

Potential nonmonotonous association between di(2-ethylhexyl) phthalate exposure and atopic dermatitis in Korean children

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Summary

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Conflicts of interest

None declared.

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Background Concerns have emerged about the adverse effect of phthalates on human health.

Objectives The aim of this study was to investigate the association between di(2-ethylhexyl) phthalate (DEHP) exposure and atopic dermatitis (AD) in Korean children, focusing on the potential dose–response relationship.

Methods A matched case–control study was conducted from May to October 2012. Subjects from 3 to 6 years of age were recruited from kindergartens and daycare centres in Seoul, Korea. The clinical diagnosis of AD was made by dermatologists. A total of 224 cases and 224 age- and sex-matched controls were included. The levels of two phthalate metabolites [mono(2-ethyl-5-hydrohexyl) phthalate (MEHHP) and mono(2-ethyl-5-oxohexyl) phthalate (MEOHP)] of DEHP in urine samples were measured.

Results The effects of DEHP varied by age, and an increased risk for AD was associated with DEHP at age 3 years (odds ratio 2.51, 95% confidence interval 1.02–6.20). The association was in the opposite direction in the other age groups although there was no statistical significance. The effects of DEHP on AD was observed differently by the level of the body burden. The predicted risk for AD based on the results of multiple logistic regression analysis showed a nonmonotonous association (U-shaped) between the level of DEHP and the risk of AD.

Conclusions This finding might suggest that the effects of DEHP on AD may be different according to the exposure level or age of the subject. Further longitudinal investigations with a suitable design to investigate the nonmonotonous association should be encouraged.

What's already known about this topic?

- Concerns have emerged about the adverse effect of phthalates on human health.
- Recent studies have suggested that exposure to phthalates may increase the risk of eczema.

What does this study add?

- The association between the level of di(2-ethylhexyl) phthalate (DEHP) and atopic dermatitis (AD) was neither linear nor monotonous.
- This finding might suggest that the effect of DEHP on AD may be different according to the exposure level.

Phthalates are primarily used as plasticizers in the manufacture of flexible polyvinyl chloride products such as flooring, medical devices and personal care products such as perfumes and cosmetics.^{1,2} Because of the noncovalent bonding structure, they easily leach out into the environment.³ Among various species of phthalate, di(2-ethylhexyl) phthalate (DEHP) has been the most widely used.⁴

Concerns have emerged about the adverse effects of phthalates on human health.^{5,6} The primary endpoints of interest have been developmental abnormalities,⁷ reproductive health^{8–10} and respiratory health including asthma.¹¹

Recently, the number of investigations of the association between phthalate exposure and allergic diseases in children has increased.¹² Although recent studies have suggested that exposure to phthalates may increase the risk of asthma or eczema,^{13,14} only limited species of phthalates demonstrated the association. This finding may be primarily because of the diversity of phthalate esters or the nonmonotonous dose–response relationship.¹⁵ Generally, a nonmonotonic dose–response relationship is defined as a nonlinear relationship between dose and effect in which the direction of relationship changes somewhere within the range of doses examined.¹⁶ It is quite well known that endocrine hormones or endocrine-disrupting chemicals may demonstrate a nonmonotonous dose–response curve.¹⁷

The aim of this study was to investigate the association between DEHP exposure and atopic dermatitis (AD) in Korean children, focusing on the potential dose–response relationship.

Methods

Subjects and study design

A matched case–control study was conducted from May to October 2012. Subjects were recruited from the ‘Seoul Atopy Free School’ as part of the ‘Atopy Free Seoul Project’.

In 2012, 359 kindergartens including daycare centres participated in the project. Among those, we asked 50 facilities (two for each of the 25 districts, considering the feasibility of performance of the research, including the number of kindergartens, location and accessibility) to participate in the study, and we provided the purpose and expected process of the study. Forty-three facilities had 100 or more children (range 100–222), and seven facilities had fewer than 100 children (range 63–99). The parents or guardians of each child decided whether to participate and, if they did, provided written informed consent. Among 6829 subjects, 4259 subjects participated. Ten dermatologists carried out the examinations of all the participants for the presence of AD. The diagnosis of AD was based on the physical examination of several major and minor criteria proposed by Hanifin and Rajka.¹⁸ Persistent or relapsing symptoms of AD lasting at least 6 months were screened by a questionnaire. To share the diagnostic criteria of AD in this study and to discuss how to standardize the diagnosis, all dermatologists had at least three technical meetings before the survey. There were 416 potential cases of physi-

cian-diagnosed AD. Potential controls were recruited, for a total number of 1000 subjects. All subjects completed questionnaires about demographic characteristics, parental history of allergic diseases, breastfeeding and environmental factors including exposure to second-hand smoke and moving to a new house within the child’s first year of life. Those who did not answer the basic questions including age and sex were excluded ($n = 273$). Of the remaining 727 subjects, 681 children from 3 to 6 years of age were included. There were 224 cases and 457 eligible controls. Among the 457 eligible controls, 224 age- and sex-matched controls were selected randomly. A total of 224 cases and 224 controls were included in the analysis. The study procedure was reviewed and approved by the Ethics Committee of the Seoul Medical Center.

Collection of urine samples and measurement of phthalate metabolites

We asked parents to collect the first morning voided urine sample of the children at home using a polypropylene specimen cup on the morning of collection day and to store the sample in the freezer until submission. The samples were transferred to polystyrene conical tubes between 09:30 and 11:30 at each facility on the designated day and immediately transported on ice to the laboratory. Two metabolites from DEHP [mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) and mono(2-ethyl-5-oxohexyl) phthalate (MEOHP)] were measured in the urine by high-performance liquid chromatography with tandem mass spectrometer (Agilent Triple Quad 6410; Agilent Technologies, Santa Clara, CA, U.S.A.). The analyses were performed using the method reported by the Centers for Disease Control and Prevention.¹⁹ The limit of detection for phthalate metabolites was as follows: $0.116 \mu\text{g L}^{-1}$ for MEHHP and $0.080 \mu\text{g L}^{-1}$ for MEOHP. None of the urine samples had concentrations of phthalate metabolites below the detection limit.

The concentration of creatinine was analysed by kinetic colorimetric assay (Hitachi 7600 II; Hitachi High-Technologies Corporation, Tokyo, Japan). The concentrations of phthalate metabolites were adjusted for creatinine levels and expressed as microgram per gram of creatinine.

Statistical analysis

We performed *t*-tests for the continuous variables and chi-square tests for categorical variables. Considering the parent phthalate, MEHHP and MEOHP were combined into their molar sum (ΣDEHP). The individual metabolite concentrations in microgram per gram of creatinine were divided by the molecular weight of the metabolite and totalled. The conditional logistic regression models were built considering a matched case–control design. We fitted the logistic regression model by the level of ΣDEHP (stratified by quartiles). In each stratum, ΣDEHP was treated as a continuous variable. In the second model, ΣDEHP was treated as a categorical variable

(below the median as reference), after stratification by age. In the multiple logistic regression models, breastfeeding, exposure to second-hand smoke, parental history of allergic diseases and moving to a new house within the child's first year of life were adjusted. A scatter plot was drawn using the predicted probabilities for AD calculated from the multiple logistic regression models to examine the relationship between the predicted risk of AD and the level of DEHP exposure. A line on the scatter plot was drawn using the smoothing spline method, and P-values ≤ 0.05 were considered statistically significant. All analyses were performed using SAS 9.2 (SAS Institute, Cary, NC, U.S.A.).

Results

The demographic characteristics of the subjects are presented in Table 1. Among 224 pairs of cases and controls, there were 40 three-year-old pairs (17.9%), 48 four-year-old pairs (21.4%), 71 five-year-old pairs (31.7%) and 65 six-year-old pairs (29.0%). There were 123 pairs (54.9%) of boys and 101 pairs (45.1%) of girls. A total of 87 case subjects (38.8%) and 66 control subjects (29.5%) reported that at least one parent had a history of allergic diseases. Other variables such as breastfeeding, exposure to second-hand smoking and moving to a new house within the child's first year of life were not significantly different between cases and controls.

The geometric mean concentrations and distributions of MEHHP, MEOHP and Σ DEHP are presented in Table 2. The creatinine-adjusted concentrations were higher than the unad-

justed concentrations. The concentrations of phthalate metabolites decreased with increasing age. The mean concentrations of phthalate metabolites were statistically significantly higher in the controls than in the cases. The differences varied by age and AD status (Fig. 1). In the cases, the concentration of Σ DEHP was inversely associated with age (P-for-trend < 0.01). The concentration of Σ DEHP was not associated with age in controls (P-for-trend 0.99).

The odds ratios (ORs) of phthalate for AD were different by the level of Σ DEHP, although there was no statistical significance (Table 3). The unadjusted ORs for AD were as follows: first quartile, OR 0.60, 95% confidence interval (CI) 0.35–1.03; second quartile, OR 0.56, 95% CI 0.24–1.29; third quartile, OR 0.89, 95% CI 0.52–1.54; fourth quartile, OR 1.04, 95% CI 0.96–1.14. After adjustment for breastfeeding, exposure to second-hand smoke, parental history of allergic disease and moving to a new house within the child's first year of life, the adjusted ORs were similar to the unadjusted ORs.

The predicted risk for AD based on the results of multiple logistic regression analysis is presented in Figure 2. A curve derived from the smoothing spline method was approximately U-shaped.

The association between AD and Σ DEHP differed by age (Table 4). The concentrations of Σ DEHP above the median value were associated with an increased risk of case status at age 3 years (OR 2.45, 95% CI 1.01–5.98). The association was in the opposite direction in the other age groups (age 4 years: OR 0.51, 95% CI 0.23–1.15; age 5 years: OR 0.67, 95% CI 0.34–1.30; age 6 years: OR 0.50, 95% CI 0.25–1.01). The adjusted ORs were similar to the unadjusted ORs, but AD was no longer statistically significantly associated with the concentration of Σ DEHP.

Discussion

In this study, we investigated the relationship between the body burden of phthalate (DEHP) and AD in children between the ages of 3 and 6 years. The effect of DEHP on AD was observed differently by the level of the body burden. There was a nonmonotonous association between the level of DEHP and the risk of AD. The effect of DEHP was different by age, and an increased risk for AD was associated with DEHP at 3 years of age.

The levels of urinary phthalate metabolites in this study appear to be different from previous studies. In this study, the geometric mean for MEHHP was $67.0 \mu\text{g L}^{-1}$, and the geometric mean for MEOHP was $75.5 \mu\text{g L}^{-1}$. These values are in agreement with a recent Korean study.²⁰ German children (3–5 years) showed lower levels of DEHP metabolites (geometric mean of $51.1 \mu\text{g L}^{-1}$ for MEHHP and $38.3 \mu\text{g L}^{-1}$ for MEOHP).²¹ In a Norwegian study of 623 children aged 8.8–12.5 years, the geometric mean was $76.8 \mu\text{g L}^{-1}$ for MEHHP and $49.8 \mu\text{g L}^{-1}$ for MEOHP.¹³ This inconsistency may be because of the difference in age or ethnicity. This study was not a population-based study, but a case-control study. Fur-

Table 1 General characteristics of the cases and controls

Variables	Cases (n = 224), n (%)	Controls (n = 224), n (%)	P-value
Age (years)			
3	40 (17.9)	40 (17.9)	
4	48 (21.4)	48 (21.4)	
5	71 (31.7)	71 (31.7)	
6	65 (29.0)	65 (29.0)	
Sex			
Boys	123 (54.9)	123 (54.9)	
Girls	101 (45.1)	101 (45.1)	
Breastfeeding			
Yes	176 (78.6)	173 (77.2)	0.90
No	10 (4.5)	12 (5.4)	
Missing	38 (17.0)	39 (17.4)	
Exposure to second-hand smoking			
Yes	35 (15.6)	38 (17.0)	0.33
No	189 (84.4)	184 (82.1)	
Missing	0 (0.0)	2 (0.9)	
Parental history of allergic diseases			
Either parent	87 (38.8)	66 (29.5)	0.11
None	101 (45.1)	118 (52.7)	
Missing	36 (16.1)	40 (17.9)	
Moving to a new house within the child's first year of life			
Yes	37 (16.5)	31 (13.8)	0.43
No	187 (83.5)	193 (86.2)	

Table 2 Geometric means and distribution of phthalates

Phthalate	Group	N	GM	Unit ^a	Percentile			P-value
					25th	50th	75th	
MEHHP	Total	448	67.0	$\mu\text{g L}^{-1}$	41.4	66.8	109.0	0.02^c
	Age ^b	3	81.7	$\mu\text{g g}^{-1} \text{ cr}$	58.0	82.2	117.8	
			89.0	$\mu\text{g g}^{-1} \text{ cr}$	61.7	86.6	132.6	
			85.8	$\mu\text{g g}^{-1} \text{ cr}$	57.1	84.4	129.6	
			81.1	$\mu\text{g g}^{-1} \text{ cr}$	58.9	85.0	117.7	
	Case	6	75.2	$\mu\text{g g}^{-1} \text{ cr}$	53.0	77.0	105.9	
			75.9	$\mu\text{g g}^{-1} \text{ cr}$	51.5	74.8	109.1	
			87.8	$\mu\text{g g}^{-1} \text{ cr}$	61.9	87.2	126.4	
			87.8	$\mu\text{g g}^{-1} \text{ cr}$	61.9	87.2	126.4	
	Control	224	87.8	$\mu\text{g g}^{-1} \text{ cr}$	61.9	87.2	126.4	
MEOHP	Total	448	75.5	$\mu\text{g L}^{-1}$	47.7	76.1	121.5	0.04
	Age	3	92.0	$\mu\text{g g}^{-1} \text{ cr}$	64.7	89.1	128.2	
			104.3	$\mu\text{g g}^{-1} \text{ cr}$	71.5	99.5	145.5	
			98.5	$\mu\text{g g}^{-1} \text{ cr}$	67.4	92.7	142.2	
			89.6	$\mu\text{g g}^{-1} \text{ cr}$	64.7	91.9	128.1	
	Case	6	83.4	$\mu\text{g g}^{-1} \text{ cr}$	61.2	82.9	112.4	
			87.1	$\mu\text{g g}^{-1} \text{ cr}$	61.8	87.1	121.1	
			97.2	$\mu\text{g g}^{-1} \text{ cr}$	70.5	93.3	134.2	
			97.2	$\mu\text{g g}^{-1} \text{ cr}$	70.5	93.3	134.2	
	Control	224	97.2	$\mu\text{g g}^{-1} \text{ cr}$	70.5	93.3	134.2	
ΣDEHP	Total	448	0.49	$\mu\text{g L}^{-1}$	0.30	0.49	0.78	0.01
	Age	3	0.59	$\mu\text{g g}^{-1} \text{ cr}$	0.42	0.58	0.84	
			0.66	$\mu\text{g g}^{-1} \text{ cr}$	0.45	0.63	0.95	
			0.63	$\mu\text{g g}^{-1} \text{ cr}$	0.42	0.61	0.92	
			0.58	$\mu\text{g g}^{-1} \text{ cr}$	0.41	0.60	0.84	
	Case	6	0.54	$\mu\text{g g}^{-1} \text{ cr}$	0.40	0.55	0.72	
			0.56	$\mu\text{g g}^{-1} \text{ cr}$	0.38	0.55	0.79	
			0.63	$\mu\text{g g}^{-1} \text{ cr}$	0.46	0.62	0.89	
			0.63	$\mu\text{g g}^{-1} \text{ cr}$	0.46	0.62	0.89	
	Control	224	0.63	$\mu\text{g g}^{-1} \text{ cr}$	0.46	0.62	0.89	

DEHP, di(2-ethylhexyl) phthalate; GM, geometric mean; MEHHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono(2-ethyl-5-oxohexyl) phthalate; ΣDEHP , molar sum of MEHHP and MEOHP, which are metabolites of DEHP ($\mu\text{mol g}^{-1} \text{ cr}$). Bold indicates significance.

^aUnits: $\mu\text{g L}^{-1}$ for unadjusted concentrations; $\mu\text{g g}^{-1} \text{ cr}$ for creatinine-adjusted concentrations. ^bAge in years. ^cP-value for trend test.

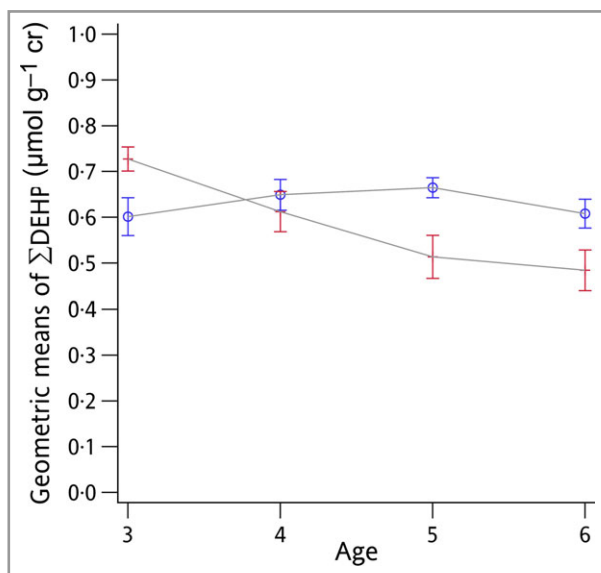


Fig 1. Geometric means of ΣDEHP by age. In the cases, the concentration of ΣDEHP decreased with age (red crosses, P -for-trend < 0.01). In contrast, the concentration of ΣDEHP was not associated with age in the controls (blue circles, P -for-trend 0.99). DEHP, di(2-ethylhexyl) phthalate.

ther investigations with additional comparability are needed to investigate the factors affecting the difference in the distribution of DEHP metabolites in children.

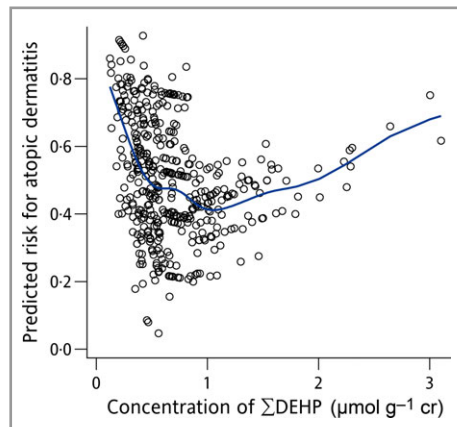
A number of studies have shown the relationship between phthalates and allergic diseases in children. In allergic diseases, the primary outcome associated with DEHP is typically asthma.^{13,22} An association between eczema and n-butylbenzyl phthalate (BBzP) was reported in Swedish children.²³ In that study, the OR for eczema was 2.26 (95% CI 1.24–5.32) for the highest BBzP quartile compared with the lowest quartile. The OR for eczema increased with quartiles for DEHP, although there was no statistical significance in each quartile compared with the lowest quartile. In this study, the OR for AD was 2.45 (95% CI 1.01–5.98) for the above median value of ΣDEHP compared with the below median value. Although the adjusted OR was similar to the unadjusted OR (aOR 2.03), there was no statistical significance (95% CI 0.78–5.30; $P = 0.14$).

The underlying mechanism of the effect of phthalate on atopic disorders has not been fully understood. It has been suggested that DEHP may have an adjuvant effect on the immunological response.²⁴ These findings were reported in experimental animal studies^{25,26} as well as in *in vitro* studies.^{27,28} An alternation of inflammatory reactions including

Table 3 Unadjusted and adjusted ORs for atopic dermatitis by the level of Σ DEHP using age- and sex-matched conditional logistic regression analysis (per 0.1 $\mu\text{mol g}^{-1}$ cr increase in Σ DEHP)

Σ DEHP	Case	Control	Unadjusted OR (95% CI)	Adjusted OR ^a (95% CI)
1st Q (≤ 0.42)	67	46	0.60 (0.35–1.03)	0.59 (0.33–1.05)
2nd Q (> 0.42 –0.58)	54	58	0.56 (0.24–1.29)	0.57 (0.23–1.37)
3rd Q (> 0.58 –0.84)	55	57	0.89 (0.52–1.54)	0.97 (0.55–1.71)
4th Q (> 0.84)	48	63	1.04 (0.96–1.14)	1.05 (0.96–1.15)

DEHP, di(2-ethylhexyl) phthalate; OR, odds ratio; CI, confidence intervals; Q, quartile. ^aAdjustment for breastfeeding, exposure to second-hand smoking, parental history of allergic disease and moved to a new house within the child's first year of life.

**Fig 2.** Circles are the predicted risk for atopic dermatitis based on the results of logistic regression analysis. The solid line was drawn by the smoothing spline method.

inactivation of the peroxisome proliferator-activated receptor- γ (PPAR- γ) or enhancement of dendritic cell differentiation may increase susceptibility to atopic disorders.^{27,29}

The association between the level of DEHP and AD was neither linear nor monotonous. The predicted risk for AD based on the results of the multiple logistic regression analysis showed a nonmonotonous dose-response curve that was approximately U-shaped (Fig. 2). A nonmonotonous dose-response relationship has been commonly found in research in the field of endocrine and endocrine-disrupting chemicals.^{16,17,30} There are reports about the nonmonotonic effects

of DEHP such as the effects on bacterial growth in a cell culture experiment³¹ and induced allergic reaction in an animal study.²⁶ A number of supporting mechanisms for the nonmonotonous dose-response relationship such as cytotoxicity, specific receptors and cofactors, receptor selectivity, receptor downregulation, receptor competition and endocrine feedback loops have been identified.¹⁶ Although it is difficult to specify the mechanism in this study, it is remarkable that there may be the possibility of a nonmonotonous relationship between DEHP exposure and AD in children. Further investigations are needed to clarify the underlying mechanisms.

In this study, an increased risk for AD was associated with DEHP in the age 3 years pairs. The association was in the opposite direction in the other age groups, although there was no statistical significance. These findings appear to be plausible with the possibility of the nonmonotonous relationship described above. The children of age 3 years pairs were the highest exposure group in this study. The mean concentrations of DEHP metabolites decreased with age. This is consistent with other previous reports.²¹ The risk for AD was increased with the level of DEHP in the relatively high-dose exposure group. So it makes sense that the odds for above median concentration of DEHP were greater than the odds for below median concentration in the age 3 years group. Although it is difficult to verify this possibility because the distributions were overlapped with each other and there is a lack of knowledge about the effects of age on phthalate metabolism and AD, it is noteworthy that younger children may be at greater risk for AD due to DEHP exposure.

Table 4 Unadjusted and adjusted odds ratios for atopic dermatitis by the level of Σ DEHP

Age	Concentration of Σ DEHP	Cases (n)	Controls (n)	Unadjusted OR (95% CI)	Adjusted OR ^a (95% CI)
3	$\leq$ Median ^b	12	21	1.00	1.00
	$>$ Median	28	19	2.45 (1.01–5.98)	2.03 (0.78–5.30)
4	$\leq$ Median	26	15	1.00	1.00
	$>$ Median	22	33	0.51 (0.23–1.15)	0.78 (0.31–1.98)
5	$\leq$ Median	37	25	1.00	1.00
	$>$ Median	34	46	0.67 (0.34–1.30)	0.73 (0.36–1.48)
6	$\leq$ Median	38	27	1.00	1.00
	$>$ Median	27	38	0.50 (0.25–1.01)	0.50 (0.24–1.03)

DEHP, di(2-ethylhexyl) phthalate; OR, odds ratio; CI, confidence intervals. Bold indicates significance. ^aAdjustment for breastfeeding, exposure to second-hand smoking, parental history of allergic disease and moved to a new house within the child's first year of life. ^bMedian concentration of Σ DEHP = 0.58 $\mu\text{mol g}^{-1}$ cr.

There is a difference in distribution of phthalate metabolites by case status. In this study, the mean DEHP metabolite concentrations decreased with age in the cases, but not in the controls. Age independency in the levels of DEHP metabolites was also observed in 233 unselected controls (data not shown). It is difficult to explain these findings clearly, but the interval of age may be one of the possible explanations. In the above-cited German study about measurement of phthalate metabolites in children, the authors reported that secondary metabolites of DEHP decreased with age, within a range of ages from 3 to 14 years.²¹ Within a narrow range, the mean concentrations of DEHP metabolites were higher in the 6–8-year-old children than in the 3–5-year-old children (geometric mean for MEHHP, $51.1 \mu\text{g L}^{-1}$ in 3–5-year olds and $54.3 \mu\text{g L}^{-1}$ in 6–8-year olds; geometric mean for MEOHP, $38.3 \mu\text{g L}^{-1}$ in 3–5-year olds and $42.3 \mu\text{g L}^{-1}$ in 6–8-year olds). Although this finding may explain, at least in part, the distribution of DEHP metabolites in controls, an additional explanation of the decrease of DEHP metabolites with age in the cases is needed.

There are a number of reports about the subtypes of AD that are extrinsic and intrinsic types according to immunologically distinctive features.^{32,33} Generally, in contrast to extrinsic atopic dermatitis (EAD), intrinsic atopic dermatitis (IAD) demonstrates nonallergic features such as normal serum total IgE, undetectable specific IgE antibodies and a negative skin prick test.³⁴ In a Korean study with infants (age 0–2 years), IAD was more prevalent (57%) than EAD.³⁵ It has been reported that IAD changed to EAD with increasing age.^{35,36} It may be postulated that DEHP affects AD with nonallergic mechanisms in children with IAD. In such cases, a relatively high level of DEHP may be required. If IAD decreases and EAD increases with age, it may indicate that there will be cases of relatively low body burden of DEHP in older children because it requires a relatively low dose with allergic or immunological mechanisms. It may be hypothesized that the effect of DEHP on AD may vary with age (or the subtype of AD) and the level of DEHP. We could not assess the subtypes of clinical AD in this study. More detailed experimental evidence about the mechanism of action of DEHP in relation to human immune responses and DEHP levels is needed to verify this hypothesis.

This study has several strengths. Because of the matched case–control study design, improved comparability could be expected. The cases were defined by dermatologist-diagnosed AD. AD is diagnosed by physical examination, and a dermatologist examined the eligible subjects to diagnose AD in the study cases. We measured urinary concentrations of oxidative metabolites (MEHHP and MEOHP) from DEHP to determine quantitatively the level of internal exposure. Generally, the urinary levels of MEHHP and MEOHP were a number of times higher than the levels of the hydrolytic metabolite [mono(2-ethylhexyl) phthalate].³⁷ The oxidized phthalate metabolites have longer half-lives than the monoesters and are resistant to contamination.¹³

There are several limitations in this study. If a subject had AD with temporary improvement at the time of examination,

he/she might have been ignored as a case. AD is a chronic condition which is defined as persisting for a minimum of 6 months. Although the condition may vary in intensity, the typical signs of AD can be detected by dermatologists. The possibility of misclassification does not appear to be a critical weakness. Although spot urine samples have comparability with corresponding 24-h urine samples for concentration testing,³⁸ considering the variability of phthalate exposure and the short biological half-life of phthalates,³⁹ a single urine analysis may be insufficient to measure the exposure over a relatively long time.⁴⁰ It is difficult to determine the relationship between the DEHP level and AD at extremely low exposures such as those below the detection limit, and more sensitive methods to detect low-dose exposure are needed.

The possibility of reverse causation is an additional concern. Parents of children with AD may avoid or reduce the use of phthalate-containing products. Considering that AD is a chronic condition, avoidance behaviour is expected to be observed to a greater extent as children grow older. Information about parental effort to avoid or reduce daily exposure to phthalate products by the children in the study may be beneficial to evaluate this hypothesis. We could not gather relevant information and cannot confirm or eliminate the possibility of reverse causation. Regardless of the presence of reverse causation, an increased risk of DEHP was observed, at least in a specific subgroup. The facilities in the study were selected for convenience, and not randomly. Although the surveys and investigations did not precede the selection of facilities, the lack of randomness may limit the generalizability. Although generalizability depends on how the underlying biological mechanism of the relationship between the exposure and outcome is similar among the populations of interest,⁴¹ further investigations should be encouraged.

In this study, there was a nonmonotonous dose–response relationship between DEHP exposure and AD. An increased risk for AD was positively associated with DEHP in the 3-year-old group. This finding might suggest that the effect of DEHP on AD may be different according to the exposure level or age of the subject. Further longitudinal investigations with a suitable design to investigate the nonmonotonous association should be encouraged.

References

- 1 Agency for Toxic Substances and Disease Registry. *Toxicological Profile for di-n-butyl phthalate (DBP)*. Atlanta, GA: ATSDR, 2001.
- 2 Agency for Toxic Substances and Disease Registry. *Toxicological Profile for di(2-ethylhexyl)phthalate (DEHP)*. Atlanta, GA: ATSDR, 2002.
- 3 Bornehag CG, Lundgren B, Weschler CJ *et al.* Phthalates in indoor dust and their association with building characteristics. *Environ Health Perspect* 2005; **113**:1399–404.
- 4 Cadogan DF, Howick CJ. Plasticizers. In: Kirk-Othmer *Encyclopedia of Chemical Technology* (Kroschwitz JI, Howe-Grant M, eds), 4th edn, Vol. 19. New York: John Wiley and Sons, 1996; 258–90.
- 5 Hauser R, Calafat AM. Phthalates and human health. *Occup Environ Med* 2005; **62**:806–18.

- 6 Duderstadt KG. Chemicals in daily life: emerging evidence on the impact on child health. *J Pediatr Health Care* 2012; **26**:155–7.
- 7 Gray LE Jr, Ostby J, Furr J *et al.* Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. *Toxicol Sci* 2000; **58**:350–65.
- 8 Duty SM, Silva MJ, Barr DB *et al.* Phthalate exposure and human semen parameters. *Epidemiology* 2003; **14**:269–77.
- 9 Cobellis L, Latini G, De Felice C *et al.* High plasma concentrations of di-(2-ethylhexyl)-phthalate in women with endometriosis. *Hum Reprod* 2003; **18**:1512–15.
- 10 Jurewicz J, Hanke W. Exposure to phthalates: reproductive outcome and children health. A review of epidemiological studies. *Int J Occup Med Environ Health* 2011; **24**:115–41.
- 11 Tsai MJ, Kuo PL, Ko YC. The association between phthalate exposure and asthma. *Kaohsiung J Med Sci* 2012; **28**:S28–36.
- 12 Jaakkola JJ, Knight TL. The role of exposure to phthalates from polyvinyl chloride products in the development of asthma and allergies: a systematic review and meta-analysis. *Environ Health Perspect* 2008; **116**:845–53.
- 13 Bertelsen RJ, Carlsen KC, Calafat AM *et al.* Urinary biomarkers for phthalates associated with asthma in Norwegian children. *Environ Health Perspect* 2013; **121**:251–6.
- 14 Hsu NY, Lee CC, Wang JY *et al.* Predicted risk of childhood allergy, asthma, and reported symptoms using measured phthalate exposure in dust and urine. *Indoor Air* 2012; **22**:186–99.
- 15 Andrade AJ, Grande SW, Talsness CE *et al.* A dose–response study following in utero and lactational exposure to di-(2-ethylhexyl)-phthalate (DEHP): non-monotonic dose–response and low dose effects on rat brain aromatase activity. *Toxicology* 2006; **227**:185–92.
- 16 Vandenberg LN, Colborn T, Hayes TB *et al.* Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. *Endocr Rev* 2012; **33**:378–455.
- 17 Cook R, Calabrese EJ. The importance of hormesis to public health. *Environ Health Perspect* 2006; **114**:1631–5.
- 18 Hanifin J, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol Suppl (Stockh)* 1980; **92**:44–7.
- 19 Centers for Disease Control and Prevention. *Phthalate Metabolites in Urine*. Atlanta, GA: CDC, 2010.
- 20 Song NR, On JW, Lee J *et al.* Biomonitoring of urinary di(2-ethylhexyl) phthalate metabolites of mother and child pairs in South Korea. *Environ Int* 2013; **54**:65–73.
- 21 Becker K, Goen T, Seiwert M *et al.* GerES IV: phthalate metabolites and bisphenol A in urine of German children. *Int J Hyg Environ Health* 2009; **212**:685–92.
- 22 Kolarik B, Naydenov K, Larsson M *et al.* The association between phthalates in dust and allergic diseases among Bulgarian children. *Environ Health Perspect* 2008; **116**:98–103.
- 23 Bornehag CG, Sundell J, Weschler CJ *et al.* The association between asthma and allergic symptoms in children and phthalates in house dust: a nested case–control study. *Environ Health Perspect* 2004; **112**:1393–7.
- 24 Larsen ST, Lund RM, Nielsen GD *et al.* Adjuvant effect of di-n-butyl-, di-n-octyl-, di-iso-nonyl- and di-iso-decyl phthalate in a subcutaneous injection model using BALB/c mice. *Pharmacol Toxicol* 2002; **91**:264–72.
- 25 Yanagisawa R, Takano H, Inoue K *et al.* Effects of maternal exposure to di-(2-ethylhexyl) phthalate during fetal and/or neonatal periods on atopic dermatitis in male offspring. *Environ Health Perspect* 2008; **116**:1136–41.
- 26 Takano H, Yanagisawa R, Inoue K *et al.* Di-(2-ethylhexyl) phthalate enhances atopic dermatitis-like skin lesions in mice. *Environ Health Perspect* 2006; **114**:1266–9.
- 27 Koike E, Inoue K, Yanagisawa R *et al.* Di-(2-ethylhexyl) phthalate affects immune cells from atopic prone mice in vitro. *Toxicology* 2009; **259**:54–60.
- 28 Lee J, Oh PS, Lim KT. Allergy-related cytokines (IL-4 and TNF- α) are induced by di(2-ethylhexyl) phthalate and attenuated by plant-originated glycoprotein (75 kDa) in HMC-1 cells. *Environ Toxicol* 2011; **26**:364–72.
- 29 Vetrano AM, Laskin DL, Archer F *et al.* Inflammatory effects of phthalates in neonatal neutrophils. *Pediatr Res* 2010; **68**:134–9.
- 30 Vandenberg LN, Maffini MV, Sonnenschein C *et al.* Bisphenol-A and the great divide: a review of controversies in the field of endocrine disruption. *Endocr Rev* 2009; **30**:75–95.
- 31 Sandy EH, Yao J, Zheng S *et al.* A comparative cytotoxicity study of isomeric alkylphthalates to metabolically variant bacteria. *J Hazard Mater* 2010; **182**:631–9.
- 32 Schmid-Grendelmeier P, Simon D, Simon HU *et al.* Epidemiology, clinical features, and immunology of the ‘intrinsic’ (non-IgE-mediated) type of atopic dermatitis (constitutional dermatitis). *Allergy* 2001; **56**:841–9.
- 33 Bos JD, Brenninkmeijer EE, Schram ME *et al.* Atopic eczema or atopiform dermatitis. *Exp Dermatol* 2010; **19**:325–31.
- 34 Akdis CA, Akdis M, Simon D *et al.* T cells and T cell-derived cytokines as pathogenic factors in the nonallergic form of atopic dermatitis. *J Invest Dermatol* 1999; **113**:628–34.
- 35 Park JH, Choi YL, Namkung JH *et al.* Characteristics of extrinsic vs. intrinsic atopic dermatitis in infancy: correlations with laboratory variables. *Br J Dermatol* 2006; **155**:778–83.
- 36 Novembre E, Cianferoni A, Lombardi E *et al.* Natural history of ‘intrinsic’ atopic dermatitis. *Allergy* 2001; **56**:452–3.
- 37 Kato K, Silva MJ, Reidy JA *et al.* Mono(2-ethyl-5-hydroxyhexyl) phthalate and mono-(2-ethyl-5-oxohexyl) phthalate as biomarkers for human exposure assessment to di-(2-ethylhexyl) phthalate. *Environ Health Perspect* 2004; **112**:327–30.
- 38 Christensen KL, Lorber M, Koch HM *et al.* Population variability of phthalate metabolites and bisphenol A concentrations in spot urine samples versus 24- or 48-h collections. *J Expo Sci Environ Epidemiol* 2012; **22**:632–40.
- 39 Frederiksen H, Kranich SK, Jorgensen N *et al.* Temporal variability in urinary phthalate metabolite excretion based on spot, morning, and 24-h urine samples: considerations for epidemiological studies. *Environ Sci Technol* 2013; **47**:958–67.
- 40 Preau JL Jr, Wong LY, Silva MJ *et al.* Variability over 1 week in the urinary concentrations of metabolites of diethyl phthalate and di(2-ethylhexyl) phthalate among eight adults: an observational study. *Environ Health Perspect* 2010; **118**:1748–54.
- 41 Hu F. *Obesity Epidemiology*. New York: Oxford University Press, 2008.