



Exposure to phthalates in house dust and associated allergies in children aged 6–12 years

Yu Ait Bamai^a, Atsuko Araki^a, Toshio Kawai^b, Tazuru Tsuboi^b, Ikue Saito^c, Eiji Yoshioka^{a,d}, Shi Cong^a, Reiko Kishi^{a,*}

^a Hokkaido University Center for Environmental and Health Sciences, Kita 12, Nishi 7, Kita-ku, Sapporo 060-0812, Japan

^b Osaka Occupational Health Service Center, Japan Industrial Safety and Health Association, 2-3-8, Tosabori, Nishi-ku, Osaka 550-0001, Japan

^c Department of Environmental Health and Toxicology, Division of Environment Health, Tokyo Metropolitan Institute of Public Health, 3-24-1 Hyakunincho, Shinjuku-ku, Tokyo 169-0073, Japan

^d Asahikawa Medical University for Department of Health Science, E2-1-1-1, Midorigaoka, Asahikawa, Hokkaido 078-8510, Japan

ARTICLE INFO

Article history:

Received 7 May 2016

Received in revised form 25 August 2016

Accepted 25 August 2016

Available online xxxx

Keywords:

Allergy

Child

Daily intake

House dust

Phthalates

Urinary metabolites

ABSTRACT

Phthalates are widely used as plasticizers in household products. Several studies have reported an association between phthalate exposure and an increased risk of allergies. The present study estimated phthalate exposure in children aged 6–12 years and assessed potential correlations with allergies. House dust samples were collected from floors and multi-surface objects >35 cm above the floor. Urine samples were collected from the first morning void of the day. Daily phthalate intake (DI_{dust} and DI) was estimated using both house dust and urinary metabolite concentrations. Exposure to di-2-ethylhexyl phthalate (DEHP) in floor dust was associated with parental-reported rhino-conjunctivitis. After stratification by gender, this trend was found to only occur in boys. Furthermore, urinary mono-isobutyl phthalate was inversely associated with parental-reported wheeze in boys. DI_{dust} of benzyl butyl phthalate (BBzP) and DEHP were significantly correlated with DI_{BBzP} and DI_{DEHP}, respectively. These correlations were stronger with floor than with multi-surface dust. Our results suggest that, among Japanese children, house dust from low surfaces, such as living room floors, might play a meaningful role in the indoor environmental exposure pathway for BBzP and DEHP.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

The adverse health effects of phthalates as endocrine disruptors are a growing public concern. Phthalates are widely used as plasticizers in household products, such as polyvinyl chloride (PVC) flooring and building materials. Although di(2-ethylhexyl) phthalate (DEHP) is regulated for use in toys and food packaging, and Japanese consumption and production of phthalates have decreased over the past 10 years (Bizzari et al., 2013), DEHP is still the most widely used phthalate, used mainly as a plasticizer in PVC. Several studies, in both animals and humans, have reported that phthalate exposure is associated with adverse health effects in children, including effects on reproductive, neurological, and pubertal development, and obesity (Bornehag and

Nanberg, 2010; Lovekamp-Swan and Davis, 2003; Martino-Andrade and Chahoud, 2010). Furthermore, phthalates found in house dust are considered to increase the risk of allergies (Ait Bamai et al., 2014; Bornehag et al., 2004; Kolarik et al., 2008). Although the major routes of phthalate exposure are thought to be through diet and the use of personal care products (PCP) (Koch et al., 2013), house dust may also be important.

Animal studies have indicated that some phthalates may enhance allergic responses by acting as adjuvants for the production of cytokines and/or immunoglobulins responsible for allergic sensitization (Koike et al., 2010; Larsen et al., 2001a; Lee et al., 2004; Tanaka et al., 2013). In an epidemiological study, Beko et al. (2015) evaluated the association between allergies in children and the levels of both urinary phthalate metabolites and phthalates in house dust. They reported that allergic sensitization was significantly associated with certain house dust phthalates, but not with urinary phthalate metabolite levels (Beko et al., 2015). Other previous studies reported that the highest quartile of benzyl butyl phthalate (BBzP) metabolites in Taiwanese children aged 2 years old was associated with an increased risk of atopic dermatitis among children at 2 years, but not at 5 years of age (Wang et al., 2014). Similarly, the highest quartiles of di-iso-nonyl phthalate (DiNP) and di-iso-decyl phthalate metabolites increased the risk of current asthma in 10-year-old Norwegian children (Bertelsen et al., 2013).

Abbreviations: BBzP, benzyl butyl phthalate; DBP, dibutyl phthalate; DEHP, di-2-ethylhexyl phthalate; DEP, diethyl phthalate; DiBP, di-iso-butyl phthalate; DiNP, di-iso-nonyl phthalate; DMP, dimethyl phthalate; DnBP, di-n-butyl phthalate; MBzP, mono benzyl phthalate; MCNP, mono-(carboxynonyl) phthalate; MCP, mono-(3-carboxypropyl) phthalate; MECPP, mono-(2-ethyl-5-carboxypentyl) phthalate; MEHP, mono-(2-ethylhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MiBP, mono-isobutyl phthalate; MnBP, mono-n-butyl phthalate; PVC, polyvinyl chloride; SVOC, semi-volatile organic compounds.

* Corresponding author.

E-mail address: rkishi@med.hokudai.ac.jp (R. Kishi).

Gender-specific responses to phthalate exposure were considered by Ku et al. (2015), who reported that postnatal DEHP and diethyl phthalate (DEP) exposures were associated with asthma among Taiwanese boys at 8 years of age (Ku et al., 2015). However, data regarding the role of routes of phthalate exposure are still limited.

One way to assess indoor phthalate contamination epidemiologically is by measuring phthalate concentrations in house dust. Numerous studies have detected phthalates in house dust (Abb et al., 2009; Bornehag et al., 2005; Jaakkola et al., 1999; Langer et al., 2010; Oie et al., 1997), but reports on the association with allergies are limited. A Swedish study found that DEHP exposure via house dust was associated with asthma, and BBzP was associated with eczema and rhinitis in children aged 3–8 years old (Bornehag et al., 2004). Consistent results were reported for DEHP in Bulgarian children (Kolarik et al., 2008). Callesen et al. (2014b) did not find a significant association between phthalates in dust and diagnosed allergies among children in Denmark, but they did report a positive association between DEHP in dust and parental-reported wheeze. In most studies, dust samples were only collected from surfaces above floor level (Beko et al., 2015; Bornehag et al., 2004; Callesen et al., 2014b; Hsu et al., 2012; Kolarik et al., 2008). We previously reported that the concentration of phthalates in house dust collected from surfaces above floor level were higher than in dust collected from the floor (Ait Bamai et al., 2013). However, dose-response associations between self-reported allergies and phthalates, including DEHP with allergic conjunctivitis, both BBzP and di-iso-butyl phthalate (DiBP) with atopic dermatitis, and DiNP with allergic rhinitis, were only found in floor surface dust (Ait Bamai et al., 2014). Reported phthalate exposure levels, such as levels of DEHP in house dust, differ among Swedish, Bulgarian, Danish, and Japanese studies. House dust levels of DEHP in Sweden were low, and those in Denmark were very low; however, levels in Bulgaria were high, and those in Japan were very high (Ait Bamai et al., 2013). These studies had different designs, and in particular, studies differed in the type of buildings from which dust was collected for analysis. Our previous findings indicated that allergies in children showed a clearer association with phthalates in floor dust than those in adults (Ait Bamai et al., 2014), and that younger children had higher phthalate intake levels than older family members (Ait Bamai et al., 2015). No study has assessed the effect of phthalate exposure, indicated by daily phthalate intake estimated from both urine metabolite and house dust levels, on the risk of allergies in children. The aim of this study was to investigate the association between allergies and phthalate exposure in Japanese children aged 6–12 years old, estimating daily phthalate intakes through both urinary phthalate metabolite level and the phthalate levels in house dust collected from surfaces at floor level and above.

2. Materials and methods

The study design and methods are described in detail elsewhere (Ait Bamai et al., 2013; Ukawa et al., 2013); therefore, only brief descriptions are provided here.

2.1. Study population

The study enrolled 184 elementary school children from 128 families in Sapporo, Japan. The study was conducted in two phases: a baseline questionnaire survey conducted in 2008, and a questionnaire and environmental measurements survey conducted in October and November of 2009 and 2010. The details of the baseline questionnaire survey in 2008 have been reported previously (Ukawa et al., 2013). Briefly, 6393 school children from 12 public elementary schools in Sapporo were asked to participate in the study. Of these, 4408 children responded to the questionnaire (response rate 69%). A total of 951 children (832 families) agreed to a home visit to conduct environmental measurements. After excluding those who had omissions on the baseline questionnaire regarding gender, school grade, and allergies, and

those who already graduated from elementary school at the time point of home visit, we contacted the parents of 681 children in 2009 and 2010. As a result, 128 families were available for home visits for environmental measurements (80 in 2009 and 48 in 2010). This selection procedure identified a total of 184 children from 128 families who completed a questionnaire survey in October and November of 2009 and 2010.

2.2. Questionnaire

Self-administered questionnaires were distributed and collected by the investigators during the home visit. The questionnaires consisted of two sections concerning personal and dwelling characteristics. The personal questionnaire gathered information on gender, age, school grade, current body weight and height, history of allergies, parental history of allergies, and household income. Parental-reported allergies were defined by using the International Study of Asthma and Allergies in Childhood (ISAAC) core questionnaire (The ISAAC Steering Committee 1998). Wheeze was defined as “Having wheezing or whistling in the chest in the past 12 months.” Allergic rhino-conjunctivitis was defined as (a) “In the past 12 months, having a problem with sneezing or runny or blocked nose when he/she did not have a cold or the flu,” or (b) “In the past 12 months, having this nose problem with accompanying itchy watery eyes.” Atopic dermatitis was defined as (a) “Having an itchy rash that comes and goes for at least 6 months,” or (b) “Having the aforementioned itchy rash at any time during the last 12 months,” or (c) “Having the aforementioned itchy rash that affects one or several of the following areas: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears, or eyes.”

The dwelling questionnaire included questions about type of building (single-family house/multi-family house); building structure (wooden/other); building age; renovation; floor materials (PVC/compressed wood/wall-to-wall carpet/other); smoker at home; usage of mechanical ventilation system for 24 h (yes/no); dampness-related problems such as condensation (yes/no), moldy odor (yes/no), visible mold (yes/no), high humidity in the bathroom (yes/no), and water leakage problems within past 5 years (yes/no).

2.3. Measurements of phthalates in settled dust

The dust collection methods, gas chromatography/mass spectrometry (GC/MS) analytical methods, quality control and quality assurance, and dwelling characteristics investigations have been described in detail previously (Ait Bamai et al., 2013; Kanazawa et al., 2010).

Briefly, dust samples were categorized as either floor dust or multi-surface dust. Floor dust samples were collected from the living room floor surface and from objects <35 cm above the floor, such as the carpet and skirting board. Samples of multi-surface dust were collected from the surfaces of objects that were >35 cm above the floor, including shelves, cupboards, moldings, frames, TV sets, and interior materials such as the corner of the walls and ceiling. The same type of hand-held vacuum cleaner (National HC-V15, Matsushita Electric works, Ltd., Osaka, Japan; 145W) equipped with a paper dust bag (Nichi-nichi Pharmaceutical Co., Ltd., Mie, Japan) was used at all dwellings. Background phthalate levels of the vacuum cleaner and the filter were examined and confirmed to be negligible.

The collected dust was weighed after the removal of unwanted substances, such as human and animal hair, food scraps, scrap paper, etc. Samples were stored in stoppered glass test tubes that were sealed with fluoroc-tape, wrapped with aluminum foil, and kept at -20°C until analysis.

GC/MS in SIM mode was used to analyze phthalate levels from collected dust. The target compounds were: dimethyl phthalate (DMP), DEP, di-n-butyl phthalate (DnBP), DiBP, BBzP, DEHP, and DiNP. The analyses were conducted at the Tokyo Metropolitan Institute of Public

Health and Osaka Occupational Health Service Center, Japan Industrial Safety and Health Association.

2.4. Measurement of phthalate metabolites in urine

Parents were asked to collect the child's urine from the first void on the morning of the day of the home visit. Collected urine was stored in the freezer until our visit. Each urine sample was dispensed into a stoppered glass test tube which had been cleaned with acetone, and was sealed with fluoroc-tape, wrapped with aluminum foil, and stored at -20°C until analysis. Of the 184 samples, two were not analyzed because the volume was deficient. The urinary phthalate metabolite concentrations were measured using GC/MS. The analytical, quality control, and quality assurance methods employed have been reported previously (Ait Bamai et al., 2015).

2.5. Estimated daily intake calculations from phthalate metabolites in urine

The details of the daily intake calculations from phthalate metabolites have been reported previously (Ait Bamai et al., 2015). Briefly, the daily intake of phthalates was estimated from urinary phthalate metabolite levels for each child using the following equation:

$$DI = (C \times CE \times MW_p) / (F_{ue} \times W), \quad (1)$$

where DI ($\mu\text{g/kg/day}$) is the total daily intake of phthalate normalized for body weight; C ($\mu\text{mol/gCr}$) is the urinary phthalate metabolite concentration; CE (g/day) is the creatinine clearance rate, estimated based on the method by Mage et al. (2008) (Mage et al., 2008); MW_p (g/mol) is the molecular weight of the parent phthalate; F_{ue} is the urinary excretion factor; and W (kg) is body weight. F_{ue} describes the molar ratio between the excreted amount of a metabolite in relation to the intake of the parent phthalate, reported to be 0.69, 0.69, 0.73, 0.059, 0.15, and 0.185 for MiBP, MnBP, MBzP, MEHP, MEOHP, and MECPP, respectively (Koch et al., 2004; Kohn et al., 2000).

2.6. Estimated daily exposure calculations for phthalates in house dust

The daily intake of phthalates through dust ingestion and dermal absorption were estimated using Eqs. (2) and (3), respectively (Guo and Kannan, 2011; U.S. EPA, 2011; Wormuth et al., 2006). Eqs. (2) and (3) were calculated separately using the levels of floor dust and multi-surface dust.

$$DI_{\text{ingest dust}} = (C_{\text{dust}} \times M_{\text{ingest dust}}) / W, \quad (2)$$

where $DI_{\text{ingest dust}}$ ($\mu\text{g/kg/day}$) is the daily intake of phthalates through dust ingestion by body weight; C_{dust} ($\mu\text{g/g dust}$) is the concentration of phthalates in house dust; $M_{\text{ingest dust}}$ (mg/day) is the daily dust ingestion rate, which was assumed to be 60 mg/day (U.S. EPA, 2011); and W (kg) is body weight.

$$DI_{\text{dermal dust}} = (C_{\text{dust}} \times A/3 \times Ms \times f \times 0.15 \times t/24) / W, \quad (3)$$

where $DI_{\text{dermal dust}}$ ($\mu\text{g/kg/day}$) is the daily intake of phthalates through absorption from dust adhering to the skin by body weight; $A/3$ (m^2/day) is the skin surface area including hands, legs, and arms representing one-third of the affected skin surface area in contact with dust (Wormuth et al., 2006); Ms (mg/cm^2) is the amount of dust adhered to the skin, which was assumed to be 0.96 g/m^2 (U.S. EPA, 2011); f is the dermal uptake rate of phthalates for children from PCPs, reported to be, 0.0243, 0.0314, 0.0143, 0.0021, and 0.0013 for DiBP, DnBP, BBzP, DEHP, and DiNP, respectively (Wormuth et al., 2006), as the dust matrix reduces the uptake of chemicals to approximately 15%; and $t/24$ (day) is the time spent at home. The skin surface area (A) was calculated from the child's

weight and height using Eq. (4) by Gehan and George (1970) (U.S. EPA, 2011).

$$A = (W^{0.51456} \times H^{0.42246} \times 0.0235), \quad (4)$$

where W (kg) and H (cm) represent the child's weight and height, respectively. The daily intake of phthalates through house dust was estimated using the following equation:

$$DI_{\text{dust}} = DI_{\text{ingest dust}} + DI_{\text{dermal dust}}, \quad (5)$$

2.7. Data analysis

The chi-squared test was used to analyze the association between children's allergies and their personal and dwelling characteristics. If the concentrations of phthalates in dust and phthalate metabolites in urine samples were lower than the detection limit, then half values of the detection limit were used. Logistic regression was used to determine the relationships between children's allergies and the levels of urinary phthalate metabolites and daily intake estimated from urine and house dust. The results are presented as odds ratios (OR) with 95% confidence intervals (CI) for the risk of allergies with Ln-transformed phthalate levels because the data for parent phthalate concentrations and their metabolites were not normally distributed according to the Shapiro-Wilk W-test. Potential confounding factors considered in the analysis were: gender; age; parental allergic history; environmental tobacco smoke exposure; household income; and dampness index (0–5), which was calculated by summing the “presence” of the five dampness indicators in each dwelling including condensation (yes/no), visible mold (yes/no), moldy odor (yes/no), water leakage (yes/no), and high air humidity in the bathroom (yes/no) (Kishi et al., 2009; Saijo et al., 2011). The covariates in the analysis were selected based on a review of the literature and on the change in estimate criteria, which were set to $>10\%$. Therefore, the logistic regression analysis was adjusted for gender, age, and parental history of allergies. DI_{DEHP} is estimated from the average of the three intakes ($DI_{\text{DEHP}_{\text{MEHP}}}$, $DI_{\text{DEHP}_{\text{MEOHP}}}$, and $DI_{\text{DEHP}_{\text{MECPP}}}$).

The correlations between daily phthalate intakes estimated from urinary and house dust levels were tested using Spearman's correlation. Regarding DI_{dust} -DiNP, since we did not measure any metabolites of DiNP, correlations of DI_{dust} -DiNP with other total intakes were not demonstrated in this study.

For all statistical analyses, a two-tailed test and a 5% level of significance were used. Analyses were performed using SPSS 19 for Macintosh (SPSS Inc., Chicago, IL, USA) and SAS software version 9.4 (SAS Institute, Inc., Cary, NC).

2.8. Ethical considerations

All participants gave their written informed consent. The study protocol was approved by the ethical board for epidemiological studies at Hokkaido University Graduate School of Medicine and at all of the regional universities involved in the study.

3. Results

Table 1 shows the parental-reported allergic prevalence and characteristics of participants and their dwellings. The children with parental-reported wheeze, allergic rhino-conjunctivitis, and atopic dermatitis had a higher prevalence of parental history of allergies. In addition, wheeze was more prevalent among those in dwellings with an older building age, higher dampness index, and presence of environmental tobacco smoke. Allergic rhino-conjunctivitis was more prevalent among those with a higher household income (of more than 5 million/year).

Table 1
Parental-reported allergic prevalence and characteristics of participants and their dwellings (n = 184).

		n	Wheeze			Allergic rhino-conjunctivitis			Atopic dermatitis		
			Yes %	No %	p value	Yes %	No %	p value	Yes %	No %	p value
<i>Personal characteristics</i>											
Gender	Male	101	20.8	79.2	1.000	27.7	72.3	0.422	31.7	68.3	0.516
	Female	83	21.7	78.3		33.7	66.3		26.5	73.5	
Age (yr.)	Mean (±SD)		9.2 (±1.9)	9.3 (±1.7)	0.859	9.6 (±1.8)	9.1 (±1.7)	0.080	9.1 (±1.6)	9.3 (±1.8)	0.480
Parental history of allergies	Yes	139	100	69.0	<0.001	94.6	67.2	<0.001	92.6	68.5	<0.001
	No	45	0	31.0		5.4	32.8		7.4	31.5	
Household income(yen/year) ^a	<5 million	44	25.0	80.5	0.745	18.2	81.8	0.028	20.5	79.5	0.142
	≥5 million	113	19.5	75.0		31.0	69.0		34.5	65.5	
	Unknown	27	22.2	77.8		48.1	51.9		22.2	77.8	
<i>Dwelling characteristics</i>											
Type of dwelling	Single-family house	87	23.0	77.0	0.593	26.4	73.6	0.336	25.3	74.7	0.262
	Multi-family house	97	19.6	80.4		34.0	66.0		33	67.0	
Building structure	Wooden	82	28.0	72.0	0.047	28.0	72.0	0.629	24.4	75.6	0.197
	Other	102	15.7	84.3		32.4	67.6		33.3	66.7	
Age of building (year)	≤5.0	48	20.5	79.5	0.045	28.9	71.1	0.498	24.1	75.9	0.612
	6.0–10.0	43	12.8	87.2		19.6	80.4		18.5	81.5	
	11.0–20.0	54	30.8	69.2		25.0	75		31.5	68.5	
	21.0+	39	35.9	64.1		26.8	73.2		25.9	74.1	
Floor materials	Compressed wood	137	17.5	82.5	0.101	29.2	70.8	0.477	27.0	73.0	0.131
	PVC	13	38.5	61.5		38.5	61.5		23.1	76.9	
	Wall-to-wall carpet	15	20.0	80.0		20.0	80.0		26.7	73.3	
	Others ^b	19	36.8	63.2		42.1	57.9		52.6	47.4	
Renovation	Yes	40	25.0	75.0	0.506	35.0	65.0	0.561	17.5	82.5	0.078
	No	144	20.1	79.9		29.2	70.8		32.6	67.4	
Dampness Index (1–5)	Mean (±SD)		2.8 (±1.2)	2.1 (±1.2)	0.002	2.3 (±1.2)	2.2 (±1.2)	0.814	2.5 (±1.3)	2.1 (±1.2)	0.062
Pet keeping	Yes	60	18.3	81.7	0.509	26.7	73.3	0.497	26.7	73.3	0.609
	No	124	22.6	77.4		32.3	67.7		30.6	69.4	
Environmental tobacco smoke	Yes	52	30.8	69.2	0.046	30.8	69.2	1.000	34.6	65.4	0.370
	No	132	17.4	82.6		30.3	69.7		27.3	72.7	
Usage of mechanical ventilation system	Yes	84	17.9	82.1	0.310	35.7	64.3	0.198	25.0	75.0	0.258
	No	100	24.0	76.0		26.0	74.0		33.0	67.0	

p values were calculated using χ^2 test.

^a 1 USD = 100 JPY (as of 2016.07.07).

^b Others mean natural wood, tatami, or tiles.

The phthalate distributions in urine and dust have previously been described (Ait Bamai et al., 2013, 2015) and are shown in Supplementary Table 1. We measured and quantified the metabolites MiBP, MnBP, MBzP, MEHP, MEOHP, and MECPP in all 184 urine samples. MEOHP was the most abundant metabolite measured in urine, followed by MiBP, MECPP, MEHP, and MBzP (Ait Bamai et al.,

2015). DEHP was found at the highest concentration in both floor and multi-surface dust. DEHP and DiNP were found in 100% of floor dust samples and 99.2% and 100% of multi-surface dust samples, respectively.

Table 2 shows the associations between parental-reported allergic symptoms and daily phthalate intake estimated from urinary

Table 2
The associations between parental-reported allergic symptoms and daily phthalate intake estimated from urinary metabolite and house dust concentrations.

	Wheeze				Rhino-conjunctivitis				Atopic dermatitis			
	OR	95% CI		p value	OR	95% CI		p value	OR	95% CI		p value
<i>Urinary metabolites</i>												
DI_DiBP	0.77	0.60	1.00	0.052	0.97	0.78	1.19	0.737	0.85	0.68	1.05	0.132
DI_DnBP	1.02	0.62	1.69	0.937	0.98	0.63	1.54	0.936	0.98	0.62	1.54	0.921
DI_BBzP	1.12	0.81	1.54	0.503	0.99	0.74	1.33	0.944	0.99	0.74	1.33	0.936
DI_DEHP _{MEHP}	0.91	0.69	1.19	0.494	0.89	0.70	1.14	0.350	1.03	0.81	1.31	0.826
DI_DEHP _{MEOHP}	0.90	0.53	1.55	0.714	1.18	0.74	1.89	0.479	1.16	0.73	1.84	0.520
DI_DEHP _{MECPP}	0.94	0.57	1.55	0.803	0.73	0.46	1.14	0.168	0.75	0.48	1.18	0.212
DI_DEHP	0.84	0.53	1.34	0.450	0.96	0.65	1.43	0.850	1.02	0.69	1.52	0.921
<i>Floor dust</i>												
DI _{dust} _DiBP	0.97	0.73	1.30	0.843	1.16	0.89	1.51	0.277	1.27	0.97	1.65	0.081
DI _{dust} _DnBP	1.17	0.87	1.59	0.301	1.25	0.96	1.63	0.092	1.01	0.78	1.30	0.944
DI _{dust} _BBzP	1.13	0.88	1.46	0.339	1.03	0.81	1.31	0.792	1.20	0.95	1.52	0.134
DI _{dust} _DEHP	1.33	0.78	2.26	0.300	1.97	1.19	3.25	0.008	1.38	0.85	2.22	0.190
DI _{dust} _DiNP	0.83	0.56	1.22	0.339	1.01	0.71	1.41	0.979	0.90	0.64	1.26	0.520
<i>Multi-surface dust</i>												
DI _{dust} _DiBP	0.90	0.52	1.55	0.690	1.40	0.85	2.31	0.188	0.84	0.51	1.38	0.481
DI _{dust} _DnBP	0.98	0.73	1.31	0.877	1.18	0.91	1.54	0.217	1.00	0.77	1.30	0.992
DI _{dust} _BBzP	1.18	0.90	1.55	0.228	0.96	0.75	1.23	0.750	1.07	0.84	1.35	0.607
DI _{dust} _DEHP	1.03	0.80	1.32	0.819	0.81	0.63	1.03	0.088	0.89	0.71	1.11	0.299
DI _{dust} _DiNP	0.73	0.53	1.00	0.052	0.92	0.71	1.18	0.492	0.84	0.65	1.10	0.206

The odds ratios were calculated using Ln transformed variables. The bold shows a statistical significant association. Adjusted for gender, age, and parental history of allergies. DI_DEHP is estimated from the average of the three intakes (DI_DEHP_{MEHP}, DI_DEHP_{MEOHP}, and DI_DEHP_{MECPP}).

metabolite and house dust concentrations. A significant relationship was found between allergic rhino-conjunctivitis and $DI_{\text{dust-DEHP}}$ from floor dust (OR, 1.97; 95% CI, 1.19–3.25; $p = 0.008$) after adjusting for confounding factors. An inverse association was found between wheeze and DI_{DiBP} , but this association was not statistically significant (OR, 0.77; 95% CI, 0.60–1.00; $p = 0.052$). The results of dust concentrations (Supplementary Table 4) were nearly the same as those for intake from dust, since intake via dust ingestion ($DI_{\text{ingest dust}}$) depends only on the dust concentration of phthalates (C_{dust}) and body weight (W). Moreover, body weight, depending on the age of children, might not vary widely and therefore weight may have a relatively small effect on $DI_{\text{ingest dust}}$ and intake from dust adhering to the skin ($DI_{\text{dermal dust}}$) most likely has a negligible contribution (Beko et al., 2013; Guo and Kannan, 2011).

Table 3 shows the association between parental-reported allergic symptoms and daily phthalate intake estimated from urinary metabolites stratified by gender. DI_{DiBP} was inversely associated with wheeze among boys (OR, 0.62; 95% CI, 0.40–0.97; $p = 0.035$). $DI_{\text{DEHP}_{\text{MECPP}}}$ was also inversely associated with atopic dermatitis among boys (OR, 0.45; 95% CI, 0.21–0.95; $p = 0.036$). However, there were no significant associations observed between phthalate intakes and allergic symptoms in girls.

Table 4 shows the associations between parental-reported allergic symptoms and estimated daily phthalate intake from house dust stratified by gender. In boys, $DI_{\text{dust-DEHP}}$ in floor dust was significantly associated with allergic rhino-conjunctivitis (2.05, 1.03–4.08; $p = 0.040$). In girls, $DI_{\text{dust-DnBP}}$ from multi-surface dust was significantly associated with allergic rhino-conjunctivitis (OR, 1.85; 95% CI, 1.12–3.06; $p = 0.016$).

Table 5 shows the correlations between daily phthalate intake estimated from urinary and house dust levels. Estimated $DI_{\text{dust-BBzP}}$ and $DI_{\text{dust-DEHP}}$ from both floor and multi-surface dust had significant positive correlations with estimated DI_{BBzP} and DI_{DEHP} .

Table 3

The association between parental-reported allergic symptoms and daily phthalate intake estimated from urinary metabolites stratified by gender.

	Boys				Girls			
	OR	95% CI	p-Value		OR	95% CI	p-Value	
<i>Wheeze</i>								
DI_{DiBP}	0.62	0.40	0.97	0.035	0.90	0.65	1.26	0.543
DI_{DnBP}	0.89	0.42	1.89	0.761	1.15	0.58	2.29	0.687
DI_{BBzP}	1.45	0.88	2.36	0.142	0.90	0.56	1.43	0.642
$DI_{\text{DEHP}_{\text{MEHP}}}$	0.93	0.64	1.35	0.705	0.89	0.59	1.34	0.578
$DI_{\text{DEHP}_{\text{MEOHP}}}$	0.87	0.39	1.94	0.733	0.95	0.45	1.98	0.882
$DI_{\text{DEHP}_{\text{MECPP}}}$	0.98	0.44	2.18	0.952	0.92	0.48	1.76	0.799
DI_{DEHP}	0.91	0.48	1.71	0.910	0.77	0.39	1.53	0.459
<i>Allergic rhino-conjunctivitis</i>								
DI_{DiBP}	0.96	0.71	1.29	0.770	0.98	0.73	1.31	0.878
DI_{DnBP}	0.84	0.43	1.65	0.620	1.09	0.59	2.02	0.785
DI_{BBzP}	1.26	0.81	1.96	0.298	0.79	0.52	1.20	0.271
$DI_{\text{DEHP}_{\text{MEHP}}}$	0.97	0.70	1.34	0.849	0.79	0.55	1.15	0.217
$DI_{\text{DEHP}_{\text{MEOHP}}}$	1.33	0.69	2.56	0.388	1.03	0.53	1.99	0.925
$DI_{\text{DEHP}_{\text{MECPP}}}$	0.67	0.33	1.37	0.273	0.75	0.42	1.34	0.334
DI_{DEHP}	1.08	0.63	1.85	0.785	0.82	0.45	1.49	0.513
<i>Atopic dermatitis</i>								
DI_{DiBP}	0.73	0.52	1.02	0.061	0.96	0.71	1.30	0.796
DI_{DnBP}	0.74	0.37	1.45	0.375	1.35	0.71	2.56	0.356
DI_{BBzP}	0.86	0.56	1.30	0.465	1.17	0.77	1.78	0.453
$DI_{\text{DEHP}_{\text{MEHP}}}$	1.13	0.82	1.56	0.473	0.93	0.64	1.36	0.712
$DI_{\text{DEHP}_{\text{MEOHP}}}$	1.31	0.68	2.52	0.421	1.10	0.56	2.14	0.781
$DI_{\text{DEHP}_{\text{MECPP}}}$	0.45	0.21	0.95	0.036	1.11	0.62	1.97	0.725
DI_{DEHP}	1.14	0.67	1.96	0.625	0.95	0.52	1.74	0.861

The odds ratios were calculated using Ln transformed variables. The bold shows statistical significant associations. Adjusted for age and parental history of allergies. DI_{DEHP} was estimated from the average of the three intakes from the three metabolites ($DI_{\text{DEHP}_{\text{MEHP}}}$, $DI_{\text{DEHP}_{\text{MEOHP}}}$, and $DI_{\text{DEHP}_{\text{MECPP}}}$).

Table 4

The associations between parental-reported allergic symptoms and estimated daily phthalate intake from house dust stratified by gender.

	Boys				Girls			
	OR	95% CI	p-Value		OR	95% CI	p-Value	
<i>Wheeze</i>								
<i>Floor dust</i>								
$DI_{\text{dust-DiBP}}$	0.86	0.58	1.26	0.425	1.21	0.76	1.93	0.428
$DI_{\text{dust-DnBP}}$	1.07	0.68	1.67	0.777	1.32	0.86	2.04	0.205
$DI_{\text{dust-BBzP}}$	1.06	0.74	1.52	0.757	1.24	0.86	1.79	0.258
$DI_{\text{dust-DEHP}}$	1.30	0.64	2.66	0.470	1.39	0.62	3.14	0.427
$DI_{\text{dust-DiNP}}$	0.83	0.50	1.37	0.463	0.84	0.44	1.59	0.583
<i>Multi-surface dust</i>								
$DI_{\text{dust-DiBP}}$	0.55	0.24	1.29	0.170	1.57	0.68	3.62	0.289
$DI_{\text{dust-DnBP}}$	0.83	0.56	1.23	0.344	1.27	0.79	2.06	0.329
$DI_{\text{dust-BBzP}}$	1.14	0.77	1.68	0.512	1.25	0.85	1.84	0.250
$DI_{\text{dust-DEHP}}$	0.94	0.70	1.26	0.679	1.25	0.76	2.06	0.374
$DI_{\text{dust-DiNP}}$	0.80	0.53	1.20	0.283	0.65	0.38	1.08	0.098
<i>Allergic rhino-conjunctivitis</i>								
<i>Floor dust</i>								
$DI_{\text{dust-DiBP}}$	1.37	0.95	1.97	0.088	0.91	0.60	1.38	0.670
$DI_{\text{dust-DnBP}}$	1.31	0.90	1.90	0.165	1.19	0.82	1.73	0.365
$DI_{\text{dust-BBzP}}$	1.15	0.82	1.61	0.424	0.92	0.66	1.30	0.644
$DI_{\text{dust-DEHP}}$	2.05	1.03	4.08	0.040	1.87	0.88	3.94	0.103
$DI_{\text{dust-DiNP}}$	0.91	0.58	1.42	0.676	1.15	0.66	2.01	0.617
<i>Multi-surface dust</i>								
$DI_{\text{dust-DiBP}}$	1.04	0.52	2.08	0.913	2.08	0.92	4.70	0.078
$DI_{\text{dust-DnBP}}$	0.89	0.62	1.28	0.539	1.85	1.12	3.06	0.016
$DI_{\text{dust-BBzP}}$	0.79	0.55	1.13	0.202	1.15	0.81	1.64	0.424
$DI_{\text{dust-DEHP}}$	0.73	0.52	1.04	0.084	0.92	0.64	1.32	0.635
$DI_{\text{dust-DiNP}}$	0.67	0.45	1.00	0.051	1.20	0.85	1.69	0.312
<i>Atopic dermatitis</i>								
<i>Floor dust</i>								
$DI_{\text{dust-DiBP}}$	1.15	0.82	1.61	0.411	1.57	0.98	2.51	0.063
$DI_{\text{dust-DnBP}}$	1.06	0.74	1.51	0.770	0.98	0.67	1.44	0.916
$DI_{\text{dust-BBzP}}$	1.04	0.75	1.45	0.797	1.40	0.99	1.99	0.061
$DI_{\text{dust-DEHP}}$	1.38	0.73	2.63	0.323	1.47	0.70	3.09	0.309
$DI_{\text{dust-DiNP}}$	0.75	0.48	1.17	0.203	1.23	0.70	2.15	0.469
<i>Multi-surface dust</i>								
$DI_{\text{dust-DiBP}}$	0.97	0.50	1.88	0.916	0.60	0.26	1.41	0.244
$DI_{\text{dust-DnBP}}$	1.04	0.74	1.48	0.808	0.93	0.61	1.40	0.712
$DI_{\text{dust-BBzP}}$	1.05	0.75	1.46	0.790	1.08	0.76	1.54	0.661
$DI_{\text{dust-DEHP}}$	0.86	0.64	1.14	0.295	0.93	0.64	1.34	0.690
$DI_{\text{dust-DiNP}}$	0.90	0.63	1.28	0.542	0.77	0.51	1.15	0.200

The odds ratios were calculated using Ln transformed variables. The bold shows statistical significant associations. Adjusted for age and parental history of allergies.

4. Discussion

To our knowledge, this is the first study to assess the effect of phthalate exposure, indicated by daily phthalate intake estimated from both urine metabolite and house dust levels, on the risk of allergies in children. Our results indicate that $DI_{\text{dust-DEHP}}$ from floor, but not multi-surface dust, was associated with parental-reported allergic rhino-conjunctivitis in children. After stratification by gender, this association was still significant for boys, while a significant association between $DI_{\text{dust-DnBP}}$ from multi-surface dust and parental-reported allergic rhino-conjunctivitis was observed in girls. Our previous study, which evaluated newly-built detached houses in 6 regions of Japan, reported dose-response relationships between concentrations of DEHP in floor dust and allergic conjunctivitis. Significant associations between children's allergic symptoms and phthalate exposure were only found for concentrations of phthalates in floor dust, but not multi-surface dust (Ait Bamai et al., 2014). Those results are consistent with the findings of the present study.

In the present study, our finding of an association between allergies and daily intake estimated from urinary metabolites was contrary to the finding of the association with dust. None of the daily intake levels estimated from urinary metabolites were statistically significant, but after stratifying by gender, DI_{DiBP} and $DI_{\text{DEHP}_{\text{MECPP}}}$ were associated with a decrease in the OR for wheeze and atopic dermatitis, respectively, in boys. Similar associations have been reported from the National

Table 5

The correlations between daily phthalate intakes estimated from urinary and house dust levels.

		Floor dust				Multi-surface dust			
		DI _{dust} _DiBP	DI _{dust} _DnBP	DI _{dust} _BBzP	DI _{dust} _DEHP	DI _{dust} _DiBP	DI _{dust} _DnBP	DI _{dust} _BBzP	DI _{dust} _DEHP
Urine	DI _{dust} _DiBP	−0.05	0.08	−0.11	−0.05	−0.01	0.01	−0.12	−0.09
	DI _{dust} _DnBP	−0.01	0.05	0.03	0.08	0.06	0.06	0.08	−0.02
	DI _{dust} _BBzP	0.02	−0.04	0.31**	0.19*	0.00	0.16*	0.23**	0.11
	DI _{dust} _DEHP _{MEOHP}	0.08	0.00	0.04	0.17*	0.01	−0.05	−0.08	0.05
	DI _{dust} _DEHP _{MEOHP}	0.10	0.14	0.05	0.28**	0.06	0.07	0.09	0.16*
	DI _{dust} _DEHP _{MEOHP}	0.08	0.07	0.06	0.06	0.00	0.05	0.02	0.02
	DI _{dust} _DEHP	0.10	0.08	0.07	0.27**	0.01	0.02	−0.03	0.10

Spearman's ρ . DI_{dust}_DEHP was estimated from the average of the three intakes from the three metabolites (DI_{dust}_DEHP_{MEOHP}, DI_{dust}_DEHP_{MEOHP}, and DI_{dust}_DEHP_{MEOHP}).* $p < 0.05$.** $p < 0.01$.

Health and Nutrition Examination Survey (NHANES) and the Danish Indoor Environment and Children's Health (IECH) study. Urinary MnBP was inversely associated with current wheeze among children (6–17 years old) (Hoppin et al., 2013), and increased levels of urinary MiBP were associated with decreases in the OR for a diagnosis of asthma in children aged 3–5 years old (Callesen et al., 2014a). One possible explanation is that DiBP or DnBP (the parent compounds of MiBP and MnBP) are used for medicines and PCPs (Hernandez-Diaz et al., 2009), so allergy medications may contain these phthalates, thus resulting in an inverse relationship. We did not collect data on medication and PCP use; hence, we could not adjust for these confounders in our analysis. Regarding the association between DI_{dust}_DEHP_{MEOHP} and atopic dermatitis, Callesen et al. reported decreased ORs for the occurrence of atopic dermatitis, but this association was not statistically significant. On the other hand, the NHANES III, Environment and Childhood Asthma (ECA) study, and Taiwan Maternal and Infant Cohort Study reported inconsistent results. Higher levels of urinary DiNP, DnBP, DEHP, and BBzP metabolites were associated with increased ORs for asthma, wheeze, or pulmonary function (Bertelsen et al., 2013; Hoppin et al., 2004; Ku et al., 2015). The lack of consistency between these previous studies may be related to the cross-sectional design.

After stratifying by gender, significant positive associations were observed between wheeze and DI_{dust}_DiBP; atopic dermatitis and DI_{dust}_DEHP_{MEOHP} (Table 3); and allergic rhino-conjunctivitis and DI_{dust}_DEHP in floor dust among boys; and between allergic rhino-conjunctivitis and DI_{dust}_DnBP in multi-surface dust among girls (Table 4). There has been only one previous study that has reported gender-specific effects of phthalates on allergies. Although most studies adjust for gender when evaluating the association between exposure to phthalates and allergies, to our knowledge, there has been only one study focused on gender-specific associations to phthalate exposure. Ku et al. (2015) demonstrated that the Σ3DEHP metabolites were associated with asthma in 5-year-old boys, but not in girls (Ku et al., 2015). Therefore, the mechanisms involved in decreasing the risk of allergies and gender-specific effects are still unclear.

So why were allergies significantly associated with DEHP in house dust but not urinary phthalate metabolites? One possible explanation is that the adverse effects from phthalates in house dust were caused by direct, regional stimulation of the nasal mucosa and/or conjunctiva, and their immune response system. Dust ingestion may be the major route of indoor environmental DEHP exposure (not accounting for diet), while it may be a much weaker contributor for the other phthalates. Deutschle et al. (2008) reported that nasal exposure to house dust with DEHP had attenuating effects on human nasal immune responses (Deutschle et al., 2008). This direct, regional stimulation may cause rhino-conjunctivitis. Animal studies have indicated that the mechanism underlying the role of phthalates in allergic and immune responses may involve phthalates acting as adjuvants for the production of cytokines and/or immunoglobulins responsible for allergic sensitization (Larsen et al., 2001a; Larsen et al., 2001b; Tanaka et al., 2013). This

indirect stimulation and a wide range of in vivo immune responses may potentially cause asthma and wheeze. However, little is known about the mechanisms of phthalates involved in both direct and indirect stimulation and/or immune response system. This could be investigated in future animal studies.

The correlations between DI_{dust}_BBzP and DI_{dust}_BBzP and between DI_{dust}_DEHP and DI_{dust}_DEHP were obtained from both floor and multi-surface dust, and these correlations were stronger for floor dust than multi-surface dust; no correlation was found for the other investigated phthalates (Table 5). These results are consistent with previous reports. Significant correlations have been demonstrated between BBzP in settled dust from surfaces above the floor and MBzP in the child's urine (Hsu et al., 2012; Langer et al., 2013). Significant correlations were also reported for BBzP between personal air and urinary measures (Adibi et al., 2003; Adibi et al., 2008). Further, significant correlations were reported between the total daily intake of BBzP estimated from urine and the corresponding day-specific daily intake from three indoor exposure routes (Beko et al., 2013). High molecular weight phthalates, such as BBzP and DEHP, and DiNP tend to have low air and/or gas-phase but high dust-phase concentrations (Kanazawa et al., 2010), while DnBP and DEP, which are categorized as low molecular weight phthalates, contribute more to dermal and inhalation uptake from air than from dust (Weschler et al., 2015). Moreover, we found that DI_{dust}_BBzP was correlated with DI_{dust}_DEHP in floor dust and DI_{dust}_DnBP in multi-surface dust. One possible explanation for the correlation between different phthalates is that they might share the same indoor exposure source (e.g., floor material, wall paper, glue, etc.). Despite a number of studies indicating that the major source of DEHP exposure in humans is dietary (Guo and Kannan, 2011; Koch et al., 2013; Rudel et al., 2011; Wormuth et al., 2006), associations between phthalates in house dust rather than urinary metabolites were observed in this study. In addition, whereas our previous study found higher concentrations of phthalates in multi-surface dust than in floor dust, most of the significant results in this study were related to floor dust. This suggests that house dust from low surfaces, such as the living room floor, has an effect on the prevalence of allergic symptoms in children, and might play a meaningful role in indoor environmental exposure to DEHP. In particular, Japanese children generally sit and play on the floor when spending time at home, so it is reasonable that floor dust showed stronger correlations in this study.

Our results suggest that house dust is an important indoor environmental exposure source for total BBzP and DEHP intake. The correlation was higher for floor dust than for multi-surface dust for DI_{dust}_DEHP and DI_{dust}_BBzP, and no correlation was found for the other investigated phthalates. In this study, neither DI_{dust}_DiBP nor DI_{dust}_DnBP (both low molecular weight phthalates) were correlated with any daily intake through house dust. Similar results were reported in a study from Denmark: The contributions of indoor exposure through dust ingestion to total intake estimated from urinary metabolites for DEP, DnBP, and DiBP were smaller than those for

BBzP and DEHP (Beko et al., 2013). However, contributions of indoor exposure (sum of dust ingestion, inhalation, and dermal exposure through air) to total intake estimated from urinary metabolites for DEP, DnBP, and DiBP were larger than those for BBzP and DEHP (Beko et al., 2013). A recent study also indicated that dermal uptake from air can be a meaningful exposure pathway for both DEP and DnBP, and is likely to be more important than dust ingestion for these two phthalates (Weschler et al., 2015). Phthalate levels in air were not measured in this study; therefore, we could not assess inhalation and dermal uptake from air (gas-phase) and their effects on allergies, which might be needed in further studies.

Even though the main exposure route for DEHP is thought to be diet, in this study, the impact of DEHP on allergies might be stronger through indoor exposure via house dust ingestion. We have previously reported that higher levels of DEHP in house dust are obtained in dwellings with PVC flooring, and dose-response relationships are obtained for DEHP in house dust and numbers of PVC materials for floor, wall, and ceiling (Ait Bamai et al., 2013). Our previous findings showed the frequency of cleaning and concentration of phthalates in floor and multi-surface dust were not significantly correlated (Ait Bamai et al., 2013). Hence, even frequent cleaning does not impact floor and multi-surface dust; since the emission source of phthalates indoors does not change much, the concentration of phthalates in house dust allows for stable and long-term assessment of indoor exposure. On the other hand, the concentration of urinary phthalate metabolites vary during the day, depending on diet and cosmetics and/or PCP use, etc., due to the characteristics of their short-half lives. Several studies have provided information on intra-individual temporal variability in urinary phthalate concentrations. Teitelbaum et al. (2008) indicated that urinary concentrations of phthalate metabolites are reproducible and do not change much over long periods (2 to 24 weeks) (Teitelbaum et al., 2008). Frederiksen et al. (2013) reported high interclass correlation coefficients for the metabolites of DEP, DBP, and BBzP, indicating that a single urine sample may provide a reasonably good estimate of exposure to these phthalates, but low interclass correlation coefficients for the metabolites of DEHP and DiNP, indicating a high risk of misclassification of exposures to these two phthalates in population studies (Frederiksen et al., 2013). Hence, daily intake estimated from house dust might be more strongly related to allergies than intake from urinary metabolites, especially for DEHP.

The limitations of this study need to be considered. First, this study had a cross-sectional design, and the measurement of phthalates in house dust and urine were conducted only once. Therefore, any causal relationships between phthalate levels and health outcomes were not discernible. Second, the participants in this study were those who were interested in their home environment and health because they agreed to participate in a home visit survey. The prevalence of wheeze, allergic rhino-conjunctivitis, atopic dermatitis, and parental history of allergies in the present study were higher, but the presence of a smoker at home was lower than in the baseline survey (Supplementary Table 5). Participants who are concerned about allergies and/or health problems were more likely to participate in this study, thus selection bias may have occurred. Third, exposure source data, such as PCP and medicine use and indoor air, were not measured. These are all major routes of exposure to phthalates. A lack of such exposure source data limited our investigation. Fourth, although we assumed a daily dust ingestion rate ($M_{\text{ingest dust}}$: 60 mg/day) for all children for floor and multi-surface dust, the actual $M_{\text{ingest dust}}$ is the total (combination of floor and surface dust) ingested dust rate and the amount of ingested floor and surface dust may differ from child to child. Therefore, our estimations of DI_{dust} estimated from either floor or multi-surface dust may underestimate or overestimate the exposure to phthalates. A further limitation is that health outcomes were only assessed using questionnaires, no biological markers to assess outcomes, such as immunoglobulin E, were measured. In this study, allergic diseases were defined using ISAAC questionnaires, which are internationally standardized procedures; these

provided validity for the criteria for developing illness. However, it does not fully ensure validity of the health data because self-assessment is subjective and difficult to perform by parents. Misclassification of outcomes may have thus occurred. The main strength of this study is that we assessed the estimated daily intake of phthalates through different exposure sources using floor dust and multi-surface dust, and estimated the total phthalate intake using urine samples.

In conclusion, this was the first study to assess the effects of phthalate exposure on the risk of parental-reported allergies in children by using daily phthalate intake estimated from both urinary metabolite concentrations and phthalate levels in two types of house dust (floor and multi-surface). This study demonstrated that high levels of exposure to DEHP ($DI_{\text{dust-DEHP}}$) in floor dust increases the risk of allergic rhino-conjunctivitis, especially among boys. However, DI_{DiBP} estimated from MiBP was inversely related to wheeze among boys. The findings of this study suggest that dust from low surfaces, such as living room floors, might play a meaningful role in the indoor environmental exposure pathway for DEHP and BBzP. Additionally, the intake from dust ingestion may be more strongly related to health than total intake from urinary metabolites. Further studies are needed to understand the mechanisms involved in allergic sensitization.

Acknowledgments

The authors would like to acknowledge Ayako Kanazawa at the Hokkaido University Center for Environmental and Health Sciences and members of this project. Furthermore, we thank all the study participants and their family members.

This work was supported in part by a Grant-in-Aid for scientific research from the Ministry of Health Labor and Welfare, Japan (H20-Kenki Ippan-009), and the Ministry of the Environment, Japan (5C-1151).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.envint.2016.08.025>.

References

- Abb, M., Heinrich, T., Sorkau, E., Lorenz, W., 2009. Phthalates in house dust. *Environ. Int.* 35, 965–970 (2009).
- Adibi, J.J., Perera, F.P., Jedrychowski, W., Camann, D.E., Barr, D., Jacek, R., Whyatt, R.M., 2003. Prenatal exposures to phthalates among women in New York City and Krakow, Poland. *Environ. Health Perspect.* 111, 1719–1722.
- Adibi, J.J., Whyatt, R.M., Williams, P.L., Calafat, A.M., Camann, D., Herrick, R., Nelson, H., Bhat, H.K., Perera, F.P., Silva, M.J., Hauser, R., 2008. Characterization of phthalate exposure among pregnant women assessed by repeat air and urine samples. *Environ. Health Perspect.* 116, 467–473.
- Ait Bamai, Y., Araki, A., Kawai, T., Tsuboi, T., Saito, I., Yoshioka, E., Kanazawa, A., Tajima, S., Shi, C., Tamakoshi, A., Kishi, R., 2013. Associations of phthalate concentrations in floor dust and multi-surface dust with the interior materials in Japanese dwellings. *Sci. Total Environ.* 468–469C, 147–157.
- Ait Bamai, Y., Shibata, E., Saito, I., Araki, A., Kanazawa, A., Morimoto, K., Nakayama, K., Tanaka, M., Takigawa, T., Yoshimura, T., Chikara, H., Saijo, Y., Kishi, R., 2014. Exposure to house dust phthalates in relation to asthma and allergies in both children and adults. *Sci. Total Environ.* 485–486C, 153–163.
- Ait Bamai, Y., Araki, A., Kawai, T., Tsuboi, T., Yoshioka, E., Kanazawa, A., Cong, S., Kishi, R., 2015. Comparisons of urinary phthalate metabolites and daily phthalate intakes among Japanese families. *Int. J. Hyg. Environ. Health* 218, 461–470.
- Beko, G., Weschler, C.J., Langer, S., Callesen, M., Toftum, J., Clausen, G., 2013. Children's phthalate intakes and resultant cumulative exposures estimated from urine compared with estimates from dust ingestion, inhalation and dermal absorption in their homes and daycare centers. *PLoS One* 8, e62442.
- Beko, G., Callesen, M., Weschler, C.J., Toftum, J., Langer, S., Sigsgaard, T., Host, A., Kold Jensen, T., Clausen, G., 2015. Phthalate exposure through different pathways and allergic sensitization in preschool children with asthma, allergic rhinoconjunctivitis and atopic dermatitis. *Environ. Res.* 137, 432–439.
- Bertelsen, R.J., Carlsen, K.C., Calafat, A.M., Hoppin, J.A., Haland, G., Mowinckel, P., Carlsen, K.H., Lovik, M., 2013. Urinary biomarkers for phthalates associated with asthma in Norwegian children. *Environ. Health Perspect.* 121, 251–256.
- Bizzari, S.N., Blagoev, M., Kishi, A., 2013. Chemical Economics Handbook Plasticizers.
- Bornehag, C.G., Nanberg, E., 2010. Phthalate exposure and asthma in children. *Int. J. Androl.* 33, 333–345.

- Bornehag, C.G., Sundell, J., Weschler, C.J., Sigsgaard, T., Lundgren, B., Hasselgren, M., Hagerhed-Engman, L., 2004. The association between asthma and allergic symptoms in children and phthalates in house dust: a nested case-control study. *Environ. Health Perspect.* 112, 1393–1397.
- Bornehag, C.G., Lundgren, B., Weschler, C.J., Sigsgaard, T., Hagerhed-Engman, L., Sundell, J., 2005. Phthalates in indoor dust and their association with building characteristics. *Environ. Health Perspect.* 113, 1399–1404.
- Callesen, M., Beko, G., Weschler, C.J., Langer, S., Brive, L., Clausen, G., Toftum, J., Sigsgaard, T., Host, A., Jensen, T.K., 2014a. Phthalate metabolites in urine and asthma, allergic rhinoconjunctivitis and atopic dermatitis in preschool children. *Int. J. Hyg. Environ. Health* 217, 645–652.
- Callesen, M., Beko, G., Weschler, C.J., Sigsgaard, T., Jensen, T.K., Clausen, G., Toftum, J., Norberg, L.A., Host, A., 2014b. Associations between selected allergens, phthalates, nicotine, polycyclic aromatic hydrocarbons, and bedroom ventilation and clinically confirmed asthma, rhinoconjunctivitis, and atopic dermatitis in preschool children. *Indoor Air* 24, 136–147.
- Deutschle, T., Reiter, R., Butte, W., Heinzow, B., Keck, T., Riechelmann, H., 2008. A controlled challenge study on di(2-ethylhexyl) phthalate (DEHP) in house dust and the immune response in human nasal mucosa of allergic subjects. *Environ. Health Perspect.* 116, 1487–1493.
- Frederiksen, H., Kranich, S.K., Jorgensen, N., Taboureaux, O., Petersen, J.H., Andersson, A.M., 2013. Temporal variability in urinary phthalate metabolite excretion based on spot, morning, and 24-h urine samples: considerations for epidemiological studies. *Environ. Sci. Technol.* 47, 958–967.
- Gehan, E., George, G.L., 1970. Estimation of human body surface area from height and weight. *Cancer Chemother. Rep.* 54 (4), 225–235.
- Guo, Y., Kannan, K., 2011. Comparative assessment of human exposure to phthalate esters from house dust in China and the United States. *Environ. Sci. Technol.* 45, 3788–3794.
- Hernandez-Diaz, S., Mitchell, A.A., Kelley, K.E., Calafat, A.M., Hauser, R., 2009. Medications as a potential source of exposure to phthalates in the U.S. population. *Environ. Health Perspect.* 117, 185–189.
- Hoppin, J.A., Ulmer, R., London, S.J., 2004. Phthalate exposure and pulmonary function. *Environ. Health Perspect.* 112, 571–574.
- Hoppin, J.A., Jaramillo, R., London, S.J., Bertelsen, R.J., Salo, P.M., Sandler, D.P., Zeldin, D.C., 2013. Phthalate exposure and allergy in the U.S. population: Results from NHANES 2005–2006. *Environ. Health Perspect.* 121, 1129–1134.
- Hsu, N.Y., Lee, C.C., Wang, J.Y., Li, Y.C., Chang, H.W., Chen, C.Y., Bornehag, C.G., Wu, P.C., Sundell, J., Su, H.J., 2012. Predicted risk of childhood allergy, asthma, and reported symptoms using measured phthalate exposure in dust and urine. *Indoor Air* 22, 186–199.
- Jaakkola, J.J.K., Oie, L., Nafstad, P., Botten, G., Samuelsen, S.O., Magnus, P., 1999. Interior surface materials in the home and the development of bronchial obstruction in young children in Oslo, Norway. *Am. J. Public Health* 89, 188–192.
- Kanazawa, A., Saito, I., Araki, A., Takeda, M., Ma, M., Saijo, Y., Kishi, R., 2010. Association between indoor exposure to semi-volatile organic compounds and building-related symptoms among the occupants of residential dwellings. *Indoor Air* 20, 72–84.
- Kishi, R., Saijo, Y., Kanazawa, A., Tanaka, M., Yoshimura, T., Chikara, H., Takigawa, T., Morimoto, K., Nakayama, K., Shibata, E., 2009. Regional differences in residential environments and the association of dwellings and residential factors with the sick house syndrome, a nationwide cross-sectional questionnaire study in Japan. *Indoor Air* 19, 243–254.
- Koch, H.M., Bolt, H.M., Angerer, J., 2004. Di(2-ethylhexyl)phthalate (DEHP) metabolites in human urine and serum after a single oral dose of deuterium-labelled DEHP. *Arch. Toxicol.* 78, 123–130.
- Koch, H.M., Lorber, M., Christensen, K.L., Palmke, C., Koslitz, S., Bruning, T., 2013. Identifying sources of phthalate exposure with human biomonitoring, results of a 48 h fasting study with urine collection and personal activity patterns. *Int. J. Hyg. Environ. Health* 216, 672–681.
- Kohn, M.C., Parham, F., Masten, S.A., Portier, C.J., Shelby, M.D., Brock, J.W., Needham, L.L., 2000. Human exposure estimates for phthalates. *Environ. Health Perspect.* 108, A440–A442.
- Koike, E., Yanagisawa, R., Sadakane, K., Inoue, K., Ichinose, T., Takano, H., 2010. Effects of diisononyl phthalate on atopic dermatitis in vivo and immunologic responses in vitro. *Environ. Health Perspect.* 118, 472–478.
- Kolarik, B., Naydenov, K., Larsson, M., Bornehag, C.G., Sundell, J., 2008. The association between phthalates in dust and allergic diseases among Bulgarian children. *Environ. Health Perspect.* 116, 98–103.
- Ku, H.Y., Su, P.H., Wen, H.J., Sun, H.L., Wang, C.J., Chen, H.Y., Jaakkola, J.J., Wang, S.L., 2015. Prenatal and postnatal exposure to phthalate esters and asthma, a 9-year follow-up study of a Taiwanese birth cohort. *PLoS One* 10, e0123309.
- Langer, S., Weschler, C.J., Fischer, A., Beko, G., Toftum, J., Clausen, G., 2010. Phthalate and PAH concentrations in dust collected from Danish homes and daycare centers. *Atmos. Environ.* 44, 2294–2301.
- Langer, S., Beko, G., Weschler, C.J., Brive, L.M., Toftum, J., Callesen, M., Clausen, G., 2013. Phthalate metabolites in urine samples from Danish children and correlations with phthalates in dust samples from their homes and daycare centers. *Int. J. Hyg. Environ. Health* 213.
- Larsen, S.T., Hansen, J.S., Thygesen, P., Begtrup, M., Poulsen, O.M., Nielsen, G.D., 2001a. Adjuvant and immuno-suppressive effect of six monophthalates in a subcutaneous injection model with BALB/c mice. *Toxicology* 169, 37–51.
- Larsen, S.T., Lund, R.M., Nielsen, G.D., Thygesen, P., Poulsen, O.M., 2001b. Di-(2-ethylhexyl) phthalate possesses an adjuvant effect in a subcutaneous injection model with BALB/c mice. *Toxicol. Lett.* 125, 11–18.
- Lee, M.H., Park, J., Chung, S.W., Kang, B.Y., Kim, S.H., Kim, T.S., 2004. Enhancement of interleukin-4 production in activated CD4+ T cells by dipthalate plasticizers via increased NF-AT binding activity. *Int. Arch. Allergy Immunol.* 134, 213–222.
- Lovekamp-Swan, T., Davis, B.J., 2003. Mechanisms of phthalate ester toxicity in the female reproductive system. *Environ. Health Perspect.* 111, 139–145.
- Mage, D.T., Allen, R.H., Kodali, A., 2008. Creatinine corrections for estimating children's and adult's pesticide intake doses in equilibrium with urinary pesticide and creatinine concentrations. *J. Expo. Sci. Environ. Epidemiol.* 18, 360–368.
- Martino-Andrade, A.J., Chahoud, I., 2010. Reproductive toxicity of phthalate esters. *Mol. Nutr. Food Res.* 54, 148–157.
- Oie, L., Magnus, P., Johansen, B.V., 1997. Suspended particulate matter in Norwegian dwellings in relation to fleece and shelf factors, domestic smoking, air exchange rate, and presence of hot wire convection heaters. *Environ. Int.* 23, 465–473.
- Rudel, R.A., Gray, J.M., Engel, C.L., Rawsthorne, T.W., Dodson, R.E., Ackerman, J.M., Rizzo, J., Nudelman, J.L., Brody, J.G., 2011. Food packaging and bisphenol A and bis(2-ethylhexyl) phthalate exposure, findings from a dietary intervention. *Environ. Health Perspect.* 119, 914–920.
- Saijo, Y., Kanazawa, A., Araki, A., Morimoto, K., Nakayama, K., Takigawa, T., Tanaka, M., Shibata, E., Yoshimura, T., Chikara, H., Kishi, R., 2011. Relationships between mite allergen levels, mold concentrations, and sick building syndrome symptoms in newly built dwellings in Japan. *Indoor Air* 21, 253–263.
- Tanaka, M., Inoue, K., Momoi, T., Takano, H., 2013. In vivo immunoamplifying effects of di-(2-ethylhexyl) phthalate on cytokine response. *Immunopharmacol. Immunotoxicol.* 35, 147–150.
- Teitelbaum, S.L., Britton, J.A., Calafat, A.M., Ye, X., Silva, M.J., Reidy, J.A., Galvez, M.P., Brenner, B.L., Wolff, M.S., 2008. Temporal variability in urinary concentrations of phthalate metabolites, phytoestrogens and phenols among minority children in the United States. *Environ. Res.* 106, 257–269.
- U.S. EPA, 2011. Exposure Factors Handbook, 2011 Edition (Final). U.S. Environmental Protection Agency, Washington, DC (EPA/600/R-09/052F).
- Ukawa, S., Araki, A., Kanazawa, A., Yuasa, M., Kishi, R., 2013. The relationship between atopic dermatitis and indoor environmental factors, a cross-sectional study among Japanese elementary school children. *Int. Arch. Occup. Environ. Health* 86, 777–787.
- Wang, I.J., Lin, C.C., Lin, Y.J., Hsieh, W.S., Chen, P.C., 2014. Early life phthalate exposure and atopic disorders in children, a prospective birth cohort study. *Environ. Int.* 62, 48–54.
- Weschler, C.J., Beko, G., Koch, H.M., Salthammer, T., Schripp, T., Toftum, J., Clausen, G., 2015. Transdermal uptake of diethyl phthalate and di(n-butyl) phthalate directly from air: experimental verification. *Environ. Health Perspect.* 123, 928–934.
- Wormuth, M., Scheringer, M., Vollenweider, M., Hungerbühler, K., 2006. What are the sources of exposure to eight frequently used phthalic acid esters in Europeans? *Risk Anal.* 26, 803–824.

Supplementary table 1

Distributions of phthalates in dust (µg/g dust: n=128) and their metabolites in urine (µg/L: n=184).

Urine	LOD	Min	25%	Median	75%	Max	> LOD (%)
MiBP	5.0	< LOD	18.8	44.3	162	6995	96.7
MnBP	5.0	< LOD	< LOD	< LOD	5.25	25	27.5
MBzP	5.0	< LOD	7.36	16.0	31.3	1616	80.8
MEHP	5.0	< LOD	6.74	21.2	60.9	522	82.4
MEOHP	5.0	< LOD	33.1	49.7	87.8	3000	99.5
MECPP	5.0	< LOD	19.6	35.4	54.2	5357	92.3
Σ3DEHP		24.1	77.2	132	204	8840	

Dust	LOD	Floor						Multi-surface					
		Min	25%	Median	75%	Max	> LOD (%)	Min	25%	Median	75%	Max	> LOD (%)
DMP	0.5	<LOD	<LOD	<LOD	<LOD	4.6	5.5	<LOD	<LOD	<LOD	<LOD	4.15	5.47
DEP	0.5	<LOD	<LOD	<LOD	<LOD	58.7	16.4	<LOD	<LOD	<LOD	<LOD	9.76	11.7
DiBP	0.5	<LOD	1.5	3.1	6.1	97.4	93.0	0.6	1.8	2.5	3.6	26.6	100
DnBP	2.0	<LOD	7.5	16.6	32.4	1670	95.3	<LOD	17.2	34.0	75.2	1380	97.7
BBzP	1.0	<LOD	<LOD	2.0	5.4	139	68.0	<LOD	1.76	3.9	10.5	267	85.2
DEHP	1.0	213	786	1110	1740	7090	100	<LOD	1140	2290	4460	44000	99.2
DiNP	2.0	11.9	66.1	139	276	2100	100	18	99.7	203	443	15500	100

LOD: Limit of detection

Supplementary table 2

Distributions of daily intakes of phthalates estimated from urine, dust ingestion and dermal uptake from dust adhered to skin ($\mu\text{g/kg/day}$: n=184).

	Min	25%	Median	75%	Max					
Daily intakes from phthalate metabolites										
DI_DnBP	0.02	0.05	0.07	0.15	0.84					
DI_DiBP	0.05	0.62	1.51	4.86	336.28					
DI_BBzP	0.03	0.23	0.48	0.94	23.13					
DI_DEHP _{MEHP}	0.57	3.17	9.80	27.90	216.14					
DI_DEHP _{MEOHP}	0.45	5.66	8.61	14.71	447.45					
DI_DEHP _{MECPP}	0.20	2.54	3.90	6.55	614.17					
DI_DEHP	0.00	3.84	7.68	15.00	216.45					
Floor dust						Multi-surface dust				
	Min	25%	Median	75%	Max	Min	25%	Median	75%	Max
Daily intakes through dust ingestion										
DI _{dusting} _DiBP	0.00	0.00	0.01	0.01	0.28	0.00	0.00	0.01	0.01	0.07
DI _{dusting} _DnBP	0.00	0.01	0.03	0.07	6.26	0.00	0.03	0.07	0.14	3.32
DI _{dusting} _BBzP	0.00	0.00	0.00	0.01	0.18	0.00	0.00	0.01	0.02	0.76
DI _{dusting} _DEHP	0.32	1.51	2.38	4.02	17.02	0.00	2.46	4.61	8.25	125.60
DI _{dusting} _DiNP	0.02	0.15	0.31	0.64	5.49	0.04	0.19	0.41	0.94	42.41
Daily intakes through absorption from dust adhered to skin										
DI _{duster} _DiBP	4.3E-06	4.4E-05	8.4E-05	1.5E-04	3.3E-03	1.6E-05	4.7E-05	6.8E-05	1.0E-04	8.2E-04
DI _{duster} _DnBP	3.0E-05	2.7E-04	5.3E-04	1.2E-03	7.9E-02	2.8E-05	5.8E-04	1.2E-03	2.5E-03	5.0E-02
DI _{duster} _BBzP	1.3E-04	2.7E-04	1.0E-03	3.0E-03	8.3E-02	5.0E-06	3.0E-05	6.3E-05	1.7E-04	4.7E-03
DI _{duster} _DEHP	4.5E-04	1.8E-03	2.6E-03	4.5E-03	1.6E-02	1.9E-06	2.7E-03	5.0E-03	1.0E-02	1.3E-01
DI _{duster} _DiNP	1.7E-05	1.0E-04	2.1E-04	4.3E-04	3.5E-03	2.7E-05	1.4E-04	2.7E-04	6.0E-04	2.6E-02
Daily intakes through dust										
DI _{dust} _DiBP	0.00	0.00	0.01	0.01	0.28	0.00	0.00	0.01	0.01	0.07
DI _{dust} _DnBP	0.00	0.02	0.03	0.07	6.34	0.00	0.03	0.07	0.14	3.37
DI _{dust} _BBzP	0.00	0.00	0.01	0.01	0.26	0.00	0.00	0.01	0.02	0.76
DI _{dust} _DEHP	0.32	1.51	2.38	4.03	17.04	0.00	2.46	4.62	8.26	125.73
DI _{dust} _DiNP	0.02	0.15	0.31	0.64	5.50	0.04	0.19	0.41	0.94	42.43

DI_DEHP was estimated from the average of the three intakes from the three metabolites (DI_DEHP_{MEHP}, DI_DEHP_{MEOHP}, and DI_DEHP_{MECPP}).

Supplementary table 3.

The associations between parental-reported allergic symptoms and phthalate metabolite in urine (µg/L)

	Wheeze				Rhino-conjunctivitis				Atopic dermatitis			
	OR	95% CI		p value	OR	95% CI		p value	OR	95% CI		p value
MiBP	0.77	0.59	1.00	0.050	0.93	0.75	1.16	0.528	0.83	0.67	1.04	0.106
MnBP	1.03	0.56	1.92	0.917	0.76	0.43	1.35	0.762	0.88	0.50	1.54	0.646
MBzP	1.13	0.81	1.57	0.470	0.95	0.70	1.28	0.727	0.99	0.73	1.34	0.944
MEHP	0.90	0.68	1.20	0.481	0.84	0.65	1.08	0.172	1.01	0.79	1.29	0.952
MEOHP	0.92	0.54	1.57	0.758	1.05	0.66	1.68	0.834	1.16	0.73	1.84	0.540
MECPP	1.01	0.66	1.55	0.958	1.06	0.73	1.54	0.746	1.27	0.88	1.83	0.199
Σ3DEHP	1.10	0.67	1.82	0.698	0.89	0.57	1.39	0.603	1.07	0.69	1.65	0.774

The odds ratios were calculated using Ln transformed variables.

Adjusted for gender, age, parental history of allergies, and creatinine.

Supplementary table 4.

The association between parental-reported allergic symptoms and phthalate in house dust (µg/g dust)

	Wheeze				Rhino-conjunctivitis				Atopic dermatitis			
	OR	95% CI	p value		OR	95% CI	p value		OR	95% CI	p value	
<i>Floor dust</i>												
DiBP	0.98	0.73	1.31	0.890	1.14	0.87	1.49	0.332	1.26	0.96	1.64	0.093
DnBP	1.20	0.88	1.63	0.260	1.25	0.95	1.63	0.110	1.00	0.77	1.30	0.988
BBzP	1.14	0.88	1.48	0.311	1.02	0.80	1.29	0.882	1.19	0.94	1.51	0.155
DEHP	1.41	0.81	2.45	0.231	1.96	1.16	3.29	0.011	1.36	0.83	2.24	0.227
DINP	0.83	0.56	1.24	0.365	0.98	0.69	1.39	0.892	0.87	0.62	1.23	0.438
<i>Multi-surface dust</i>												
DiBP	0.91	0.51	1.63	0.754	1.36	0.80	2.29	0.255	0.78	0.45	1.33	0.355
DnBP	0.99	0.73	1.33	0.922	1.17	0.89	1.53	0.260	0.99	0.76	1.29	0.940
BBzP	1.19	0.91	1.57	0.205	0.95	0.74	1.21	0.659	1.06	0.83	1.34	0.663
DEHP	1.04	0.81	1.33	0.769	0.80	0.62	1.02	0.071	0.89	0.71	1.11	0.285
DINP	0.73	0.53	1.01	0.055	0.90	0.70	1.16	0.414	0.83	0.63	1.09	0.172

The odds ratios were calculated using Ln transformed variables.

Adjusted for gender, age, and parental history of allergies.

Supplementary table 5.

Characteristics comparison between the present home environment survey and base-line questionnaire survey

		Base-line questionnaire survey		Home environment survey	
		(n= 4408)		(n=184)	
		n (%)		n (%)	
Gender					
	Male	2121	(48.1)	101	(54.9)
	Female	2187	(49.6)	83	(45.1)
Age (yr.)					
	Mean ($\pm$ SD)	9.2	(1.8)	9.3	(1.7)
School year					
	1st	708	(16.4)	19	(10.3)
	2nd	726	(16.9)	23	(12.5)
	3rd	738	(17.1)	43	(23.4)
	4th	753	(17.5)	32	(17.4)
	5th	685	(15.9)	32	(17.4)
	6th	698	(16.2)	35	(19)
Parental-reported wheeze					
	Yes	551	(13.2)	39	(21.2)
	No	3610	(86.8)	145	(78.8)
Parental-reported allergic rhino-conjunctivitis					
	Yes	651	(15.5)	56	(30.4)
	No	3538	(84.5)	128	(69.6)
Parental-reported atopic dermatitis					
	Yes	710	(16.7)	54	(29.3)
	No	3545	(83.3)	130	(70.7)
Environmental tobacco smoke					
	Yes	2055	(47.7)	52	(28.3)
	No	2250	(52.3)	132	(71.7)
Parental history of allergies					
	Yes	2762	(64.8)	139	(75.5)
	No	1500	(35.2)	45	(24.5)
Type of house					
	Detached house	1910	(43.5)	87	(47.3)
	Apartment	2463	(54.7)	97	(52.7)
	Others	80	(1.8)	0	(0.0)
Structure of house					
	Wooden house	2248	(51.4)	82	(44.6)
	Reinforced concrete	2083	(47.6)	102	(55.4)
	Others	41	(0.9)		
Age of building (yr.)					
	Mean ($\pm$ SD)	14.1	(10.0)	13.1	(10.2)
Dampness index (1-5)					
	Mean ($\pm$ SD)	1.0	(1.0)	2.2	(1.2)
House income (yen/yr.)					
	< 3 million			8	(4.3)
	3 -< 5 million			36	(19.6)
	5 -< 8 million	ND		74	(40.2)
	>= 8 million			39	(21.2)
	Unknown			27	(14.7)

ND: no data