Environmental Research 172 (2019) 569–577

Contents lists available at ScienceDirect

Environmental Research

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

Prenatal and childhood exposure to phthalate diesters and neurobehavioral development in a 15-year follow-up birth cohort study

Han-Bin Huang[a,*], Po-Hsiu Kuo[b], Pen-Hua Su[c], Chien-Wen Sun[d], Wei J. Chen[b], Shu-Li Wang[a,d,e,**]

[a] School of Public Health, National Defense Medical Center, Taipei, Taiwan
[b] Institute of Epidemiology and Preventive Medicine, College of Public Health, National Taiwan University, Taipei, Taiwan
[c] Department of Pediatrics, Chung-Shan Medical University Hospital, School of Medicine, Taichung, Taiwan
[d] National Institute of Environmental Health Sciences, National Health Research Institutes, Miaoli, Taiwan
[e] Department of Safety, Health, and Environmental Engineering, National United University, Miaoli, Taiwan

ARTICLE INFO

Keywords:
Phthalates
Neurobehavioral development
Pregnant women
Children
Behavioral syndrome

ABSTRACT

Objective: Longitudinal studies on neurobehavioral development in relation to prenatal and postnatal exposure to phthalates in school-age children and adolescents are limited. We investigated the association of prenatal and childhood phthalate exposure with the development of behavioral syndromes in 8–14-year-old children.

Method: We recruited 430 pregnant women from 2000 to 2001 and followed their children at the ages of 2, 5, 8, 11, and 14 years, yielding 153 mother–child pairs in total. Urine samples from pregnant women in the third trimester and from children at 2–8 years of age were analyzed for the concentrations of seven urinary phthalate metabolites: monomethyl phthalate, monoethyl phthalate, monobutyl phthalate, monobenzyl phthalate (MBzP), and three di(2-ethylhexyl) phthalate (DEHP) metabolites, namely mono-2-ethylhexyl phthalate (MEHP), mono (2-ethyl-5-hydroxyhexyl) phthalate, and mono(2-ethyl-5-oxohexyl) phthalate. Behavioral syndromes in children aged 8–14 years were assessed using the Child Behavior Checklist. We constructed mixed models to examine these associations after adjustments for potential covariates.

Results: Maternal urinary MEHP levels were associated with higher scores for internalizing problems (β = 0.028, 95% confidence interval [CI]: 0.0004, 0.055) and externalizing problems (β = 0.040, 95% CI: 0.013, 0.066). Associations of the maternal urinary sum of DEHP metabolite levels with delinquent behavior scores and externalizing problems scores were positive (β = 0.035, 95% CI: 0.013, 0.057 for delinquent behavior; β = 0.026, 95% CI: 0.001, 0.050 for externalizing problems). Furthermore, children's urinary MBzP levels were associated with higher scores for social problems (β = 0.018, 95% CI: 0.001, 0.035). Similar patterns were observed for borderline and clinical ranges.

Conclusion: Early-life exposure to phthalates may influence behavioral syndrome development in children. Future studies are needed to replicate these findings, and efforts to reduce exposures to phthalates during critical early life stages may be warranted.

1. Introduction

Phthalates are a family of industrial chemicals used in plastics and other products, including cosmetics, toys, packaging film, food containers, intravenous delivery systems, and personal care products (Koch and Calafat, 2009). Because they are not covalently bound to plastics, humans are exposed to them through multiple routes, including dust ingestion, inhalation, and dermal absorption (Beko et al., 2013). Once absorbed, phthalates are rapidly metabolized into monoesters or hydrophilic oxidative metabolites before being excreted through urine (Heudorf et al., 2007). Phthalate metabolites are detected in the urine of all age groups in humans, including pregnant women and children (Chen et al., 2008; Huang et al., 2017; Silva et al., 2004).

Because the use of phthalates is widespread, concerns regarding the adverse health effects of phthalate exposure, particularly for susceptible populations, including its effects on children's neurodevelopment, have been raised. Experimental studies have indicated that phthalate exposure can have adverse effects on neurobehavioral development by impairing the dopamine system (Chen et al., 2011; Dhanya et al., 2003; Tully et al., 2000; Wang et al., 2016), disturbing thyroid hormone

* Correspondence to: School of Public Health, National Defense Medical Center, 161 Minchuan East Road, Sec. 6, Taipei, Taiwan.
** Correspondence to: National Health Research Institutes, 35 Keyan Road, Zhunan, Miaoli County 35035, Taiwan.
E-mail addresses: slwang@nhri.org.tw (H.-B. Huang), slwang@nhri.org.tw (S.-L. Wang).

https://doi.org/10.1016/j.envres.2019.02.029
Received 28 August 2018; Received in revised form 15 February 2019; Accepted 19 February 2019
Available online 20 February 2019
0013-9351/

homeostasis (Liu et al., 2015), and altering lipid metabolism (Xu et al., 2007).

Several prospective cohort studies have examined the associations between prenatal exposure to phthalates and neurodevelopment in children at 1–10 years of age and have reported inconsistent results (Engel et al., 2010; Kim et al., 2017; Kobrosly et al., 2014; Whyatt et al., 2012). Our previous report demonstrated adverse effects in children until the age of 8 years (Lien et al., 2015). However, whether certain phthalates may affect neurobehavioral development during adolescence remains unclear. No study has addressed the association between prenatal and postnatal exposure to phthalates and the neurobehavioral development in children older than 10 years of age. Therefore, we conducted this longitudinal study to explore the association of maternal phthalate exposure in the third trimester and childhood phthalate exposure at the ages of 2–8 years with neurobehavioral development in these children at the ages of 8–14 years.

2. Methods

2.1. Study population

This prospective birth cohort study examining the effects of exposure to endocrine-disrupting chemicals was part of the nationwide Taiwan Maternal and Infant Cohort Study, a pilot study as described elsewhere (Lin et al., 2011). In brief, 430 pregnant women in the third trimester were invited between December 1, 2000, and November 30, 2001; their urine samples and information were collected, including demographics, dietary habits, and reproductive and medical histories. We also collected urine samples of children at 2–3, 5–6, and 8–9 years of age separately. We assessed full-scale intelligence quotient (IQ) scores at the ages of 8–9 years by using the Chinese version of the Wechsler Intelligence Scale for Children-version III (Wechsler, 1997) and 11–12 and 14–15 years by using the Wechsler Intelligence Scale for Children-Fourth Edition (Wechsler, 2007). Each included mother–child pair met the following criteria: (1) the mother provided a urine sample at baseline, (2) the child had at least one follow-up visit at 2–8 years of age and provided a urine sample, and (3) the child had at least one follow-up visit at 8–14 years of age where their Child Behavior Checklist (CBCL) scores were collected. In total, 153 pregnant women participated, comprising 153 mother–child pairs. A flowchart of the participant recruitment and evaluation process is presented in Fig. 1. This study was approved by the Ethics Review Committee of the National Health Research Institutes in Taiwan (available codes: EC0980405 and EC1010505). Written informed consent was obtained before enrollment from the guardian, normally the parent, and child assent was obtained when children were 6 years of age or older.

2.2. Assessment of the CBCL

Mothers completed the CBCL at each visit when their children were aged 8–9, 11–12, and 14–15 years. The mothers' evaluations were based on the status of their children in the preceding 6 months. The CBCL/4–18 by Achenbach (Achenbach, 1991) comprises 20 competence items and 118 behavioral/emotional items rated on a 3-point scale (0, 1, and 2 correspond to not true, somewhat or sometimes true, and very or often true, respectively). Eight narrowband behavioral syndromes, namely withdrawn, somatic complaints, anxious or depressed, social problems, thought problems, attention problems, delinquent behavior, and aggressive behavior, were defined by Achenbach (1991). For the scores of broadband internalizing problems, we summed withdrawn, somatic complaints, and anxious or depressed. For the scores of externalizing problems, we summed delinquent behavior and aggressive behavior. All scores in the CBCL were shown as T-scores based on well-established normative data in Taiwan (Yang et al., 2000). The reliability and validity of the Chinese version of the CBCL were determined to be very good in relation to Taiwanese adolescents (Yang et al., 2000).

2.3. Phthalate metabolites

We quantified seven phthalate metabolites in the maternal and child urine samples: monomethyl phthalate (MMP), monoethyl phthalate (MEP), monobutyl phthalate (MBP), monobenzyl phthalate (MBzP), monoethylhexyl phthalate (MEHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), and mono(2-ethyl-5-oxohexyl) phthalate (MEOHP). We selected these seven phthalate metabolites due to the frequent use and their parental compounds known to be high in this population (Chen et al., 2008). Maternal and children's urinary phthalate metabolite concentrations were assessed through liquid chromatography–tandem mass spectrometry (Lin et al., 2011). The sum of the di(2-ethylhexyl) phthalate (DEHP) metabolites (ΣMEHP) was calculated by adding the corresponding concentrations (molecular-based): MEHP, MEHHP, and MEOHP. The creatinine-corrected urinary concentrations of the seven phthalate metabolites were observed. To account for urine dilution, we corrected urinary phthalate metabolite concentrations for creatinine. Urinary creatinine level was measured by the spectrophotometric method in central laboratory of Kaohsiung Medical Center. The limit of detection (LOD) of MMP, MEP, MBP, MBzP, MEHP, MEHHP, and MEOHP were 0.3, 0.3, 1, 0.3, 0.7, 0.1, and 0.1 ng mL^{-1}, respectively. When the phthalate metabolite levels were below the LOD, we replaced these values with half of the LOD value (Hornung and Reed, 1990). A blank, repeated quality control (QC) sample, was included in each batch of the analyzed samples. The concentrations of the QC samples were to be less than 2 folds the method detection limit. The intra-day variations were < 10%, and intra-day recoveries were at 100 ± 20% at three different concentrations, 25%, 50%, and 75%, of the individual substances.

2.4. Statistical analyses

We used chi-square and independent *t*-tests to compare the demographic characteristics of the included and excluded pregnant women. We used natural log (ln)–transformed values in the analysis because the distribution of urinary phthalate metabolites and the T-scores of CBCL syndromes were skewed to the right. The associations between the T-scores of CBCL syndromes and the maternal and children's urinary phthalate metabolite levels were determined using mixed-model repeated-measure analyses after adjustment for fixed covariates of the children's sex, IQ, family income, and study visit (for example, Y = intercept + β1 (sex) + β2 (IQ) + β3 (family income) + β4 (study visit) + β5 ln (maternal creatinine-corrected phthalates)i + β6 ln (children's creatinine-corrected phthalates)i, i: the same urinary phthalate metabolite). These covariates in the models were selected on the basis of being associated with exposure, associated with outcomes, and not intermediate variables between exposure and outcome (Szklo and Nieto, 2013), a 10% change-in-estimated criterion (such as study visit) (Rothman et al., 2008), and the previous research (Lien et al., 2015) (such as children's sex, IQ, and family income). These models treated the participants as random effect, and the first-order autoregressive and variance components were constructed as covariance structures. The models were selected on the basis of Akaike's information criterion. We applied Shapiro–Wilk test to assess and verify the normality of residual distributions. We also conducted residual and influence analyses using Cook's D statistic and did not observe any outliers and influential data points (Littell and Statistical Analysis System, 2006).

According to the percentiles in the normative population in Taiwan, the CBCL scores can be classified into the normal (< 90th percentile of T-scores), borderline (90–95th percentile of T-scores), or clinical (> 95th percentile of T-scores) range (Yang et al., 2000). Generalized linear mixed models were applied to estimate odds ratios (ORs) comparing exposure levels between the children classified as having

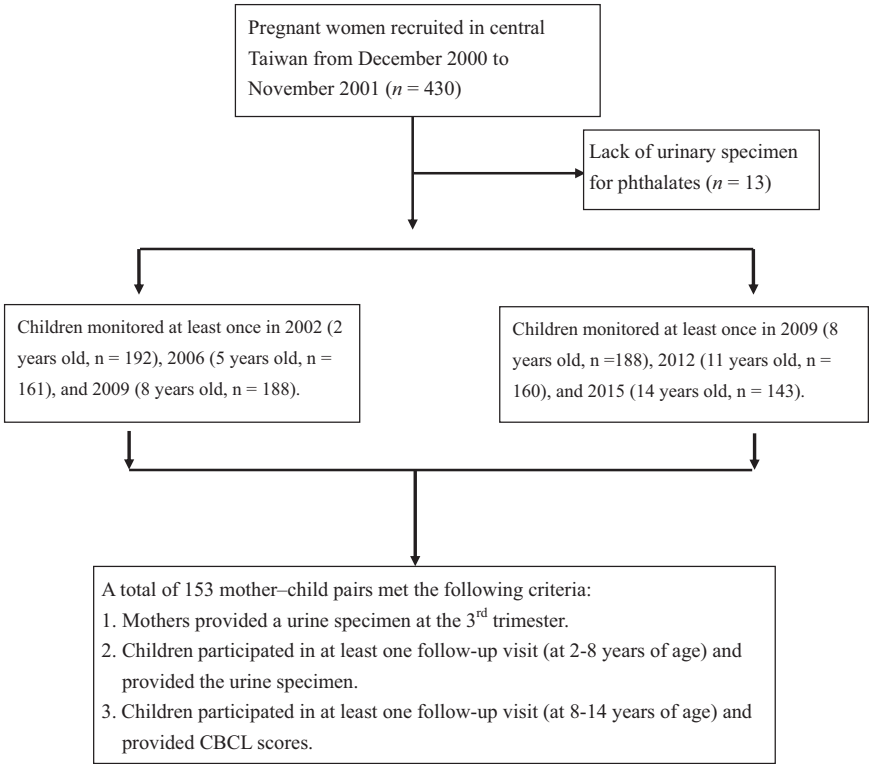

Fig. 1. Flow of participant follow-up procedures for birth cohort study.

borderline behavior (90–95th percentile) with those who had normal behavior (< 90th percentile) and to estimate ORs comparing exposure levels between the children classified as having clinical behavior (> 95th percentile) with those who had normal behavior (< 90th percentile). We also applied generalized linear mixed models to estimate ORs to compare exposure levels between children classified as having combined borderline or clinical and normal behaviors.

To examine the effect modifications of the maternal and children's urinary phthalate metabolite levels and children's sex on the T-scores of CBCL syndromes, we stratified the data by sex for the subsequent analysis. For sensitivity analysis, urinary creatinine levels were included as the covariate in the models. A two-sided P value of < 0.05 was considered as statistically significant unless otherwise specified. We also used the $P < 0.05$ significance criteria for effect modifications. All the statistical analyses were conducted using SAS (version 9.1.3; SAS Institute Inc., Carry, NC).

3. Results

3.1. Characteristics of study population

Except for maternal education levels, demographic characteristics between those mother-child pairs included in our analysis as compared to those excluded due to loss to follow-up were similar (Table 1). Mothers of children in the present analyses were more highly educated and had lower levels of MBzP. Though non-significant, the group included in the present analysis generally had lower maternal concentrations of MMP, MBP, MEHP and sum of MEHP (primary metabolite of DEHP), MEHHP (secondary metabolite) and MEOHP (tertiary metabolite) in molecular base, and higher levels of MEHHP and MEOHP compared to those excluded from the present analysis.

3.2. Distributions of children's urinary phthalate metabolites at 2–8 years of age and CBCL scores at 8–14 years of age

The detection rate of urinary phthalate metabolites for pregnant women ranged from 84% to 100%. For the children's urinary samples, the detection rate of urinary phthalate metabolites at 2–3, 5–6 and 8–9 years were 90–100%, 95–100%, and 99–100%, respectively. Urinary MBzP levels in boys aged 5–6 years (study visit 2) were significantly higher than the concentrations of urinary phthalate metabolites in boys aged 2–3 years (study visit 1) (Tables 2 and S1). In addition, urinary MMP, MEP, MBP, MEHP, MEHHP, and ΣMEHP levels were significantly lower and urinary MBzP levels were significantly higher at 8–9 years of age (study visit 3) than at 2–3 years of age in boys (study visit 1). The patterns of urinary phthalate metabolite levels in girls at 2–8 years of age were similar to those in boys in the same age range. However, concentrations of urinary phthalate metabolites were not significant different between boys and girls in the same age range except that the urinary MBzP levels in boys were higher than those in girls at 5–6 years of age. We observed that spearman correlations of urinary phthalate metabolite levels within pregnant women and children at the same age group were significantly low to moderate (Table S2). Regarding the correlations among pregnant women and children at different ages, most of the coefficients were nonsignificant, probably because of the different exposure scenarios among the adult, young, and older children groups.

The withdrawal, somatic complaint, social problem, and internalizing problem scores in boys aged 11–12 years were significantly higher than those in boys at 8–9 years of age (Table 3). Furthermore, the scores of withdrawn, somatic complaints, and thought problems in boys at 14–15 years of age were significantly higher than those in boys at 8–9 years of age, whereas the scores of aggressive behavior and externalizing problems in boys at 14–15 years of age were significantly lower. We also observed similar patterns of CBCL scores in girls at 8–14 years of age. Moreover, we noted significant differences in the scores of delinquent behavior between girls and boys at 11–14 years of age, in

Table 1
Demographic characteristics of study population.

	Subjects included (n = 153)			Subjects excluded (n = 277)		
Categorical variables		***n***	**%**		***n***	**%**
Maternal age[a] (yr)	29.2 ± 4.0	153		28.5 ± 4.6	251	
Children's sex						
Male		73	47.7		123	45.6
Female		80	52.3		103	54.4
Active smoker (before pregnancy)						
Yes		6	4.2		24	9.6
No		137	98.5		227	90.4
Passive smoke (before pregnancy)						
Yes		59	41.3		120	48.0
No		84	58.7		130	52.0
Active smoker (during pregnancy)						
Yes		1	0.7		7	2.8
No		142	99.3		245	97.2
Alcohol consumption						
Yes		6	4.2		7	2.8
No		137	95.8		245	97.2
Pesticide use at home						
Yes		43	30.1		84	33.3
No		100	69.6		168	66.7
Lactation						
Yes		124	92.5		204	89.5
No		10	7.5		24	10.5
Maternal education[c]	≤**12** yrs	57	37.3		140	53.8
	> 12 yrs	96	62.7		120	46.2
Paternal education	≤**12 yrs**	59	38.8		122	48.6
	> 12 yrs	93	61.2		129	51.4
Family annual income ($ US/year)	< 20,000	59	39.3		116	47.0
	≥20,000	91	60.7		131	53.0
Continuous variables	***n***	GM	95% CI	***n***	GM	95% CI
Maternal urinary phthalate metabolite (μg/g creatinine)						
MMP	153	52.38	44.00, 62.36	239	55.48	49.09, 62.69
MEP	153	63.70	55.07, 73.67	239	63.81	57.14, 71.26
MBP	153	67.29	57.78, 78.38	239	75.22	66.66, 84.88
MBzP[c]	153	14.99	13.23, 16.98	239	19.38	17.62, 21.32
MEHP	153	16.73	14.46, 19.36	239	20.53	18.11, 23.27
MEHHP	153	7.07	5.19, 9.64	239	6.08	4.68, 7.90
MEOHP	153	12.79	9.88, 16.57	239	9.40	7.54, 11.73
ΣMEHP[b] (μmole/g creatinine)	153	0.17	0.15, 0.20	239	0.19	0.17, 0.22

Abbreviations: GM, geometric mean.
[a] Mean ± SD.
[b] ΣMEHP = (MEHP + MEHHP + MEOHP) at molecular base.
[c] Significant difference ($P < 0.05$) in the distribution of maternal education and urinary MBzP levels among follow-up subjects compared with lost-to-follow-up subjects.

the scores of aggressive behavior between girls and boys at 8–11 years of age, and in the scores of externalizing problems between girls and boys at 11–14 years of age.

3.3. Relations between urinary phthalate metabolite levels and CBCL scores

The associations between maternal and children's urinary phthalate metabolite levels and CBCL scores determined using mixed models adjusted for children's sex, IQ, family income, and study visit are presented in Table 4. Children's urinary MBzP levels were positively associated with the scores of social problems (β = 0.018, 95% confidence interval [CI]: 0.001, 0.035). Maternal urinary MEHP levels were positively associated with the scores of anxious or depressed (β = 0.029, 95% CI: 0.004, 0.054), social problems (β = 0.033, 95% CI: 0.003, 0.063), thought problems (β = 0.038, 95% CI: 0.006, 0.070), attention problems (β = 0.040, 95% CI: 0.008, 0.072), delinquent behavior (β = 0.044, 95% CI: 0.019, 0.069), aggressive behavior (β = 0.034, 95% CI: 0.008, 0.061), internalizing problems (β = 0.028, 95% CI: 0.0004, 0.055), and externalizing problems (β = 0.040, 95% CI: 0.013, 0.066). We observed positive associations between maternal ΣMEHP levels and delinquent behavior scores (β = 0.035, 95% CI: 0.013, 0.057). We also noted a positive relationship between maternal ΣMEHP levels and the scores of externalizing problems (β = 0.026, 95% CI: 0.001, 0.050). In other words, a one-fold increase in maternal ΣMEHP or MEHP levels increased the 1.8–3.1% scores of externalizing problems in children (formula: {eˆ[Ln(2) x *Beta*] -1}x100). In addition, we used urinary creatinine levels as the covariates in the mixed models and observed similar results (Table S3).

The ORs of the borderline or clinical range scores on the CBCL internalizing and externalizing domain behaviors for each Ln-unit increase in maternal and children's urinary phthalate metabolite concentrations are presented in Table 5. Maternal MEHP and ΣMEHP levels were associated with increased ORs on the borderline or clinical range of internalizing problems (OR = 1.69; 95% CI: 1.12, 2.56 for maternal MEHP levels; OR = 1.52, 95% CI: 1.02, 2.28 for maternal ΣMEHP levels). By contrast, maternal MBP, MEHP, and ΣMEHP levels were associated with increased ORs on the borderline or clinical range of externalizing problems (OR = 1.72, 95% CI: 1.03, 2.89 for maternal MBP levels; OR = 2.39, 95% CI: 1.44, 3.97 for maternal MEHP; OR = 2.19, 95% CI: 1.34, 3.57 for maternal ΣMEHP levels). We observed that most of the maternal or children's urinary metabolite levels were of similar magnitudes and directions about the scores of internalizing and externalizing problems in girls and boys. We did not observe any sex-specific associations between maternal or childhood phthalate

Table 2
Concentrations of children's urinary phthalate (μg/g creatinine) at three follow-up time-points.

Variables	1st (2–3 years)			2nd (5–6 years)			3rd (8–9years)		
	> LOD (%)	n	GM (95% CI)	> LOD (%)	n	GM (95% CI)	> LOD (%)	n	GM (95% CI)
Boys									
MMP	100	45	14.84 (11.48, 19.18)	100	51	17.25 (11.48, 25.93)	100	60	6.82 (5.16, 9.01)*
MEP	100	45	24.11 (17.71, 32.83)	100	51	22.80 (16.49, 31.53)	100	60	13.30 (10.30, 17.18)*
MBP	100	45	162.12 (128.72, 204.17)	100	51	151.78 (108.55, 212.23)	100	60	84.75 (70.23, 102.27)*
MBzP	87	45	7.14 (4.73, 10.80)	98	51	17.30 (12.76, 23.45)*,#	98	60	11.41 (8.38, 15.54)*
MEHP	100	45	15.29 (12.11, 19.32)	100	51	12.59 (9.63, 16.46)	100	60	10.36 (7.76, 13.84)*
MEHHP	100	45	87.71 (69.26, 111.06)	100	51	84.67 (64.26, 111.57)	100	60	48.10 (37.65, 61.43)*
MEOHP	98	45	59.61 (44.37, 80.08)	100	51	47.98 (37.74, 61.01)	100	60	41.82 (33.01, 52.98)
ΣMEHP[a] (μmole/g creatinine)		45	0.59 (0.47, 0.73)		51	0.54 (0.42, 0.68)		60	0.35 (0.28, 0.45)*
Girls									
MMP	98	43	15.29 (12.44, 18.78)	100	53	16.15 (12.68, 20.57)	99	71	7.82 (6.16, 9.92)*
MEP	100	43	39.38 (27.82, 55.75)	100	53	18.15 (13.77, 23.94)*	100	71	17.97 (13.78, 23.42)*
MBP	100	43	165.96 (129.64, 212,45)	100	53	104.86 (89.91, 122.29)*	100	71	102.34 (85.19, 122.95)*
MBzP	93	43	7.56 (5.44, 10.50)	100	53	13.18 (10.75, 16.16)*	100	71	10.37 (7.87, 13.65)
MEHP	98	43	16.79 (12.38, 22.77)	91	53	11.32 (7.65, 16.74)	97	71	7.51 (5.52, 10.20)*
MEHHP	100	43	88.66 (65.63, 119.77)	100	53	78.86 (59.17, 105.12)	100	71	40.44 (33.34, 49.07)*
MEOHP	100	43	66.39 (50.84, 86.68)	100	53	44.55 (33.66, 58.95)*	100	71	34.05 (28.01, 41.39)*
ΣMEHP[a] (μmole/g creatinine)		43	0.61 (0.47, 0.81)		53	0.52 (0.40, 0.67)		71	0.30 (0.24, 0.37)*

Abbreviations: GM, geometric mean; LOD, limit of detection.
* Significant difference ($P < 0.05$) in urinary phthalate metabolite levels compared with visit 1 (reference) using linear mixed models.
# Significant difference ($P < 0.05$) in urinary phthalate metabolite levels between girls and boys at the same study visit using independent *t*-test.
[a] ΣMEHP = (MEHP + MEHHP + MEOHP) at molecular base.

metabolite levels and the scores of internalizing and externalizing problems (Table S4).

4. Discussion

In this prospective birth cohort study, we demonstrated that maternal urinary MEHP levels were associated with increasing scores on the CBCL symptoms (i.e., anxiety or depression, social problems, thought problems, attention problems, delinquent behavior, aggressive behavior, internalizing problems, and externalizing problems) in children aged 8–14 years. We also observed positive relations between maternal urinary ΣMEHP levels and delinquent behavior scores and externalizing problem scores. Furthermore, in children, urinary MBzP levels at the age of 2–8 years were associated with their increasing scores for social problems at the age of 8–14 years. These results indicate that prenatal and childhood phthalate exposure is associated with the development of child behavior in later life and that prenatal exposure effects are independent of exposures during childhood.

In a previous birth cohort study, Lien et al. (2015) reported positive associations between maternal urinary DEHP metabolite levels and externalizing domain behavioral problem scores in 8-year-old children. We also observed that these associations were retained for 6 years

Table 3
Children's CBCL scores at three follow-up time points.

Variables	1st (8–9 years)		2nd (11–12 years)		3rd (14–15 years)	
	n	GM (95% CI)	n	GM (95% CI)	n	GM (95% CI)
Boys						
Withdraw	70	44.56 (43.28, 45.88)	60	47.02 (44.91, 49.23)*	50	48.19 (46.10, 50.37)*
Somatic Complaints	70	45.96 (44.51, 47.45)	60	49.62 (47.12, 52.25)*	50	50.43 (48.14, 52.83)*
Anxious/Depressed	70	47.49 (45.92, 49.11)	60	49.41 (47.33, 51.59)	50	45.57 (44.08, 47.11)
Social Problems	70	50.27 (48.43, 52.19)	60	54.02 (51.25, 56.95)*	50	48.05 (45.65, 50.59)
Thought Problems	70	46.14 (44.30, 48.06)	60	47.45 (45.10, 49.92)	50	54.94 (50.92, 59.26)*
Attention Problems	70	48.98 (47.10, 50.94)	60	49.52 (47.09, 52.09)	50	51.03 (48.12, 54.11)
Delinquent Behavior	70	50.59 (48.79, 52.46)	60	50.38 (48.31, 52.53)#	50	51.08 (48.77, 53.51)#
Aggressive Behavior	70	51.60 (49.56, 53.72)#	60	50.79 (48.60, 53.08)#	50	46.59 (44.84, 48.41)*
Internalizing Problems	70	45.68 (44.27, 47.13)	60	48.77 (46.58, 51.05)*	50	47.50 (45.72, 49.35)
Externalizing Problems	70	51.41 (49.40, 53.51)	60	50.81 (48.58, 53.12)#	50	47.91 (46.05, 49.85)*,#
Girls						
Withdraw	74	45.28 (43.89, 46.72)	63	46.24 (44.32, 48.24)	54	46.00 (43.86, 48.24)
Somatic Complaints	74	47.18 (45.44, 48.97)	63	47.56 (45.59, 49.62)	54	48.67 (46.57, 50.87)
Anxious/Depressed	74	48.68 (46.71, 50.72)	63	48.73 (46.83, 50.70)	54	47.73 (45.80, 49.74)
Social Problems	74	50.92 (48.97, 52.95)	63	51.46 (49.19, 53.82)	54	48.64 (45.97, 51.47)*
Thought Problems	74	46.05 (44.68, 47.47)	63	47.71 (45.86, 49.64)	54	52.20 (48.86, 55.77)*
Attention Problems	74	46.54 (45.00, 48.14)	63	46.39 (44.40, 48.48)	54	49.29 (46.77, 51.94)*
Delinquent Behavior	74	48.74 (47.16, 50.37)	63	47.12 (45.48, 48.83)*	54	46.16 (44.67, 47.70)*
Aggressive Behavior	74	48.74 (46.90, 50.66)	63	46.99 (45.25, 48.78)*	54	45.30 (43.60, 47.07)*
Internalizing Problems	74	46.93 (45.21, 48.73)	63	47.43 (45.54, 49.41)	54	47.30 (45.29, 49.41)
Externalizing Problems	74	48.74 (47.02, 50.53)	63	46.90 (45.17, 48.70)*	54	45.32 (43.65, 47.05)*

Abbreviations: GM, geometric mean.
* Significant difference ($P < 0.05$) in CBCL scores compared with visit 1 (reference) using linear mixed models.
# Significant difference ($P < 0.05$) in CBCL scores between girls and boys at the same study visit using independent *t*-test.

Table 4
Regression coefficients (95% CIs) for 1-unit increase in Ln concentrations of maternal and children's urinary phthalate metabolites (*ug/g* creatinine) in children aged 2–8 years on Ln CBCL scores in children aged 8–14 years determined using mixed models (N = 243)[a,b].

Metabolite/behavior	Withdrawn Beta (95% CI)	Somatic complaints Beta (95% CI)	Anxious/ depressed Beta (95% CI)	Social problems Beta (95% CI)	Thought problems Beta (95% CI)	Attention problems Beta (95% CI)	Delinquent behavior Beta (95% CI)	Aggressive behavior Beta (95% CI)	Internalizing problems Beta (95% CI)	Externalizing problems Beta (95% CI)
Maternal MEHP	0.023 (−0.005, 0.051)	0.017 (−0.013, 0.048)	**0.029* (0.004, 0.054)**	**0.033* (0.003, 0.063)**	**0.038* (0.006, 0.070)**	**0.040* (0.008, 0.072)**	**0.044* (0.019, 0.069)**	**0.034* (0.008, 0.061)**	**0.028* (0.0004, 0.055)**	**0.040** (0.013, 0.066)**
Children's MEHP	−0.0004 (−0.018, 0.017)	−0.009 (−0.028, 0.010)	0.003 (−0.014, 0.020)	0.0001 (−0.019, 0.019)	−0.006 (−0.029, 0.017)	−0.004 (−0.021, 0.013)	0.002 (−0.013, 0.017)	0.002 (−0.014, 0.018)	−0.002 (−0.019, 0.015)	0.001 (−0.015, 0.017)
Maternal ΣMEHP[C] (μmole/g creatinine)	0.016 (−0.010, 0.041)	0.012 (−0.016, 0.039)	0.016 (−0.008, 0.039)	0.025 (−0.003, 0.053)	0.024 (−0.006, 0.055)	0.023 (−0.007, 0.052)	**0.035** (0.013, 0.058)**	0.020 (−0.005, 0.045)	0.015 (−0.010, 0.041)	**0.026* (0.002, 0.051)**
Children'sΣMEHP[C] (μmole/g creatinine)	−0.009 (−0.031, 0.013)	−0.003 (−0.027, 0.022)	−0.005 (−0.027, 0.017)	0.002 (−0.022, 0.026)	−0.008 (−0.038, 0.022)	−0.007 (−0.029, 0.015)	0.008 (−0.011, 0.027)	0.006 (−0.015, 0.027)	−0.009 (−0.031, 0.013)	0.006 (−0.014, 0.026)
Maternal MBP	0.011 (−0.015, 0.038)	0.0003 (−0.028, 0.029)	0.019 (−0.005, 0.043)	0.017 (−0.012, 0.046)	0.021 (−0.010, 0.052)	0.020 (−0.011, 0.050)	0.022 (−0.002, 0.047)	0.017 (−0.009, 0.043)	0.014 (−0.012, 0.040)	0.020 (−0.006, 0.045)
Children's MBP	−0.003 (−0.025, 0.019)	−0.017 (−0.041, 0.007)	−0.009 (−0.030, 0.012)	−0.006 (−0.030, 0.018)	−0.005 (−0.035, 0.024)	−0.002 (−0.023, 0.019)	−0.001 (−0.020, 0.017)	0.004 (−0.016, 0.025)	−0.010 (−0.031, 0.011)	0.002 (−0.017, 0.021)
Maternal MBzP	−0.023 (−0.056, 0.011)	0.003 (−0.033, 0.038)	0.005 (−0.026, 0.035)	0.005 (−0.033, 0.042)	−0.012 (−0.052, 0.028)	−0.002 (−0.041, 0.037)	0.004 (−0.027, 0.035)	−0.005 (−0.038, 0.028)	−0.006 (−0.039, 0.027)	−0.002 (−0.035, 0.030)
Children's MBzP	−0.005 (−0.021, 0.012)	−0.013 (−0.031, 0.005)	−0.005 (−0.021, 0.011)	**0.018* (0.001, 0.035)**	−0.008 (−0.031, 0.014)	−0.005 (−0.020, 0.011)	−0.006 (−0.020, 0.008)	−0.005 (−0.021, 0.010)	−0.008 (−0.023, 0.008)	−0.006 (−0.021, 0.008)
Maternal MMP	−0.010 (−0.033, 0.014)	−0.018 (−0.043, 0.007)	−0.016 (−0.037, 0.006)	−0.007 (−0.033, 0.018)	−0.013 (−0.040, 0.015)	0.0001 (−0.027, 0.027)	−0.013 (−0.035, 0.008)	−0.018 (−0.041, 0.004)	−0.017 (−0.040, 0.006)	−0.018 (−0.041, 0.004)
Children's MMP	−0.005 (−0.023, 0.013)	−0.009 (−0.030, 0.011)	−0.015 (−0.033, 0.003)	0.007 (−0.013, 0.027)	0.007 (−0.019, 0.032)	0.003 (−0.015, 0.020)	0.0002 (−0.015, 0.016)	−0.004 (−0.022, 0.013)	−0.012 (−0.029, 0.006)	−0.004 (−0.020, 0.013)
Maternal MEP	−0.004 (−0.032, 0.025)	0.012 (−0.020, 0.043)	−0.0003 (−0.027, 0.026)	−0.022 (−0.053, 0.010)	0.015 (−0.019, 0.050)	−0.007 (−0.041, 0.026)	0.009 (−0.018, 0.036)	−0.007 (−0.035, 0.022)	0.001 (−0.028, 0.030)	−0.003 (−0.031, 0.026)
Children's MEP	−0.010 (−0.028, 0.007)	0.010 (−0.009, 0.029)	−0.005 (−0.022, 0.013)	−0.003 (−0.022, 0.016)	0.010 (−0.014, 0.033)	0.003 (−0.014, 0.021)	0.001 (−0.014, 0.016)	−0.001 (−0.018, 0.015)	−0.001 (−0.018, 0.016)	−0.001 (−0.017, 0.014)

Abbreviations: N, number of observations.
[a] Adjusted for children's sex, IQ, family income, and study visit.
[b] Maternal and childhood urinary phthalate levels were mutually adjusted.
[c] ΣMEHP = (MEHP + MEHHP + MEOHP) at molecular base.
* $P < 0.05$.
** $P < 0.01$.

Table 5
ORs (95% CIs) for CBCL scores in the borderline and/or clinical range compared with normal range for each Ln increase in concentrations of maternal and children's urinary phthalate metabolites determined using generalized linear mixed models (N = 243)[a,b].

Metabolite/behavior	Borderline OR (95% CI)	Clinical OR (95% CI)	Borderline/clinical OR (95% CI)
Internalizing problems	N = 9	N = 19	N = 28
Maternal MEHP	**2.33 (1.20, 4.55)***	1.61(0.99, 2.60)	**1.69 (1.12, 2.56)***
Children's MEHP	0.51 (0.25,1.07)	1.30 (0.85,1.98)	1.03 (0.70, 1.51)
Maternal ΣMEHP[c]	1.77 (0.88, 3.56)	1.49 (0.93, 2.39)	**1.52 (1.02, 2.28)***
Children'sΣMEHP[c]	0.89 (0.41, 1.94)	1.19 (0.69, 2.06)	1.11 (0.69, 1.77)
Maternal MBP	1.03 (0.47, 2.24)	1.55 (0.91, 2.66)	1.36 (0.85, 2.18)
Children's MBP	0.80 (0.30, 2.16)	0.92 (0.51, 1.65)	0.88 (0.53, 1.47)
Maternal MBzP	0.99 (0.37, 2.66)	0.96 (0.49,1.88)	0.95 (0.53, 1.71)
Children's MBzP	1.22 (0.60, 2.48)	0.79 (0.50,1.24)	0.90 (0.62, 1.32)
Maternal MMP	0.90 (0.45, 1.24)	0.87 (0.55, 1.39)	0.88 (0.58, 1.33)
Children's MMP	0.66 (0.31, 1.38)	0.82 (0.50, 1.33)	0.79 (0.51, 1.20)
Maternal MEP	0.98 (0.45, 2.12)	1.05 (0.63, 1.77)	1.05 (0.66, 1.67)
Children's MEP	1.16 (0.62, 2.19)	0.80 (0.50, 1.29)	0.94 (0.64, 1.39)
Externalizing problems	N = 15	N = 13	N = 28
Maternal MEHP	**2.24 (1.29, 3.89)****	**2.41 (1.34, 4.34)****	**2.39 (1.44, 3.97)****
Children's MEHP	0.69 (0.39, 1.21)	1.31 (0.75, 2.31)	0.88 (0.56, 1.38)
Maternal ΣMEHP[c]	**2.20 (1.25, 3.89)****	**2.12 (1.17, 3.84)***	**2.19 (1.34, 3.57)****
Children'sΣMEHP[c]	0.74 (0.36, 1.53)	1.35 (0.68, 2.68)	0.93 (0.53,1.61)
Maternal MBP	**1.90 (1.03, 3.51)***	1.56 (0.82, 2.95)	**1.72 (1.03, 2.89)***
Children's MBP	0.94 (0.49, 1.82)	0.81 (0.41, 1.63)	0.87 (0.52, 1.46)
Maternal MBzP	1.30 (0.58, 2.90)	2.07 (0.84, 5.10)	1.60 (0.78, 3.30)
Children's MBzP	0.82 (0.51, 1.32)	0.70 (0.41, 1.19)	0.78 (0.52, 1.17)
Maternal MMP	1.00 (0.59, 1.69)	0.64 (0.38, 1.08)	0.77 (0.50, 1.19)
Children's MMP	1.01 (0.59, 1.74)	0.71 (0.40, 1.28)	0.84 (0.54, 1.29)
Maternal MEP	0.83 (0.43, 1.62)	1.93 (1.09, 3.43)	1.29 (0.78, 2.13)
Children's MEP	1.20 (0.75, 1.92)	0.77 (0.42, 1.42)	0.98 (0.64, 1.49)

Abbreviations: N, number of observations.
[a] Adjusted for children's sex, IQ, family income, and study visit.
[b] Maternal and childhood levels of urinary phthalate were mutually adjusted.
[c] ΣMEHP = (MEHP + MEHHP + MEOHP) at molecular base.
* $P < 0.05$.
** $P < 0.01$.

(8–14 years of age) and that internalizing behavioral problems was significant. Kobrosly et al. (2014) demonstrated that maternal DEHP metabolite concentrations were associated with higher scores for somatic problems among children at 6–10 years of age. Messerlian et al. (2017) indicated that maternal and paternal preconception concentrations of DEHP metabolites were associated with internalizing behavior scores assessed using the Behavior Assessment System for Children (BASC) among children at 2–9 years of age. Whyatt et al. (2012) did not observe any associations between maternal DEHP metabolite levels and the CBCL outcomes, whereas they observed positive associations between maternal MnBP and MBzP levels and scores for withdrawn and internalizing behaviors in 3-year-old children. Kim et al. (2017) reported that maternal urinary MEP levels were higher in 1- to 2-year-old children with behavioral problems than in other children. Engel et al. (2010) reported that low-molecular-weight phthalate concentrations were positively associated with the BASC domains (i.e., aggression, attention problems, conduct problems, and depression), whereas high-molecular-weight phthalate concentrations were not associated with most of the BASC domains. The inconsistencies between the findings of the previous studies and those of the present study are potentially attributable to differences in the profiles of maternal phthalate exposure, outcomes assessed at the different children's ages, population size, sample collection timing during the pregnancy, population demographic characteristics, and postnatal phthalate exposure.

Here, we noted that in children, urinary MBzP levels at 2–8 years of age were associated with higher scores for social problems at 8–14 years of age, considering maternal phthalate exposure. Although cross-sectional and case–control studies have reported that DEHP metabolites may be associated with attention-deficit–hyperactivity disorder symptom in school-age children (Hu et al., 2017; Kim et al., 2009; Won et al., 2016), these studies have not examined the influence of prenatal exposure. The current study is the first to report that certain phthalate exposure (i.e. MBzP) during childhood might also be associated with increasing scores for social problems in later life after adjustment for maternal phthalate exposure simultaneously.

The biological mechanisms underlying the adverse effects of phthalate exposure on neurobehavioral development are unclear. Experimental research indicated that phthalates could alter the lipid metabolism of the rat fetal brain mediated by the peroxisome proliferator-activated receptor, which may cause aberrant neurodevelopment (Xu et al., 2007). Moreover, phthalates could disturb dopamine receptor D2, tyrosine hydroxylase, and homeostasis of calcium-dependent neurotransmitters, leading to a reduction in the release of dopamine (Chen et al., 2011; Dhanya et al., 2003; Tully et al., 2000; Wang et al., 2016). Exposure to DEHP could cause brain neurodegeneration and degeneration of dopaminergic neurons, as revealed in an in vivo study (Dhanya et al., 2003). However, phthalates can affect thyroid hormones not only through biosynthesis and biotransport but also through biotransformation and metabolism (Liu et al., 2015). Moreover, phthalates can affect the homeostasis of thyroid hormones which are important during childhood neurobehavioral developments (Boas et al., 2010; Huang et al., 2017; Johns et al., 2016).

Birth cohort studies have reported conflicting observations regarding sex-specific associations between phthalate exposure and neurobehavioral development. Engel et al. (2010) reported positive associations between low-molecular-weight phthalates and externalizing problem scores in boys (Engel et al., 2010). However, Whyatt et al. (2012) observed positive associations between MBzP and internalizing behavior scores in girls. Neither Kobrosly et al. (2014) nor Lien et al. (2015) observed any significant sex-specific associations between phthalate exposure and internalizing behavior or externalizing behavior scores. Although our results do not reveal any interaction effects of maternal and childhood phthalate exposure and children's sex on internalizing behavior and externalizing behavior scores, future studies are required to confirm whether there are sex-dependent effects and the underlying modulating effects of sex.

This study has several limitations. First, our sample size was small, which could have affected the sex-specific associations between phthalate exposure and neurobehavioral development in children. Second, spot urine in pregnant women from their third trimester was assessed for phthalate metabolite concentrations, which may not be representative of the average body burden of the participants because of the short half-lives of these chemicals. Nonetheless, phthalates measured in spot urine samples from pregnant women can have moderate reliability for a period ranging from weeks to months (Suzuki et al., 2009). Our previous data about third-trimester urinary phthalate metabolite levels supported this result (Wu et al., 2018). Thirdly, we observed no significant differences in all studied characteristics and phthalate concentrations between the subjects included and excluded from the present study, except for higher maternal educational levels and lower maternal MBzP levels in the follow-up group. However, as compare to the excluded group, the included tend to have higher paternal educations and family income, and exposed to less maternal smoking behaviors though non-significant. Thus, we might have subjects with higher social economic status with more awareness of avoiding phthalate exposure than the excluded. We thought this potential selection bias might make the hypothesis towards to null due to the possibly narrowed social environment with the lower exposure in included group. Overall, generalizing the results in this study to the overall Asian population may be impeded by the limited sample size

and number of ethnic backgrounds. Fourth, the correlations of urinary phthalate metabolite levels within pregnant women, and children at the same age group were significantly low to moderate. Mutual adjustment for the correlated phthalate may lead to type II error (Braun et al., 2016). Thereby to certain extent we could not point out which phthalate compound played the specific causal role. Finally, we conducted multiple comparisons for the 8 phthalate compounds and thus Type I error was considered. Due to correlation of the chemical mixtures, we decided not to perform the adjustment (e.g., Bonferroni one) to prevent type II error from the lack of independence among the chemicals (Perneger, 1998). Furthermore, after adjustments for all maternal and children's urinary phthalate metabolites mutually in the same models (Table S5), most of these results were similar to those in the present study (Table 4). Future newer method to deal with such situation is warranted (Braun et al., 2016). Despite these limitations, our study has several strengths. For example, the follow-up design owns the temporality for causal inference. Besides, both prenatal and postnatal exposures were evaluated with same methods to observe relative important of the effect associations. In addition, the repeated-measurement approach in this study favored statistical modeling techniques to more accurately detect the associations.

5. Conclusions

Our results provide evidence that prenatal and/or postnatal exposure to environmental phthalates can influence development of children's and adolescence's behaviors. Further large-scale research is required to confirm these associations and investigate sex-specific effects.

Acknowledgments

We are greatly indebted to the clinics of the Department of Obstetrics and Gynecology and the Chung-Shan Medical University Hospital, particularly for assistance with subject recruitment and follow-up from Dr. Tsung-Ho Ying and Dr. Ching-Yi Lin, respectively. We appreciate the assistance of Mr. Chien-Jen Wang in phthalate metabolite quantification and Ms. Shih-Fen Tsai, Hsiao-Yen Chen, and Ms. Carol Huichun Chen in subject recruitment and data and specimen collection at the National Health Research Institutes, Miaoli, Taiwan.

Sources of financial support

This study was funded by the National Health Research Institutes, Taiwan (EO-104-SP-01, and EM-107-PP-05), and the Ministry of Science and Technology, Taiwan (MOST 106-2314-B-400-014-MY3, 106-3114-B-400-002, and 107-2321-B-400-010).

Competing interests

The authors declare no competing interests.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envres.2019.02.029.

References

Achenbach, T.M., 1991. Manual for the Child Behavior Checklist/4-18 and 1991 Profile. University of Vermont Department of Psychiatry, Burlington, VT.
Beko, G., Weschler, C.J., Langer, S., Callesen, M., Toftum, J., Clausen, G., 2013. Children's phthalate intakes and resultant cumulative exposures estimated from urine compared with estimates from dust ingestion, inhalation and dermal absorption in their homes and daycare centers. PLoS One 8, e62442.
Boas, M., Frederiksen, H., Feldt-Rasmussen, U., Skakkebaek, N.E., Hegedus, L., Hilsted, L., Juul, A., Main, K.M., 2010. Childhood exposure to phthalates: associations with thyroid function, insulin-like growth factor I, and growth. Environ. Health Perspect. 118, 1458–1464.
Braun, J.M., Gennings, C., Hauser, R., Webster, T.F., 2016. What can epidemiological studies tell us about the impact of chemical mixtures on human health? Environ. Health Perspect. 124, A6–A9.
Chen, M.L., Chen, J.S., Tang, C.L., Mao, I.F., 2008. The internal exposure of Taiwanese to phthalate–an evidence of intensive use of plastic materials. Environ. Int. 34, 79–85.
Chen, T., Yang, W., Li, Y., Chen, X., Xu, S., 2011. Mono-(2-ethylhexyl) phthalate impairs neurodevelopment: inhibition of proliferation and promotion of differentiation in PC12 cells. Toxicol. Lett. 201, 34–41.
Dhanya, C.R., Indu, A.R., Deepadevi, K.V., Kurup, P.A., 2003. Inhibition of membrane Na (+)-K+ Atpase of the brain, liver and RBC in rats administered di(2-ethyl hexyl) phthalate (DEHP) a plasticizer used in polyvinyl chloride (PVC) blood storage bags. Indian J. Exp. Biol. 41, 814–820.
Engel, S.M., Miodovnik, A., Canfield, R.L., Zhu, C., Silva, M.J., Calafat, A.M., Wolff, M.S., 2010. Prenatal phthalate exposure is associated with childhood behavior and executive functioning. Environ. Health Perspect. 118, 565–571.
Heudorf, U., Mersch-Sundermann, V., Angerer, J., 2007. Phthalates: toxicology and exposure. Int. J. Hyg. Environ. Health 210, 623–634.
Hornung, R.W., Reed, L.D., 1990. Estimation of average concentration in the presence of nondetectable values. Appl. Occup. Environ. Hyg. 5, 46–51.
Hu, D., Wang, Y.X., Chen, W.J., Zhang, Y., Li, H.H., Xiong, L., Zhu, H.P., Chen, H.Y., Peng, S.X., Wan, Z.H., Zhang, Y., Du, Y.K., 2017. Associations of phthalates exposure with attention deficits hyperactivity disorder: a case-control study among Chinese children. Environ. Pollut. 229, 375–385.
Huang, H.B., Chuang, C.J., Su, P.H., Sun, C.W., Wang, C.J., Wu, M.T., Wang, S.L., 2017. Prenatal and childhood exposure to phthalate diesters and thyroid function in a 9-year follow-up birth cohort study: Taiwan maternal and infant cohort study. Epidemiology 28 (Suppl 1) (S10-s18).
Johns, L.E., Ferguson, K.K., McElrath, T.F., Mukherjee, B., Meeker, J.D., 2016. Associations between repeated measures of maternal urinary phthalate metabolites and thyroid hormone parameters during pregnancy. Environ. Health Perspect. 124, 1808–1815.
Kim, B.N., Cho, S.C., Kim, Y., Shin, M.S., Yoo, H.J., Kim, J.W., Yang, Y.H., Kim, H.W., Bhang, S.Y., Hong, Y.C., 2009. Phthalates exposure and attention-deficit/hyperactivity disorder in school-age children. Biol. Psychiatry 66, 958–963.
Kim, S., Eom, S., Kim, H.J., Lee, J.J., Choi, G., Choi, S., Kim, S., Kim, S.Y., Cho, G., Kim, Y.D., Suh, E., Kim, S.K., Kim, S., Kim, G.H., Moon, H.B., Park, J., Kim, S., Choi, K., Eun, S.H., 2017. Association between maternal exposure to major phthalates, heavy metals, and persistent organic pollutants, and the neurodevelopmental performances of their children at 1 to 2years of age- CHECK cohort study. Sci. Total Environ. 624, 377–384.
Kobrosly, R.W., Evans, S., Miodovnik, A., Barrett, E.S., Thurston, S.W., Calafat, A.M., Swan, S.H., 2014. Prenatal phthalate exposures and neurobehavioral development scores in boys and girls at 6–10 years of age. Environ. Health Perspect. 122, 521–528.
Koch, H.M., Calafat, A.M., 2009. Human body burdens of chemicals used in plastic manufacture. Philos. Trans. R. Soc. Lond. B Biol. Sci. 364, 2063–2078.
Lien, Y.J., Ku, H.Y., Su, P.H., Chen, S.J., Chen, H.Y., Liao, P.C., Chen, W.J., Wang, S.L., 2015. Prenatal exposure to phthalate esters and behavioral syndromes in children at 8 years of age: Taiwan maternal and infant cohort study. Environ. Health Perspect. 123, 95–100.
Lin, L.C., Wang, S.L., Chang, Y.C., Huang, P.C., Cheng, J.T., Su, P.H., Liao, P.C., 2011. Associations between maternal phthalate exposure and cord sex hormones in human infants. Chemosphere 83, 1192–1199.
Littell, R.C., Statistical Analysis System, I, 2006. SAS System for Mixed Models, Mixed Model Diagnostics. SAS Inst., Cary, NC, pp. 414–436.
Liu, C., Zhao, L., Wei, L., Li, L., 2015. DEHP reduces thyroid hormones via interacting with hormone synthesis-related proteins, deiodinases, transthyretin, receptors, and hepatic enzymes in rats. Environ. Sci. Pollut. Res. Int. 22, 12711–12719.
Messerlian, C., Bellinger, D., Minguez-Alarcon, L., Romano, M.E., Ford, J.B., Williams, P.L., Calafat, A.M., Hauser, R., Braun, J.M., 2017. Paternal and maternal preconception urinary phthalate metabolite concentrations and child behavior. Environ. Res. 158, 720–728.
Perneger, T.V., 1998. What's wrong with Bonferroni adjustments. BMJ 316, 1236–1238.
Rothman, K.J., Greenland, S., Lash, T.L., I, O.T., 2008. Instroduction to stratified analysis. In: Modern Epidemiology, pp. 258–282.
Silva, M.J., Barr, D.B., Reidy, J.A., Malek, N.A., Hodge, C.C., Caudill, S.P., Brock, J.W., Needham, L.L., Calafat, A.M., 2004. Urinary levels of seven phthalate metabolites in the U.S. population from the National Health and Nutrition Examination Survey (NHANES) 1999–2000. Environ. Health Perspect. 112, 331–338.
Suzuki, Y., Niwa, M., Yoshinaga, J., Watanabe, C., Mizumoto, Y., Serizawa, S., Shiraishi, H., 2009. Exposure assessment of phthalate esters in Japanese pregnant women by using urinary metabolite analysis. Environ. Health Prev. Med. 14, 180–187.
Szklo, M., Nieto, F.J., 2013. Epidemiology: Beyond the Basics, Identifying Noncausal Associations: Confounding. Jones and Bartlett, Mundford, pp. 153–183.
Tully, K., Kupfer, D., Dopico, A.M., Treistman, S.N., 2000. A plasticizer released from IV drip chambers elevates calcium levels in neurosecretory terminals. Toxicol. Appl. Pharmacol. 168, 183–188.
Wang, R., Xu, X., Zhu, Q., 2016. Pubertal exposure to di-(2-ethylhexyl) phthalate influences social behavior and dopamine receptor D2 of adult female mice. Chemosphere 144, 1771–1779.
Wechsler, D., 1997. Wechsler Intelligence Scale for Children–III. Chinese Behavioral Science Corporation, Taipei, Taiwan (Chinese version).
Wechsler, D., 2007. (Chinese version). Wechsler Intelligence Scale for Children, 4th ed. Chinese Behavioral Science Corporation, Taipei, Taiwan.
Whyatt, R.M., Liu, X., Rauh, V.A., Calafat, A.M., Just, A.C., Hoepner, L., Diaz, D., Quinn,

J., Adibi, J., Perera, F.P., Factor-Litvak, P., 2012. Maternal prenatal urinary phthalate metabolite concentrations and child mental, psychomotor, and behavioral development at 3 years of age. Environ. Health Perspect. 120, 290–295.
Won, E.K., Kim, Y., Ha, M., Burm, E., Kim, Y.S., Lim, H., Jung, D.E., Lim, S., Kim, S.Y., Kim, Y.M., Kim, H.C., Lee, K.J., Cheong, H.K., Kang, H.T., Son, M., Sakong, J., Oh, G.J., Lee, C.G., Kim, S.Y., Ryu, J.M., Kim, S.J., 2016. Association of current phthalate exposure with neurobehavioral development in a national sample. Int. J. Hyg. Environ. Health 219, 364–371.
Wu, C.F., Chen, H.M., Sun, C.W., Chen, M.L., Hsieh, C.J., Wang, S.L., Wu, M.T., 2018. Cohort profile: the Taiwan Maternal and Infant Cohort Study (TMICS) of phthalate exposure and health risk assessment. Int. J. Epidemiol.
Xu, Y., Agrawal, S., Cook, T.J., Knipp, G.T., 2007. Di-(2-ethylhexyl)-phthalate affects lipid profiling in fetal rat brain upon maternal exposure. Arch. Toxicol. 81, 57–62.
Yang, H.J., Soong, W.T., Chiang, C.N., Chen, W.J., 2000. Competence and behavioral/emotional problems among Taiwanese adolescents as reported by parents and teachers. J. Am. Acad. Child Adolesc. Psychiatry 39, 232–239.