Environment International 145 (2020) 106134

Contents lists available at ScienceDirect

Environment International

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

# Prenatal and childhood exposure to phthalic acid esters and vaccination antibodies in children: A 15-year follow-up birth cohort study

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ARTICLE INFO

Handling editor: Da Chen

*Keywords:*
Birth cohort
Phthalate
Children
Antibody
Vaccine
Hepatitis B

ABSTRACT

Phthalic acid esters (PAE) are widely used during chemical synthesis and do not form covalent linkages with products. It has been reported that exposure to PAE affects the immune response. However, their effect on antibody concentrations in children is still under investigation. We aimed to examine the association between early-life phthalate exposure and antibody concentrations in children in a longitudinal birth cohort established in 2000–2001.

We recruited 398 neonates in central Taiwan and followed them up every 2–3 years, with various antibody-related studies at 11- and 14-year follow-ups. Seven urinary phthalate metabolites were quantified in mothers during pregnancy and children aged 11 years. Four antibody concentrations were analyzed in children aged 11 and 14 years. The percent change in antibody concentrations from ages 11 to 14 years was calculated and its association with phthalate exposure was evaluated via multivariate regression analysis.

Eighty-one followed-up children were with sufficient data. After adjusting for prenatal exposure and other confounders, double concentrations of the urinary sum of di-2-ethylhexyl phthalate (ΣDEHPm) and mono-n-butyl phthalate (MnBP) were associated with a 18.06% (95% CI = 3.34%, 32.78%) and 22.53% decrease (95% CI = 3.39%, 41.66%) in antibody concentration against hepatitis B, respectively.

Phthalate exposure was found to be related to decreased antibody concentrations against hepatitis B (DEHP, DBnP) in the early teens. This exposure is suggested to be considered for clinical re-booster vaccines among junior high school students. Further verification with additional cohorts and studies on the underlying mechanisms of phthalate exposure are warranted.

## 1. Introduction

Phthalic acid esters (PAEs) are widely used as plasticizers, stabilizers, and/or solvents for manufacturing numerous items of daily use (Wittassek et al., 2011, Wormuth et al., 2006). PAEs are prominently released into air, water, and/or dust owing to their non-linkage with materials (Wormuth et al., 2006). Therefore, humans are potentially frequently exposed to PAEs through ingestion, inhalation, dermal contact, and/or use of medical devices.

Immune development in childhood is potentially associated with the

*Abbreviations:* AM, arithmetic mean; AntiHBs, antibody against hepatitis B; BBzP, benzyl butyl phthalate; CI, confidence interval; DAG, DAGitty; DEP, diethyl phthalate; DEHP, di-2-ethylhexyl phthalate; DMP, dimethyl phthalate; DnBP, Di-n-butyl phthalate; EDC, endocrine-disrupting chemicals; GM, geometrical mean; HBV, hepatitis B virus; INF, interferon; IQR, interquartile range; LD, limit of detection; MEP, mono-ethyl phthalate; MMP, mono-methyl phthalate; MnBP, mono-n-butyl phthalate; MBzP, mono-benzyl phthalate; MEHP, mono-2-ethylhexyl phthalate; MEHHP, mono-2-ethyl-5-hydroxyhexyl phthalate; MEOHP, mono-2-ethyl-5-oxohexyl phthalate; PAE, phthalic acid esters; pDCs, plasmacytoid dendritic cells; PFOA, Perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; SD, standard deviation.

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[1] RID: C-1367-2010.

https://doi.org/10.1016/j.envint.2020.106134
Received 30 January 2020; Received in revised form 28 August 2020; Accepted 9 September 2020
Available online 1 October 2020

endocrine system. Immune cells have hormone receptors and can thus receive and respond to endocrine signals (DeWitt and Patisaul 2018). Environmental endocrine-disrupting chemicals (EDCs) can mimic hormones, disrupt normal hormone function, and affect endocrine regulation (Diamanti-Kandarakis et al., 2009, Yilmaz et al., 2019). EDC exposure may therefore potentially affect the immune system.

For protection against infectious diseases, neonates are vaccinated from birth till preschool (vaccines against diphtheria, tetanus, rubella, hepatitis B, and mumps). For example, infants receive the first dose of the routine hepatitis B vaccination within 24 h postpartum, and the second and third dose at 1 and 6 months, respectively. However, the immune response against hepatitis B declines with time after routine vaccination (Gold et al., 2003). The fall-off patterns of antitoxic immunity for diphtheria and tetanus were also observed in an 8-year follow-up study (Simonsen et al., 1996). It is important to investigate the factors associated with this decreased antibody concentration. Age, gender, diet, nutrition, body mass index, and environmental exposure are all related to the humoral immune response and may affect antibody concentrations (Lin et al., 2017, MacGillivray and Kollmann 2014, Van Loveren et al., 2001).

In previous studies, EDC exposure [i.e. per- and polyfluoroalkyl substances (PFASs) and heavy metals] was observed to be associated with decreased antibody concentrations against vaccines (Grandjean et al., 2017, Lin et al., 2017). Exposure to higher perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) level in the serum at 5 years of age was associated with lower antibody levels against tetanus and diphtheria in children aged 7 years (Grandjean et al., 2017). In a follow-up study on e-waste-exposed preschool children, a negative association was found between higher blood levels of lead, copper, and zinc and antibody titer against diphtheria, pertussis, tetanus, hepatitis B, Japanese encephalitis, polio, and measles (Lin et al., 2017).

PAEs are well-established EDCs and were found to be associated with immune response and allergies (Ait Bamai et al., 2018, Ku et al., 2015). Li et al. (2017) reported a positive correlation between benzyl butyl phthalate (BBzP) exposure and an increased risk of childhood asthma in a systematic review and *meta*-analysis. We previously reported that phthalate exposure, particularly diethyl phthalate (DEP) and di-2-ethylhexyl phthalate (DEHP), is associated with asthma development in 8-year-old children (Ku et al., 2015). Phthalate was observed to interfere with the balance of the Th1/Th2 immune response (Han et al., 2014), enhance secretion of inflammatory biomarkers (i.e., eosinophils, interleukin [IL]-6, tumor necrosis factor α) (Bolling et al., 2012, Deutschle et al., 2008), and increase production of IL-4 and IL-13 (Shen et al., 2017, You et al., 2016). Kuo et al. (2013) found that phthalates suppress type I interferons (INFs) in human plasmacytoid dendritic cells (pDCs) that are important for protection against infections and regulate adaptive immunity. However, the effect of phthalate exposure on antibody concentration in children remains understudied.

Children are more susceptible and vulnerable to environmental exposure than adults. They are still in their early growth and developmental stages; therefore, they are more susceptible to the health impact of environmental exposure. Their hand-to-mouth behavior and lack of knowledge of health risks associated with environmental exposure may augment their exposure to various environment pollutants, such as the widely used phthalate plasticizers. Antibody responses against infections through childhood vaccination potentially reflect immune function in children. A lower antibody concentration decreases the ability of the immune system to counter infectious diseases, thus increasing the risk of infections and diseases. Hence, this study aimed to examine the association between phthalate exposure and antibody concentrations resulting from mass vaccination. Two basic types of vaccines are used for vaccination: inactivated vaccine (i.e. vaccine against diphtheria, tetanus, and hepatitis B) and live attenuated vaccine (i.e. vaccine against measles, mumps, and rubella). With references of previous studies of EDC exposure and antibody concentrations, we investigated the association between prenatal and childhood phthalate exposure and concentrations of antibodies against diphtheria, tetanus, rubella, and hepatitis B in children through a 15-year longitudinal follow-up birth cohort study established in 2000–2001.

## 2. Material and methods

### 2.1. Study cohort

Pregnant women without clinical complications between the ages of 25 and 35 years, who visited our collaborated medical center for pregnancy consultations, were invited to participate in the study between 2000 and 2001. In total, 427 women in their third trimester of pregnancy were invited and interviewed after providing written informed consent. Among them, 398 neonates were enrolled in the follow-up study (Fig. 1). Children were followed-up every 2–3 years. When children were 6 years of age or at later follow up, along with the primary caretaker, they also provided written informed consent. The study

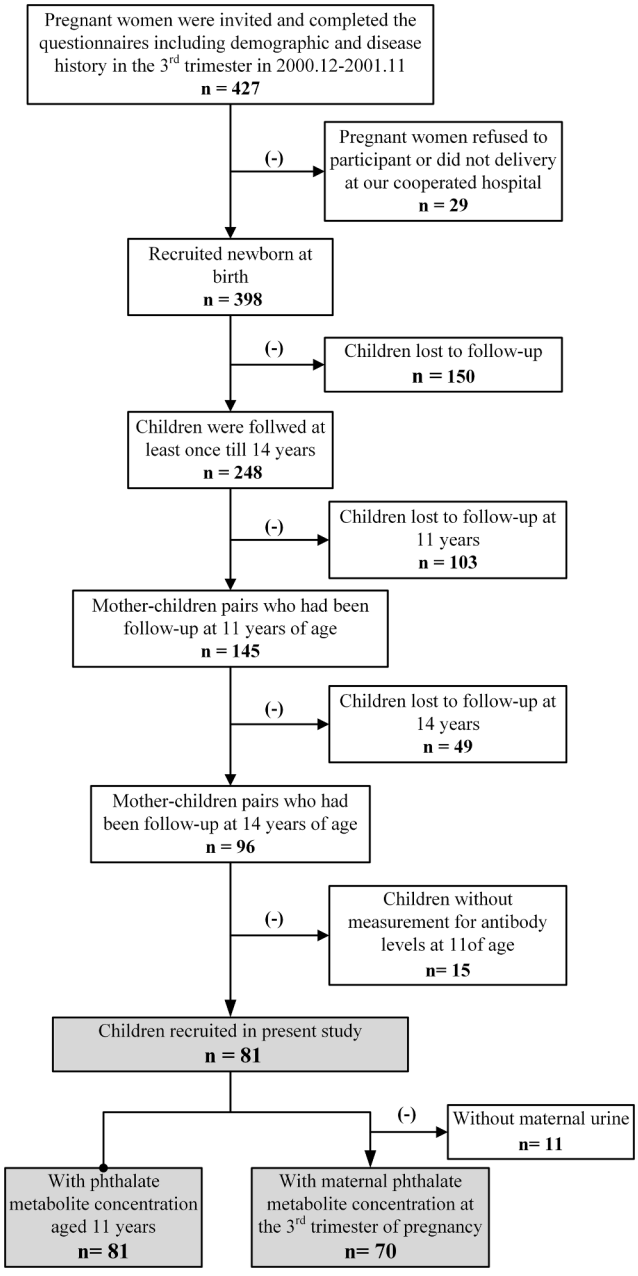


**Fig. 1.** Schematic representation of subject recruitment.

process was approved by the Research Ethics Committee of the National Health Research Institutes and Chung Shan Medical University Hospital in Taiwan. Children followed up at both ages of 11 and 14 years (i.e., 5th and 8th grade, respectively) were enrolled herein.

### 2.2. Data collection

Structured questionnaires were completed by pregnant women in the third trimester of pregnancy. Children or their primary caretakers also filled out the questionnaire at each follow-up visit. Data regarding demographic characteristics of the participants, dietary habits, medical status, and environmental conditions were obtained from these questionnaires. Maternal urine was collected from subjects during the third trimester of pregnancy (28–38 weeks). Peripheral blood and urine samples were collected from the children at each follow-up visit. The procedure for obtaining questionnaire data and specimen collection for children has been previously described in detail (Lin et al., 2011b).

### 2.3. Covariates

The potential covariates included gender of the child, BMI of 11-year-old children, parental education, gestational week, maternal age at childbirth, and serum PFOS concentration in pregnant women and 11-year-old children. The child's gender, parental education (<12 years and ≥ 12 years), gestational week, and maternal age at childbirth were obtained from the questionnaire. BMI was calculated as body weight (g) divided by the square of the height (m). In addition, per- and polyfluoroalkyl substances (PFASs) were observed to be associated with immune response. According to a previous study (Grandjean et al., 2012), total PFAS (tPFAS) exposure can be calculated via the sum of PFASs. Due to high correlation between PFASs and perfluorooctane sulfonic acid (PFOS) and that a majority of tPFASs are derived from PFOS, we therefore used PFOS as an indicator of tPFAS exposure factor. These covariates were considered because they were related to phthalate metabolite concentration (Tsai et al., 2016, Wen et al., 2017) and antibody levels (Grandjean et al., 2017, MacGillivray and Kollmann 2014, Van Loveren et al., 2001). We then put these factors in the directed acyclic graph (DAG) analysis for selecting potential confounders of multiple regression model.

### 2.4. Measurement of phthalate metabolites

Urinary monoesters are the major urinary metabolites of PAEs and are commonly used as internal exposure indices. Herein, urine concentrations (μg/L) of seven phthalate metabolites of the five most commonly used PAEs were analyzed via quantitative liquid chromatography-tandem mass spectrometry (LC-MS/MS), as previously described (Lin et al., 2011a), among pregnant women and their children aged 11 years; these included mono-ethyl phthalate (MEP) for diethyl phthalate (DEP), mono-methyl phthalate [MMP] for dimethyl phthalate (DMP), mono-n-butyl phthalate [MnBP] for di-n-butyl phthalate (DnBP), mono-benzyl phthalate (MBzP) for benzyl butyl phthlate (BBzP), and mono-2-ethylhexyl phthalate (MEHP), mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP) and mono-2-ethyl-5-oxohexyl phthalate (MEOHP) for di-2-ethyl-hexyl phthalate (DEHP). In brief, we prepared 0.1-mL urine sample aliquots containing 1 M ammonium acetate (20 μL), β-glucuronidase (10 μL), and a mixture of isotopic phthalate metabolite standards. The samples were incubated at 37 °C for 1.5 h and treated with 270 μL solvent (0.1% formic acid and 5% acetonitrile) in glass screw-cap vials and mixed for quantitative LC-MS/MS after hydrolysis.

For urinary dilution correction, measured phthalate metabolite levels were divided on the basis of urinary creatinine levels and expressed as "μg/g creatinine." Urinary creatinine levels were measured using a spectrophotometric method at Kaohsiung Medical University Chung-Ho Memorial Hospital. The sum of the DEHP metabolite levels (∑DEHPm) (nmol/g creatinine) was estimated as the sum of MEHP, MEHHP, and MEOHP. The measured concentration of the blank sample below two-fold of the detection limit value for urinary metabolites was the standard for quality control. The limit of detection (LOD) values of phthalate metabolites were 0.55, 0.23, 0.26, 0.99, 1.6, 3.4, and 2.2 μg/mL for MEHP, MEHHP, MEOHP, MBzP, MnBP, MMP, and MEP, respectively. The recovered amounts measured for the internal standards added was required to be 80% to 120%. Phthalate metabolite levels under the detection limits were replaced by a value equal to half the LOD value. Furthermore, we annually participated in the intercomparison program in the German External Quality Assessment Scheme for Biological Monitoring (G-EQUAS) for external quality assurance of the measurement of urinary phthalate metabolites.

### 2.5. Antibody assessment

The concentration of antibodies against diphtheria (IU/mL), tetanus (IU/mL), rubella (IU/mL), and hepatitis B (AntiHBs) (IU/L) in venous blood was measured in children aged 11 and 14 years. Antibodies against diphtheria and tetanus were assessed using enzyme-linked immunosorbent assay (TECAN ELISA Reader, EUROIMMUN) and rubella and AntiHBs were measured using an electrochemiluminescence immunoassay (Cobas e601, Cobas 6000). All experiments were performed at the clinical diagnostic laboratory.

### 2.6. Statistical analysis

All statistical analyses were performed by using JMP version 13.0 (SAS Institute Inc., Cary, NC, USA). The correlation between urinary phthalate metabolite concentrations and antibody levels was assessed using Pearson's correlation coefficient. The percent change (%) of antibody concentrations (AC) in children aged 11 and 14 years was calculated using the following equation:

$$\text{Percent change of AC } (\%) = \frac{\text{AC at 14 years old} - \text{AC at 11 years old}}{\text{AC at 11 years old}} \times 100$$

DAGitty (DAG) analysis was performed to select potential covariates for multivariate analysis. On DAG analysis, gender of child, BMI at 11 years of age, maternal education, serum PFOS in pregnant women/11-year-old children were considered potential covariates and therefore adjusted through multivariable analysis (Fig. s2). Multivariant regression analysis was performed to evaluate the association between phthalate metabolites and antibody concentrations in children. Phthalate metabolite concentrations were $\log_2$-transformed owing to their skewed distribution, and the effect was assessed as doubling of phthalate exposure. A $P$-value of $< 0.05$ in a two-sided test was considered statistically significant.

## 3. Results

In total, 248 children were followed up at least once until the age of

**Table 1**
Characteristics of mothers and their children (n = 81).

| Variables | Mean (SD)[¶]/n (%) |
|---|---|
| Maternal age at childbirth (year) | 29.78 (3.66)[¶] |
| Missing | 0 |
| Maternal weight gain during pregnancy (kg) | 11.33 (4.91)[¶] |
| Missing | 3 (3.7) |
| *Gender of child* | |
| Boys | 37 (45.7) |
| Girls | 44 (54.3) |
| Missing | 0 |
| Birthweight (g) | 3138.06 (414.93)[¶] |
| Low birth weight (<2500 g) | 2 (2.5) |
| Missing | 1 (1.2) |
| Gestational week (weeks) | 38.93 (1.47)[¶] |
| Preterm birth (<37 weeks) | 3 (3.7) |
| Missing | 1 (1.2) |
| *Birth order* | |
| 1st | 43 (53.1) |
| 2nd | 27 (33.3) |
| ≥3rd | 8 (9.9) |
| Missing | 3 (3.7) |
| Child's BMI at 11 years of age | 18.93 (3.97)[¶] |
| Missing | 0 |
| *Maternal education* | |
| ≤12 years | 32 (39.5) |
| >12 years | 45 (55.6) |
| Missing | 4 (4.9) |
| *Paternal education* | |
| ≤12 years | 30 (37.0) |
| >12 years | 46 (56.8) |
| Missing | 5 (6.2) |
| *Smoking before pregnancy* | |
| Yes | 3 (3.7) |
| No | 74 (91.4) |
| Missing | 4 (4.9) |
| *Smoking during pregnancy* | |
| Yes | 0 |
| No | 80 (98.8) |
| Missing | 1 (1.2) |
| *Alcohol drinking during pregnancy* | |
| Yes | 1 (1.2) |
| No | 77 (95.1) |
| Missing | 3 (3.7) |
| *ETS before pregnancy* | |
| Yes | 38 (46.9) |
| No | 38 (46.9) |
| Missing | 5 (6.2) |

14 years. Data on titers of antibodies at age 11 and 14 years, together with data of phthalate metabolites at 11 years of age were available for 81 children. Furthermore, 11 children were excluded owing to lack of maternal urine samples (Fig. 1).

The characteristics of recruited children and their parents are illustrated in Table 1. The prevalence of low birth weight and preterm delivery were 2.5% and 3.7%, respectively. More than half of the parents had > 12 years of education, representing education levels beyond college or university level. Table s1 outlines the characteristics of children included (n = 81) and excluded (n = 167) from the study among those followed up at least once. Mothers of the included children were slightly older than those of the excluded children. The concentration of antibody against diphtheria in children aged 11 years was significant higher in included children than in excluded ones. No significant difference in phthalate metabolite concentrations was observed between included children and excluded children (Table s1). Influential outlier points for antibody concentrations were excluded from further analysis, based on value 3-fold those of the interquartile range (Table s2). The descriptive statistics of urinary phthalate metabolites in mothers in the third trimester of pregnancy and 11-year-old children and serum antibodies in 11- and 14-year-old children are provided in Table 2.

The association between prenatal and childhood phthalate metabolites and antibody concentration in children aged 11 and 14 years are shown in Table 3. Among the maternal urinary phthalate metabolites, MEP positively correlated with antibody concentration against tetanus in children aged 11 years, after adjusting for the child's gender, BMI at 11 years of age, maternal education, and maternal PFOS concentration. MEHP was associated with increased antibody concentration against tetanus in children aged 14 years. Among phthalate metabolites in 11-year-old children, MEOHP was negatively associated with the concentration of antibodies against diphtheria in children aged 11 years, after adjusting for the child's gender, BMI at 11 years of age, maternal education, and PFOS concentration of 11-year-old children. MEHHP, MEOHP, and ∑DEHPm were also negatively associated with antibodies against diphtheria in children aged 14 years. The association between phthalate metabolite concentration and antibody levels without adjusted covariates is shown in Table s3.

Furthermore, regarding the percent change in antibody concentration from 11 to 14 years of age, we further excluded children with no change (the percent change = 0) in antibody concentrations. Specifically, there were two children for diphtheria, one child for tetanus, one child for rubella, and twenty children for AntiHBs excluded for the above reason. Table s4 demonstrates the relationship between covariates selected by DAG analysis and the percent change in antibody concentrations in children aged 11 to 14 years. Boys and children of parents with > 12 years of education had the percent change in the more decrease of antibody against diphtheria. The percent change in the increase of antibody concentration against tetanus was observed in children aged 11 years with higher BMI.

After adjusting for covariates, no significant association was observed between maternal phthalate metabolite concentrations and the percent change in the four antibody concentrations in children aged 11–14 years (Table 3). Nevertheless, urinary MnBP, MEHHP, MEOHP, and ∑DEHPm were associated with the percent change in the decrease of antibody concentration against HBV at 11–14 years of age, after adjusting for the child's gender, BMI at 11 years of age, serum PFOS concentrations at 11 years of age, maternal education, and maternal urinary phthalate metabolite concentrations. Double the concentrations of urinary MnBP, MEHHP, MEOHP, and ∑DEHPm were associated with a 22.53% (95% confidence interval, CI = 3.96–41.66%), 18.36% (95% CI = 3.33–33.38%), 10.88% (95% CI = 2.77–18.99%), and 18.06% (95% CI = 3.34–32.78%) decrease of antibody concentration against HBV, respectively (Table 3). Scatter plots of linear regression and the quadratic model of MEHP, MEHHP, and ΣDEHPm in children at 11 years of age and the percent change in antibody concentrations in children are further illustrated in Fig. s1. The association between phthalate metabolite concentration and percent change in antibody concentrations without adjusted covariates is shown in Table s3.

## 4. Discussion

This study describes a 15-year follow-up evaluation to investigate the association between prenatal and childhood phthalate exposure and changes in antibody concentrations in children. The present results show that childhood phthalate exposure was associated with a decrease in antibody concentrations. DBP and DEHP at 11 years of age were associated with a decrease in antibody concentration against HBV at 11–14 years of age. Moreover, prenatal PAE exposure was not significantly associated with changes in antibody concentration in children. To our knowledge, this is the first study to determine an association between childhood phthalate exposure and the change in the vaccinated antibody concentration upon routine vaccination.

In May 2011 in Taiwan, an episode of phthalate exposure occurred owing to the illegal supplementation of DEHP and diisononyl phthalate (DiNP) in foodstuffs as a clouding agent to replace palm oil (Li and Ko 2012). The foodstuffs that children frequently used, including tea

**Table 2**
Concentration of urinary phthalate metabolites in mothers and 11-year-old children and serum antibodies in 11- and 14-year-old children.

| Variables | n | GM | 95% CI | Median | IQR | Min ~ Max | % <LD |
|---|---|---|---|---|---|---|---|
| *Phthalate metabolites (μg/g creatinine)* | | | | | | | |
| Mothers | | | | | | | |
| MMP | 70 | 49.03 | 37.62, 63.91 | 48.34 | 26.01 ~ 99.59 | 0.57 ~ 377.70 | 4.3 |
| MEP | 70 | 63.16 | 50.73, 78.65 | 55.41 | 36.29 ~ 100.73 | 10.32 ~ 681.82 | 0 |
| MnBP | 70 | 72.76 | 57.87, 91.48 | 70.48 | 38.94 ~ 165.63 | 8.10 ~ 613.29 | 0 |
| MBzP | 70 | 14.95 | 12.1, 18.48 | 16.77 | 9.20 ~ 26.43 | 0.17 ~ 109.60 | 2.9 |
| MEHP | 70 | 20.19 | 16.05, 25.40 | 18.82 | 10.72 ~ 36.83 | 3.89 ~ 706.03 | 0 |
| MEHHP | 70 | 7.61 | 4.81, 12.04 | 13.30 | 3.85 ~ 21.77 | 0.06 ~ 491.33 | 14.3 |
| MEOHP | 70 | 14.03 | 9.70, 20.29 | 16.23 | 7.52 ~ 33.73 | 0.10 ~ 1011.56 | 4.3 |
| ΣDEHPm (nmole/g creatinine) † | 70 | 190.96 | 150.13, 242.90 | 197.34 | 113.51 ~ 344.87 | 17.95 ~ 7666.16 | – |
| 11-year-old children | | | | | | | |
| MMP | 81 | 8.22 | 5.89, 11.47 | 12.07 | 4.01 ~ 21.70 | 0.06 ~ 125.89 | 11.1 |
| MEP | 81 | 7.35 | 4.91, 11.00 | 12.57 | 1.86 ~ 23.51 | 0.10 ~ 184.67 | 23.5 |
| MnBP | 81 | 49.13 | 43.13, 55.97 | 48.88 | 36.44 ~ 72.09 | 13.02 ~ 212.83 | 0 |
| MBzP | 81 | 3.37 | 2.63, 4.31 | 3.36 | 1.68 ~ 6.97 | 0.19 ~ 77.13 | 12.3 |
| MEHP | 81 | 10.20 | 8.31, 12.52 | 11.58 | 5.72 ~ 19.25 | 0.73 ~ 91.59 | 6.2 |
| MEHHP | 81 | 33.34 | 28.41, 39.13 | 31.29 | 21.11 ~ 52.90 | 2.68 ~ 261.17 | 0 |
| MEOHP | 81 | 21.44 | 15.87, 28.96 | 25.74 | 17.54 ~ 37.35 | 0.09 ~ 229.78 | 6.2 |
| ΣDEHPm (nmole/g creatinine) † | 81 | 244.71 | 208.18, 287.65 | 240.79 | 154.93 ~ 352.36 | 19.75 ~ 1925.31 | – |
| *Antibody* | | | | | | | |
| 11 years | | | | | | | |
| Diphtheria (IU/mL) | 81 | 0.82 | 0.68, 0.98 | 0.96 | 0.59 ~ 1.45 | 0.03 ~ 2.00 | 3.7 |
| Tetanus (IU/mL) | 81 | 1.74 | 1.44, 2.09 | 1.77 | 0.95 ~ 3.66 | 0.05 ~ 5.00 | 1.2 |
| Rubella (IU/mL) | 81 | 197.17 | 164.07, 236.94 | 231.30 | 118.70 ~ 377.10 | 3.92 ~ 500.00 | 1.2 |
| AntiHBs (IU/L) | 76 | 6.96 | 4.60, 10.55 | 6.25 | 1.00 ~ 42.59 | 1.00 ~ 188.10 | 39.5 |
| 14 years | | | | | | | |
| Diphtheria (IU/mL) | 81 | 0.59 | 0.50, 0.69 | 0.61 | 0.42 ~ 1.02 | 0.03 ~ 2.00 | 2.5 |
| Tetanus (IU/mL) | 81 | 1.36 | 1.12, 1.64 | 1.50 | 0.89 ~ 2.29 | 0.03 ~ 5.00 | 1.2 |
| Rubella (IU/mL) | 81 | 169.36 | 140.08, 204.77 | 198.00 | 95.32 ~ 307.9 | 4.30 ~ 500.00 | 1.2 |
| AntiHBs (IU/L) | 71 | 6.36 | 4.24, 9.54 | 5.60 | 1.00 ~ 31.69 | 1.00 ~ 181.80 | 36.6 |
| % change from 11 to 14 years old¶ | | | | | | | |
| Diphtheria | 79 | – | – | −31.90 | −44.32 ~ −20.99 | −66.95 ~ 47.06 | – |
| Tetanus | 77 | – | – | −27.54 | −38.90 ~ −14.33 | −79.2 ~ 93.41 | – |
| Rubella | 79 | – | – | −16.77 | −28.33 ~ −0.36 | −48.30 ~ 50.78 | – |
| AntiHBs | 66 | – | – | −37.21 | −62.36 ~ 0 | −93.62 ~ 167.44 | – |

Some numbers do not add up to total n because of excluded outliers.
"–", not applicable.

¶ $\%\ change = \left(\frac{\text{levels at 14 years} - \text{levels at 11 years}}{\text{levels at 11 years}}\right) \times 100$

† $\sum$DEHPm (nmole/g creatinine) = MEHP + MEHHP + MEOHP.

drinks, fruit beverages, sport drinks, fruit juice or jelly, and dietary supplements in capsule or powdered form, were contaminated by phthalate according to the Taiwan Food and Drug Administration (TFDA) (Wu et al., 2012). Wu et al.(2013) reported that the concentration of urinary DEHP metabolites in children measured immediately after the episode was decreased to baseline levels 6 months after terminating the usage of contaminated products. Nonetheless, an adverse health effect of phthalate exposure was observed in children suspected to be exposed to higher phthalate levels even 1 year after the incident (Wen et al., 2017). High follicle-stimulating hormone and sex hormone-binding globulin levels were observed in girls with elevated DEHP exposure. Herein, children were followed up at 11 years of age approximately 3 months after the episode in 2011. The median of urinary MEHP levels were slightly higher than those reported by Wen et al. (2017) (boys: 10.56 [μg/g creatinine] and girls: 7.93 [μg/g creatinine]). Median MEHP level in the present study was 11.6 (μg/g creatinine) (Table 4).

Besides, urinary phthalate metabolite concentrations in our recruited children were slightly different from those in other countries (Table 4). In Denmark (Frederiksen et al., 2013) and in the United States (US CDC, 2019), lower urinary MEHP, MEHHP, and MEOHP levels were reported in children aged 6–11 years than those in our study. Lower urinary MEHP and MEOHP levers were observed in Australian children aged 7–15 years (Hartmann et al., 2015). In Germany, higher urinary MEHHP and MEOHP levels and lower urinary MEHP levels were observed in children aged 3–14 years (Becker et al., 2004). However, Teitelbaum et al. (2008) reported higher urinary DEHP metabolite levels in American children aged 6–10 years. In addition, urinary DEHP metabolite levels in the recriuited children of the present study were similar to that of children aged 1–10 years recruited immediately after the phthalate-exposure episode at Kaohsiung and tested 6 months subsequent to the incident (Wu et al., 2013).

For urinary phthalate metabolite concentrations in women, higher urinary MEHP, MEHHP, and MEOHP levels and lower urinary MEP, MnBP, and MBzP levels were reported in pregnant women in France according to a study by Zeman et al. (2013) as compared to those recruited in the present study (Table s6). Lower urinary MEHP and higher urinary MEHHP and MEOHP were observed in pregnant women aged 18–41 years in Netherlands (Ye et al., 2008) and women aged 20–39 years in South Korea (Song et al., 2013). In previous studies, lower levels of MEP, MnBP, MBzP, MEHP, MEHHP, and MEOHP were reported in pregnant Japanese women (Suzuki et al., 2009), in German women with an average age of 39.2 years (Kasper-Sonnenberg et al., 2012), in Danish women aged 31–52 years (Frederiksen et al., 2013), and in Australian adults aged 18–64 years (Hartmann et al., 2015) (Table s6). Hence, owing to a high level of prior potential phthalate exposure, the health effects of phthalate on the present study cohort needed to be investigated.

**Table 3**
Association between urinary phthalate metabolites and antibody concentrations in 11- and 14-year-old children (multivariate regression analysis).

| Phthalate metabolite (μg/g creatinine)[¶] | β (95%CI) | | | | | |
|---|---|---|---|---|---|---|
| | Diphtheria (IU/mL) | | | Tetanus (IU/mL) | | |
| | 11 years | 14 years | % change[§] | 11 years | 14 years | % change[§] |
| *Mothers*[†] | | | | | | |
| n | 62 | 62 | 61 | 62 | 62 | 59 |
| MMP | 0.012 (−0.083, 0.107) | −0.005 (−0.075, 0.064) | −1.589 (−5.695, 2.518) | **0.251 (0.002, 0.501) (p = 0.049)** | 0.063 (−0.160, 0.285) | −1.134 (−6.427, 4.160) |
| MEP | 0.041 (−0.076, 0.157) | 0.033 (−0.052, 0.117) | −2.038 (−7.208, 3.131) | 0.193 (−0.120, 0.505) | 0.139 (−0.132, 0.410) | −2.840 (−9.409, 3.730) |
| MnBP | 0.007 (−0.094, 0.107) | 0.001 (−0.072, 0.074) | −0.697 (−5.127, 3.732) | 0.061 (−0.210, 0.332) | 0.025 (−0.209, 0.260) | −2.134 (−7.919, 3.652) |
| MBzP | 0.064 (−0.047, 0.175) | 0.028 (−0.054, 0.110) | −2.924 (−7.800, 1.953) | 0.047 (−0.258, 0.352) | 0.011 (−0.253, 0.274) | −2.297 (−8.514, 3.920) |
| MEHP | 0.074 (−0.025, 0.173) | 0.037 (−0.036, 0.110) | −3.139 (−7.522, 1.243) | 0.222 (−0.044, 0.489) | **0.243 (0.016, 0.470) (p = 0.036)** | −1.652 (−7.348, 4.044) |
| MEHHP | 0.014 (−0.039, 0.066) | 0.012 (−0.026, 0.050) | 0.108 (−2.288, 2.505) | −0.044 (−0.186, 0.098) | −0.002 (−0.125, 0.120) | −0.858 (−3.819, 2.103) |
| MEOHP | 0.016 (−0.047, 0.080) | 0.011 (−0.036, 0.057) | −0.885 (−3.719, 1.950) | −0.025 (−0.198, 0.148) | −0.002 (−0.151, 0.147) | −1.376 (−4.948, 2.196) |
| ΣDEHPm (nmole/g creatinine)[&] | 0.045 (−0.051, 0.141) | 0.028 (−0.042, 0.099) | −1.162 (−5.559, 3.234) | 0.007 (−0.256, 0.269) | 0.089 (−0.136, 0.314) | −1.108 (−6.629, 4.412) |
| *11-year-old children*[†] | | | | | | |
| n | 67 | 67 | 66 | 67 | 67 | 63 |
| MMP | −0.045 (−0.113, 0.023) | −0.033 (−0.082, 0.015) | 0.007 (−2.987, 3.000) | 0.009 (−0.182, 0.200) | −0.049 (−0.215, 0.118) | −3.433 (−7.516, 0.650) |
| MEP | 0.026 (−0.026, 0.079) | 0.019 (−0.019, 0.057) | −0.297 (−2.653, 2.060) | 0.123 (−0.018, 0.263) | 0.036 (−0.092, 0.164) | −0.204 (−3.409, 3.001) |
| MnBP | −0.086 (−0.245, 0.073) | −0.081 (−0.194, 0.032) | −1.729 (−8.665, 5.207) | −0.187 (−0.632, 0.259) | −0.156 (−0.542, 0.230) | −2.772 (−12.311, 6.767) |
| MBzP | −0.037 (−0.124, 0.050) | −0.017 (−0.080, 0.046) | 1.021 (−2.850, 4.892) | −0.029 (−0.275, 0.217) | 0.115 (−0.096, 0.326) | 3.359 (−1.896, 8.614) |
| MEHP | −0.022 (−0.123, 0.079) | −0.019 (−0.093, 0.054) | −0.951 (−5.330, 3.428) | 0.145 (−0.136, 0.426) | −0.125 (−0.363, 0.113) | −5.635 (−12.226, 0.956) |
| MEHHP | −0.097 (−0.226, 0.032) | **−0.101 (−0.192, −0.010) (p = 0.031)** | −4.577 (−10.314, 1.159) | 0.206 (−0.161, 0.573) | 0.123 (−0.195, 0.441) | −3.975 (−11.792, 3.842) |
| MEOHP | **−0.083 (−0.151, −0.016) (p = 0.016)** | **−0.079 (−0.125, −0.033) (p = 0.001)** | −1.880 (−4.905, 1.145) | 0.057 (−0.141, 0.254) | 0.083 (−0.087, 0.253) | −0.288 (−4.466, 3.890) |
| ΣDEHPm (nmole/g creatinine)[&] | −0.104 (−0.231, 0.022) | **−0.105 (−0.194, −0.016) (p = 0.022)** | −4.197 (−9.822, 1.428) | 0.198 (−0.164, 0.560) | 0.109 (−0.204, 0.422) | −4.041 (−11.784, 3.702) |

| Phthalate metabolite (μg/g creatinine)[¶] | β (95% CI) | | | | | |
|---|---|---|---|---|---|---|
| | Rubella (IU/mL) | | | AntiHBs (IU/L) | | |
| | 11 years | 14 years | % change[§] | 11 years | 14 years | % change[§] |
| *Mothers*[†] | | | | | | |
| n | 62 | 62 | 59 | 57 | 54 | 35 |
| MMP | −12.826 (−38.338, 12.687) | −5.369 (−31.304, 20.565) | 1.682 (−1.521, 4.884) | 0.980 (−5.652, 7.613) | 0.986 (−6.162, 8.134) | 9.383 (−4.661, 23.427) |
| MEP | −3.412 (−34.929, 28.106) | 10.429 (−42.121, 21.263) | 1.002 (−3.026, 5.030) | −0.449 (−8.459, 7.560) | 0.153 (−8.570, 8.876) | 6.721 (−13.862, 27.304) |
| MnBP | −2.609 (−29.652, 24.434) | −3.682 (−30.959, 23.596) | 0.991 (−2.409, 4.392) | 5.443 (−1.248, 12.134) | 5.889 (−2.354, 14.132) | 9.941 (−7.044, 26.927) |
| MBzP | −25.842 (−55.467, 3.784) | −19.670 (−49.911, 10.571) | 2.071 (−1.703, 5.845) | 2.354 (−5.250, 9.958) | −0.916 (−9.418, 7.586) | −2.727 (−19.614, 14.160) |
| MEHP | 9.080 (−17.999, 36.158) | 5.947 (−21.438, 33.333) | 1.574 (−1.841, 4.989) | 0.553 (−6.286, 7.392) | 2.229 (−6.218, 10.676) | 3.608 (−14.000, 21.216) |
| MEHHP | −6.388 (−20.442, 7.665) | −9.223 (−23.293, 4.847) | 0.689 (−1.166, 2.544) | 1.278 (−2.225, 4.779) | 2.225 (−1.625, 6.074) | −1.042 (−9.424, 7.341) |
| MEOHP | 6.302 (−10.823, 23.427) | −0.128 (−17.491, 17.234) | −0.481 (−2.788, 1.826) | 1.710 (−2.573, 5.993) | 3.588 (−1.189, 8.364) | −11.499 (−24.575, 1.577) |
| ΣDEHPm (nmole/g creatinine)[&] | 2.034 (−24.057, 28.125) | −2.784 (−29.105, 23.537) | 0.994 (−2.345, 4.334) | 0.920 (−5.589, 7.429) | 3.497 (−4.281, 11.274) | −4.495 (−22.225, 13.236) |
| *11-year-old children*[†] | | | | | | |
| n | 67 | 67 | 64 | 62 | 58 | 37 |
| MMP | 1.962 (−17.206, 21.129) | −5.186 (−23.747, 13.375) | −1.916 (−4.123, 0.292) | −2.908 (−7.627, 1.812) | −1.632 (−6.990, 3.726) | 0.202 (−8.993, 9.397) |
| MEP | 12.413 (−2.401, 27.228) | 12.567 (−1.633, 26.767) | 1.032 (−0.670, 2.734) | 0.127 (−3.673, 3.928) | 2.019 (−1.998, 6.036) | −1.152 (−9.385, 7.080) |
| MnBP | 9.239 (−35.809, 54.287) | 6.848 (−36.408, 50.104) | −1.428 (−6.476, 3.620) | −2.393 (−13.243, 8.456) | −3.487 (−15.708, 8.735) | **−22.526 (−41.658, −3.394) (p = 0.023)** |
| MBzP | −3.039 (−26.955, 20.876) | −0.058 (−23.435, 23.320) | 0.540 (−2.272, 3.352) | 0.172 (−6.180, 6.524) | 0.376 (−6.207, 6.958) | −8.596 (−19.713, 2.521) |
| MEHP | | | | | | −6.896 (−21.827, 8.034) |

(*continued on next page*)

**Table 3** (*continued*)

| Phthalate metabolite (μg/g creatinine)[¶] | β (95% CI) | | | | | |
|---|---|---|---|---|---|---|
| | Rubella (IU/mL) | | | AntiHBs (IU/L) | | |
| | 11 years | 14 years | % change[§] | 11 years | 14 years | % change[§] |
| | 25.935 (−2.171, 54.041) | 13.659 (−14.114, 41.433) | −1.931 (−5.341, 1.478) | 3.834 (−3.252, 10.919) | 0.900 (−7.018, 8.818) | |
| MEHHP | 22.787 (−13.874, 59.449) | 7.447 (−28.055, 42.949) | −1.695 (−5.910, 2.519) | 7.021 (−1.898, 15.940) | 0.322 (−9.279, 9.923) | **−18.355 (−33.376, −3.334) (p = 0.018)** |
| MEOHP | 12.046 (−7.494, 31.586) | 12.529 (−6.347, 31.405) | 1.142 (−1.052, 3.335) | 4.497 (−0.669, 9.663) | 2.045 (−3.520, 7.609) | **−10.878 (−18.989, −2.767) (p = 0.010)** |
| ΣDEHPm (nmole/g creatinine)[&] | 26.650 (−9.512, 62.813) | 13.102 (−22.261, 48.465) | −1.309 (−5.524, 2.905) | 6.695 (−2.213, 15.604) | 0.364 (−9.249, 9.976) | **−18.059 (−32.778, −3.341) (p= 0.018)** |

[¶] Phthalate metabolite concentrations were log2-transformed.

[§] $\%\ change = \left(\frac{\text{levels at 14 years} - \text{levels at 11 years}}{\text{levels at 11 years}}\right) \times 100$

[†] Model was adjusted for gender, BMI at 11 years old, maternal education, and maternal PFOS concentration.

[‡] Model was adjusted for gender, BMI at 11 years old, maternal education, 11-year-old PFOS, and maternal phthalate.

[&] DEHPm (nmole/g creatinine) = MEHP + MEHHP + MEOHP.

**Table 4**
Median of urinary phthalate metabolites (μg/g creatinine) in children from different countries.

| Country | City/Region; recruited year | Subjects | Median | | | | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | MMP | MEP | MnBP | MBzP | MEHP | MEHHP | MEOHP | |
| Taiwan | **This study; 2011** | **11–12 years** | **12.1** | **12.6** | **48.9** | **3.4** | **11.6** | **31.3** | **25.7** | |
| | Kaohsiung; 2011 | 1–10 years (n = 29) During episode | | | | | 84.7 | 78.0 | 121.9 | (Wu et al., 2013) |
| | | 6 months later | | | | | 11.9 | 23.4 | 24.8 | |
| | Across Taiwan; 2012–3 | ≤ 12 years old (n = 222)[†] | | | | | B/G: 10.6/ 7.9 | B/G: 51.5/ 38.9 | B/G: 34.4/ 28.6 | (Wen et al., 2017) |
| Denmark | Gentofte and Viby Sj; 2001 | 6–11 years (n = 143) | | 19.0 | 33.0 | 7.4 | 2.2 | 22.0 | 11.0 | (Frederiksen et al., 2013) |
| Germany | Berlin; 2001–2 | 3–14 years (n = 259) | | | | | 5.9 | 39.9 | 30.5 | (Becker et al., 2004) |
| U.S.A. | New York; 2004–5 | 6–10 years (n = 35) | | | | | 11.7 | 76.4 | 50.9 | (Teitelbaum et al., 2008) |
| U.S.A. | CDC; 2009–10 | 6–11 years (n = 415) | | | | | 2.08 | 18.6 | 12.6 | (US CDC, 2019) |
| Australia | –; 2010–2 | 7–15 years (n = 215) | | 21.0 | 12.0 | 2.5 | 0.9 | 31.0 | 3.3 | (Hartmann et al., 2015) |

"–" indicated no information.

[†]B/G, boys/girls.

EDC exposure reportedly influences the immune response. Certain potential EDCs, e.g., PFASs and heavy metals, were associated with a reduction in antibody concentrations in children. Lin et al. (2017) reported that the exposure to multiple heavy metals including lead, zinc, and copper was negatively associated with 7 antibody concentrations, including those determined herein, in children aged 3–7 years. Children residing near the electrical waste recycling area and exposed to higher Pb levels also presented a reduction in the antibody concentration against HBV and approximately half of them failed to develop sufficient antibody concentration against HBV after vaccination (Xu et al., 2015). PFOA and PFOS, two well-known PFASs, suppressed immunity in children; their exposure to children aged 5 years was reportedly negatively associated with antibodies against tetanus and diphtheria at 7 years of age in a prospective birth cohort study (Grandjean et al., 2012). According to an extended follow-up in the study, children exposed to perfluorodecanoate at 7 years and PFOA at 13 years of age displayed a reduction in the antibody concentration against diphtheria (Grandjean et al., 2017). Furthermore, titers of antibodies against mumps and rubella were low in 12–19-year-old children with higher serum PFOS concentration from the US National Health and Nutrition Examination Survey (Stein et al., 2016). These studies provide robust evidence regarding immunosuppression due to environmental toxicant exposure.

Herein, childhood DBP and DEHP exposure was associated with a decrease in antibody concentration against hepatitis B in children. Although the results did not achieve statistical significance, the decreased pattern of antibody levels against diphtheria, tetanus, and rubella associated with DBP and DEHP exposure in children aged 11–14 years was also observed (Table 3). Thus, immune suppression as a result of phthalate exposure may be a possibility.

The trigger of the immune response via vaccination after birth is related to adaptive immunity and requires both B cells and T cells. Upon repeated exposure to the pathogen, immune memory facilitates the production of antibodies and protection against infection. Phthalate exposure influences the production of B and T cells and may be the potential factor associated with antibody titers. In an *in vitro* study, Bissonnette et al. (2008) observed that a low dose MEHP exposure is associated with induction of B-cell apoptosis and suppression of B-cell proliferation. Phthalate exposure may interfere with the balance of the Th1/Th2 immune response (Han et al., 2014), enhance secretion of inflammatory biomarkers (i.e., eosinophils, interleukin [IL]-6, tumor necrosis factor α (Bolling et al., 2012, Deutschle et al., 2008), and increase production of IL-4 and IL-13 (Shen et al., 2017, You et al., 2016). Besides, the development of the immune system in children is closely related to gut microbiota (Ahern et al., 2014), which may be associated with phthalate exposure. In a case-control study of neonates with respiratory distress, DEHP exposure through intravenous infusion alters the diversity and composition of gut microbiota (Yang et al., 2019). In addition, pDCs play an important role in the immune response,

including secretion of type I INFs for protection against infection and regulation of adaptive immunity (McKenna et al., 2005). Koyama et al. (2010) observed that pDCs are essential for the immunogenicity of the inactivated whole-virus vaccine against influenza virus. DEHP and BBP results in the suppression of type I INFs in human pDCs by epigenetic regulation (Kuo et al., 2013). Taken together, DEHP exposure may potentially affect the vaccination-triggered immune response. The biological mechanism of this phenomenon is still unclear and further investigations are needed in this direction.

Prenatal phthalate exposure was not associated with the change in antibody concentration in children aged 11–14 years in our study. The development of the fetal immune system begins early in the first trimester of pregnancy (Park et al., 2020). Adaptive immunity, a part of immune function, continues to develop during childhood. Vaccination triggers the immune response and is an example of adaptive immunity, and is affected by both prenatal and postnatal/childhood environmental exposure (Grandjean et al., 2017, MacGillivray and Kollmann 2014, Van Loveren et al., 2001). However, Grandiean et al. observed that prenatal PFAS exposure was associated with antibody concentration at the age of 5 years and PFAS exposure at the age of 7 years was associated with current antibody concentration (Grandjean et al., 2012, Mogensen et al., 2015). Similar to the report by Grandiean et al., we also found that the effect of phthalate exposure on the change in antibody concentration in 11–14 year-old children was more evident in 11-year exposure than prenatal exposure. Confirmation of the association between prenatal phthalate exposure and antibody concentration in early childhood in the other cohort studies is warranted.

This study has some strengths. Because of the longitudinal study design, we observed a temporal sequence between perinatal phthalate exposure and antibody concentrations in children at later ages. We also adjusted the regression models for prenatal and childhood PFOS exposure and other potential confounders when estimating the association between childhood phthalate exposure and the change in antibody concentrations. Finally, the final routine vaccine was administered to children before attaining 7 years of age in Taiwan. Herein, antibody concentrations were assessed in children aged 11 and 14 years. We therefore reduced the interference of routine vaccination and assessed the change in antibody concentrations during a stable period of immune response resulting from vaccination.

This study has several limitations. First, urine phthalate metabolites were quantified only once in pregnant women and children aged 11 years, which may not represent long-term PAE exposure owing to their short half-life. Thus, we assumed that the usage of phthalate-related materials was quite constant in the daily life of pregnant mothers and children. Urine phthalate metabolite concentrations were considered their steady-state concentrations. Second, only 81 children, upon follow-up, were recruited owing to the dearth of specimens for measurement. Thus, a selection bias may be a concern. However, the characteristics of children (e.g., birth outcomes, gender, or birth order) and parents (e.g., maternal age at childbirth and parental education) and the urine phthalate metabolite concentrations in mothers and children did not significantly differ between followed-up children that were included and those who were not (Table s1). Furthermore, since the interviewers and the interviewees were unaware of the primary study hypothesis, a selection bias due to differential participation was less likely. Third, we measured antibody concentrations in children aged 11 and 14 years, which was 4–6 years after the last booster vaccination at the age of 5–7 years. The antibody concentration declines naturally over time and therefore may not be attributed to environmental exposure to toxicants. However, our assumption that exposure to environmental toxicants may accelerate the decline of antibody concentration was supported by our results. Fourth, caution should be exercised while extrapolating our results to the general population because children recruited from central Taiwan may not represent all children in Taiwan. In addition, the limited sample size in our study may have reduced the statistical power and is also insufficient for further stratified analyses. An external validation study with a more representative population and larger cohort is needed.

In conclusion, the present study shows that phthalate exposure is associated with a reduction in antibody concentrations in children. Through a vaccination-triggered immune response, children with sufficiently stimulated antibody concentrations effectively counter infections and prevent disease pathogenesis. However, long-term PAE exposure may continually decrease antibody concentration and affect the potential to counter infections or disorders. Individuals with high phthalate exposure should be considered first for the clinical management of booster vaccinations for teenagers. Further follow-up studies are required for other populations and delineation of the potential mechanisms of phthalate exposure are warranted.

## CRediT authorship contribution statement

**Hui-Ju Wen:** Methodology, Formal analysis, Resources, Data curation, Writing - original draft, Visualization. **Yue Leon Guo:** Conceptualization, Resources, Funding acquisition. **Pen-Hua Su:** Investigation, Resources. **Chien-Wen Sun:** Investigation, Project administration. **Shu-Li Julie Wang:** Conceptualization, Methodology, Resources, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgement

We thank all children and their mothers who participated in this study, the interviewers who supported data collection, and all of the study groups who participated in the present longitudinal birth cohort study. This study was supported by a research grant from National Health Research Institutes (PH-106-PP-09, EM-108-PP-05, EM-109-PP-05), Chung-Shan Medical University (CSH-2017-C-004), and Ministry of Science and Technology (MOST 106-3114-B-400-002, MOST 107-2321-B-400-010, MOST 108-2321-B-400-007), Taiwan. The authors have no conflict of interest to declare.

## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.106134.

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