A benchmark dose study of prenatal exposure to di(2-ethylhexyl) phthalate and behavioral problems in children

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ABSTRACT

Introduction: Prenatal exposure to di(2-ethylhexyl) phthalate (DEHP) has been reported to be associated with adverse effects on neurodevelopment that yield behavior syndromes in young children with an estimated median exposure lower than the currently recommended tolerable daily intake (TDI) and reference dose (RfD).
Objectives: Our aim was to derive the benchmark dose for prenatal exposure to DEHP for the neurodevelopmental health in children.
Methods: A total of 122 mother-child pairs from the Taiwan Maternal and Infant Cohort Study were analyzed for the dose-response relationship between maternal exposure to DEHP and children's behavioral syndromes evaluated at 8 years (n = 122, 2009), 11 years (n = 96, 2012), and 14 years (n = 78, 2015) of age. We employed a multivariate regression model to assess the statistical associations between the estimated maternal average daily intake of DEHP and child's individual CBCL scores for boys and girls at each separate age, followed by a mixed model for all the children across three ages accounting for individual variations. We then employed structural equation models by combining the children's specific behavioral problem scores at different ages and obtained a simulated overall latent score in relation to maternal exposure. Based on the established dose-response relationship, we derived the benchmark dose (BMD) and the lower limit (BMDL).
Results: Associations of maternal DEHP exposure (median $4.54 \mu g/kg\_bw/day$) with the Child Behavior Checklist (CBCL) scores were all significant, except for somatic complaints, adjusting for child's age, gender, IQ, and family income. The BMDL, given a benchmark response of 0.10 (0.05) and a background response of 0.05, was 6.01 (2.16) $\mu g/kg\_bw/day$ for an integrated CBCL score.
Conclusions: The current TDI (RfD) of 50 (20) $\mu g/kg\_bw/day$ for DEHP might not protect pregnant women for their children from behavioral problems. There remains the lack of comparable toxicological data. Further investigations are needed.

1. Introduction

Phthalates esters are a family of chemicals that are ubiquitous in the daily living environment. High molecular weight phthalates (HMWP), such as di(2-ethylhexyl) phthalate (DEHP) and butylbenzyl phthalate (BBzP), are mainly used as plasticizers and adhesives in polyvinyl chloride (PVC) products (Ejaredar et al., 2015; Guo et al., 2011). Low molecular weight phthalates (LMWP), such as dimethyl phthalate (DMP), dibutyl phthalate (DBP), and diethyl phthalate (DEP), are used in cosmetic products, lotions, and pharmaceutical products (Ejaredar et al., 2015). Exposure to various phthalates mainly occurs through the pathways of plastic containers, foods, plastic packaging for foods, indoor environment, and personal care products (Chen et al., 2017; Schecter et al., 2013).

Mounting evidence from human observational studies concerning the negative effects of prenatal phthalates exposure on neurodevelopment in children has been reported. In a multiethnic birth cohort study, significant negative associations between HMWP exposure and Orientation score and Quality of Alertness score were found in female infants. However, there were opposite patterns between boys and girls

(Engel et al., 2009). In outcomes from prospective follow-up studies, higher somatic problems in boys aged 6–10 years old with prenatal exposure to molar sum of DEHP metabolites was found (Kobrosly et al., 2014). In a birth cohort study in Taiwan, positive associations between maternal DEHP exposure (median 4.54 $\mu g/kg\_bw/day$) and externalizing domain behavior problems in children at 8 years of age were found (Lien et al., 2015). Recently, prenatal exposure to molar sum of DEHP metabolites was found to be significantly associated with increased risk of emotional symptoms and internalizing behavior in boys at 3 years old (Philippat et al., 2017).

The current regulatory limit for DEHP is the tolerable daily intake (TDI) of 50 $\mu g/kg\_bw/day$ recommended by the European Food Safety Authority (EFSA), based on the study of testicular and developmental toxicity in rats (EFSA, 2005). Also, a reference dose (RfD) of 20 $\mu g/kg\_bw/day$ is recommended by the US Environmental Protection Agency, based on the study of increased liver weights in guinea pigs (US EPA, 1987). The TDI of 50 $\mu g/kg\_bw/day$ for DEHP was recently further endorsed by the French Agency for Food, Environmental and Occupational Health (ANSES and Opinion, 2015) and Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) of the European Commission (2016). However, to date, none of the human epidemiological studies with reported negative health effects had median exposure to DEHP that exceeded 20 or 50 $\mu g/kg\_bw/day$ (see Table 3), which suggests that the current recommended reference levels might not be able to safeguard public health, especially neurobehavioral development in children due to prenatal exposure.

For animal studies, perinatal exposure to DEHP was shown to be associated with anxiety- and depression-like behaviors in rats (Holahan and Smith, 2015; Xu et al., 2015). Such prenatal exposure was also shown to suppress aromatase enzyme activity in male rat pups but not female pups, suggesting that there might be a sex-specific effect on brain development (Andrade et al., 2006; Holahan and Smith, 2015). In an experimental study, male pups prenatally and postnatally exposed to DEHP showed significantly decreased brain weight and decreased dopaminergic activity at 6 weeks, suggesting that prenatal exposure might be related to hyperactive behaviors through the dopaminergic pathway (Miodovnik et al., 2014; Tanida et al., 2009).

In this study, using the same birth cohort of Lien et al. (2015) but with two further follow-up visits when the children were 11 and 14 years old, we intended to derive the prenatal DEHP exposure level that is acceptable without adverse effects on children's neurobehavioral development using a benchmark dose approach.

2. Materials and methods

2.1. Study population

The participants were recruited for a birth cohort study of prenatal environmental exposure and child health outcomes, which became the pilot for the nationwide Taiwan Maternal and Infant Cohort Study (TMICS) (Huang et al., 2015; Lien et al., 2015; Lin et al., 2011). A total of 610 pregnant women aged 25–34 years without clinical complications of eclampsia or preeclampsia at a medical center in central Taiwan between December 1, 2000 and November 30, 2001 were invited to join the study. Among them, 430 women completed information about demographic and dietary habits and provided urine samples during the 3rd trimester. A follow-up study to evaluate the associations between prenatal exposure to DEHP and children's behavior problems was performed when the children were 8 years of age (n = 122) (Lien et al., 2015); they were then successfully followed at 11 (n = 96) and 14 (n = 78) years of age. This study was approved by the Institutional Review Board of the National Health Research Institutes of Taiwan. Informed consent was obtained from each of the pregnant participants before the commencement of the study.

2.2. Phthalate measurements

Seven phthalate metabolites, including mono-2-ethylhexyl phthalate (MEHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP), mono-hydroxyhexyl phthalate (MEHHP), mono-methyl phthalate (MMP), mono-ethyl phthalate (MEP), mono-butyl phthalate (MBP), and mono-benzyl phthalate (MBzP), were measured for the study. Maternal urine samples were collected during the 3rd trimester, and children's urine samples were collected at the follow-up when they were 8, 11, and 14 years old. The details of quality control procedures and assurance of the analysis of the collected urine samples were described previously (Lien et al., 2015; Lin et al., 2011). In brief, β-glucuronidase enzymatic deconjugation of the metabolites was used from their glucuronidated form. The procedure was followed by solid-phase extraction and qualification using liquid chromatography-electrospray ionization-tandem mass spectrometry. The spectro-photometric method was used to measure urinary creatinine level in the central laboratory of Kaohsiung Medical Center (Lien et al., 2015).

2.3. Child behavior checklist (CBCL) and IQ scores

The behavior problems of the participated children were evaluated by the CBCL, which was completed by their mothers based on the status of the children during the previous 6 months when the children were 8, 11, and 14 years old at the visits. Eight behavior syndromes, namely, Withdrawn, Somatic Complains, Anxious/Depressed, Social Problems, Thought Problems, Attention Problems, Delinquent Behavior, and Aggressive Behavior, were obtained based on cross-information analysis of the CBCL/4–18 (Achenbach, 1991). Of these syndromes, the broad-band Internalizing Problems scores were derived from the sum of Withdrawn, Somatic Complaints, and Anxious/Depressed; and Externalizing Problems were derived from the sum of Delinquent Behavior and Aggressive Behavior (Achenbach, 1991). The Chinese version of the CBCL (CBCL-C, with a normalized mean of 50 and a SD of 10) was validated, and the reliability was satisfactory for adolescents in Taiwan (Yang et al., 2000). Full-scale IQ scores of the children were evaluated using the Chinese version of the Wechsler Intelligence Scale for Children-version III (WISC-III) (Wechsler, 1991) when they were 8, 11, and 14 years old, separately (Huang et al., 2015).

Estimation of average daily intake for the study participants. The average daily intake (AvDI) of maternal exposure to DEHP was estimated with following the equation:

$$\text{AvDI } (\mu g/kg\_bw/d) = \frac{U_{sum}(\mu mol/g) \times CE\,(mg/d)}{F_{UE} \times 1000\,(mg/g) \times BW\,(kg)} \times MW_{DEHP}, \quad [1]$$

where U_{sum} is the sums of urinary excretion of MEHP, MEOHP, and MEHHP in micromoles per gram creatinine, CE is the age, body weight, and height-specific urinary creatinine excretion value (molecular weight-transformed):

$$CE = 1.93 \times (140 - age) \times weight^{1.5} \times \frac{height^{0.5}}{1000}\,(mg/d) \quad [2]$$

(Mage et al., 2008), F_{UE} (=0.33) is the molar fraction of excreted metabolites MEHP, MEOHP, and MEHHP relative to total intake at 24-h post-dosing (Anderson et al., 2011), and MW_{DEHP} is the molecular weight of DEHP.

2.4. Statistical analyses

We employed a multivariate regression model to assess the statistical associations between the estimated maternal AvDI (natural log-transformed) and child's individual CBCL scores at ages 8, 11, and 14 years old, with adjustment for their IQ scores, family income at the time, and gender. We performed the analyses for boys and girls– separately first and then together adjusting for gender effect. To assess the

overall effect across the different ages of the participating children, we applied a mixed model with the participating children as random effects. The approach is able to differentiate between inter-individual variation from intra-individual variation. However, a consistent association across different ages was assumed.

Let $\mathbf{Y}_{it} = (Y_{i1t}, ..., Y_{i8t})'$ be the vector of behavior problem scores of the i-th subject at time t, Y_{ijt}, $j = 1, ..., 8$ corresponds to the scores of Withdrawn, Somatic Complaints, Anxious/Depressed, Social Problems, Thought Problems, Attention Problems, Delinquent Behavior, and Aggressive Behavior, respectively, and $t = 8, 11, 14$ of age. We combined the individual behavioral problem scores of children evaluated at 8, 11, and 14 years of age and the latent CBCL scores at different layers by using structural equation models (SEMs). An overall latent CBCL score U was simulated for every child. See Supplemental Material (SM), Part 1. A diagram of the SEMs of the multilevel latent CBCL scores and their relationship with maternal DEHP exposure is given in Fig. S1 (SM).

A preliminary analysis showed that the CBCL scores were linearly associated with the log-transformed maternal exposure of DEHP. This association was similar for both boys and girls: see Fig. S2 (SM). Therefore, we related the overall latent CBCL score.

U to maternal exposure of (natural log-transformed creatinine-corrected) DEHP concentrations x as follows:

$$U_i = \eta_0 + \eta_1 \ln(x_i) + \boldsymbol{\gamma}' \mathbf{Z}_i + \varepsilon_i. \tag{3}$$

Gender, family income, and IQ score were adjusted for in the model as covariables $\mathbf{Z}$ for potential confounding effects. Similar to the integrated CBCL scores, we obtained a combined latent family income and IQ score at ages 8, 11, and 14 years old for each child by using SEMs. Given a benchmark response (BMR) level and a background response (abnormal probability) p_0, we derived the corresponding BMD with equation [3]: see SM, Part 2.

To ensure that the associations between CBCL scores and maternal exposure were robust to individual variations, we estimated the longitudinal risk of child's behavior problem for different levels of prenatal DEHP exposures. At each age, a child's score was classified as (sub-)clinical if the simulated overall CBCL score for that child exceeded the 85th percentile. The 85th percentile cutoff was determined based on the range of proportions of the participated children with the standardized CBCL scores fell within the sub-clinical and clinical categories: see Table S1 (SM). We used a mixed effect logistic model with the participating children as random effects to estimate and compare the longitudinal risks of clinical behavior problem of the prenatal DEHP exposure subgroups < 25th (low), 25th-66th (medium), and > 66th (high) percentiles. The cutoff was set at the 25th percentile instead of 33rd percentile to avoid potential misclassification. Covariates adjusted in the model included mother's age at childbirth, family income, gender, child's age, and IQ measured at the time.

Because of the hierarchical structure shown in Fig. S1, a Bayesian approach using MCMC simulations for the distributions of latent variables at different stages to avoid cumbersome likelihood derivations appears to be a natural way to derive the corresponding BMDs and BMDLs. We employed Markov chain Monte-Carlo (MCMC) simulations for the posterior distributions of the latent CBCL scores at different layers and the parameter estimates of the SEMs. We then related the simulated overall CBCL score to the estimated maternal average daily intake AvDI of DEHP to derive the BMD. The 95% lower bound (BMDL) was obtained as the 5th percentile of the simulated BMDs after convergence of MCMC simulations. We performed the MCMC simulations using WinBUGS 1.4.3 (MRC Biostatistics Unit, Cambridge, UK), and the analyses of statistical associations using SAS software version 9.4 (SAS Institute Inc., Cary, NC). A P-value less than 0.05 was considered significant.

2.5. Sensitivity analyses

The children's DEHP exposure may show different associations with the CBCL scores at different ages. We performed an additional analysis incorporating the estimated DEHP exposures of the children into the model to derive the corresponding BMDL, as well as a separate analysis by deleting the two extremely high maternal exposures, 61.8 and 188.7 $\mu g/kg\_bw/day$. Spot urine samples are known to have intra-individual variations of metabolite levels. We performed a separate analysis substituting the individual's estimated DEHP daily intake by a lognormal distribution with a mean of the calculated AvDI and a variance of AvDI/6 (i.e., coefficient of variation = 40.8%) in the model and derived the corresponding BMD.

Studies have shown that prenatal exposures to heavy metals (Budtz-Jørgensen et al., 2002; Kim et al., 2018), other phthalates (Kobrosly et al., 2014; Lien et al., 2015; Whyatt et al., 2012), and mother's physiological and psychological stress during pregnancy (Tearne et al., 2015) have an effect on children's neurodevelopment. We assessed maternal urine samples for phthalates including DMP, DEP, DBP, and BBzP, and heavy metals including vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), gallium (Ga), arsenic (As), cadmium (Cd), indium (In), thallium (Tl), and lead (Pb) separately for their associations with the overall CBCL score and their possible effects on the derived BMD (BMDL) level. We obtained a summary stress score of mother on the degree of sadness, tiredness, exhaustion, and nervousness, etc. based on the questionnaire collected during pregnancy. A separate sensitivity analyses was performed by incorporating these covariates into the model and deriving the adjusted BMDs (BMDLs) to assess the potential confounding effects.

3. Results

3.1. Baseline characteristics

A total of 122 women were followed when their children were 8 years old. Of the 122 children followed at age 8 years old, 96 were followed when they were 11 years old, and 78 were followed at 14 years old, with slightly more girls than boys. Table 1(a) summarizes the urinary DEHP metabolite levels, estimated DEHP exposures, family income, IQ scores, and CBCL scores of these children. The metabolite levels (adjusted for creatinine) of MEHP, MEHHP, and MEOHP as well as the estimated DEHP exposures tended to decrease with age, which might have resulted from the growing body weight of the children. For the CBCL scores of the children, there was a significant increasing trend in thought problems and attention problems and a decreasing trend in aggressive behavior. Comparisons between the groups of children lost to follow-up at ages 11 and 14 years old for the CBCL scores showed no significant differences: see Table S2 (SM). Among the pregnant women, 7 were older than 35 years at delivery, 65.6% had an education level higher than junior college, and only 1 smoked during pregnancy (Lien et al., 2015). The percentiles of the phthalate metabolite levels of MEHP, MEHHP, and MEOHP in urine collected during the third trimester and the estimated daily intakes of DEHP of these women are given in Table 1(b). Except for 2 women with very high DEHP exposures (61.8 and 188.7 $\mu g/kg\_bw/day$), most of the women had an exposure level lower than the suggested TDI or RfD of DEHP, with a median of 4.54 $\mu g/kg\_bw/day$.

3.2. Associations between DEHP exposure and CBCL scores

We regressed the children's CBCL scores with the estimated maternal DEHP exposure (after log-transformation) by age and gender separately. All the individual CBCL scores had a positive association with prenatal DEHP exposure for both boys and girls at different ages except for Somatic Complaints and Thought Problems in girls at 8 years old. Consistent with the previous study when the children were 8 years

Table 1

Maternal DEHP exposures and children's CBCL scores at different ages. (a) Baseline characteristics and CBCL scores of the participating children at ages 8, 11, and 14 years old; (b) Distribution of the urinary DEHP metabolites of the pregnant women (n = 122).

(a)

Age of Children				
	8 (n = 122)	11 (n = 96)	14 (n = 78)	p-value
Study Year	2009	2012	2015	
Number of Boys[a]	58 (47%)	46 (48%)	37 (47%)	0.98
Mean ± SD[b] (2.5%, 97.5%)				
Body Weight (kg)	29.3 ± 7.6 (21.0, 48.0)	42.4 ± 11.3 (27.1, 70.9)	55.3 ± 12.3 (39.9, 84.7)	< 0.0001
Height (cm)	130.8 ± 5.7 (120.8, 141.0)	148.2 ± 7.3 (134.1, 162.4)	163.8 ± 7.6 (153.5, 182.0)	< 0.0001
BMI (kg/m^2)	17.0 ± 3.2 (12.9, 25.2)	19.1 ± 3.8 (13.6, 28.2)	20.5 ± 3.7 (16.3, 30.4)	< 0.0001
IQ Score	108.8 ± 12.2 (86.0, 133.0)	110.9 ± 14.5 (87.0, 140.0)	112.8 ± 14.8 (84.0, 140.0)	0.15
Annual Family Income (× 10^3 USD)	40.1 ± 24.0 (6.9, 96.5)	38.7 ± 25.5 (8.6, 120.7)	40.9 ± 22.3 (8.6, 77.6)	0.47
GM[b] (95% CI)				
DEHP metabolite				
MEHP (μg/g creatinine)	8.29 (6.61, 10.38)	8.91 (7.35, 10.81)	2.86 (2.20, 3.74)	< 0.0001
MEHHP	43.24 (36.82, 50.77)	31.84 (28.40, 35.69)	13.13 (11.67, 14.79)	< 0.0001
MEOHP	37.21 (31.73, 43.65)	23.37 (19.25, 28.36)	9.10 (8.02, 10.33)	< 0.0001
Daily Intake (μg/kg-BW/day)	6.82 (5.77, 8.07)	5.12 (4.52, 5.80)	2.07 (1.81, 2.37)	< 0.0001
Mean[c] (2.5%, 97.5%)				
CBCL scores				
Withdrawn	45.19 (38.71, 65.33)	46.47 (38.62, 69.38)	47.05 (38.62, 65.54)	0.21
Somatic Complaints	48.14 (42.22, 76.18)	49.66 (42.02, 80.64)	49.56 (42.02, 72.06)	0.41
Anxious/Depressed	49.64 (40.82, 68.94)	49.33 (40.78, 71.78)	46.41 (40.78, 61.45)	0.02
Social Problems	51.61 (40.50, 76.04)	52.85 (40.44, 76.00)	48.31 (40.44, 80.44)	0.01
Thought Problems	47.55 (42.45, 68.73)	47.67 (42.27, 69.55)	54.63 (42.27, 96.82)	< .0001
Attention Problems	47.84 (36.90, 68.27)	47.71 (36.72, 65.71)	50.81 (36.72, 74.41)	0.05
Delinquent Behavior	49.29 (42.31, 70.55)	48.82 (42.37, 70.28)	49.11 (42.37, 70.28)	0.91
Aggressive Behavior	50.05 (39.39, 72.88)	48.91 (39.41, 70.78)	45.70 (39.41, 64.90)	0.002
Internalizing Problems	47.58 (38.98, 70.50)	48.34 (38.67, 73.05)	47.10 (38.67, 64.80)	0.59
Externalizing Problems	49.81 (39.60, 72.20)	48.87 (39.64, 70.76)	46.54 (39.64, 64.83) 0.03	

(b)

Percentiles							
N = 122	GM	Mean ± SD	Min.	10%	50%	90%	Max.
MEHP (μg/g creatinine)	16.93	30.06 ± 67.63	1.79	5.65	15.89	57.65	706.10
MEHHP	7.91	26.77 ± 57.70	0.05	0.30	11.95	51.32	489.28
MEOHP	13.59	39.18 ± 99.65	0.16	1.99	16.26	69.78	1010.72
DEHP (μg/kg bw/day)	4.56	8.59 ± 18.47	0.21	1.38	4.54	14.12	188.71

CBCL, child behavior checklist; CI, confidence interval; DEHP, di(2-ethylhexyl) phthalate; GM, geometric mean; MEHP, mono-2-ethylhexyl.
phthalate; MEHHP, mono-2-ethyl5-hydroxyhexyl phthalate; MEOHP, mono-2-ethyl-5-oxohexyl phthalate; SD, standard deviation.

[a] Age difference was assessed by chi-square test.
[b] Age difference was assessed by Kruskal-Wallis test.
[c] Age difference was assessed by analysis of variance (ANOVA).

old (Lien et al., 2015), we found that the associations between maternal exposure to DEHP and children's Delinquent, Aggressive Behavior, and Externalizing Problems were significant when they were 11 and 14 years old. Because the associations were similar across different ages, a mixed model was employed for the overall association adjusting for age effect. Except for Somatic Complaints, all the CBCL scores were significant in the mixed model. However, incorporating Somatic Complaints as one of the components, Internalizing Problems was significant for both boys and girls at age 14 and in the mixed model. Alternatively, the associations for boys and girls at different ages were similar. Therefore, we performed a separate mixed model analysis first adjusted for gender effect for different age in the combined sample of boys and girls, and then further adjusted for age effect in the model. The outcomes were all similar to those for different age and gender separately, but the statistical associations were even more significant. We did not find statistical associations at any age between children's CBCL scores, as well as their IQ scores, and their DEHP metabolite concentrations measured at ages 8, 11, and 14. Table 2 summarizes the regression coefficients for each individual CBCL score for boys and girls at different ages and the outcomes for mixed models adjusting for child's age and gender separately and together. Fig. 1 shows the linear association between the children's integrated CBCL scores and the

estimated maternal exposure of DEHP daily intakes after log-transformation, which is parallel for boys and girls. To ensure that the associations between maternal exposure and child's CBCL scores were unbiased, we also assessed the Spearman correlations between mother's DEHP exposure and child's exposure at ages 2, 5, 8, 11, and 14 years old. The results showed that the maternal DEHP levels were uncorrelated with the children's DEHP levels across all ages. See Table S3 (SM). We did not include the data of the children at ages 2 and 5 years old in the analysis because CBCL scores were not applicable for children at younger ages.

Compared with children who had lower percentiles of prenatal exposures, children of the high exposure group had consistently higher overall CBCL scores than those of the medium and low exposure groups at ages 8, 11, and 14 years old. Similarly, children of the medium exposure group had consistently higher CBCL scores than those of the low exposure group (Fig. 2A). The trajectory model predictions of the three exposure subgroups for the probability of (sub-) clinical at different ages showed similar pattern as the overall CBCL scores. That is, children in the higher prenatal exposure group had a trajectory of higher probability of being (sub-) clinical when they grew older (Fig. 2B).

To compare with the exposures of other epidemiological studies, Table 3 summarizes the DEHP metabolite levels and the estimated daily

Table 2
Coefficient estimates of regression models for associations between log-transformed maternal DEHP. exposures and children's CBCL scores.

		Children's Age (years)[a]			Overall[b]
		8	11	14	
Boys		(n = 58)	(n = 43)	(n = 31)	
	Withdrawn	0.67 (−1.43, 2.77)	2.87 (−0.16, 5.91)	3.85 (0.95, 6.75)*	1.99 (0.26, 3.72)*
	Somatic Complaints	2.55 (0.29, 4.80)*	0.91 (−3.16, 4.98)	1.65 (−2.28, 5.58)	1.98 (−0.28, 4.24)
	Anxious/Depressed	1.06 (−1.30, 3.43)	2.08 (−0.95, 5.11)	1.18 (−1.00, 3.36)	1.36 (−0.33, 3.05)
	Social Problems	3.00 (0.27, 5.73)*	3.41 (−0.57, 7.39)	0.05 (−3.78, 3.87)	2.45 (0.01, 4.88)*
	Thought Problems	1.85 (−1.92, 5.62)	2.85 (−1.31, 7.01)	2.80 (−4.23, 9.83)	2.98 (−0.24, 6.20)
	Attention Problems	1.49 (−1.29, 4.26)	2.31 (−0.99, 5.61)	1.75 (−2.30, 5.80)	2.02 (−0.39, 4.43)
	Delinquent Behavior	4.18 (1.76, 6.59)**	2.89 (−0.50, 6.29)	3.52 (0.20, 6.84)*	4.40 (2.05, 6.74)***
	Aggressive Behavior	1.97 (−0.99, 4.93)	1.51 (−1.89, 4.91)	1.33 (−0.86, 3.53)	1.88 (−0.46, 4.22)
	Internalizing Problems	1.56 (−0.64, 3.77)	2.45 (−0.74, 5.65)	2.53 (0.01, 5.06)*	2.14 (0.28, 4.00)*
	Externalizing Problems	2.80 (−0.05, 5.65)	2.06 (−1.45, 5.57)	2.13 (−0.20, 4.46)	2.83 (0.45, 5.20)*
Girls		(n = 64)	(n = 49)	(n = 35)	
	Withdrawn	1.43 (−1.03, 3.88)	1.32 (−1.32, 3.96)	2.37 (−1.91, 6.65)	1.39 (−0.69, 3.46)
	Somatic Complaints	−0.38 (−3.71, 2.95)	2.17 (−1.61, 5.95)	2.57 (−1.98, 7.13)	0.88 (−1.67, 3.43)
	Anxious/Depressed	0.51 (−2.90, 3.93)	3.12 (0.13, 6.11)*	3.94 (0.41, 7.47)*	2.00 (−0.49, 4.50)
	Social Problems	0.91 (−2.52, 4.34)	2.62 (−1.01, 6.26)	4.22 (−1.87, 10.31)	1.93 (−0.98, 4.83)
	Thought Problems	−0.44 (−3.04, 2.16)	1.25 (−1.27, 3.76)	6.02 (−0.19, 12.24)	1.32 (−0.91, 3.56)
	Attention Problems	0.35 (−2.14, 2.85)	1.86 (−0.77, 4.49)	2.56 (−2.68, 7.80)	1.18 (−1.18, 3.54)
	Delinquent Behavior	2.16 (−0.46, 4.77)	1.92 (−0.83, 4.68)	3.15 (0.42, 5.88)*	2.26 (0.38, 4.14)*
	Aggressive Behavior	4.16 (1.12, 7.21)**	2.87 (0.42, 5.32)*	2.42 (−0.70, 5.54)	3.13 (1.06, 5.20)**
	Internalizing Problems	0.64 (−2.45, 3.73)	2.73 (−0.25, 5.71)	3.77 (0.00, 7.53)*	1.80 (−0.59, 4.20)
	Externalizing Problems	3.81 (1.00, 6.62)**	2.78 (0.20, 5.37)*	2.83 (−0.18, 5.85)	3.06 (1.05, 5.07)**
All		(n = 122)	(n = 92)	(n = 66)	
	Withdrawn	1.10 (−0.46, 2.65)	2.12 (0.18, 4.06)*	3.17 (0.79, 5.55)**	1.79 (0.47, 3.11)**
	Somatic Complaints	1.59 (−0.38, 3.56)	1.29 (−1.49, 4.07)	2.41 (−0.37, 5.19)	1.63 (−0.02, 3.28)
	Anxious/Depressed	1.04 (−0.97, 3.04)	2.53 (0.49, 4.57)*	2.14 (0.22, 4.05)*	1.68 (0.23, 3.12)*
	Social Problems	2.28 (0.16, 4.40)*	3.01 (0.42, 5.60)*	1.87 (−1.40, 5.13)	2.27 (0.44, 4.10)*
	Thought Problems	1.08 (−1.19, 3.35)	2.10 (−0.25, 4.45)	4.44 (0.01, 8.87)*	2.33 (0.39, 4.27)*
	Attention Problems	1.15 (−0.70, 2.99)	2.13 (0.10, 4.16)*	2.13 (−0.88, 5.14)	1.68 (0.03, 3.33)*
	Delinquent Behavior	3.56 (1.81, 5.32)***	2.46 (0.35, 4.56)*	3.31 (1.28, 5.33)**	3.31 (1.89, 4.73)***
	Aggressive Behavior	3.00 (0.94, 5.07)**	2.06 (0.03, 4.10)*	1.86 (0.08, 3.64)*	2.42 (0.88, 3.97)**
	Internalizing Problems	1.40 (−0.43, 3.24)	2.49 (0.37, 4.61)*	3.04 (0.98, 5.11)**	2.02 (0.58, 3.46)**
	Externalizing Problems	3.38 (1.43, 5.33)***	2.34 (0.23, 4.45)*	2.46 (0.68, 4.24)**	2.88 (1.35, 4.40)***

CBCL, child behavior checklist; DEHP, di(2-exylhexyl) phthalate. *P < 0.05. **P < 0.01. ***P < 0.001.

[a] The models for boys and girls were adjusted for child's family income and IQ score, and the models for all were adjusted for child's gender, family income, and IQ score.

[b] The mixed models for boys and girls were adjusted for child's age, and the models for all were adjusted for child's age and gender, in addition to family income and IQ score.
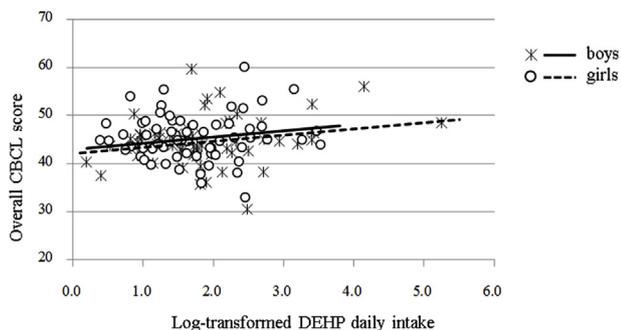

Fig. 1. Plot of the integrated child behavior checklist (CBCL) scores versus the estimated log-transformed maternal DEHP exposure for boys and girls. The CBCL scores were generated from the mean of 30,000 Monte Carlo Markov chain (MCMC) simulation runs after convergence for each of the 122 children.

intakes from studies on associations between prenatal exposure and children's neurodevelopment. All the listed studies had an estimated median daily intake of DEHP less than the RfD of $20 \mu g/kg\_bw/day$. Additionally, all of these studies reported negative effects of prenatal DEHP exposure to children's neurobehavioral problems in one way or another except for one study with no association (Whyatt et al., 2012).
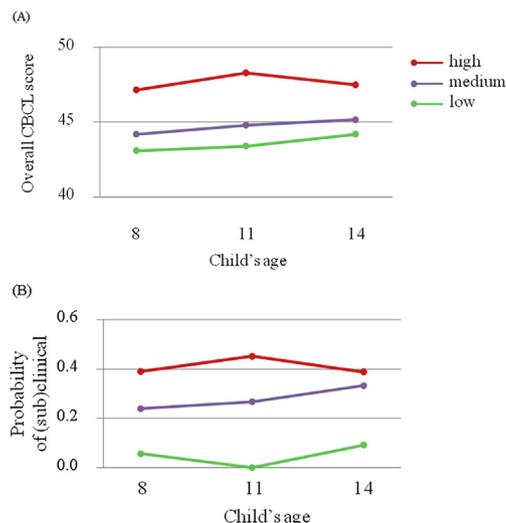

Fig. 2. Adjusted trajectories of simulated overall child behavior checklist (CBCL) scores (A) and probability of (sub-) clinical (B) of the children with prenatal DEHP exposure < 25th (low), 25th-66th (medium), and > 66th (high) percentile at ages 8, 11, and 14 years old. Covariates adjusted in the model included mother's age at childbirth, family income, child's age, gender, and IQ.

Table 3

Epidemiological studies of prenatal exposure to DEHP and children's neurodevelopmental outcomes.

Study	Country	Stage of pregnancy	Age of study children	Maternal DEHP exposure[a]	Association with neurodevelopment of children
Engel et al. (2009)	US	25–40 weeks	within 5 days of delivery (295)	MEHP 6.1 μg/L (50th) MEHHP 19.6 μg/L (50th) MEOHP 17.9 μg/L (50th) MECPP 35.8 μg/L (50th) DEHP[b] 6.27 μg/kg.bw/day	↓ Orientation and quality of alertness scores in girls
Whyatt et al. (2012)	US	3rd trimester	27–42 months for BSID-II; 33–48 months for CBCL (n = 319)	MEHP 5.1 ng/mL (GM)	No association.
				MEHHP 23.0 ng/mL (GM) MEOHP 19.2 ng/mL (GM) MECPP 40.2 ng/mL (GM) DEHP[c] 6.75 (5.95, 7.73) μg/kg.bw/day	
Kobrosly et al. (2014)	US	1st -3rd trimesters	6–10 years old (n = 153)	MEHP 3.3 ng/mL (50th) MEHHP 11.4 ng/mL (50th) MEOHP 11.1 ng/mL (50th)[d] DEHP[e] 2.90 μg/kg.bw/day	↓ Anxious/Depressed and anxiety problem scores in girls with ΣDEHP
Lien et al. (2015)	Taiwan	3rd trimester	8 years old (n = 122)	MEHP 16.93 μg/g creatinine (GM) MEHHP 7.91 μg/g creatinine (GM) MEOHP 13.59 μg/g creatinine (GM)	↑Delinquent Behavior and Externalizing Problems ↑ Social Problems, Delinquent Behavior, and Externalizing Problems ↑ Social Problems, Delinquent Behavior, Aggressive Behavior, and Externalizing Problems
				DEHP 4.54 (0.60, 31.10) μg/kg.bw/day	
Philippat et al. (2017)	France	24–28 weeks	Boys at 3 or 5 years old (n = 529)	MEHP 7.44 μg/L (50th) MEHHP 26.8 μg/L (50th) MEOHP 22.3 μg/L (50th) MECPP 38.4 μg/L (50th) ΣDEHP 0.32 μmol/L (50th) DEHP[f] 6.57 μg/kg.bw/day	↑ Emotional symptoms and Internalizing Behavior
Current study	Taiwan	3rd trimester	8 years old (n = 122) 11 years old (n = 96) 14 years old (n = 78)	DEHP 4.54 (0.60, 31.10) μg/kg.bw/day	↑ Delinquent Behavior, Aggressive Behavior, and Externalizing Problems

DEHP, di(2-exylhexyl) phthalate; GM, geometric mean; MEHP, mono-2-ethylhexyl phthalate; MEHHP, mono-2-ethyl5-hydroxyhexyl phthalate.
MEOHP, mono-2-ethyl-5-oxohexyl phthalate.

[a] The estimated DEHP daily intake was the median of the study, with 95% confidence interval given in the parenthesis.
[b] Mothers' mean age 23.5, body weight 74.7 kg, height 157.5 cm were obtained from the US CDC report (CDC, 2016); urinary creatinine 73 mg/dL (Baba et al., 2017).
[c] Mothers' mean age 25.5; body weight 74.7 kg, height = 157.5 cm were obtained from the US CDC report (CDC, 2016); urinary creatinine = 73 mg/dL (Baba et al., 2017).
[d] Metabolite levels MEHP, MEHHP, and MEOHP were obtained from Swan et al. (2005) of the same study cohort.
[e] Mothers' mean age 31.1, body weight 78.4 kg, height 163.3 cm were obtained from the US CDC report (CDC, 2016); urinary creatinine 73 mg/dL (Baba et al., 2017).
[f] Mothers' mean age 29.7, body weight 61.3 kg, height 162.5 cm were based on average human data obtained from Wikipedia (https://en.wikipedia.org/wiki/List_of_average_human_height_worldwide).

Table 4

Benchmark dose (BMD) ($\mu g/kg\_bw/day$) and 95% lower limit of BMD (BMDL) ($\mu g/kg\_bw/day$) . from Markov-chain Monte Carlo (MCMC) simulation outcomes under different scenarios.

(µg/kg_bw/day)	BMR = 0.10				BMR = 0.05			
	P₀ = 0.05		P₀ = 0.03		P₀ = 0.05		P₀ = 0.03	
	BMD	BMDL	BMD	BMDL	BMD	BMDL	BMD	BMDL
Model 1[a]								
Integrated by ages (n = 122)								
Overall CBCL score	16.80	6.01	33.46	9.96	4.47	2.16	8.64	3.63
Internalizing Problems	16.92	7.36	33.76	12.60	4.49	2.50	8.69	4.32
Externalizing Problems	15.69	5.35	30.85	8.71	4.27	1.98	8.16	3.28
Separately by age								
Overall CBCL score age 8 (n = 122)	19.51	8.65	40.03	15.24	4.95	2.81	9.77	4.95
age 11 (n = 96)	25.66	9.96	55.63	17.98	5.94	3.11	12.24	5.58
age 14 (n = 78)	10.26	4.17	18.62	6.54	3.17	1.64	5.72	2.64
Adjusted for children's exposure (n = 122)								
Overall CBCL score	16.76	5.91	33.37	9.77	4.46	2.13	8.62	3.58
Deleted two extremes (n = 120)								
Overall CBCL score	17.82	6.13	35.91	10.20	4.65	2.19	9.07	3.69
Maternal's exposure as lognormal distribution (n = 122)								
Overall CBCL score	16.15	6.02	31.93	9.98	4.35	2.16	8.36	3.64
Model 2[b]								
Adjusted for mother's SBP, stress (n = 113)								
Overall CBCL score		7.36	39.48	12.62	4.91	2.50	9.68	4.32
Model 3[c]								
Overall CBCL score (n = 107)								
Adjusted for DEP, Cr	17.67	6.42	35.55	10.75	4.63	2.26	9.01	3.84
Adjusted for DEP, As	19.86	7.17	40.90	12.24	5.01	2.46	9.92	4.22
Adjusted for DEP, Tl	18.45	6.78	37.44	11.47	4.76	2.36	9.33	4.03
Adjusted for DBP, Cr	19.59	6.84	40.23	11.57	4.96	2.37	9.81	4.05
Adjusted for DBP, As	23.56	7.30	50.20	12.49	5.61	2.49	11.41	4.29
Adjusted for DBP, Tl	22.55	7.61	47.62	13.11	5.45	2.56	11.01	4.44

As, arsenic; BMD, benchmark dose; BMDL, 95% lower limit of benchmark dose; BMR, benchmark response; CBCL, child behavior checklist; Cr, chromium; DEHP, di (2-exylhexyl) phthalate; DBP, di-butyl phthalate; DEP, diethyl phthalate; SBP, systolic blood pressure; Tl, thallium.

[a] Adjusted for child's gender, family income, and IQ score.

[b] Adjusted for child's gender, family income, and IQ score, maternal's SBP, psychological stress at urine sample collection.

[c] Adjusted for child's gender, family income, and IQ score, maternal's SBP, psychological stress at urine sample collection, and various combinations of DEP, DBP, Cr, As, and Tl.

3.3. Benchmark dose for maternal DEHP exposures

We calculated the BMD (BMDL) under different scenarios of background response p_0 and BMR separately for the combined simulated latent overall CBCL scores, Internalizing Problems, and Externalizing Problems. A single linear dose-response model was used to derive the BMDL by taking the natural logarithm of the estimated daily DEHP intake as the exposure variable. The associations between individual CBCL scores and the prenatal exposures in the original scale or after log-transformation also supports the latter relationship: See Fig. S2 (SM). To determine the background response p_0, we divided the estimated maternal DEHP exposures into $\leqq 3 \, \mu g/kg\_bw/day$ and $> 3\mu g/kg\_bw/day$ groups and the proportions of children fallen within the subclinical (CBCL scores 65–69) and clinical (CBCL scores > 69) range for each of the CBCL categories in these two groups. Depending on the CBCL problem, the percentages of the children in the $\leqq 3 \, \mu g/kg\_bw/day$ group then fell within the subclinical/clinical categories ranged from 0% to 17%, with most of the percentages ranging from 0% to 6%: see Table S1 (SM). Based on the dichotomous outcomes, we chose two scenarios, $p_0 = 0.03$ and 0.05, for the background response. Two scenarios, BMR = 0.05 and 0.10, were determined for the benchmark response. The background abnormal percentage was supported by a Canadian study (Arbuckle et al., 2016) and previous studies in Taiwan (Chen et al., 2017; Lien et al., 2015). Similarly, the scenario BMR = 0.05 and $p_0 = 0.05$ was chosen to determine the BMDL for methylmercury based on the rationale that children who function at or below 5% are considered significantly developmentally compromised, and a BMR of 0.05 would double that percentage (US EPA (2001)).

In general, given BMR = 0.10, the derived BMD levels were close to

the RfD of $20\mu g/kg\_bw/day$ when the background response $p_0 = 0.05$ and the TDI of $50\mu g/kg\_bw/day$ when $p_0 = 0.03$. However, the corresponding BMDLs, lower bound of 95% CI of BMD, were much lower than these regulation levels. The BMDL for the overall effect was $6.01\mu g/kg\_bw/day$, given BMR = 0.10 and $p_0 = 0.05$, which increased to $9.96\mu g/kg\_bw/day$ when $p_0 = 0.03$. Given BMR = 0.05, the BMDL levels lowered to 2.16 and $3.63\mu g/kg\_bw/day$ for $p_0 = 0.05$ and 0.03, respectively. The BMDLs for the Internalizing Problems and Externalizing Problems were slightly higher and lower than the levels above for different scenarios, respectively. When evaluated separately for the overall effect at different ages, the BMDLs were slightly higher at ages 8 and 11 years old, but was lower at 14 years old. The BMD estimates were the medians of the 30,000 MCMC simulated samples with a burn-in period of 20,000, and the BMDLs corresponded to the lower 5 percentiles. The other parameter estimates for the overall model are summarized in Table S4 (SM). Finally, the BMDs (BMDLs) for individual CBCL scores are given in Table S5 (SM). The individual BMD (BMDL) levels were heterogeneous and were in general smaller than those of the overall CBCL scores. All the MCMC simulation outcomes for the model parameters showed evidence of convergence.

3.4. Sensitivity analyses

The BMDs (BMDLs) by incorporating the estimated DEHP exposures of the children into the model, deleting the two extremely high maternal exposures, and treating the estimated exposures as lognormal distribution were essentially the same as those of model 1. The BMDLs of the analyses by adjusting children's exposure further in the model were also of essentially the same level. In model 2, in addition to child's

gender, IQ, and family income, we adjusted further mother's systolic blood pressure (SBP) and psychological stress score at 3rd trimester that were (marginally) significant or marginally significant in the model. The BMDL of 7.36 $\mu g/kg\_bw/day$ given BMR = 0.10 and p_0 = 0.05 was slightly higher than that of model 1. Prenatal exposures to other phthalates and heavy metals might also have an effect on child's neurodevelopment behavior problems. Among these, DEP, DBP, Cr, As, and Tl had (marginally) significant associations with the overall CBCL score. In model 3, in addition to the covariates in model 2, we adjusted further various combinations of DEP, DBP, Cr, As, and Tl in the model. For different combinations of phthalates and heavy metals, the derived BMDs (BMDLs) were of approximately the same levels or of 1 unit higher. Table 4 summarizes the simulated BMDs and BMDLs corresponding to BMR = 0.05, 0.10 and p_0 = 3%, 5%, respectively, for the integrated CBCL scores, Internalizing Problems, Externalizing Problems, different ages of model 1, and different scenarios of models 2 and 3.

4. Discussion

We found consistent and significant associations between maternal exposure to DEHP and children's Delinquent Behavior, Aggressive Behavior, and Externalizing Problems at ages 11 and 14 years old, as when they were 8 years old (Lien et al., 2015). The overall mixed model adjusting for age, gender, family income, and child's IQ also showed that, except for somatic complaints, all the associations with each of the CBCL scores were significant. The trajectory analysis comparing different subgroups for the probability of (sub-) clinical abnormal provided further suggestion of the neurodevelopment effect of prenatal exposure to DEHP on children's behavioral problems (Fig. 2). After conversion to daily intake of DEHP, over 50% of the women were exposed at a level lower than $7\mu g/kg\_bw/day$ during the 3rd trimester of pregnancy, as with all the other studies listed in Table 3. Based on the established dose-response relationship, we derived the corresponding BMD (BMDL) for allowable level of prenatal exposure under various scenarios via MCMC simulations. As shown in Table 4, the derived BMDL levels for different scenarios were several times to one order lower than the current TDI of $50\mu g/kg\_bw/day$ recommended by the EU EFSA and the RfD of $20\mu g/kg\_bw/day$ by the US EPA. To the best of our knowledge, this is the first study to use repeated CBCL scores at different ages of children and determine the allowable exposure level of DEHP for pregnant women.

We adopted a benchmark dose approach that makes better use of dose-response information (Crump et al., 2000). This approach was based on the significant association between the integrated children's CBCL scores for overall scores across ages 8, 11, and 14 years old and maternal DEHP exposures of the present study. A baseline abnormal probability p_0 was used instead of a predetermined abnormal score x_0 for threshold because the integrated CBCL scores might have a mean and scale different from the original standardized CBCL scores. The approach by specifying p_0 also has the advantage of independence on the covariates (Crump et al., 2000).

The BMD derivation for prenatal DEHP exposure is similar to the derivation of RfD for methylmercury by the US EPA, 2001, which is also based on epidemiological studies of the effects of prenatal exposure on children's neurobehavioral deficits (Budtz-Jørgensen et al., 2000). However, rather than evaluating the BMD separately for each endpoint, we adopted a SEM approach to derive the BMD for an integrated overall effect (Budtz-Jørgensen et al., 2002; Budtz-Jørgensen, 2007). An estimation of the point of departure based on the most significant effect might be overprotective and fails to take into account multiple testing problems (Budtz-Jørgensen, 2007). In contrast, SEMs allow for measurement errors for multiple predictors, can accommodate multivariate study outcomes within one model, and allow regression coefficients between latent variables, which is more general than random-effects models (Sánchez et al., 2005). Combining related endpoints by pooling

them together could increase precision for the best estimates of dose corresponding to the specified response level (Allen et al., 1996; Budtz-Jørgensen, 2007). Furthermore, the combined CBCL scores across different ages can account for intra-individual variations for every child. However, SEMs are sensitive to model misspecification and may be biased in the mean parameters by incorrect covariance specification (Sánchez et al., 2005). The hierarchical model structure we adopted for the combined CBCL score was based on *a priori* knowledge of child behavioral problems (Achenbach, 1991). Furthermore, the covariance structure among individual CBCL scores is of natural assumption. Therefore, there is no model misspecification nor covariance misspecification of the underlying study.

Ponsonby et al. (2016) proposed a potential causal pathway that phthalates might induce an epigenetic change such as in the brain-derived neurotrophic factor (BNDF) gene, a key neurodevelopmental gene in the hippocampus. Early postnatal exposure to DEHP has been shown to be associated with higher methylation of BNDF with reduced gene expression in male but not female rats (Ponsonby et al., 2016; Smith et al., 2011). A more recent experiment suggested that sex steroid endocrine disruptors might also impair normal behavior by generating oxidative stress perturbation in the male adult rat brain (Tabassum et al., 2017). Human study showed that prenatal urinary phthalate metabolite concentrations was associated with placental *LINE-1* methylation (Zhao et al., 2015). In a longitudinal birth cohort study of pregnant Mexican-American women, Huen et al. (2016) found a consistent reverse association between prenatal mono-ethyl phthalate concentrations with cord blood methylation of *Alu* repeats during the early and late stage of pregnancy. The authors also found that higher urinary DEHP metabolite concentrations during late pregnancy were associated with lower levels of the methylation of *Alu* repeats in blood of 9-year-old children.

There are some limitations of the present study. The sample size of this study is small. However, there were two repeated measurements of the children's CBCL at the ages of 11 and 14 years old. After adjusting for intra-individual variations, the study findings have provided even stronger evidence of associations with limited sample size. The BMD approach also has the advantage that it is relatively robust to the sample size for the derived BMDL (Crump, 1984).

The maternal exposure to DEHP was determined by spot urine sample collected during the 3rd trimester. Activities related to phthalate exposures may vary slightly within a day (Watkins et al., 2014). However, spot urine sample has also been shown to be a reasonable estimate for DEHP to characterize population distributions of intakes (Christensen et al., 2012), although there might be a risk of misclassification (Frederiksen et al., 2013). Multiple spot urine samples for DEHP have been shown to have an advantage over 24-h voided urine collection (Preau et al., 2010), and is preferred across the course of pregnancy rather than a single spot measure (Fisher et al., 2015). An example for our nationwide sample of 166 pregnant women with three trimester urinary phthalate levels all available showed that there were slight variations across the three trimesters (see SM, Fig. S3). The urine samples collected at the 3rd trimester also had best correlations with the urine samples collected at the 1st and 2nd trimesters (data not shown). Furthermore, statistically speaking, the presence of measurement error in the exposure will actually bias the results toward higher exposure limit (Budtz-Jørgensen, 2007). Therefore, the derived BMDL levels under different scenarios might be even lower with more precise urine sample measurement.

The children's CBCL scores were assessed by their mothers, which might have yielded some bias due to parent-child discrepancies, especially when the children had grown to adolescence. One large sample size study in Taiwan (n = 1421) showed that parent-adolescent agreement in emotional and behavioral problems was moderate with a Pearson correlation coefficient of 0.37 (P < 0.001) (Chen et al., 2017). Therefore, the problem of disagreement might not be a serious concern. The consistent associations observed for the participated children at age

14 years old with those when they were 8 and 11 years old also supported the conclusion.

Lastly, we did not adjust for multiple exposures to bispherol A, methyl-mercury, etc that have potential effects on child's behavioral development, because there were no significant correlations among the toxicants.

5. Conclusions

In summary, the longitudinal follow-up study outcomes showed persistent effects of maternal DEHP exposure on the neurobehavioral development of children, even to adolescence, which has strengthened the evidence for the possible causal inference. Based on the established dose-response relationship, the derived BMDL levels under some scenarios were several times to one order lower than the current recommended TDI by the EU EFSA and RfD by the US EPA. However, there is a lack of comparable toxicological data. Nevertheless, it is noted that DEHP exposure has dropped considerably in the US and Europe over the last 10–15 years due to regulatory measures and/or marked changes (Koch et al., 2017). The same declining trend was observed in another study cohort in Taiwan following the same protocol of this study cohort (Wu et al., 2018), as well as in another Asian country (Choi et al., 2017). However, vulnerable subpopulations such as pregnant women in particular still need to be educated to have the DEHP exposure as low as possible. Given the ubiquitous existence of DEHP in the environment and its impacts on public health, further research is required.

Declarations of interest

None.

Conflicts of interest

The authors declare NO competing financial interest in relation to the work described.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2019.06.002.

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