Effects of prenatal di(2-ethylhexyl) phthalate exposure on childhood allergies and infectious diseases: the Hokkaido Study on Environment and Children's Health

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Abstract

Phthalates are widely used in consumer products, and experimental studies suggest that exposure to phthalates increase the risk of allergies. However, epidemiologic evidence on the associations between prenatal phthalate exposure and allergies/infectious diseases and cord blood immunoglobulin E (IgE) levels is limited. This study aimed to evaluate the associations between maternal mono(2-ethylhexyl) phthalate (MEHP) levels and cord blood IgE levels at birth (n = 127), as well as the risk of allergies/infectious diseases in participants up to 7 years of age; the participants were 1.5 (n = 248), 3.5 (n = 222), 7 (n = 184) years of age. Maternal blood MEHP level in the second trimester was measured using gas chromatography-mass spectrometry. Participant characteristics were obtained from the medical birth records and self-administered questionnaires during pregnancy and after delivery. Wheeze and eczema were defined according to the Japanese version of the International Study of Asthma and Allergies in Childhood and the American Thoracic Society-Division of Lung Diseases questionnaire, respectively. Infectious diseases were defined using questionnaires for each specified age. To evaluate the associations between maternal MEHP and allergies or infectious diseases, we used logistic regression analysis and generalized estimating equations analysis. Maternal MEHP levels were negatively associated with cord blood IgE levels and increased risks of allergies and infectious diseases up to 7 years of age. This is the first study to investigate the effects of prenatal MEHP exposure on IgE levels at birth and allergies/infectious diseases up to 7 years of age. Further epidemiological studies should focus on other phthalate metabolites and their health effects on larger populations.

Running title

Maternal DEHP and childhood allergies and infectious diseases

Keywords

di(2-ethylhexyl) phthalate, allergies, infectious diseases, immunoglobulin E, prenatal exposure, children

Highlights

- Effects of prenatal DEHP exposure on IgE levels in cord blood were investigated.
- Prenatal DEHP exposure and allergy/infection up to 7 years of age were investigated.
- Cord blood IgE levels were negatively associated with maternal MEHP levels.
- High maternal MEHP increased the risk of wheeze, otitis media, and chicken pox.
- Prenatal DEHP exposure may promote the risk of allergy/infection during childhood.

Abbreviations: CIs, confidence intervals; DEHP, di(2-ethylhexyl) phthalate; IgE, immunoglobulin E; ETS, environmental tobacco smoke; GEE, generalized estimating equations; IQR, interquartile range; ISAAC, the International Study of Asthma and Allergies in Childhood; MBzP, mono-benzyl phthalate; MECPP, mono(2-ethyl-5-carboxypentyl) phthalate; MEHP, mono(2-ethylhexyl) phthalate; MEOHP, mono(2-ethyl-5-oxohexyl) phthalate; NOAEL, no-observed-adverse-effect level; OR, odd ratios; RR, relative risk; RSV, respiratory syncytial virus

1. Introduction

Phthalates are widely used in consumer products, such as building materials, toys, food packaging, cosmetics, and personal care products. Because of concerning adverse health effects of di(2-ethylhexyl) phthalate (DEHP), it is regulated for use in toys and food packaging and its consumption and production in Japan have decreased over the past 10 years (Bizzari, 2013). However, DEHP is still the most widely used phthalate, mainly as a plasticizer in polyvinyl chloride.

Since phthalates are ubiquitous in the environment, humans are exposed to phthalates from the point of conception. Phthalates are detected in environments such as air, atmospheric aerosols (Kanazawa et al., 2010), house dust (Ait Bamai et al., 2014), drinking water, and soil (Tang et al., 2017), and human specimens, such as urine, blood, and the placenta (Frederiksen et al., 2010; Hines et al., 2009; Hogberg et al., 2008; Li et al., 2013). Based on evidence from animal and in vitro studies, DEHP can cause immunotoxicity, immune suppression, allergic responses, and adjuvant effects (Bornehag and Nanberg, 2010; Han et al., 2014; Robinson and Miller, 2015). Positive dose-response relationships between allergen-combined DEHP and serum total immunoglobulin E (IgE), interleukin (IL)-4, interferon-γ (IFN-γ), and eosinophil levels have been reported in mice (Guo et al., 2012; Larsen et al., 2001a; Larsen et al., 2001b). One animal study reported that maternal exposure to DEHP during pregnancy in mice reduces the levels of IgE and ovalbumin (OVA)-specific IgE in the serum of their offspring and decreases the levels of IL-4, IL-13, and

eotaxin in the bronchoalveolar lavage fluid (BALF) of the offspring. This lead to the suppression of Th2 immune responses in the offspring (Shin et al., 2014). However, there have been few animal studies investigating the correlation between maternal exposure to DEHP during pregnancy and immunotoxicity and/or allergic responses in offspring. Moreover, extrapolation from animal studies to humans has limitations because humans are exposed to low chemical levels but are constantly compared with experimental studies conducted in animals.

Recent cross-sectional studies have relatively consistent results, in which high levels of urinary DEHP metabolites in children increased the risk of asthma or wheeze (Beko et al., 2015; Bertelsen et al., 2013; Ku et al., 2015), and high levels of urinary mono-benzyl phthalate (MBzP) in children were associated with an increased risk of atopic dermatitis in those aged 2 years (Wang et al., 2014). In regard to prenatal exposure to phthalates, several prospective studies reported that increased maternal MBzP levels were associated with early-onset eczema, diagnosed asthma, and wheezing among children (Just et al., 2012; Ku et al., 2015; Whyatt et al., 2014). Gascon et al. (2014) reported that prenatal exposure to DEHP and BBzP increases the risk of asthma symptoms and respiratory tract infections throughout childhood (Gascon et al., 2014). However, epidemiological findings regarding the effects of prenatal exposure to DEHP on infectious diseases and immune function are still limited and unclear. To our knowledge, there are two pieces of epidemiological evidence regarding the associations between prenatal phthalate exposure and immune system function among

children: one is from the Canadian birth cohort study (Ashley-Martin et al., 2015), and the other is from a Taiwanese birth cohort (Wang et al., 2014). Inverse and non-linear associations have been reported between maternal urinary mono-carboxypropyl phthalate levels and cord blood total IgE levels (Ashley-Martin et al., 2015). In contrast, it has been reported that levels of maternal urinary phthalate metabolites mono-ethyl phthalate, mono-butyl phthalate, MBzP, and mono(2-ethylhexyl) phthalate [MEHP]) were not associated with either cord blood total IgE or serum total IgE levels in children aged 2 years (Wang et al., 2014). The associations between maternal phthalates levels and cord blood IgE are still not clear.

This study aimed to evaluate the association between maternal MEHP levels and the risk of allergies and infectious diseases throughout childhood until 7 years of age. A secondary aim was to evaluate the association between maternal MEHP levels and cord blood total IgE levels at birth.

2. Materials and methods

2.1 Study population

This prospective study was based on the Sapporo cohort of the Hokkaido Study on Environment and Children's Health (Kishi et al., 2013; Kishi et al., 2011). Briefly, between July 2002 and October 2005, we recruited pregnant Japanese women at 23–35 weeks gestation from Sapporo Toho Hospital in Hokkaido, Japan. Of the 1796 women asked to participate in this study in

their second or third trimester during regular antenatal visits, 514 agreed (28.6%). The baseline questionnaire included information on tobacco smoking history, alcohol consumption, caffeine intake, family income, and educational levels of the participants and their partners. Among the 514 women, 10 were excluded because they experienced miscarriage or stillbirth, because they relocated, or because they voluntarily withdrew from the study prior to the birth. Medical records, which contained information on infant birth weight, height, and gestational age, were obtained from 504 participants (98.1%). Four hundred and ninety-three maternal blood samples for MEHP measurement were collected from each pregnant woman after the second trimester or after delivery, while 268 umbilical cord blood samples were collected at the time of delivery. In the follow-up, among the 504 participants, 389 completed the self-administered questionnaire at 1.5 years post-delivery (76.3%), 345 participants at 3.5 years (67.6%), and 280 participants at 7 years (54.9%). In this study, because of the relatively short biological half-life of DEHP, 179 maternal blood samples collected after delivery were excluded; thus, 314 maternal blood samples for MEHP measurement were included in the study. Among the 314 participants, 258 had available data on both maternal MEHP levels and allergies and infectious diseases for at least one of 1.5, 3.5, or 7 years of age. Of these, 248 participants had data available for when they were 1.5 years old. Similarly, 222 participants had data available for when they were 3.5 years old and 184 participants had data available for when they were 7 years old. In addition, 127 participants had available data on

umbilical cord blood IgE levels.

This study was conducted with written informed consent from all participants. The protocol used was approved by the Institutional Ethical Board for epidemiological studies at the Hokkaido University Graduate School of Medicine, Hokkaido University Center for Environmental and Health Sciences, and the Ethics Review Committee of Nagoya University Graduate School of Medicine.

2.2 MEHP measurement

Measurement of maternal blood MEHP has been previously reported elsewhere (Araki et al., 2014; Jia et al., 2015). In this study, approximately 40 mL of blood was taken from each woman. All samples were stored at -80°C until analysis to avoid hydrolysis by enzyme activity. MEHP concentrations in maternal blood were measured using gas chromatography-mass spectrometry (GC/MS) at Nagoya University under analytical conditions (Jia et al., 2015). Briefly, 30 μL of blood samples was mixed with 120 μL of 1 M HCL to deactivate the serum enzymes, and 350 μL of saturated saline solution and 50 μL of 10 μM MEHP-d was used as an internal standard. MEHP was then extracted twice with 500 μL ethyl acetate after shaking for 15 min. There was no incubation process until extraction. The ethyl acetate layer was evaporated and then the residue was dissolved into 40 μL ethyl acetate. After the addition of 20 μL of N-methyl-N-(*tert*-butyldimethylsilyl) trifluoroacetamide (GL science, Tokyo, Japan), the reaction was incubated for 60 min at room temperature. The concentration of the MEHP *tert*-butyldimethylsilyl derivative was measured using

GC/MS (6890N, 5973N; Agilent Technologies, CA, USA). Two ions, *m/z* 227 as the quantification ion and 339 as the conformation ion, were used to detect MEHP (Jia et al., 2015). The limit of detection (LOD) was 0.278 ng/mL (1 pmol/mL). For each sample, duplicate analysis was performed. To determine background levels, MEHP levels in a tube containing the same medium as the reaction vial were measured. All glassware was heated at 200^{o}C for 2 h to exclude the possibility of environmental contamination. The coefficient of validation (CV) of MEHP measurements within a day was 2.0 – 7.8 % for 6 days, and the CV from day to day for 6 days was 6.2% at 5 pmol/mL of MEHP (Jia et al., 2015).

2.3 Outcome assessment

A self-administered questionnaire was distributed to the home of each of the children at ages 1.5, 3.5, and 7 years. To assess the children's wheeze, we used a modified part of the Japanese version of the American Thoracic Society-Division of Lung Diseases questionnaire (Nishima et al., 2009): "Having wheezing or whistling in the chest in the past?" Eczema was defined using the following questions from the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire (Asher et al., 1995): (a) "Having an itchy rash that comes and goes for at least 6 months?" (b) "Having the aforementioned itchy rash at any time during the last 12 months?" and (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?" Food allergy was defined as a positive response to the following question: "Has your child ever had symptoms such as hives, swelling of the lips, emesis, diarrhoea, or respiratory distress when they ate food allergens including milk, egg rice gruel, egg-drop, shrimp, or other foods?" Moreover, we defined "any allergies" as cases with at least one allergic symptom, including eczema, wheeze, and food allergy. Infectious diseases were assessed when infants had a positive response to the following medical question: "Has your child ever had a doctor's diagnosis, hospitalization, or medical treatment for the following diseases: otitis media, respiratory syncytial virus (RSV) diseases, chicken pox, and bronchitis/pneumonia?" "Any infectious diseases" was defined as having at least one of these four infectious diseases. The questionnaire also included information related to covariates, such as home environment, environmental tobacco smoke (ETS), wall-to-wall carpeting, condensation, visible mould, water leakage, pet keeping at home, and day care attendance.

Total IgE concentrations were measured from cord blood samples. All samples were stored at -80 °C until analysis. IgE levels were measured using an enzyme-linked immunosorbent assay (IMx analyser, Abbott Japan Co., Ltd., Tokyo, Japan) at SRL, Inc. (Tokyo, Japan).

2.4 Data analysis

The chi-squared test was used to analyse the associations between allergies/infectious diseases and maternal/child characteristics. Because the distribution of MEHP was skewed and not normally distributed according to the Shapiro-Wilk W-test, the MEHP concentration was converted into a

natural log scale. Logistic regression was used to determine the relationship between maternal MEHP levels and childhood allergies and infectious diseases at the ages of 1.5, 3.5, and 7 years. The results were presented as odd ratios (ORs) with 95% confidence intervals (CIs) for the risk of each outcome using natural log-transformed MEHP levels. To evaluate sex interactions, the interaction terms of sex (assigned as male=1 and female=0, respectively) × MEHP were added in each model. To assess the relative risk (RR) between maternal MEHP exposure level and the risk of each outcome from birth to the age of 7 years, we used generalized estimating equations (GEE) with an unstructured correlation matrix and an interaction term between the child's age at follow-up and maternal MEHP levels. Chicken pox was excluded from the GEE analysis because we did not collect the data on chicken pox at the age of 1.5 years. We selected potential confounding factors according to reviews of the literature and changes in the estimate criteria, which were set to values greater than 10%. We calculated the residual of potential confounding variables to natural log-transformed cord blood IgE levels. Polynomial regression analysis was performed to calculate the residual of potential confounding variables for dependent variables and the natural log-transformed maternal MEHP levels for the independent variable. Confounding variables were selected based on the covariates that influenced cord blood IgE levels in univariate analyses and possible risk factors reported in previous studies, as well as the changes in the estimate criteria. For the fully adjusted model, polynomial regression analysis was applied, adjusting for maternal age, maternal allergic history (yes/no), parity

(primiparous/multiparous), and infant sex. We further performed stratified analysis by infant sex. 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 JMP Pro 10 for Macintosh (SAS Institute, Inc., Cary, NC).

3. Results

The characteristics of the parents during pregnancy are shown in Table 1. The mean of maternal age at delivery was 31.0 ± 4.5 years. MEHP in maternal blood in pregnancy was detected in 100% of the samples and the median level was 10.54 ng/mL (interquartile range [IQR], 5.84–17.33). The characteristics of the children are shown in Table 2. At ages 1.5, 3.5, and 7 years, the prevalence of eczema was 14.9%, 19.4%, and 22.8%, the prevalence of wheeze was 16.1%, 17.6%, and 21.7%, the prevalence of food allergy was 18.5%, 35.9%, and 20.2%, the prevalence of any allergies was 32.6%, 42.3%, and 63.5%, and the prevalence of otitis media was 19.0%, 42.3%, and 42.4%, respectively. We did not collect data on chicken pox at the age of 1.5 years. The prevalence of chicken pox in children aged 3.5 and 7 years was 29.3% and 52.2%, respectively. The prevalence of any infectious diseases was 47.5%, 62.5%, and 71.2% in children aged 1.5, 3.5, and 7 years, respectively. The median level of IgE in cord blood was 0.43 IU/mL (IQR, 0.19–2.13). No

significant relationships were observed between maternal MEHP concentrations and maternal and children characteristics at each specified age (Supplementary Table 1).

The associations between the characteristics of the study participants and the prevalence of allergies and infectious diseases at each age are shown in Supplementary Table 2. At 1.5 years of age, eczema was associated with weight and height ($p < 0.01$). Wheeze was associated with parity ($p < 0.01$), day care attendance, mould growth, and water leakage ($p < 0.05$). Otitis media was associated with parity ($p < 0.05$) and day care attendance ($p < 0.01$). At 3.5 years of age, eczema was associated with maternal smoking during early pregnancy ($p < 0.01$), wheeze was associated with water leakage ($p < 0.05$), otitis media was associated with ETS in early pregnancy ($p < 0.05$) and day care attendance ($p < 0.01$), and chicken pox was associated with parity and wall-to-wall carpet at home ($p < 0.05$). At 7 years of age, chicken pox was associated with parity ($p < 0.05$).

Associations between maternal natural log-transformed MEHP levels (ng/mL) and the residual of potential confounding variables to natural log-transformed cord blood IgE levels (IU/mL) are shown in Table 3 and Figure 1. Cord blood IgE levels were negatively associated with maternal MEHP levels ($\beta = -0.40$; 95% CI, -0.74 – -0.05; $p = 0.024$). After stratification by sex, cord blood IgE levels tended to decrease linearly in both boys and girls; however, the p-values were not statistically significant.

The associations between maternal MEHP exposure and allergies and infectious diseases in

their children are shown in Table 4. After adjustment for confounders, maternal MEHP levels were found to increase the risk of food allergy at 3.5 (OR, 1.83; 95% CI, 1.12–3.00; p = 0.016) and 7 years of age (OR, 1.92; 95% CI, 1.21–3.14; p = 0.007), and to increase the risk of any allergies at 7 years of age (OR, 1.95; 95% CI, 1.20–3.26; p = 0.009). Furthermore, maternal MEHP levels significantly increased the risk of food allergy (RR, 2.10; 95% CI, 1.46–3.01; p < 0.001), any allergies (RR, 1.44; 95% CI, 1.06–1.98; p = 0.021), otitis media (RR, 1.53; 95% CI, 1.09–2.16; p = 0.015), chicken pox (RR, 1.52; 95% CI, 1.10–2.10; p = 0.011), and any infectious diseases (RR, 2.00; 95% CI, 1.41–2.83; p < 0.001) during the study period (from birth to 7 years old). Maternal MEHP levels were negatively associated with the risk of eczema at 3.5 years of age (OR, 0.57; 95% CI, 0.32–0.97; p = 0.046). Significant interactions were identified between sex and the risks of chicken pox at 3.5 years of age (p for interaction = 0.041) and any infectious diseases at 7 years of age (p for interaction = 0.007). After stratification by sex, maternal MEHP levels significantly increased the risk of chicken pox at 3.5 years of age among girls and any infectious diseases at 7 years of age among boys. However, p-values for the MEHP × sex interaction were not statistically significant, except for chicken pox at 3.5 years of age and any infectious diseases at 7 years of age. Because cord blood IgE is thought to be one of the confounder of allergies/infectious diseases and environmental chemicals, we included cord blood IgE levels into adjusted model. Although no significant difference was apparent between the adjusted model with and without cord blood IgE

levels, some of multivariate analyses could not converge because of lack of numbers of sample (Supplementary Table 4).

4. Discussion

We investigated the effects of prenatal MEHP exposure on the IgE level at birth and allergies and infectious diseases until the age of 7 years in a birth cohort study. We found that the maternal MEHP concentration was associated with an increased risk of otitis media and chicken pox during the study periods. After stratification by sex, significant associations were observed with wheeze and chicken pox at 3.5 years of age and from birth to 7 years of age among girls. Furthermore, cord blood IgE levels were negatively associated with maternal MEHP levels.

Most studies presented urinary levels of MEHP, and they are generally higher than blood levels(Hines et al., 2009; Hogberg et al., 2008; Kato et al., 2004). In our study, the maternal MEHP levels were higher than those previously reported in pregnant women in Australia (median, 1.18 ng/mL; IQR, < 0.60 to 3.10) (Hart et al., 2014) and in Swedish mothers 14–20 days after delivery (median, 0.49 ng/mL; range, 0.49–4.5) (Hogberg et al., 2008). However, one study in Italy measured the MEHP level in maternal blood and showed a mean ± SD of 0.68 ± 0.85 μg/mL (Latini et al., 2003), which was approximately 70 times higher than the MEHP level in this study. We previously reported that DEHP levels in house dust and urinary DEHP metabolite levels in the Sapporo area of

Japan were also higher than those in other countries, such as Denmark, Germany, USA, and Norway (Ait Bamai et al., 2014; Ait Bamai et al., 2015). The levels of production and usage of DEHP differ between countries. In fact, DEHP is still the dominant plasticizer/phthalate in Japan, whereas the consumption of DEHP has decreased considerably in Europe and the USA (Bizzari, 2013), which may explain the higher MEHP levels in this study than in other populations.

The potential effects of exposure to DEHP on allergies have been demonstrated by experimental studies showing that DEHP has adjuvant effects, immunosuppressive effects, and inflammatory activities (Ashley-Martin et al., 2015; Han et al., 2014; Larsen and Nielsen, 2007; Pei et al., 2014; Tanaka et al., 2013). In epidemiological evidence, several cross-sectional studies have shown that childhood exposure to DEHP increases the risk of allergies, including asthma, wheeze, and rhino-conjunctivitis among children (Ait Bamai et al., 2016; Beko et al., 2015; Bertelsen et al., 2013; Callesen et al., 2014; Hoppin et al., 2013). However, epidemiological evidence regarding the effects of prenatal exposure is still unclear. Only four studies have reported the association between prenatal exposure to DEHP and wheeze/asthma or eczema/atopic dermatitis (Gascon et al., 2014; Ku et al., 2015; Wang et al., 2014; Whyatt et al., 2014) and these findings are not consistent. Gascon et al. (2014) reported that maternal urinary ΣDEHP metabolites (MEHP, mono(2-ethyl-5-hydroxyhexyl) phthalate [MEHHP], mono(2-ethyl-5-oxohexyl) phthalate [MEOHP], and mono(2-ethyl-5-carboxypentyl) phthalate [MECPP]) in pregnancy were associated with an

increased risk of wheeze and bronchitis during childhood (Gascon et al., 2014), which is consistent with our findings. In contrast, no significant associations were reported between maternal DEHP metabolite (MEHHP) concentration and asthma-like symptoms in children aged 5–11 years from the birth cohort study in the USA (Whyatt et al., 2014). Maternal urinary ΣDEHP metabolites (MEHP, MEHHP, and MEOHP) in pregnancy were not associated with wheeze at 8 years of age (Ku et al., 2015). We observed a borderline inverse association with eczema at 3.5 years. However, two birth cohort studies from Taiwan (Wang et al., 2014) and Spain (Gascon et al., 2014) have reported that prenatal exposure to DEHP is not associated with childhood eczema or atopic dermatitis. Current evidence from epidemiological studies regarding the effects of prenatal DEHP exposure on childhood eczema or atopic dermatitis are not consistent and remain unclear; therefore, more prospective studies are warranted. To our knowledge, with regard to the effects of prenatal DEHP exposure on food allergies and/or infectious diseases, no experimental and epidemiological studies have been reported.

Prenatal and postnatal exposure to environmental chemicals has been related to reduced IgE levels in children, which suggests the suppressive effects of such chemicals on immune function and reactions (Ashley-Martin et al., 2015; Grandjean et al., 2012; Yu et al., 1998). The experimental evidence also indicates that DEHP can disrupt the immune system through the modulation of cellular proliferation, lymphocyte division, and cytokine synthesis, which leads to the potentiation of

immune or inflammatory responses and immunosuppressive activity (Kimber and Dearman, 2010). Pei et al. (2014) reported that DEHP significantly increased IL-4 and IFN-γ levels, and reduced the Th1/Th2 ratio *in vitro* (Pei et al., 2014). In animal models, the potential effect of DEHP on immune deviation, such as the activation of PPARα in disrupting the Th1/Th2 balance towards the Th2 response, may also result in allergies (Larsen and Nielsen, 2007). It has also been reported that exposure to DEHP results in immune response, such as inhibition of B cell proliferation and reduction of the IgM-secreting cells in rainbow trout (Martins et al., 2015). Han et al. (2014) reported adjuvant toxic effects on T follicular helper cells, involving the excess synthesis of Th2 cell-related cytokines (IL-21 and IL-4) and leading to the increased secretion of allergy-related IgE and IgG1 in mice (Han et al., 2014). Taken together, the evidence from *in vitro* and animal studies indicate that exposure to DEHP might induce allergies and infectious diseases. However, no clear mechanism of action has been defined for prenatal exposure to DEHP that fully explains its ability to induce allergies and infectious diseases. Therefore, further studies related to the transgenerational effects of DEHP exposure on allergies and infectious diseases are needed. We observed that high maternal MEHP levels were inversely associated with significant linearly decreased cord blood IgE levels. One recent study from a Canadian birth cohort also reported a significant non-linear inverse association between DEHP metabolites and the ORs of levels of cord blood IgE (Ashley-Martin et al., 2015). Their findings suggested that prenatal exposure to phthalates may suppress the immune

system in early life. A systematic literature review also suggested that endocrine-disrupting chemicals, including DEHP, showed non-linear associations with adverse effects in a number of experimental studies (Vandenberg et al., 2012). After considering the non-linear relationship, in this study we also observed a quadratic association between maternal MEHP and cord blood IgE levels (Supplementary Figure 1). Moreover, higher levels of IgE in cord blood increased the risk of any infectious diseases during childhood (Supplementary Table 3). However, mediation analysis showed that cord blood IgE level was not a mediator of the association between prenatal exposure to MEHP and infectious diseases; thus, prenatal exposure to MEHP may affect infectious diseases during childhood other than mechanism through IgE. The findings from cord blood IgE should be interpreted with caution because it is still debated whether the IgE is transferred from mother to foetus or whether the foetus itself produces IgE. Previous studies reported that cord blood IgE was transferred from the mother and that maternal and foetal IgE sensitization patterns are highly correlated (Bertino et al., 2006). In contrast, recent studies reported differential sensitization profiles between maternal blood and cord blood (Kamemura et al., 2012; Pfefferle et al., 2008); therefore, cord blood IgE is likely to represent the foetal immune response.

The limitations of this study need to be considered. Firstly, a single exposure measurement may not accurately reflect exposure during the whole pregnancy because of the short half-life of DEHP. Secondly, we only measured MEHP. In urine samples, approximately 70% of detected phthalates

were found in oxidized metabolite forms, such as MEHHP and MEOHP, whereas only 6% are found in the form of MEHP (Koch et al., 2006). In contrast, MEHP is detected in more than 80% of blood samples and the detection rates of oxidized metabolites are less than 40% (Hogberg et al., 2008; Lin et al., 2011). Nevertheless, secondary metabolites such as MEHHP, MEOHP, MECPP, and mono(2-carboxymethylhexyl) phthalate of DEHP should also be measured in future studies. Moreover, we used blood samples for exposure assessment. It is known that the diester is converted to the monoester after blood sampling. However, in our study, the blood samples were immediately stored at -80°C, and an acid was added to the samples right after thawing to minimize enzyme activity from external contamination that may result in MEHP (Kato et al., 2003). Besides, we measured background levels of MEHP and confirmed that there were no effects of external contamination. All samples were handled carefully to avoid *ex vivo* hydrolysis of DEHP. From the above, the analysis of MEHP in blood could provide an indication of DEHP exposure. Thirdly, the effects of other phthalates on allergies, infectious diseases, and cord blood IgE levels were not investigated. Moreover, since it is still debated whether IgE is transferred from mother to foetus or whether the foetus produces IgE. It might not be appropriate to use cord blood IgE levels as indicators of the foetal immune response. However, recent studies indicate that cord blood IgE represents the foetal immune response (Kamemura et al., 2012; Pfefferle et al., 2008). Fourthly, the sample size of this study was relatively small, which might influence the evaluation of clear

associations between maternal MEHP levels and health outcomes because of lack of statistical power. Especially in the stratification analysis, several borderline associations were observed. This may result in an underestimation of the associations between maternal MEHP levels and health effects; however, it is unlikely to lead false positive associations and our findings with statistically significant in small sample size might be robust. Loss to follow-up in this cohort study could also be a bias for the original population. However, the response rates of the questionnaire for each age group were > 70%. Moreover, the data on the maternal history of allergies and the prevalence of allergies among children between the study population in this study and the original cohort are similar; therefore, we believe that selection bias was minimized. Lastly, we collected information on allergies and infectious diseases based on maternal reports, which is prone to recall bias. Genetic factors, such as atopy related single nucleotide polymorphisms, were not considered in this study. Therefore, further studies investigating gene/environment interactions and allergy/infectious disease-related biomarkers are needed.

In conclusion, this is the first study that investigated the effects of prenatal DEHP exposure on the level of IgE at birth and the incidence of allergies and infectious diseases up to 1.5, 3.5, and 7 years of age and from birth to 7 years of age. This study suggests that prenatal DEHP exposure may increase the risk of allergies and infectious diseases during childhood. Furthermore, umbilical cord blood IgE levels were negatively associated with maternal MEHP levels. Further epidemiological

studies should focus on the underlying immune system mechanisms and their induced health effects in larger populations.

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Table 1

The characteristics of the parents during pregnancy

	N=258 Mean ±SD / N, (%)	
Parents		
Age at delivery	31.0	±4.5
Parity >=1	166	(64.3)
Maternal history of allergies	79	(30.7)
Smoking at early pregnancy	30	(11.6)
ETS at early pregnancy	172	(66.7)
Annual household income		
<5 million yen	171	(66.3)
>=5 million yen	87	(33.7)
Paternal history of allergies	40	(15.5)
MEHP (ng/mL) (Median, IQR)	10.54	(5.84 - 17.33)

Table 2.

The characteristics of the children

	1.5y (n=248) Mean ±SD / N, (%)		3.5y (n=222) Mean ±SD / N, (%)		7y (n=184) Mean ±SD / N, (%)	
Children						
Boys sex	124	(50.0)	109	(50.0)	95	(51.6)
Birth weight (g)	3079.0	±378.3	3079.9	±369.9	3097.5	±383.2
Birth height (cm)	48.0	±2.0	48.0	±2.0	48.1	±2.1
Weight at each age (kg)	10.3	±1.9	12.6	±4.2	20.7	±4.4
Height at each age (cm)	78.1	±11.5	84.9	±26.4	113.6	±22.3
History of day care attendance	51	(20.6)	128	(57.7)	101	(54.9)
Eczema	37	(14.9)	43	(19.4)	42	(22.8)
Wheeze	40	(16.1)	39	(17.6)	40	(21.7)
Otitis media	47	(19.0)	94	(42.3)	78	(42.4)
Food allergy	46	(18.5)	48	(32.6)	84	(45.7)
Any allergies	89	(35.9)	94	(42.3)	115	(62.5)
Chicken pox	-	-	65	(29.3)	96	(52.2)
Bronchitis or pneumonia	-	-	44	(19.8)	29	(15.8)
RSV diseases	5	(2.0)	6	(2.7)	5	(2.7)
Any infectious diseases	50	(20.2)	141	(63.5)	131	(71.2)
Cord blood IgE (IU/mL) (Median, IQR)	0.43	(0.19 – 2.13)				
Living conditions						
Maternal smoking	50	(20.2)	47	(21.1)	29	(15.8)
ETS	125	(50.4)	119	(53.6)	82	(44.6)
Wall-to-wall carpeting	153	(61.7)	116	(52.3)	91	(49.5)
Condensation	178	(71.8)	160	(72.1)	125	(67.9)
Visible mould	128	(51.6)	119	(53.6)	95	(51.6)
Water leakage	35	(14.1)	34	(15.3)	19	(10.3)

IgE: immunoglobulin E; ETS: environment tobacco smoke; RSV: respiratory syncytial virus

Table 3

Associations between maternal natural log-transformed MEHP (ng/mL) and residual of potential confounding variables to natural log-transformed cord blood IgE levels (IU/mL)

	β	SE	stβ	95% CI		p value
Overall (n=127)						
linear	**-0.40**	**0.17**	**-0.20**	**-0.74**	**-0.05**	**0.024***
quadratic	**-0.43**	**0.20**	**-0.22**	**-0.84**	**-0.03**	**0.034***
Boys (n=62)						
linear	-0.51	0.32	-0.20	-1.16	0.14	0.121
quadratic	-0.40	0.34	-0.15	-1.08	0.29	0.251
Girls (n=65)						
linear	-0.34	0.20	-0.21	-0.74	0.06	0.093†
quadratic	-0.46	0.25	-0.28	-0.96	0.04	0.073†

*: p< 0.05; †: p< 0.1

Adjusted for maternal age at delivery, maternal history of allergies, and parity.

Table 4

The associations between prenatal MEHP exposure and children allergies and infectious diseases at each age and from birth to 7 year of age

	total					boys				girls			
	OR[F], RR[£]	95% CI		p value	*p* for interaction[∮]	OR[F], RR[£]	95% CI		p value	OR[F], RR[£]	95% CI		p value
1.5y[a]													
Eczema	0.74	0.42	1.25	0.275	0.175	0.48	0.19	1.09	0.096†	1.08	0.50	2.32	0.835
Wheeze	1.34	0.78	2.29	0.289	0.344	0.99	0.46	2.03	0.989	1.98	0.84	4.83	0.121
Food allergy	1.43	0.90	2.29	0.133	0.999	1.57	0.77	3.23	0.216	1.67	0.85	3.27	0.138
Any allergies	1.09	0.73	1.62	0.690	0.765	1.03	0.58	1.83	0.910	1.25	0.68	2.29	0.468
Otitis media	1.62	0.96	2.76	0.073†	0.404	**2.20**	**1.06**	**4.71**	**0.036***	1.17	0.51	2.63	0.708
Any infectious diseases	1.65	0.98	2.76	0.059†	0.069†	**2.26**	**1.07**	**4.78**	**0.033***	1.36	0.64	2.90	0.424
3.5y[b]													
Eczema	**0.57**	**0.32**	**0.97**	**0.046***	0.292	0.41	0.16	0.95	0.049	0.70	0.32	1.49	0.371
Wheeze	1.39	0.83	2.32	0.209	0.073†	0.75	0.30	1.70	0.512	**2.30**	**1.10**	**5.05**	**0.030***
Food allergy	**1.83**	**1.12**	**3.00**	**0.016***	0.853	**2.10**	**1.01**	**4.58**	**0.050***	1.81	0.91	3.70	0.093†
Any allergies	0.93	0.87	1.00	0.735	0.376	0.94	0.51	1.69	0.828	1.31	0.71	2.44	0.388
Otitis media	1.42	0.93	2.19	0.108	0.391	1.29	0.68	2.48	0.435	1.69	0.93	3.17	0.092†
Chicken pox	1.37	0.87	2.17	0.176	**0.041***	0.90	0.44	1.77	0.762	**2.30**	**1.18**	**4.69**	**0.017***
Bronchitis/pneumonia	1.29	0.78	2.12	0.320	0.808	1.40	0.61	3.10	0.408	1.22	0.61	2.41	0.565
Any infectious diseases	1.51	0.97	2.41	0.073†	0.399	1.16	0.62	2.24	0.654	**1.96**	**1.02**	**4.00**	**0.043***
7y[c]													
Eczema	1.15	0.68	1.93	0.592	0.528	1.00	0.44	2.20	0.997	1.28	0.62	2.67	0.507
Wheeze	0.90	0.51	1.56	0.720	0.576	0.83	0.37	1.76	0.628	1.02	0.43	2.33	0.963
Food allergy	**1.92**	**1.21**	**3.14**	**0.007****	0.183	1.42	0.73	2.83	0.309	**2.74**	**1.38**	**5.84**	**0.006****
Any allergies	**1.95**	**1.20**	**3.26**	**0.009****	0.312	1.65	0.83	3.45	0.164	**2.46**	**1.21**	**5.39**	**0.017***
Otitis media	1.10	0.70	1.74	0.672	0.265	1.51	0.77	3.05	0.233	0.92	0.47	1.77	0.802
Chicken pox	1.31	0.83	2.07	0.249	0.195	1.82	0.93	3.73	0.090†	1.05	0.55	2.02	0.882
Bronchitis/pneumonia	0.77	0.40	1.42	0.418	0.287	1.02	0.41	2.38	0.923	0.53	0.19	1.31	0.188
Any infectious diseases	1.18	0.72	1.99	0.515	**0.007****	**2.91**	**1.30**	**7.44**	**0.015***	0.63	0.29	1.32	0.217
From birth to 7y[d]													
Eczema	0.89	0.60	1.31	0.543	0.139	0.67	0.36	1.22	0.185	1.13	0.67	1.89	0.657
Wheeze	1.23	0.85	1.79	0.271	0.077†	0.85	0.46	1.54	0.582	**1.90**	**1.12**	**3.20**	**0.017***
Food allergies	**2.10**	**1.46**	**3.01**	**<0.001****	0.368	**1.88**	**1.14**	**3.09**	**0.013***	**2.61**	**1.51**	**4.52**	**0.001****
Any allergies	**1.44**	**1.06**	**1.98**	**0.021***	0.273	1.24	0.81	1.91	0.319	**1.78**	**1.13**	**2.81**	**0.013***
Otitis media	**1.53**	**1.09**	**2.16**	**0.015***	0.697	1.50	0.93	2.42	0.094†	1.62	0.98	2.66	0.060†
Chicken pox	**1.52**	**1.10**	**2.10**	**0.011***	0.900	1.25	0.8	1.96	0.324	**1.89**	**1.16**	**3.07**	**0.011***
Any infectious diseases	**2.00**	**1.41**	**2.83**	**<0.001****	0.251	**2.18**	**1.37**	**3.48**	**0.001****	**2.07**	**1.21**	**3.56**	**0.008****

a: adjusted for gender, smoking at early pregnancy, maternal history of allergies, parity, maternal age at delivery, household income, ETS at 1.5y, and day care attendance at 1.5y.

b: adjusted for gender, maternal history of allergies, parity, maternal age at delivery, household income, ETS at 3.5y, and day care attendance at 3.5y.

c: adjusted for gender, maternal history of allergies, parity, maternal age at delivery, household income, ETS at 7y, and day care attendance at 7y.

d: adjusted for sex, history of maternal allergies, maternal age at delivery, parity, maternal smoking, day care attendance, and household income; Interaction term between child's age at follow-up and MEHP levels using generalized equation estimates.

∮: The interaction terms of sex x MEHP levels were assigned as male=1 and female=0, respectively.

*: p< 0.05; †: p< 0.1; ₣: odds ratio for each outcome at 1.5y, 3.5y, and 7y of age; £: relative risk for each outcome from birth to 7 years of age.

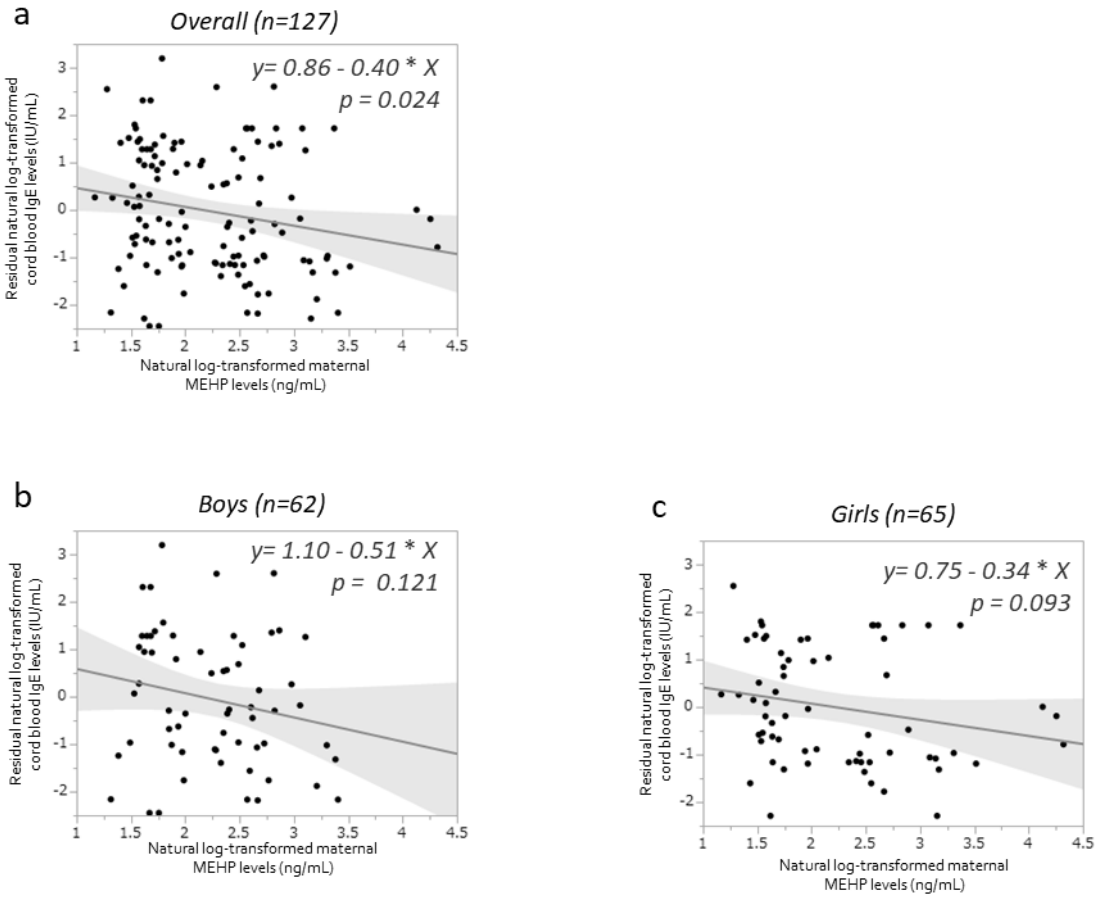
Figure legend

Associations between maternal natural log-transformed MEHP (ng/mL) and residual of potential confounding variables to natural log-transformed cord blood IgE levels (IU/mL).

- The solid lines denote the predicted fit from a linear regression model with 95% confidence interval (gray).
- Individual observations of maternal natural log-transformed MEHP (ng/mL) and residual of potential confounding variables to natural log-transformed cord blood IgE levels (IU/mL) are shown as plots.
- Corresponding estimates are presented in Table 3.
- (a) Among overall. (b) Among boys. (c) Among girls.

Figure 1

Associations between maternal natural log-transformed MEHP (ng/mL) and residual of potential confounding variables to natural log-transformed cord blood IgE levels (IU/mL)



Supplementary Table 1

Associations between maternal MEHP concentrations (ng/mL) and maternal and infant characteristics

		n	Median	25th	75th	p value
				ρ		
Smoking at early pregnancy	Yes	30	9.41	15.15	6.36	0.698
	No	228	10.75	17.58	5.79	
ETS at early pregnancy	Yes	172	9.54	16.12	5.74	0.089
	No	86	12.78	17.64	6.81	
History of allergies	Yes	79	9.69	18.02	5.32	0.550
	No	178	10.88	17.05	6.02	
Parity	0	92	10.81	18.26	5.90	0.660
	>=1	166	10.46	17.05	5.84	
Household income	<5 million yen	171	9.69	17.11	5.79	0.295
	>=5 million yen	87	12.04	17.70	6.35	
Infant sex	Boy	129	10.58	6.30	16.52	0.856
	Girl	129	10.41	5.64	17.70	
Maternal age at pregnancy		258		-0.076		0.222
Cord blood IgE (IU/mL)		130		-0.140		0.113
Infant birth weight (g)		258		-0.028		0.654
Infant birth height (cm)		258		0.025		0.694

Mann-Whitney U test or Spearman's correlation

Supplementary Table 2
Associations between characteristics of study participants and the prevalence of allergies and infectious diseases at each age

1.5y	N	**Eczema**		**Wheeze**		**Food allergy**		**Any allergies**		**Otitis media**		**RS virus**		**Any infectious diseases**	
		w/ %	w/o %	w/ %	w/o %	w/ %	w/o %	w/ %	w/o %	w/ %	w/o %	w/ %	w/o %	w/ %	w/o %
Maternal age at delivery (mean±SD)		30.8±4.5	31.1±4.6	31.7±4.8	30.9±4.5	30.2±4.6	30.9±4.7	30.8±4.5	30.7±4.8	32.2±4.3	30.8±4.6	34±3.8	31±4.6	31.6±4.5	30.6±4.7
Birth weight (g)(mean±SD)		2993.4±423.1	3094±369	3092.6±382.1	3076.4±378.5	3136.4±349.1	3144.4±302.2	3134.3±300.8	3147.5±315.6	3133.7±306.1	3066.2±392.9	3151.2±310.4	3077.5±380	3190.3±279.4	3132.7±316.1
Birth height (cm) (mean±SD)		47.6±2.1	48.1±1.9	47.8±1.8	48.1±2	48.2±3.0	48.4±1.6	48.2±2.4	48.4±1.6	48±1.6	48±2.1	47.6±1.2	48±2	48.4±1.7	48.4±1.9
Weight (kg) (mean±SD)		**9.5±3.1**	**10.4±1.6****	10.2±3.2	10.3±1.5	10.4±1.3	10.4±1.6	10.5±1.7	10.4±1.5	10.4±1.9	10.3±1.9	10.4±1.3	10.3±1.9	10.5±1.2	10.4±1.7
Height (cm) (mean±SD)		**72.9±22.1**	**79.0±8.2****	74.4±21.6	78.8±8.2	79.5±2.9	78.8±9.1	78.4±10	79.1±7.4	78.1±12	78.1±11.4	79.2±1.2	78.1±11.6	79.9±3	78.7±9.1
Parity >=1	159	75.7	62.1	**90.0**	**59.1****	76.1	61.4	77.5	56.6	**76.6**	**61.2***	**100.0**	**63.4***	78.0	60.6
Maternal history of allergies	75	43.2	28.1	35.0	29.5	37.0	28.9	37.1	26.6	31.9	30.0	20.0	30.6	32.0	30.0
Maternal smoking at early pregnancy	28	10.8	11.4	20.0	9.6	15.2	10.4	13.5	10.1	10.6	11.4	0.0	11.5	10.0	11.6
ETS at early pregnancy	162	59.5	66.4	57.5	66.8	16.55	83.5	33.8	66.2	76.6	62.7	40.0	65.8	18.6	81.4
Maternal smoking	50	18.9	20.4	25.0	19.2	17.4	82.6	32.6	67.4	21.3	19.9	20.0	20.2	15.2	84.8
Household income <5million yen	163	62.2	66.4	65.0	65.9	69.6	64.9	65.2	66.0	63.8	66.2	80.0	65.4	64.0	66.2
>=5million yen	85	37.8	33.7	35.0	34.1	30.4	35.2	34.8	34.0	36.2	33.8	20.0	34.6	36.0	33.8
Gender Boys	124	48.7	50.2	57.5	48.6	45.7	51.0	52.8	48.4	63.8	46.8	40.0	50.2	60.0	47.5
Girls	124	51.4	49.8	42.5	51.4	54.4	49.0	47.2	51.6	36.2	53.2	60.0	49.8	40.0	52.5
Day care attendance	51	18.9	20.9	**35.0**	**17.8***	15.2	21.8	24.7	18.2	**46.8**	**14.4****	40.0	20.2	**46.0**	**14.1****
ETS	125	40.5	52.1	50.0	50.5	52.2	50.0	50.6	50.3	57.5	48.8	40.0	50.6	56.0	49.0
Wall-to-wall carpet	153	54.1	63.0	60.0	62.0	60.9	61.9	59.6	62.9	55.3	63.2	80.0	61.3	80.0	61.3
Condensation	178	78.4	70.6	80.0	70.2	78.3	70.3	77.5	68.6	72.3	71.6	80.0	71.6	74.0	71.2
Mold growth	128	59.5	50.2	**67.5**	**48.6***	76.1	61.4	37.0	28.9	53.2	51.2	60.0	51.4	54.0	51.0
Water leakage	35	13.5	14.2	**25.0**	**12.0***	17.4	13.4	**20.2**	**10.7***	14.9	13.9	**60.0**	**13.2****	**20.0**	**12.6***
Pet keeping	48	24.3	18.5	20.0	19.2	23.9	18.3	22.5	17.6	27.7	17.4	20.0	19.3	28.0	17.2

X2 test or t-test
*: p<0.05; **: p<0.01

Supplementary Table 2 continued

3.5y	N	**AD**		**Wheeze**		**Food allergy**		**Any allergies**		**Otitis media**		**Chicken pox**		**Bronchitis/ pneumonia**		**RS virus**		**Any infectious diseases**	
		w/ %	w/o %	w/ %	w/o %	w/ %	w/o %	w/ %	w/o %	w/ %	w/o %	w/ %	w/o %	w/ %	w/o %	w/ %	w/o %	w/ %	w/o %
Maternal age at delivery (mean±SD)		30.4±4.9	31±4.2	30.5±4.5	31±4.3	29.9±3.9	31.2±4.5	30.3±4.4	31.4±4.3	30.5±4.6	31.2±4.2	3.09±4.5	30.9±4.3	31.2±4.5	30.8±4.3	30.2±4.2	30.9±4.4	30.9±4.5	30.9±4.1
Birth weight (g) (mean±SD)		3074.2±338.2	3081.3±377.9	3150±350.4	3065±373.1	3100.8±391.4	3074.1±364.7	3107.7±366.9	3059.5±372.2	3060.8±395.9	3093.9±350.4	3051.9±336.2	3091.5±383.4	3097.5±430.5	3075.6±354.5	3048.3±251.1	3080.8±373	3066.2±384.2	3103.8±344.5
Birth height (cm) (mean±SD)		48.1±1.8	48±2	48.2±1.8	48±2	48.1±2.9	48±1.7	48.2±2.4	47.9±1.6	47.8±1.8	48.2±2.1	47.9±1.6	48.1±2.1	48±2.1	48±2	48.2±1.5	48±2	47.9±1.8	48.2±2.3
Weight (kg) (mean±SD)		13.5±3.5	12.4±4.3	12.4±5.1	12.7±4	12.6±4.2	12.7±4.2	12.8±4.3	12.5±4.1	12.9±3.9	12.4±4.4	12.7±4.4	12.6±4.1	12.9±3.9	12.6±4.3	11.3±5.8	12.7±4.1	12.7±4.2	12.5±4.2
Height (cm) (mean±SD)		89±20.2	83.9±27.6	80.4±32.2	85.8±25	85.4±26.3	84.7±26.5	84.9±26.6	84.8±26.3	86±24.7	84.1±27.6	84.2±27.2	85.1±26.1	86.5±23.9	84.5±27	76.4±37.5	85.1±26.1	85±26.2	84.7±26.9
Parity >=1	141	63.7	62.8	62.3	69.2	64.6	63.2	63.8	63.3	62.5	64.9	**78.5**	**57.3****	59.1	64.6	63.9	50.0	68.8	54.3
Maternal history of allergies	72	31.5	37.2	31.9	35.9	50.0	27.8	39.4	27.6	33.1	31.9	32.3	32.7	36.4	31.6	31.6	66.7	33.3	31.3
Maternal smoking at early pregnancy	19	**10.6**	**0.0****	8.2	10.3	10.4	8.1	7.5	9.4	8.6	8.5	9.2	8.3	18.2	6.18	8.8	0.0	9.2	7.41
ETS at early pregnancy	151	68.2	67.4	68.9	64.1	58.3	70.7	63.8	71.1	**75.5**	**62.5***	72.3	66.2	68.2	68.0	68.5	50.0	71.6	61.7
Maternal smoking	47	22.9	14.0	20.2	25.6	20.8	21.3	21.3	21.1	18.0	25.5	20.0	21.7	18.2	21.9	21.3	16.7	21.3	21.0
Household income <5million yen	146	66.5	62.8	65.6	66.7	70.8	64.4	66.0	65.6	66.4	64.9	66.2	65.6	65.9	65.7	65.7	66.7	66.7	64.2
>=5million yen	76	33.5	37.2	34.4	33.3	29.2	35.6	34.0	34.4	33.6	35.1	33.9	34.4	34.1	34.3	34.3	33.3	33.3	35.8
Gender Boys	109	48.6	51.2	49.7	46.2	45.8	50.0	50.0	48.4	48.4	50.0	55.4	46.5	40.9	51.1	50.0	16.7	48.2	50.6
Girls	113	51.4	48.8	50.3	53.9	54.2	50.0	50.0	51.6	51.6	50.0	44.6	53.5	59.1	48.9	50.0	83.3	51.8	49.4
Day care attendance	128	57.5	58.1	56.8	61.5	56.3	58.1	55.3	59.4	**72.3**	**46.9****	66.2	54.1	72.7	53.9	57.4	66.7	66.7	42.0
ETS	119	54.8	48.8	54.6	48.7	45.8	55.8	48.9	57.0	49.2	59.6	63.1	49.7	59.1	52.3	53.7	50.0	58.2	45.7
Wall-to-wall carpet	116	53.6	46.5	50.3	61.5	43.8	54.6	55.3	50.0	55.5	47.9	**41.5**	**56.7***	52.3	52.3	52.3	50.0	49.7	56.8
Condensation	160	71.0	76.7	70.0	82.1	77.1	70.7	80.9	65.6	68.8	76.6	70.8	72.6	72.7	71.9	72.2	66.7	73.8	69.1
Mold growth	119	52.0	60.5	50.8	66.7	56.3	52.9	62.8	46.9	57.8	47.9	46.2	56.7	50.0	54.5	53.7	50.0	50.4	59.3
Water leakage	34	15.1	16.3	**28.2**	**12.6***	14.6	15.5	17.0	14.1	16.4	13.8	10.8	17.2	13.6	15.7	14.8	33.3	14.2	17.3
Pet keeping	57	25.7	25.6	24.6	30.8	31.3	24.1	27.7	24.2	25.0	26.6	23.1	26.8	40.9	21.9	25.9	16.7	27.7	22.2

X2 test or t-test

*: p<0.05; **: p<0.01

Supplementary Table 2 continued

7y	N	AD		Wheeze		Food allergy		Any allergies		Otitis media		Chicken pox		Bronchitis/ pneumonia		RS virus		Any infectious diseases	
		w/ %	w/o %	w/ %	w/o %	w/ %	w/o %	w/ %	w/o %	w/ %	w/o %	w/ %	w/o %	w/ %	w/o %	w/ %	w/o %	w/ %	w/o %
Maternal age at delivery (mean±SD)		31.9 ±4.6	31.0±4.2	31.3 ±4.9	31.2 ±4.1	30.6 ±4.0	31.7 ±4.5	30.9 ±4.4	31.6±4	30.9 ±4.3	31.4 ±4.3	31.5 ±4.5	30.8 ±4.0	31.4 ±3.9	31.2 ±4.3	30±4.1	31.2 ±4.3	31.5 ±4.3	30.5 ±4.1
Birth weight (g)(mean±SD)		3097 ±336.5	3097.7 ±397	3155.5 ±282.8	3081.4 ±406	3106.8 ±423.6	3089.7 ±347.5	3103.4 ±387	3087.7 ±379.3	3044.5 ±392.8	3136.5 ±373	3119.3 ±358.5	3073.7 ±409.2	3083.5 ±382.8	3100.1 ±384.4	2900 ±551.8	3103± 378.1	3089.6 ±385.3	3117.1 ±380.9
Birth height (cm) (mean±SD)		48.1 ±1.6	48.1±2.2	48.3±1 .3	48±2.2	48.2 ±1.9	48±2.2	48.2±1. 7	47.9±2. 5	47.7±2. 4	48.4±1. 7	48.0±2. 1	48.2±2. 0	48.2±1. 5	48.1±2. 1	47.1±1. 9	48.1±2 .1	47.9±2. 1	48.5±1. 9
Weight (kg) (mean±SD)		21.1±5. 8	20.6±3.9	21.1±5 .1	20.6 ±4.2	20.6 ±4.8	20.8 ±4.0	20.9 ±5.2	20.4 ±2.6	20.8 ±3.7	20.7 ±4.8	111.6 ±27.8	115.8 ±13.8	21.5 ±3.2	20.6 ±4.5	22.8 ±5.7	20.7 ±4.3	20.9 ±4.2	20.1 ±4.7
Height (cm) (mean±SD)		114.3±1 8.9	113.4±23. 3	108.9± 38.8	114.9± 14.8	113.5± 18.8	113.7± 25	112±27 .9	116.2± 5	112.5± 28.1	114.4± 17	20.6±4. 6	20.8±4. 1	117.8± 4.9	112.8± 24.1	112.5± 5.4	113.6± 22.6	113.3± 24.1	114.2± 17.3
Parity >=1	123	69.1	66.2	75.0	64.6	66.7	67.0	67.8	65.2	59.0	72.6	**74.0**	**59.1***	**69.0**	**66.5**	60.0	67.0	67.9	64.2
Maternal history of allergies	62	42.9	31.2	40.0	32.2	40.5	28.3	36.5	29.4	38.5	30.5	33.3	34.5	48.3	31.2	**80.0**	**32.6***	32.8	36.5
Maternal smoking at early pregnancy	15	4.8	9.2	7.5	8.3	10.7	6.0	7.8	8.7	7.7	8.5	8.3	8.0	6.9	8.4	20.0	7.8	8.4	7.6
ETS at early pregnancy	122	66.7	66.2	67.5	66.0	61.9	70.0	64.4	69.6	70.5	63.2	66.7	65.9	65.5	66.5	80.0	65.9	68.7	60.4
Maternal smoking	29	11.9	16.9	15.0	16.0	15.5	16.0	13.0	20.3	15.4	16.0	13.5	18.2	13.8	16.1	20.0	15.6	15.3	17.0
Household income <5million yen	116	61.9	63.4	65.0	62.5	59.5	66.0	63.5	62.3	61.5	64.2	64.6	61.4	65.5	62.6	60.0	63.1	64.9	58.5
>=5million yen	68	36.6	38.1	37.5	35.0	43.1	50.0	36.5	37.7	35.9	38.5	35.4	38.6	34.5	37.4	36.9	40.0	35.1	41.5
Gender Boys	95	50.0	52.1	62.5	48.6	50.0	53.0	50.4	53.6	53.9	50.0	51.0	45.5	51.7	51.6	20.0	52.5	50.4	54.7
Girls	89	50.0	47.9	37.5	51.4	50.0	47.0	49.6	46.4	46.2	50.0	55.2	54.6	48.3	48.4	80.0	47.5	49.6	45.3
Day care attendance	101	57.1	54.2	65.0	52.1	56.0	54.0	55.7	53.6	60.3	50.9	55.2	54.6	65.5	52.9	80.0	54.2	55.7	52.8
ETS	82	38.1	46.5	40.0	45.8	40.5	48.0	43.5	46.4	44.9	44.3	43.8	45.5	51.7	43.2	40.0	44.7	47.3	37.7
Wall-to-wall carpet	91	42.9	51.4	45.0	50.7	44.1	54.0	48.7	50.7	44.9	52.8	50.0	48.9	51.7	49.0	40.0	49.7	49.6	49.1
Condensation	125	66.7	68.3	70.0	67.4	73.8	63.0	70.4	63.8	69.2	67.0	69.8	65.9	65.5	68.4	60.0	68.2	67.2	69.8
Mold growth	95	50.0	52.1	50.0	52.1	57.1	47.0	53.9	47.8	48.7	53.8	53.1	50.0	**27.6**	**56.1****	60.0	51.4	49.6	56.6
Water leakage	19	9.5	10.6	15.0	9.0	14.3	7.0	13.0	5.8	10.3	10.4	8.3	12.5	10.3	10.3	20.0	10.1	8.4	15.1
Pet keeping	43	19.1	24.7	20.0	24.3	25.0	22.0	22.6	24.6	24.4	22.6	20.8	26.1	27.6	22.6	20.0	23.5	25.2	18.9

X2 test or t-test

*: p<0.05; **: p<0.01

Supplementary Table 3

The associations between cord blood IgE and children allergies and infectious diseases at each age and from birth to 7 year of age

	total					boys				girls			
	OR[F], RR[£]	95% CI		p value	p for interaction	OR[F], RR[£]	95% CI		p value	OR[F], RR[£]	95% CI		p value
1.5y[a]													
Eczema	1.07	0.79	1.43	0.667	0.776	1.05	0.72	1.54	0.784	1.07	0.63	1.82	0.802
Wheeze	0.88	0.66	1.17	0.378	0.910	0.88	0.63	1.24	0.471	0.90	0.46	1.75	0.747
Food allergy	**1.29**	**1.00**	**1.66**	**0.052†**	0.864	**1.30**	**0.96**	**1.76**	**0.086†**	1.36	0.82	2.23	0.232
Any allergies	1.04	0.84	1.28	0.723	0.632	1.04	0.81	1.34	0.771	1.07	0.72	1.60	0.721
Otitis media	1.15	0.88	1.49	0.304	0.916	1.19	0.86	1.64	0.291	1.10	0.65	1.86	0.716
Any infectious diseases	1.13	0.88	1.46	0.332	0.549	1.22	0.89	1.68	0.222	1.03	0.64	1.66	0.893
3.5y[b]													
Eczema	0.94	0.72	1.23	0.664	0.851	0.92	0.63	1.33	0.642	0.92	0.61	1.40	0.702
Wheeze	0.93	0.67	1.30	0.690	0.154	0.67	0.39	1.16	0.150	1.23	0.73	2.08	0.428
Food allergy	1.01	0.79	1.28	0.965	0.905	1.04	0.78	1.40	0.790	0.90	0.56	1.45	0.664
Any allergies	0.92	0.75	1.14	0.469	0.402	0.87	0.65	1.15	0.328	0.94	0.65	1.36	0.750
Otitis media	1.14	0.91	1.43	0.239	0.364	**1.32**	**0.95**	**1.82**	**0.093†**	0.97	0.67	1.41	0.873
Chicken pox	0.82	0.64	1.05	0.117	0.885	0.81	0.57	1.14	0.224	0.87	0.59	1.28	0.467
Bronchitis/pneumonia	0.99	0.76	1.31	0.967	0.195	1.15	0.80	1.66	0.438	0.81	0.52	1.26	0.346
Any infectious diseases	0.98	0.79	1.23	0.879	0.269	1.11	0.80	1.53	0.540	0.86	0.61	1.23	0.416
7y[c]													
Eczema	1.14	0.88	1.49	0.319	0.245	1.24	0.87	1.78	0.240	1.04	0.68	1.58	0.860
Wheeze	0.87	0.64	1.17	0.356	0.166	0.75	0.51	1.11	0.154	1.32	0.75	2.35	0.341
Food allergy	1.06	0.80	1.41	0.680	0.746	1.04	0.73	1.47	0.837	1.08	0.62	1.89	0.786
Any allergies	1.04	0.83	1.31	0.732	0.934	1.01	0.75	1.36	0.941	1.05	0.71	1.55	0.812
Otitis media	1.01	0.80	1.28	0.910	0.404	0.94	0.70	1.27	0.701	1.13	0.75	1.70	0.566
Chicken pox	0.93	0.73	1.17	0.510	0.287	1.05	0.78	1.40	0.766	0.76	0.50	1.15	0.194
Bronchitis/pneumonia	1.17	0.87	1.57	0.294	0.551	1.07	0.71	1.59	0.753	1.35	0.85	2.14	0.208
Any infectious diseases	1.05	0.81	1.36	0.737	0.701	1.09	0.79	1.51	0.601	0.95	0.60	1.49	0.817
From birth to 7y[d]													
Eczema													
Wheeze	0.85	0.66	1.09	0.205	0.058	0.74	0.54	1.00	0.052	1.08	0.74	1.59	0.683
Food allergy	1.18	0.91	1.52	0.208	0.415	1.24	0.87	1.75	0.232	1.16	0.75	1.79	0.497
Any allergies	1.03	0.85	1.23	0.791	0.259	0.95	0.72	1.25	0.730	1.11	0.85	1.45	0.440
Otitis media	**1.31**	**1.04**	**1.65**	**0.021***	0.364	1.24	0.92	1.67	0.167	**1.50**	**1.04**	**2.18**	**0.032***
Chicken pox	0.99	0.83	1.18	0.917	0.433	0.90	0.73	1.12	0.342	1.14	0.85	1.54	0.381
Bronchitis/pneumonia	0.98	0.77	1.24	0.865	0.996	1.03	0.73	1.45	0.877	0.94	0.67	1.33	0.736
Any infectious diseases	**1.50**	**1.20**	**1.87**	**<0.001****	0.577	**1.37**	**1.03**	**1.83**	**0.031***	**1.69**	**1.21**	**2.38**	**0.002****

a: adjusted for gender, smoking at early pregnancy, maternal history of allergies, parity, maternal age at delivery, household income, ETS at 1.5y, and day care attendance at 1.5y.
b: adjusted for gender, maternal history of allergies, parity, maternal age at delivery, household income, ETS at 3.5y, and day care attendance at 3.5y.
c: adjusted for gender, maternal history of allergies, parity, maternal age at delivery, household income, ETS at 7y, and day care attendance at 7y.
d: adjusted for maternal age at delivery, household income, maternal history of allergies, parity, sex, and day care attendance; Interaction term between child's age at follow-up and IgE levels using generalized equation estimates.
*: p< 0.05; †: p< 0.1; ₣: odds ratio for each outcome at 1.5y, 3.5y, and 7y of age; £: relative risk for each outcome from birth to 7 years of age.

Supplementary Table 4

The associations between prenatal MEHP exposure and children allergies and infectious diseases at each age and from birth to 7 year of age

	total					boys				girls			
	OR[₣], RR[£]	95% CI		p value	*p* for interaction[ɸ]	OR[₣], RR[£]	95% CI		p value	OR[₣], RR[£]	95% CI		p value
1.5y[a]													
Eczema	0.83	0.36	1.93	0.672	0.906	0.74	0.21	2.57	0.636	0.85	0.22	3.26	0.807
Wheeze	1.19	0.56	2.53	0.655	0.918	0.96	0.37	2.49	0.934	1.56	0.35	6.98	0.560
Food allergy	1.82	0.96	3.46	0.067	0.458	**3.40**	**1.05**	**10.96**	**0.041***	1.54	0.55	4.31	0.409
Any allergies	1.26	0.72	2.21	0.417	0.781	1.65	0.73	3.71	0.229	1.05	0.42	2.64	0.916
Otitis media	**2.65**	**1.31**	**5.39**	**0.007****	0.175	**5.72**	**1.47**	**22.33**	**0.012***	2.90	0.79	10.67	0.109
Any infectious diseases	**2.65**	**1.31**	**5.39**	**0.007****	0.464	**5.72**	**1.47**	**22.33**	**0.012***	2.80	0.92	8.52	0.071†
3.5y[b]													
Eczema	0.76	0.36	1.59	0.461	0.666	0.83	0.27	2.55	0.750	0.69	0.21	2.26	0.543
Wheeze	1.03	0.42	2.49	0.956	0.975	0.97	0.14	6.70	0.971	1.25	0.36	4.30	0.728
Food allergy	1.78	0.92	3.45	0.087	0.524	2.24	0.79	6.35	0.130	2.06	0.69	6.11	0.194
Any allergies	1.03	0.59	1.81	0.921	0.464	1.26	0.54	2.95	0.593	1.13	0.46	2.77	0.784
Otitis media	**2.45**	**1.24**	**4.86**	**0.010****	0.816	**4.37**	**1.06**	**17.96**	**0.041***	**2.95**	**1.10**	**7.86**	**0.031***
Chicken pox	1.39	0.74	2.61	0.308	**0.016**	0.47	0.16	1.40	0.175	2.48	0.94	6.51	0.066†
Bronchitis/pneumonia	1.06	0.31	3.63	0.932	0.715	n.a				1.25	0.31	5.14	0.753
Any infectious diseases	**2.82**	**1.30**	**6.12**	**0.009**	0.525	2.81	0.75	10.50	0.126	**3.53**	**1.18**	**10.53**	**0.024***
7y[c]													
Eczema	1.50	0.75	3.02	0.254	0.860	1.45	0.43	4.87	0.545	0.58	0.17	2.01	0.392
Wheeze	0.75	0.29	1.97	0.565	0.895	0.69	0.18	2.68	0.588	0.63	0.10	3.94	0.620
Food allergy	0.73	0.29	1.84	0.502	0.721	0.89	0.23	3.43	0.869	0.47	0.09	2.55	0.377
Any allergies	1.14	0.60	2.19	0.688	0.789	1.16	0.41	3.25	0.780	0.53	0.17	1.72	0.291
Otitis media	1.04	0.54	2.01	0.897	0.901	1.23	0.44	3.43	0.696	0.88	0.26	3.00	0.843
Chicken pox	1.17	0.60	2.27	0.654	0.406	1.74	0.58	5.17	0.322	0.65	0.22	1.89	0.424
Bronchitis/pneumonia	0.75	0.29	1.96	0.558	0.462	1.32	0.30	5.83	0.713	0.26	0.04	1.57	0.144
Any infectious diseases	1.32	0.62	2.80	0.476	**0.040***	3.72	0.89	15.57	0.072†	0.47	0.14	1.57	0.221
From birth to 7y[d]													
Eczema	1.18	0.74	1.88	0.481	0.814	1.08	0.57	2.03	0.819	1.39	0.62	3.12	0.420
Wheeze	n.a				n.a	0.85	0.36	1.99	0.711	n.a			
Food allergies	1.43	0.90	2.26	0.128	0.492	n.a				1.37	0.69	2.73	0.365
Any allergies	1.29	0.84	1.98	0.255	0.575	1.44	0.74	2.81	0.287	n.a			
Otitis media	**1.93**	**1.21**	**3.07**	**0.006****	0.967	1.97	0.96	4.04	0.063†	n.a			
Chicken pox	1.44	0.92	2.25	0.115	0.238	0.94	0.51	1.76	0.856	1.85	0.85	4.06	0.123
Any infectious diseases	**2.69**	**1.62**	**4.48**	**<0.001****	0.404	**3.16**	**1.49**	**6.73**	**0.003****	**2.79**	**1.27**	**6.13**	**0.011***

a: adjusted for gender, smoking at early pregnancy, maternal history of allergies, parity, maternal age at delivery, household income, ETS at 1.5y, day care attendance at 1.5y, and cord blood IgE level.

b: adjusted for gender, maternal history of allergies, parity, maternal age at delivery, household income, ETS at 3.5y, day care attendance at 1.5y, and cord blood IgE level.

c: adjusted for gender, maternal history of allergies, parity, maternal age at delivery, household income, ETS at 7y, day care attendance at 1.5y, and cord blood IgE level.

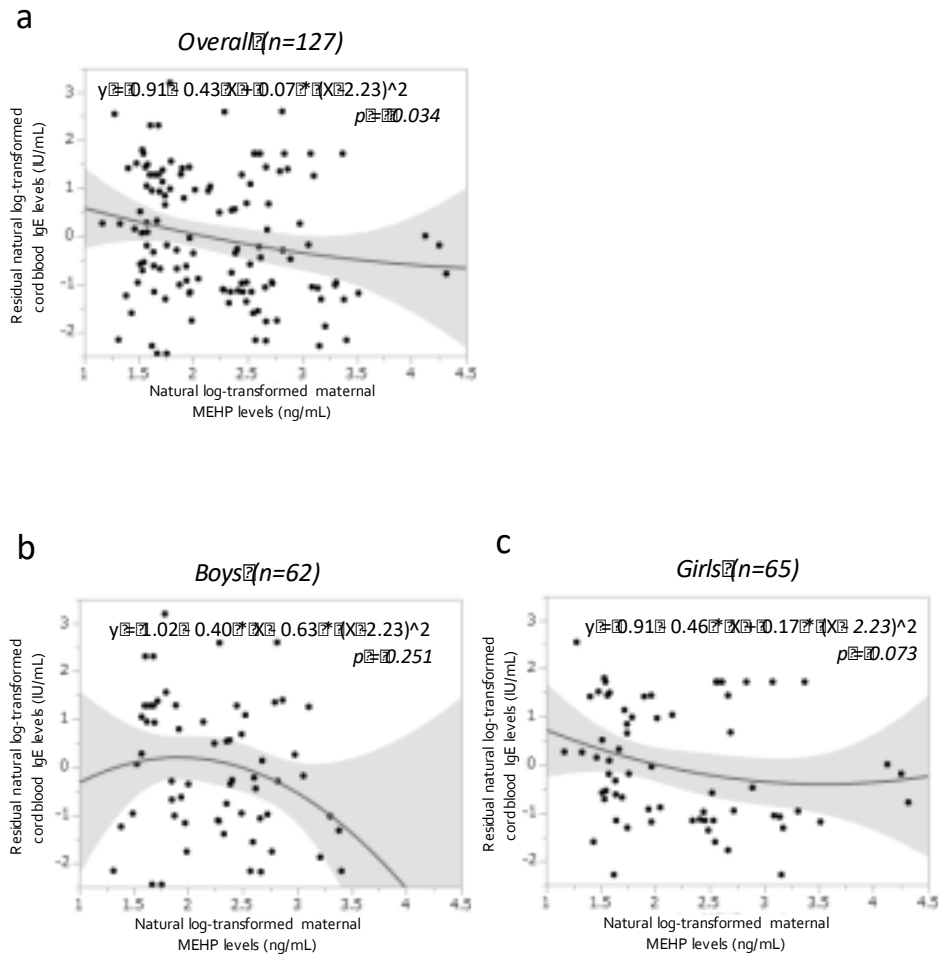
d: adjusted for sex, history of maternal allergies, maternal age at delivery, parity, maternal smoking, day care attendance, household income, and cord blood IgE level; Interaction term between child's age at follow-up and MEHP levels using generalized equation estimates.

ɸ: The interaction terms of sex x MEHP levels were assigned as male=1 and female=0, respectively.

*: p< 0.05; †: p< 0.1; ₣: odds ratio for each outcome at 1.5y, 3.5y, and 7y of age; £: relative risk for each outcome from birth to 7 years of age; n.a: could not converge the analysis because of lack of numbers of sample.

Supplementary Figure 1

Associations between maternal natural log-transformed MEHP (ng/mL) and residual of potential confounding variables to natural log-transformed cord blood IgE levels (IU/mL)



- The solid lines denote the predicted fit from a quadratic regression model with 95% confidence interval (gray).
- Individual observations of maternal natural log-transformed MEHP (ng/mL) and residual of potential confounding variables to natural log-transformed cord blood IgE levels (IU/mL) are shown as plots.
- Corresponding estimates are presented in Table 3.

(a) Among overall. (b) Among boys. (c) Among girls.