergic predisposition than for those without (DnBP: 12.7 vs 17.4 µg/g; DiBP: 26.6 vs. 30.1 µg/g).

Our analysis is based on a single urine sample collected in the morning. Earlier studies have reported day-to-day and month-to-month variations in the concentrations of phthalate metabolites in a given subject's urine samples (Fromme et al., 2007a; Hauser et al., 2004). Additionally, the elimination half-life of the lower molecular weight phthalate metabolites measured in the current study is relatively short (e.g., Koch et al., 2012). Such variability and fast elimination may have reduced our ability to detect associations between phthalate metabolites in the children's urine and allergic disease, which is a long term outcome. However, Hauser et al. (2004) concluded that "a single urine sample was moderately predictive of each subject's exposure over three months". Furthermore, other studies have found associations between levels of phthalate metabolites and various health endpoints (e.g., thyroid hormones, reproductive outcomes) using measurements based on a single urine sample (Boas et al., 2010; Huang et al., 2007; studies cited in Meeker et al., 2009).

Conclusions

Differences in phthalate metabolite levels between cases and healthy controls were small. We found only one positive association between children's urinary phthalate metabolite concentrations and allergic diseases (DEP in the 4th quartile and atopic dermatitis). Indeed, for certain metabolites we observed slightly higher levels among healthy controls than among cases. This may be a consequence of altered cleaning habits and the use of "allergy friendly" products by parents of children with allergic disease or predisposition. Thus, our study did not find an association between phthalate exposure and allergic disease. However, urinary concentration of a phthalate metabolite represents the total intake of that phthalate ester, irrespective of the exposure pathway. If a given pathway (e.g., diet) overwhelmingly dominates the total exposure, urinary metabolite concentrations would fail to reveal the potential association between a different exposure pathway (e.g., inhalation) and the development/exacerbation of an allergic disease.

Conflict of interest

The authors have no conflict of interest.

Acknowledgements

The study was supported by the Villum Foundation, TRYG Fund, the Danish Research Council (grant 95-103-21990) and the Kirsten and Freddy Johansens Fund (grant 95-103-72087). We thank Carl-Gustaf Bornehag and Jan Sundell for advice and support throughout the study. We thank the participating families for their cooperation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ijheh.2013.12.001>.

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Supporting Information

Phthalate metabolites in urine and asthma, allergic rhinoconjunctivitis and atopic dermatitis in preschool children

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Table S1. Concentrations of urinary phthalate metabolites (ng/ml) in the “wheeze ever” case group determined by the written questionnaire (WQ) and the control group obtained by excluding the children with “wheeze ever” (by the WQ) from the healthy control group. None of the differences were significant (by Mann Whitney U test as well as logistic regression on quartiles).

Phthalate metabolite	Cases (n=229)					Controls (n=164)				
	Median	GM (95% CI)	Min	Max	95%-ile	Median	GM (95% CI)	Min	Max	95%-ile
MEP	15.0	15.0 (12.9-17.6)	0.47	258.7	90.1	16.7	16.7 (13.8- 20.0)	0.47	888.5	120.7
MnBP	78.0	73.1 (65.8-81.3)	6.2	1774	240.8	87.4	82.4 (72.7-93.3)	8.4	4007	242.7
MiBP	70.2	67.7 (60.7-75.4)	6.5	594.1	277.0	74.7	71.4 (63.9-79.8)	9.0	1418	204.9
MBzP	13.7	13.2 (11.4-15.3)	0.61	597	80.0	12.0	13.0 (11.1-15.1)	0.61	388.2	69.6
MEHP	4.7	4.7 (4.1-5.3)	0.14	530.0	17.1	5.1	4.7 (4.1-5.3)	0.14	36.2	13.4
MEHHP	32.8	32.5 (28.9-36.7)	0.53	2580	114.8	33.3	32.4 (28.7-36.6)	2.5	297.0	115.9
MEOHP	18.1	16.4 (14.3-18.8)	2.9	2092	72.3	17.2	16.0 (13.8-18.5)	2.9	156.5	64.0
MECPP	34.0	37.3 (33.5-41.6)	3.2	5541	137.5	35.2	36.6 (32.7-40.9)	5.0	446.9	127.6

Table S2. Concentrations of urinary phthalate metabolites (ng/ml) in the “doctor diagnosed asthma” case group determined by the WQ and the control group obtained by excluding the children with doctor diagnosed asthma (by the WQ) from the healthy control group. None of the differences were significant (by Mann Whitney U test and logistic regression on quartiles)

Phthalate metabolite	Cases (n=117)					Controls (n=206)				
	Median	GM (95% CI)	Min	Max	95%-ile	Median	GM (95% CI)	Min	Max	95%-ile
MEP	17.3	16.7 (13.5-20.7)	0.47	258.7	94.7	15.7	15.6 (13.2-18.4)	0.47	888.5	114.1
MnBP	72.4	69.3 (59.5-80.6)	6.2	664.7	228.3	86.0	82.6 (74.0-92.2)	8.4	4007	257.4
MiBP	73.4	65.0 (56.0-75.5)	6.6	594.1	259.1	75.6	71.8 (64.8-79.4)	6.5	1418	222.6
MBzP	12.0	12.1 (9.8-14.9)	0.61	153.8	69.6	13.6	13.7 (11.9-15.8)	0.61	597	71.7
MEHP	4.7	4.8 (4.0-5.7)	0.14	529.9	21.0	5.2	4.6 (4.1-5.2)	0.14	36.2	13.6
MEHHP	30.4	32.2 (26.6-39.0)	0.53	2580	152.4	34.1	33.7 (30.4-37.5)	2.5	297.0	115.9
MEOHP	17.3	16.4 (13.4-20.1)	2.9	2092	92.0	19.3	16.8 (14.7-19.1)	2.9	156.5	64.0
MECPP	33.0	36.7 (30.9-43.7)	3.2	5541	164.1	37.8	38.1 (34.5-42.1)	5.0	446.9	131.9

Table S3. Concentrations of urinary phthalate metabolites (ng/ml) in the “doctor diagnosed hay fever” case group determined by the WQ and the control group obtained by excluding the children with doctor diagnosed hay fever (by the WQ) from the healthy control group. None of the differences were significant (by Mann Whitney U test and logistic regression on quartiles)

Phthalate metabolite	Cases (n=52)					Controls (n=221)				
	Median	GM (95% CI)	Min	Max	95%-ile	Median	GM (95% CI)	Min	Max	95%-ile
MEP	13.6	13.9 (9.8-19.6)	0.47	133.1	124.9	16.0	15.8 (13.4-18.5)	0.47	888.5	111.6
MnBP	79.2	67.9 (54.8-84.2)	7.8	193.7	180.4	84.8	82.6 (74.5-91.6)	8.4	4007	256.8
MiBP	65.4	65.4 (52.4-81.8)	7.3	331.4	259.1	74.5	70.4 (63.9-77.7)	6.5	1418	206.7
MBzP	10.6	12.6 (9.7-16.4)	1.5	92.4	80.0	13.7	13.7 (12.0-15.7)	0.61	597	71.4
MEHP	4.7	4.4 (3.4-5.8)	0.14	22.2	15.6	5.2	4.8 (4.3-5.3)	0.14	529.9	13.7
MEHHP	34.7	32.1 (25.3-40.8)	0.53	197.9	102.3	33.5	34.1 (30.6-38.0)	2.5	2580	118.1
MEOHP	16.8	17.1 (13.6-21.5)	2.9	142	62.8	19.4	17.1 (15.0-19.5)	2.9	2092	71.3
MECPP	31.9	35.0 (29.0-42.3)	6.8	227.1	103.7	37.1	38.8 (35.0-43.1)	5.0	5541	135.8

Table S4. Concentrations of urinary phthalate metabolites (ng/ml) in the “doctor diagnosed eczema” case group determined by the WQ and the control group obtained by excluding the children with doctor diagnosed eczema (by the WQ) from the healthy control group. None of the differences were significant (by Mann Whitney U test and logistic regression on quartiles)

Phthalate metabolite	Cases (n=84)					Controls (n=213)				
	Median	GM (95% CI)	Min	Max	95%-ile	Median	GM (95% CI)	Min	Max	95%-ile
MEP	13.2	12.8 (10.0-16.5)	0.47	258.7	69.3	16.6	16.2 (13.8-19.1)	0.47	888.5	114.1
MnBP	79.8	80.2 (68.7-93.5)	16.3	1774	226.9	84.8	82.1 (73.7-91.3)	8.4	4007	257.4
MiBP	66.9	70.6 (60.2-82.8)	14.7	562.1	239.2	74.5	70.6 (63.8-78.0)	6.5	1418	222.6
MBzP	12.6	12.1 (9.5-15.4)	0.61	273.2	55.2	13.7	13.8 (12.0-15.8)	0.61	597	71.7
MEHP	4.5	4.4 (3.7-5.3)	0.14	37.2	13.2	5.2	4.8 (4.3-5.4)	0.14	529.9	13.7
MEHHP	31.1	31.8 (26.7-37.7)	2.5	370.3	102.3	33.6	34.3 (30.8-38.3)	2.5	2580	118.4
MEOHP	17.5	15.1 (12.2-18.6)	2.9	186.8	58.8	19.7	17.2 (15.0-19.7)	2.9	2092	72.3
MECPP	31.1	33.3 (28.3-39.1)	3.5	505.5	103.7	37.9	39.4 (35.4-43.8)	5.0	5541	137.5