Intellectual Evaluation of Children Exposed to Phthalate-Tainted Products After the 2011 Taiwan Phthalate Episode

Po-Chin Huang[a,b,*], Chih-Hsin Tsai[a], Chu-Chih Chen[c], Ming-Tsang Wu[b,d], Mei-Lien Chen[e], Shu-Li Wang[a,], Bai-Hsiun Chen[f,g], Ching-Chang Lee[h,i], Jouni J. K. Jaakkola[j,k], Wen-Chiu Wu[l], Min-Kung Chen[m], Chao A. Hsiung,[c,**], RAPIT Group[n]

[a]National Environmental Health Research Center, National Institute of Environmental Health Sciences, National Health Research Institutes, Miaoli, Taiwan; [b]Research Center for Environmental Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; [c]Division of Biostatistics and Bioinformatics, Institute of Population Health Sciences, National Health Research Institutes, Miaoli, Taiwan; [d]Department of Family Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; [e]Institute of Environmental and Occupational Health Sciences, National Yang Ming University, Taipei, Taiwan; [f]Department of Laboratory Medicine and Pediatrics, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; [g]Graduate Institute of Clinical Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; [h]Department of Environmental and Occupational Health, College of Medicine, National Cheng Kung University, Tainan, Taiwan; [i]Research Center of Environmental Trace Toxic Substance, National Cheng Kung University, Tainan, Taiwan; [j]Center for Environmental and Respiratory Health Research, Faculty of Medicine, University of Oulu, Oulu, Finland; [k]Medical Research Center, University of Oulu and Oulu University

Hospital, Oulu, Finland; [l]Taipei Hospital, Ministry of Health and Welfare, Taiwan; [m]Department of Pediatrics, Taichung Hospital, Ministry of Health and Welfare, Taichung, Taiwan; [n]Members of the Risk Assessment of Phthalate Incident in Taiwan (RAPIT) Group.

Corresponding Authors: (*) Dr. Po-Chin Huang, National Environmental Health Research Center, National Institute of Environmental Health Sciences, National Health Research Institutes, 35 Keyan Road, Zhunan, Miaoli County 35035, Taiwan; Fax: +886 37 584 730. Tel: ext. 38507. E-mail: pchuang@nhri.org.tw; **(**)** Dr. Chao A. Hsiung, Division of Biostatistics and Bioinformatics**,** Institute of Population Health Sciences, National Health Research Institutes, 35 Keyan Road, Zhunan, Miaoli County 35035, Taiwan. Fax: +886 37 586 467; Tel: ext. 36100; E-mail: hsiung@nhri.org.tw.

Acknowledgments: We thank Ms. Yu-Ann Chen, Chien-Wen Sun, Yi-Chun Chang, Yin-Han Wang, Fang-Ru Lin, and the other research assistants at the Institute of Population Health Sciences and National Health Research Institutes in Taiwan for their assistance in data and specimen collection. We thank the RAPIT members, including Dr. Chao Agnes Hsiung (coordinating principle investigator), Dr. Po-Chin Huang, Dr. Wen-Harn Pan, Dr. Bai-Hsiun Chen, Dr. Ming-Tsang Wu, Dr. Chu-Chih Chen, Dr. Shu-Li Wang, Dr. Ching-Chang Lee, and Dr. Mei-Lien Chen (all principle investigators). This study was funded by the National Health

Research Institutes (NSC 100 - 3114 - Y - 043 - 005, EH-102-SP-01, EH-103-SP-01, EH-104-SP-01, EH-102-PP-05, EH-103-PP05, EH-104-PP05, EM-105-PP15).

Keywords: Phthalates, intellectual development, children, episode

Conflicts of Interest: The authors declare that no competing interests exist.

Author Contributions: Conception and design of experiments: PCH and CAH. Execution of experiments: MTW, SLW, BHC, and CAH. Data analysis: CHT and CCC. Contribution of reagents, materials, and analysis tools: PCH, CCL, SLW, and CAH. Manuscript writing: PCH. Collection of specimens, arrangement of samples, and preparations: MTW, WCW, MKC, SLW, BHC, and CAH. Contribution to critical revision of manuscript: MTW, MLC, BHC, JJJ, and CAH.

Abstract

Introduction. Phthalate exposure may reduce intellectual development in young children. In 2011, numerous Taiwanese children had been reported to have consumed phthalate-tainted products. We investigated the effects of phthalate exposure on the intellectual development of these children after the 2011 Taiwan di-2-ethylhexyl phthalate (DEHP) episode.

Methods. We recruited 204 children, aged 3–12 y, from 3 hospitals in Taiwan between 2012 and 2013. First-morning urine samples were collected for analyzing 5 phthalate metabolites. We applied a Bayesian model to estimate the past DEHP exposure ($_{est}DEHP_{ADD}$) of each participant before the 2011 DEHP episode. Demographic information, consumption of phthalate-tainted products, and maternal education, of each participant were obtained using a questionnaire. We used the Wechsler intelligence evaluation tools for assessing the children's and maternal intelligence quotient.

Results and Discussion. The median levels of mono-2-ethylhexyl phthalate, mono(2-ethyl-5-hydroxyhexyl) phthalate, mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-*n*-butyl phthalate, and mono-*iso*-butyl phthalate in the children were 9.97, 45.8, 32.2, 46.2, and 24.3 μg/g creatinine, respectively. Using the aforementioned urinary phthalate metabolites, we found that the children's verbal comprehension index (N = 98) was significantly negatively associated with urinary $\log_{10}$ MEOHP (β, −11.92; SE, 5.33; 95%CI, -22.52~ -1.33; P = .028) and $\log_{10}$ ΣDBP metabolites (β, −10.95; SE, 4.93; 95%CI, -20.74~

-1.16; *P* = .029) after adjustment for age, gender, maternal IQ and education, passive smoking, $_{est}DEHP_{ADD}$, active and passive smoking during pregnancy. Through a tolerable daily intake-based approach, we only found a significant negative association between past estimate $DEHP_{ADD}$ and $VIQ_{\geq 3-<6}$ in preschool children whereas no correlation was observed between current DEHP exposure and $IQ_{\geq 3-<6}$ score with/ without estimate $DEHP_{ADD}$ adjustment. It revealed that the effect of past high-DEHP exposure on verbal-related neurodevelopment of younger child are more sensitive.

Conclusion. Our results are consistent with the hypothesis that exposure to DEHP and DnBP affects intellectual development in preschool and school-aged children, particularly their language learning or expression ability.

1. Introduction

Phthalates, such as di-2-ethylhexyl phthalate (DEHP), are widely used in many daily products in plastics, toys, and medical equipment; diethyl phthalates (DEPs) are used in cosmetics and personal care products, and di-*n*-butyl phthalate (DnBP) is used in food packaging films and plastic products. Humans may be exposed to phthalates mainly through the ingestion of phthalate-tainted food or inhalation and dermal absorption of phthalate-containing products. Urinary phthalate metabolites are considered suitable biomarkers for assessing the extent of exposure (Zota et al. 2014; Huang et al. 2015a).

In 2011, many Taiwanese parents self-reported, in the clinic, that their children had ingested DEHP or phthalate-tainted products, including nutritional supplements, probiotics, beverages (teas, juices, and sport drinks), and jelly after the episode of DEHP exposure in 2011 (Wu et al. 2012, 2013; Huang et al. 2015b). An official investigation reported that DEHP levels in some DEHP-tainted nutritional supplements ranged between 100 and 1000 ppm (Wu et al. 2013). However, no information is available regarding the potential long-term effects of human exposure to high-dose DEHP-tainted food products on neurodevelopment, particularly in young children.

Epidemiological studies have revealed that low-level phthalate exposure might affect neurodevelopment and behavior in children depending on their age and gender. Some studies have indicated that exposure to low doses of certain phthalates, such as of DEHP and DnBP,

is associated with prenatal or postnatal neurodevelopment in children (Cho et al. 2010; Kim et al. 2011; Téllez-Rojo et al. 2013; Whyatt et al. 2012; Huang et al. 2015b). A few studies have reported that phthalate exposure during childhood might correlate with children's behavioral development, with a possible association with autism spectrum disorder or behavioral issues (Engel et al. 2010; Larsson et al. 2009; Testa et al. 2012; Park et al. 2014, 2015; Kobrosly et al. 2014; Lien et al. 2015).

Experimental studies have provided some indications regarding how phthalates might affect the brain and neurons through different mechanisms. In rats, postnatal exposure to phthalates, including DEHP and DnBP, altered dopamine receptors and transporters in the midbrain and striatum (Ishido et al. 2004; Tanida et al. 2009). In rats and mice, prenatal exposure to DEHP and DnBP affected reference memory, spatial learning, and surface righting reflex or impaired neurodevelopment through hormone-related receptors (Dai et al. 2015; Harris et al. 2007; Smith et al. 2011; Lin et al. 2011; Xu et al. 2015). Human and animal studies have revealed that phthalate exposure might negatively affect neurodevelopment. Thus, we evaluated the effects of phthalate exposure on the intellectual development of children exposed to phthalate-tainted products.

2. Methods

2.1 Participant Recruitment

Study participants were recruited from among individuals who obtained consultation services provided by 128 hospitals across Taiwan and who were then transferred to specialty clinics at 3 participating hospitals after plasticizer contamination was reported in 2011 (Tsai et al., 2016a, b, c; Chen et al., 2016). Briefly, a total of 347 participants were recruited by the RAPIT project, including 237 children from Taipei, Taichung Hospital, run by the Ministry of Health and Welfare, and Kaohsiung Medical University Chung-Ho Memorial Hospital in northern, central, and southern Taiwan, respectively, between August 2012 and February 2013. We included 204 children (≥3 year old and <12 year old) to evaluate their intellectual development using Wechsler tools. This research protocol was approved by the Research Ethics Committee of the National Health Research Institutes (No. EC1000903) and the collaborating hospitals. Written informed consent on behalf of the participated children was obtained from their parents after receiving written and oral information about this study.

2.2 Sample Collection

We collected a 50-mL sample of first-morning urine from each participant, in a polypropylene bottle. Each sample was immediately transferred into an amber glass bottle and stored at −20°C before analysis. The samples were analyzed for assessing kidney function, such as creatinine levels. Furthermore, 5 phthalate metabolites, including

mono-2-ethylhexyl phthalate (MEHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-*n*-butyl phthalate (MnBP), mono-*iso*-butyl phthalate (MiBP), were analyzed using a quantitative liquid chromatography and electrospray ionization tandem mass spectrometry (LC–ESI/MS–MS) system.

2.3 Questionnaire Assessment

Information regarding demographic, socioeconomic, and other factors that could confound phthalate exposure, as well as affect the children's cognitive development was collected. We used a questionnaire to obtain information regarding individual characteristics (age, gender, residence, education of their mother, and family income), health status (childbirth, lactation), environmental exposures (active and passive cigarette smoking in children and their mother during pregnancy, and insecticide usage).

2.4 Urinary Levels of Phthalate Metabolites

In each urine sample, 5 phthalate metabolites (MEHP, MEHHP, MEOHP, MnBP, and MiBP) that represented exposure to 3 reported phthalates (DEHP, DnBP, and DiBP) in phthalate-tainted foods were measured. We used an analytical method to assess (Huang et al. 2015) the levels of these metabolites. Briefly, after the urine samples were thawed and sonicated for 10–15 min, a 100-μL urine sample was loaded into a 2-mL glass vial containing ammonium acetate (20 μL), β-glucuronidase (10 μL), and a 10-isotope ($^{13}C_4$) mixture as the phthalate metabolite standard (100 μL). We used HPLC-grade H_2O (Merck, Darmstadt,

Germany) as reagent water in preparation of stock solution (like standard solution) and quality control samples (like blank sample). After the sample was incubated (37°C; 90 min), a 270-μL solution (5% acetonitrile [ACN] with 0.1% formic acid [FA]) was added. The mixture was placed in a vortex and sealed with a PTEF cap for analysis. We used an online system coupled with a LC–ESI-MS/MS (Agilent 1200/ API4000). We used 2 columns in our online system. One of the C18 column (Inertsil ODS-3; 33 mm × 4.6 mm; 5 μm) was used for extracting and cleaning the samples, whereas the other analytical column (Inertsil Ph-3; 150 mm × 4.6 mm; 5 μm) was used to separate different phthalate metabolites. The gradient program for the clean-up column was as follows: 100% solution A (5% ACN + 0.1% FA) (0–7 min), 100% solution B (90% ACN + 0.1% FA) (7–9 min), and 100% solution A (9–10 min) that was continued for 12 min. The flow rate was set at 1000 μL/min. The gradient program for the analytical column was as follows: 100% solution C (50% ACN + 0.1% FA) (0–3.6 min), 100% solution D (95% ACN + 0.1% FA) (3.6–8.6 min), and 100% solution C (8.6–9 min) that was continued for 12 min. We used a negative multiple reaction mode model for MS detection. The ion pair for each phthalate metabolite was as follows: MEHP (277/134), MEHHP (293/121), MEOHP (291/143), and MnBP/MiBP (221/71). The detection limits for the metabolites were 0.3, 0.7, 0.1, and 1.0 ng/mL, respectively. The calibration curve ranged from 0.5 ppb to 1000 ppb (correlation coefficient [R^2] > 0.995), and each batch included blank, repeat, and spiked samples for quality control. We also monitored the

recoveries of an isotopic $^{13}C_4$-labeled internal standard for each phthalate metabolite. The concentration of the blank samples was less than 2 times the detection limit. The QC sample was spiked in a pooled urine sample with a mixture of phthalate metabolite standards (20–50 ng/mL) in each sample. The relative percent difference of repeat sample and recovery of QC sample was within ±30%.

2.5 Estimated Daily Intake of DEHP Before the 2011 Taiwan DEHP Episode

For evaluating the potential effects of phthalate exposure in our participants before the 2011 Taiwan DEHP episode, we used a published method (Chen et al., 2016) to estimate the daily intake of DEHP in participants before the 2011 DEHP episode. Briefly, a detailed exposure assessment questionnaire was applied, and we obtained information regarding whether the participants were exposed, the approximate exposure frequency, and possible exposure duration. Including a self-report list provided more specific detailed information regarding the product name, producer, consumption amount, frequency, and explicit duration (particularly for nutritional supplements). Therefore, we estimated the average daily intakes (AvDIs) of DEHP separately from the questionnaire ($AvDI_{QN}$) and from the self-report list ($AvDI_{SF}$) (if provided by a participant's caregiver). Furthermore, we assumed that the lifestyle of our participants remained unchanged after the episode, and the background

exposure ($AvDI_{ENV}$) was estimated by converting the urine DEHP metabolite measurements using equation (1). Then, we applied Bayesian models to estimate their daily DEHP exposure dose, as an estimation of exposure to DEHP-tainted food products, which has been described in detail in a previously published study (Chen et al., 2016). Briefly, a Bayesian statistical procedure using MCMC simulation was employed to deal with the complexity of uncertainties in the DEHP concentrations of the contaminated foods and exposure scenarios of the participants. Different prior lognormal distributions were used to describe the measured DEHP concentrations of the tainted foods, and a mixture distribution for average concentration was obtained by weighing the proportions of various product measurements for each food category. Then, we used the following equation (2) to obtain the individual average for the exposure period to DEHP-contaminated products (ADD_{DEHP}).

$$ADI_{ENV}(\mu g/kg\_bw/day) = \frac{UE_{sum}(\mu mol/g\_Cr)\times CE(g\_Cr/day)}{F_{UE}\times BW(kg)}\times MW_{DEHP} \quad \ldots \text{Equation (1)}$$

$$ADD_{DEHP} = AvDI_{QN} + AvDI_{SF} + AvDI_{ENV} \ldots \text{Equation (2)}$$

2.6 Intellectual Evaluation

The intellectual performance of children is a result of long-term development. All intellectual evaluations were performed by qualified and certified psychologists in the hospital on the participants by using a standardized protocol. We used the Chinese version of the Wechsler Preschool and Primary Scale of Intelligence-Revised (WPPSI-R) (Wechsler

2000) and the Wechsler Intelligence Scale for Children-version IV (WISC-IV) (Wechsler 1997) to assess the participant's IQ at ages ≥3-<6 y and ≥6-<12 y, respectively. We labeled the scores $IQ_{\geq 3-<6}$, which represented the IQ score for WPPSI-R, and $IQ_{\geq 6-<12}$, which represent the IQ score for WISC-IV, to present the results obtained with different IQ evaluation tools. The WPPSI-R and WISC-III are universally acknowledged tools for evaluating the IQ of preschool children, and they also provide a thorough sampling of the abilities of children with below-average IQ scores (Lanphear et al. 2005; Huang et al. 2015b). The WPPSI-R has 5 subtests on verbal skills (arithmetic, comprehension, information, similarities, and vocabulary) and 5 subtests on visual–spatial skills (block design, geometric design, mazes, object assembly, and picture completion). Verbal $IQ_{\geq 3-<6}$ ($VIQ_{\geq 3-<6}$), which represents combined scores for the verbal tests and performance, and $IQ_{\geq 3-<6}$ ($PIQ_{\geq 3-<6}$), which represents combined scores for the visual–spatial tests, were standardized to yield the full-scale IQ ($FSIQ_{\geq 3-<6}$).

WISC-IV has 5 verbal comprehension index (VCI) subtests (vocabulary, similarities, comprehension, information, and word reasoning); 4 perceptual reasoning index (PRI) subtests (block design, picture concepts, matrix reasoning, and picture completion); 3 working memory index (WMI) subtests (digit span, letter–number sequencing, and arithmetic); and 3 processing speed index (PSI) subtests (coding, symbol search, and cancelation). Full-scale $IQ_{\geq 6-<12}$ ($FSIQ_{\geq 6-<12}$) was obtained by combining the VCI, PRI, WMI,

and PSI scores.

We also evaluated the intelligence of the children's mothers using the Wechsler Adult Intelligence Scale-III (WAIS-III; Wechsler 2002) for adjusting the children's cognitive development. The WAIS-III also has 3 VCI subtests (information, similarities, and vocabulary); 2 WMI subtests (arithmetic and digit span) to yield the verbal IQ_m (VIQ_m); 3 perceptual organization index subtests (block design, matrix reasoning, and picture completion); and 2 PSI subtests (digit–symbol-coding, symbol search) to yield a performance IQ_m (PIQ_m). Full-scale IQ_m ($FSIQ_m$) was obtained by combining the VIQ_m and PIQ_m scores.

2.7 Statistical Analysis

To evaluate intellectual performance using the Wechsler tools, we categorized the participants into age groups with a cut-off age of 6 y. We used the Student's *t* test for examining continuous variables and the χ^2 test for analyzing categorical variables. We calculated the "not detectable (ND)" levels as half the detection limit of each phthalate metabolite, and the detectable rate as the number of urine samples with levels of each phthalate metabolite higher than the detection limit divided by all the analyzed urine samples. We calculated the sum ($\sum$) of DEHP metabolites by using the molar concentrations of all DEHP metabolites, and $\sum$DBP metabolites by adding molar concentrations of MnBP and MiBP. Because the distributions of urinary phthalates were skewed in the samples, we used

logarithm-transformed values in the analysis. Pearson correlation coefficients were used to assess the associations among age, gestation, IQ values, and the level of each urinary phthalate metabolite. Principal component analysis and cluster analysis were applied to assess the exposure profiles of different phthalates compared with different populations. Physiological factors or variables that significantly correlated with urinary phthalate metabolite levels were included in the multiple regression model. We also evaluated the correlation between adjusted creatinine and unadjusted phthalate metabolite levels. The associations between the urinary phthalate level and the full-scale IQ scores were assessed using a regression model after adjusting for covariates (such as age, gender, maternal IQ, maternal education, passive smoking, maternal active and passive smoking during pregnancy, and/or estimate $DEHP_{ADD}$). A 2-sided *P* value of <.05 was considered significant. All statistical analyses were conducted using SPSS (version 22.0; IBM Corp, Armonk, NY).

3. Results

3.1 Participant Characteristics

Table 1 lists the demographic characteristics of the 204 study participants stratified by age groups, including preschool children (aged ≥3-<6 y; N = 108) and school-aged children (aged ≥6-<12 y; N = 96). The mean primary caregiver age and maternal age during pregnancy were >37 and >30 y, respectively. Nearly 70% of the mothers received a college education, >92%

of the participants were breastfed, <5% of the participants' mothers were active smokers and consumed alcohol, and approximately 20% of the participants' mothers were exposed to second-hand smoke during pregnancy.

3.2 Urinary Phthalate Metabolites Levels and Estimated DEHP Exposure

Table 2 present the levels of the 5 phthalate metabolites in the urine samples collected from the participants (N = 204) between the groups. Median levels for most of the phthalate metabolites were higher in preschool children than in school-aged children. Table 2 also lists the estimated daily dose (ADD_{est}) of DEHP for our participants before the 2011 DEHP episode. We observed that the median (range) ADD_{est} levels for DEHP were 15.1 (0.6–140.0) μg/kg/day for preschool children and 12.2 (2.8–88.5) μg/kg/day for school-aged children.

3.3 IQ Scores for the Children and Their Mothers

Table 3 lists the WPPSI-R (N = 108), WISC-IV (N = 96), and WAIS-III scores for our participants stratified by gender. The $FSIQ_{\geq 3-<6}$ scores for most preschool children and school-aged children were within the normal range, regardless of gender. We found that girls had significantly higher WISC-IV scores than did the boys for $WMI_{\geq 6-<12}$ (114 vs 107, respectively; $P = .021$) and $PSI_{\geq 6-<12}$ (105 vs 98, respectively; $P = .024$). Girls had higher $PIQ_{\geq 3-<6}$ scores than did boys (111 vs 105, respectively; $P = .051$). However, we did not

observe a significant difference among the $VIQ_{\geq 3-<6}$, $VCI_{\geq 6-<12}$, $PRI_{\geq 6-<12}$, or in the maternal FSIQ (WAIS-III) scores for the participants regardless of gender.

3.4 Association between Urinary Phthalate Metabolites and Pediatric Intellectual Development

We assessed the relationship between urinary phthalate metabolites and IQ scores and other covariates using Pearson correlation analysis (Supplementary Table 1). Table 4 and Table 5 present the multiple regression results for DEHP exposure in terms of the IQ scores (WPPSI-R) of the preschool children (N = 108). Among all models for the DEHP exposure estimation, we did not observe any significant difference between current exposure to DEHP and DBP and the IQ scores of the preschool children's (including $FSIQ_{\geq 3-<6}$, $PIQ_{\geq 3-<6}$, and $VIQ_{\geq 3-<6}$) after adjustment for covariates (such as estimated $DEHP_{ADD}$ and maternal FSIQ).

Table 5 and Figure 1 illustrate the multiple regression results for DEHP exposure regarding the $IQ_{\geq 6-<12}$ scores (WISC-IV) for the school-aged children (N = 96). We found only a marginally significant correlation between urinary MEOHP and $FSIQ_{\geq 6-<12}$ (β/SE, −7.4/4.1; 95%CI, −15.55 to 0.75; *P* = .074) in the crude model. We found that the children's $VCI_{\geq 6-<12}$ was negatively significantly associated with urinary MEOHP (β/SE, −11.92/5.33; 95%CI, −22.52 to −1.33; *P* = .028), urinary MnBP (β/SE, −11.32/5.31; 95%CI, −21.89 to −0.76; *P* = .036), and urinary MiBP (β/SE, –8.85/4.08; 95%CI, –16.96 to –0.74; *P* = .033) after

adjustment for the participants' age, gender, maternal IQ and education, estimate $DEHP_{ADD}$, passive smoking, maternal active and passive smoking during pregnancy. However, we did not observe any significant difference between current DEHP exposure and the children's IQ scores, including $FSIQ_{\geq 6-<12}$, $PRI_{\geq 6-<12}$, $WMI_{\geq 6-<12}$, and $PSI_{\geq 6-<12}$, after adjustment for covariates. We also observed a marginal yet negative correlation between urinary MEOHP and $WMI_{\geq 6-<12}$ (β/SE, −9.27/5.04; 95%CI, −19.28 to 0.75; P = .066), whereas 2 factors exhibited significant effects ($DEHP_{ADD}$: β, 11.0; P = .031; gender: β, −6.6; P = .013) after adjustment for the same factor. However, we did not observe similar phenomena in models for urinary MnBP or MiBP. We did not observe any correlations between any other urinary phthalate metabolites and $FSIQ_{\geq 6-<12}$ or any other sub-IQ indices such as $PRI_{\geq 6-<12}$ and $PSI_{\geq 6-<12}$ in our participants.

4. Discussion

We found that the current exposure to DEHP, DnBP, and DiBP for school-aged children aged ≥6–<12 y, who were exposed to phthalate-tainted products, was significantly and negatively associated with the $VCI_{\geq 6-<12}$ performance after adjustment for significant covariates. Maternal IQ was slightly positively and significantly associated with the neurodevelopment for our participants aged ≥3–<12 y. Our data revealed that the current exposure to DEHP and DBP might affect the children's nervous system that might become

visible in their language learning or expression ability.

A high detection rate of DEHP, DnBP, and DiBP metabolites indicated that our participants continued to have exposure to these phthalates after the 2011 Taiwan DEHP episode. We only found a negative association between $VCI_{\geq 6-<12}$ and the urinary MEOHP, MnBP, and MiBP levels in school-aged children aged ≥6–<12 y. We found that their current DEHP or DBP metabolite exposure levels remained higher than those of the general Taiwanese pediatric population (Huang et al. 2015). One possible explanation is that the participants in our study may still be continuously exposed to certain products that have low-dose DEHP and DBP levels.

Three possible mechanisms may underlie the effects of phthalates on children's neurodevelopment. Phthalates may interrupt the neurotransmitter system during neuronal development. Some studies have revealed that a low-dose phthalate exposure could reduce the number of midbrain dopaminergic neurons (Tanida et al. 2009), tyrosine hydroxylase immunoreactivity, and biosynthetic activity (Ishido et al. 2004) in rodents. Second, phthalates may indirectly affect cognitive development through the alteration of thyroid hormones by interacting with hormone synthesis proteins, deiodinases, and receptors (Breous et al. 2005; Sugiyama et al. 2005; Shen et al. 2011; Liu et al. 2015). Maternal or neonatal hypothyroidism and subclinical hypothyroidism permanently affect children's cognition (de Escobar 2004a, 2004b; Ohara et al. 2004). Some studies have revealed that a low-dose phthalate exposure

was associated with altered thyroid activity in children (Boas et al. 2010), pregnant women (Huang et al. 2007), adolescents, and adults (Meeker et al. 2011). Third, peroxisome proliferator activated receptors (PPARs) are found during the development of neural tubes, and they play a role in cellular proliferation (Braissant 1998; Kota et al. 2005). Phthalates may activate the PPARs (Roberts et al. 1997) by altering signal transduction during the neurodegenerative process. However, little is known about the effects of a high-dose phthalate exposure on neurodevelopment through the aforementioned mechanisms.

We only found a significant negative association between estimate $DEHP_{ADD}$ (past exposure) and $VIQ_{\geq 3-<6}$ in preschool children, but not for other IQ index at ≥3-<6 y or ≥6-<12 y in this study (Supplementary Table 2). We did not observe significant correlations between current DEHP or DBP exposure and IQ score in our subject aged ≥3-<6 y adjustment for other covariates and with or without estimate $DEHP_{ADD}$ (Supplementary Table 3). It indicated that the effect of past high-DEHP exposure on verbal-related neurodevelopment of younger child are more sensitive than other age groups. Most toxicological studies on DEHP or phthalates have focused on liver toxicity, reproductive, and endocrinologic effects in rodents or in vitro, whereas only a few have studied the neurological effects of DEHP or phthalates. Fetal or neonatal exposure to low-dose DEHP levels resulted in an irreversible change in the midbrain dopaminergic nuclei of mice (Tanida et al. 2009). One animal study showed that DBP induced neurotoxicity in the brain of immature and mature rat offspring. Perinatal DBP

exposure could induce neurotoxicity in immature offspring rats by regulating ER-β, BDNF, and p-CREB expression, whereas it did not influence mature offspring animals (Li et al. 2014). Therefore, DEHP or phthalate toxicity was likely organ dependent and further studies on the association between phthalate exposure and neurodevelopment are required.

We found that current DEHP, DnBP, and DiBP exposure in school-aged children aged ≥6–<12 y, who were exposed to phthalate-tainted products was significantly and negatively associated with $VCI_{\geq 6-<12}$ performance after adjustment for significant covariates. Our findings were consistent with those of some cross-sectional studies. One cross-sectional study reported that FSIQ and VIQ scores (WISC-III) were negatively associated with DEHP metabolites in 667 elementary school-aged Korean children. A significant negative relationship existed between urinary DEHP and DBP metabolite levels and the children's vocabulary subscores after controlling for maternal IQ (Cho et al. 2010). Another study revealed that postnatal phthalate exposure was associated with reduced cognitive development in young children in central Taiwan (Huang et al. 2015b).

However, some prospective studies have reported the effects of prenatal phthalate exposure. One prospective study revealed that prenatal maternal urinary DnBP and DiBP metabolite levels during late pregnancy were associated with deficits in the intellectual development of children at age 7 y, particularly perceptual reasoning, working memory, processing speed, and verbal comprehension (DiBP only) (Factor-Litval et al. 2014). Ejaredar et al. conducted a

systematic review of 11 medical articles regarding phthalate exposure on children's neurodevelopment and concluded that prenatal phthalate exposure is associated with adverse cognitive and behavior function in children (Ejaredar et al. 2015). Because of a lack of information regarding prenatal phthalate exposure for our subjects, we could not evaluate the effect of prenatal phthalate exposure in our results.

We included several confounders in our evaluation of children's neurodevelopment that are comparable to those of other studies (Ejaredar et al. 2015). The ages of the child and mother, gender, maternal IQ and education level, and socioeconomic status were the most frequently adjusted confounders in previous and different epidemiological studies. We included additional factors such as the active and passive smoking (during pregnancy) to assess the prenatal exposure of tobacco smoke. We also conducted an additional model to evaluate the effect of annual family income (Supplementary Table 2 and 3). We did not include breastfeeding in our model because >90% of our participants were breastfed.

The current study had some strengths. First, we included some crucial confounders such as maternal IQ and controlled for them in our models. Second, we used a Bayesian model to estimate DEHP exposure in our participants prior to the episode of DEHP exposure and controlled it in our model. It provided an estimate of the magnitude of DEHP exposure, instead of a categorical variable. Some limitations regarding data interpretation existed in this study. First, we only have around one hundred subjects in each age group for which small

sample size may limit our statistic power and interpretation. Second, the study was designed as a cross-sectional study that limited the uncertainty of causality. Third, we used only one first-morning urine sample. Variations in urinary phthalate metabolite levels in our participants were not assessed. Fourth, because of the regulations governing studies on human volunteers in Taiwan, we could not collect any specimens such as a urine or blood sample immediately after the 2011 DEHP exposure episode. Therefore, we did not have the actual data on phthalate exposure levels or duration of exposure before the DEHP episode. Fifth, we did not have any information regarding prenatal or postnatal phthalate exposure of our participants before 2011. Sixth, all the effects of multiple factors on child neurodevelopment may not have been included in the current study. Although we included some critical factors, such as maternal IQ, cigarette smoking and maternal education, we did not evaluate the effects of exposure to heavy metals or any other organic environmental pollutants.

Conclusion

Our results are consistent with the hypothesis that exposure to phthalates influences neurodevelopment in school-aged children. Further studies are warranted to follow-up the long-term effects of phthalate exposure on neurodevelopment, such as attention deficit hyperactivity disorder, in children exposed to numerous phthalate-tainted products.

References.

Boas M, Frederiksen H, Feldt-Rasmussen U, Skakkebaek NE, Hegedus L, Hilsted L, et al. Childhood exposure to phthalates: associations with thyroid function, insulin-like growth factor I, and growth. Environ Health Perspect. 2010; 118:1458–1464.

Braissant O, Wahli W. Differential expression of peroxisome proliferator-activated receptor-alpha,-beta, and-gamma during rat embryonic development. Endocrinology. 1998; 139:2748–2754.

Breous E, Wenzel A, Loos U. The promoter of the human sodium/iodide symporter responds to certain phthalate plasticizers. Mol Cell Endocrinol 2005;244:75–78.

Chen CC, Wang SL, Wu MT, Wang YH, Huang PC, Chen BH, Sun CW, Ho CK, Shih YC, Shiu MN, Pan WH, Chen ML, Lee CC, Hsiung CA. Exposure Estimation for Risk Assessment of the Phthalate Incident in Taiwan. PLoS One. 2016;11(3):e0151070.

Cho SC, Bhang SY, Hong YC, Shin MS, Kim BN, Kim JW, Kim HW, 2010. Relationship between environmental phthalate exposure and the intelligence of school-age children. Environ Health Perspect. 118;7: 1027–1032.

Dai Y, Yang Y, Xu X, Hu Y. Effects of uterine and lactational exposure to di-(2-ethylhexyl) phthalate on spatial memory and NMDA receptor of hippocampus in mice. Horm Behav. 2015;71:41-8.

de Escobar GM, Obregón MJ, del Rey FE. Maternal thyroid hormones early in pregnancy and fetal brain development. Best Pract Res Clin Endocrinol Metab. 2004a;18(2):225-48.

de Escobar MG, Obregon MJ, del Rey FE. Role of thyroid hormone during early brain development. Eur J Endocrinol. 2004b;151 Suppl 3:U25-37.

Ejaredar M, Nyanza EC, Ten Eycke K, Dewey D. Phthalate exposure and childrens neurodevelopment: A systematic review. Environ Res. 2015;142:51-60.

Engel SM, Miodovnik A, Canfield RL, Zhu C, Silva MJ, Calafat AM, Wolff MS, 2010. Prenatal phthalate exposure is associated with childhood behavior and executive functioning. Environ Health Perspect.118; 4: 565–571.

Factor-Litvak P, Insel B, Calafat AM, Liu X, Perera F, Rauh VA, Whyatt RM. Persistent Associations between Maternal Prenatal Exposure to Phthalates on Child IQ at Age 7 Years. PLoS One. 2014; 9(12):e114003.

Huang HB, Chen HY, Su PH, Huang PC, Sun CW, Wang CJ, Chen HY, Hsiung CA, Wang SL. Fetal and Childhood Exposure to Phthalate Diesters and Cognitive Function in Children Up to 12 Years of Age: Taiwanese Maternal and Infant Cohort Study. PLoS One. 2015;10:e0131910.

Huang PC, Kuo PL, Guo YL, Liao PC, Lee CC. Associations between urinary phthalate monoesters and thyroid hormones in pregnant women. Hum Reprod. 2007; 22:2715–2722.

Huang PC, Su PH, Chen HY, Huang HB, Tsai JL, Huang HI, et al. Childhood blood lead levels and intellectual development after ban of leaded gasoline in Taiwan: a 9-year prospective study. Environ Int. 2012; 40:88–96.

Huang PC, Tsai CH, Liang WY, Li SS, Pan WH, Chiang HC. Age and Gender Differences in Urinary Levels of Eleven Phthalate Metabolites in General Taiwanese Population After a DEHP Episode. PLoS One 2015;10(7):e0133782.

Guo Y, Zhang Z, Liu L, Li Y, Ren N, Kannan K. Occurrence and profiles of phthalates in foodstuffs from China and their implications for human exposure. J Agric Food Chem. 2012;60(27):6913-9.

Ishido M, Masuo Y, Sayato-Suzuki J, Oka S, Niki E, Morita M. Dicyclohexylphthalate causes hyperactivity in the rat concomitantly with impairment of tyrosine hydroxylase immunoreactivity. J Neurochem. 2004; 91:69–76.

Kim Y, Ha EH, Kim EJ, Park H, Ha M, Kim JH, Kim BN, 2011. Prenatal exposure to phthalates and infant development at 6months: prospective Mothers and Children's Environmental Health (MOCEH) study. Environ Health Perspect. 119; 10: 1495–1500.

Kobrosly RW, Evans S, Miodovnik A, Barrett ES, Thurston SW, Calafat AM, Swan SH. Prenatal phthalate exposures and neurobehavioral development scores in boys and girls at 6-10 years of age. Environ Health Perspect. 2014;122(5):521-8.

Kota BP, Huang TH, Roufogalis BD. An overview of biological mechanisms of PPARs. Pharmacol Res. 2005; 51:85–94.

Lanphear BP, Hornung R, Khoury J, Yolton K, Baghurst P, Bellinger DC, et al. Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. Environ Health Perspect 2005;113:894–9.

Larsson M, Weiss B, Janson S, Sundell J, Bornehag CG. Associations between indoor environmental factors and parental-reported autistic spectrum disorders in children 6-8 years of age. Neurotoxicology. 2009; 30(5):822-31.

Li X, Jiang L, Cheng L, Chen H. Dibutyl phthalate-induced neurotoxicity in the brain of immature and mature rat offspring. Brain Dev. 2014;36(8):653-60.

Lien YJ, Ku HY, Su PH, Chen SJ, Chen HY, Liao PC, Chen WJ, Wang SL. Prenatal exposure to phthalate esters and behavioral syndromes in children at 8 years of age: Taiwan Maternal and Infant Cohort Study. Environ Health Perspect. 2015; 123(1):95-100.

Lin CH1, Chen TJ, Chen SS, Hsiao PC, Yang RC. Activation of Trim17 by PPARγ is involved in di(2-ethylhexyl) phthalate (DEHP)-induced apoptosis on Neuro-2a cells. Toxicol Lett. 2011; 206(3):245-51.

Meeker JD, Ferguson KK. Relationship between urinary phthalate and bisphenol A concentrations and serum thyroid measures in U.S. adults and adolescents from the

National Health and Nutrition Examination Survey (NHANES) 2007-2008. Environ Health Perspect. 2011;119(10):1396-402.

Ohara N, Tsujino T, Maruo T. The role of thyroid hormone in trophoblast function, early pregnancy maintenance, and fetal neurodevelopment. J Obstet Gynaecol Can. 2004; 26:982–990.

Park S, Kim BN, Cho SC, Kim Y, Kim JW, Lee JY, Hong SB, Shin MS, Yoo HJ, Im H, Cheong JH, Han DH. Association between urine phthalate levels and poor attentional performance in children with attention-deficit hyperactivity disorder with evidence of dopamine gene-phthalate interaction. Int J Environ Res Public Health. 2014;11(7):6743-56.

Park S, Lee JM, Kim JW, Cheong JH, Yun HJ, Hong YC, Kim Y, Han DH, Yoo HJ, Shin MS, Cho SC, Kim BN. Association between phthalates and externalizing behaviors and cortical thickness in children with attention deficit hyperactivity disorder. Psychol Med. 2015; 45(8):1601-12.

Roberts RA, Nebert DW, Hickman JA, Richburg JH, Goldsworthy TL. Perturbation of the mitosis/apoptosis balance: a fundamental mechanism in toxicology. Fundam Appl Toxicol. 1997; 38:107–115.

Serrano SE, Karr CJ, Seixas NS, Nguyen RH, Barrett ES, Janssen S, Redmon B, Swan SH, Sathyanarayana S. Dietary phthalate exposure in pregnant women and the impact of consumer practices. Int J Environ Res Public Health. 2014;11(6):6193-215.

Smith CA, Macdonald A, Holahan MR. Acute postnatal exposure to di(2-ethylhexyl) phthalate adversely impacts hippocampal development in the male rat. Neuroscience. 2011;193:100-8.

Sugiyama SI, Shimada N, Miyoshi H, Yamauchi K. Detection of thyroid system-disrupting chemicals using in vitro and in vivo screening assays in Xenopus laevis. Toxicol Sci 2005;88:367–374.

Tanida T, Warita K, Ishihara K, Fukui S, Mitsuhashi T, Sugawara T, et al. Fetal and neonatal exposure to three typical environmental chemicals with different mechanisms of action: mixed exposure to phenol, phthalate, and dioxin cancels the effects of sole exposure on mouse midbrain dopaminergic nuclei. Toxicol Lett. 2009; 189:40–47.

Tellez-Rojo MM, Cantoral A, Cantonwine DE, Schnaas L, Peterson K, Hu H, Meeker, JD, 2013. Prenatal urinary phthalate metabolites levels and neurodevelopment in children at two and three years of age. Sci Total Environ. 461– 462; 386–390.

Testa C, Nuti F, Hayek J, DeFelice C, Chelli M, Rovero P, Papini AM, 2012. Di-(2-ethylhexyl) phthalate and autism spectrum disorders. ASN Neuro 4;4: 223–229.

Tsai HJ, Chen BH, Wu CF, Wang SL, Huang PC, Tsai YC, Chen ML, Ho CK, Hsiung CA, Wu MT. Intake of phthalate-tainted foods and microalbuminuria in children: The 2011 Taiwan food scandal. Environ Int. 2016a; 89-90: 129-37.

Tsai HJ, Wu CF, Tsai YC, Huang PC, Chen ML, Wang SL, Chen BH, Chen CC, Wu WC, Hsu PS, Hsiung CA, Wu MT. Intake of Phthalate-tainted Foods and Serum Thyroid Hormones in Taiwanese Children and Adolescents. Sci Rep. 2016b; 6: 30589.

Tsai YA, Lin CL, Hou JW, Huang PC, Lee MC, Chen BH, Wu MT, Chen CC, Wang SL, Lee CC, Hsiung CA, Chen ML; RAPIT Group. Effects of high di(2-ethylhexyl) phthalate (DEHP) exposure due to tainted food intake on pre-pubertal growth characteristics in a Taiwanese population. Environ Res. 2016c; 149: 197-205.

Wechsler D. The Wechsler preschool and primary scales of intelligence — revised (Chinese version). Taipei, Taiwan: Chinese Behavioral Science Corporation; 2000.

Wechsler D. Wechsler intelligence scale for children-III (Chinese version). Taipei, Taiwan: Chinese Behavioral Science Corporation; 1997.

Wechsler, D. Wechsler adult intelligence scale-third edition (Chinese version) Taipei, Taiwan: Chinese Behavioral Science Corporation; 2002.

Whyatt RM, Liu X, Rauh VA, Calafat AM, Just AC, Hoepner L, 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; 2: 290–295.

Wu MT, Wu CF, Chen BH, Chen EK, Chen YL, Shiea J, Lee WT, Chao MC, Wu JR. Intake of phthalate-tainted foods alters thyroid functions in Taiwanese children. PLoS One. 2013;8:e55005.

Wu MT, Wu CF, Wu JR, Chen BH, Chen EK, Chao MC, Liu CK, Ho CK. The public health threat of phthalate-tainted foodstuffs in Taiwan: the policies the government implemented and the lessons we learned. Environ Int. 2012;44:75-9.

Xu X, Yang Y, Wang R, Wang Y, Ruan Q, Lu Y. Perinatal exposure to di-(2-ethylhexyl) phthalate affects anxiety- and depression-like behaviors in mice. Chemosphere. 2015;124:22-31.

Zota AR, Calafat AM, Woodruff TJ. Temporal trends in phthalate exposures: findings from the National Health and Nutrition Examination Survey, 2001-2010. Environ Health Perspect. 2014;122(3):235-41.

Table 1. Characteristics of participants stratified by age (N = 204)

Characteristics	Preschool Children[a] (N= 108)	School-aged Children[a] (N= 96)
Continuing variables [Median (range)]		
Age (y)	4.6 (3.0-5.98)	7.6 (6.08-11.88)
Body Mass Index (kg/m^2)	15.7 (12.7-24.4)	16.0 (12.3-26.7)
Age of primary caregiver (y)	37 (29-70)	39 (11-68)
Maternal age at pregnancy (y)	31 (24-41)	30 (20-40)
Categorical variables [N (%)]		
Gender		
Boy	67 (62.0)	56 (41.7)
Girl	41 (38.0)	40 (58.3)
Maternal education		
<College	13 (12.0)	12 (12.5)
College	73 (67.6)	71 (74.0)
≧Graduate	22 (20.4)	13 (13.5)
Education of primary caregiver		
<College	23 (21.3)	13 (13.5)
College	65 (60.2)	69 (71.9)
≧Graduate	20 (18.5)	14 (14.6)
Annual family income (USD)[b]		
≦15,600	12 (11.2)	16 (16.7)
15,600-312,50	45 (42.1)	23 (24.0)
≧312,50	50 (46.7)	57 (59.3)
Childbirth		
Natural birth	67 (62.0)	57 (59.4)
Caesarean birth	41 (38.0)	39 (40.6)
Lactation		
Yes	105 (97.2)	89 (92.7)
No	3 (2.8)	7 (7.3)
Passive smoking		
Yes	13 (12.0)	17 (17.7)
No	95 (88.0)	79 (82.3)
Home use of pesticide		
Yes	48 (44.4)	47 (49.0)
No	60 (55.6)	49 (51.0)
During pregnancy		
Active smoker		
Yes	2 (1.9)	3 (3.1)
No	106 (98.1)	93 (96.9)
Passive smoking		
Yes	19 (17.6)	21 (21.9)
No	89 (82.4)	75 (78.1)
Alcohol		
Yes	2 (1.9)	2 (2.1)
No	106 (98.1)	94 (97.9)
Medicine		

	Yes	36 (33.3)	35 (36.5)
	No	72 (66.7)	61 (63.5)

[a] Preschool children: child aged ≥3-<6 y; School-aged children: children aged ≥6-<12 y.

[b] Currency exchange rate of USD to new Taiwan dollar is 1:32 and one preschool child did not provide annual family income.

Table 2. Creatinine-adjusted concentrations (μg/g creatinine) of urinary phthalate metabolites and estimate $DEHP_{ADD}$[a] by age groups (N = 204)

Phthalate metabolites	Preschool Child[b] (N= 108)		School-aged Children[b] (N= 96)	
	Median (Range)	Geometric Mean (95% CI)	Median (Range)	Geometric Mean (95% CI)
MEHP	8.4 (0.2-71.0)	6.2 (4.7-8.2)	10.2 (0.2-99.9)	9.0 (7.4-10.8)
MEOHP	35.8 (0.1-271.4)	33.8 (26.8-41.2)	26.8 (0.3-199.6)	28.0 (23.8-32.2)
MEHHP	53.4 (1.8-359.1)	50.0 (43.0-58.7)	37.6 (10.5-404.9)	40.7 (36.4-45.7)
ΣDEHP	103.3 (4.4-701.4)	95.7 (81.7-112.6)	73.5 (23.8-704.4)	80.7 (72.1-90.7)
MnBP	46.8 (6.0-344.7)	51.4 (44.0-59.0)	43.7 (10.0-227.1)	43.3 (37.9-50.1)
MiBP	27.6 (0.1-386.5)	24.7 (20.0-29.2)	19.9 (4.4-641.2)	22.1 (18.9-26.2)
ΣDBP	81.5 (11.0-731.2)	81.0 (70.2-92.0)	70.3 (14.3-739.4)	68.6 (59.6-79.9)
Estimate $DEHP_{ADD}$ (μg/kg/day)	15.1 (0.6-140.0)	16.5 (14.1-19.5)	12.2 (2.8-88.5)	12.0 (10.4-13.7)

Abbreviations: MEHP = mono-2-ethylhexyl phthalate; MEOHP = mono-(2-ethyl-5-oxohexyl) phthalate; MEHHP = mono-(2-ethyl-5-hydroxylhexyl) phthalate; MnBP = mono-n-butyl phthalate; MiBP = mono-*iso*-butyl phthalate; ΣDEHP metabolites = MEHP + MEOHP + MEHHP; ΣDBP = MnBP + MiBP.

[a]Estimate DEHP exposure before 2011 Taiwan DEHP food scandal.

[b]Preschool children: children aged ≥3-<6 y; School-aged children: child aged ≥ 6-<12 y.

Table 3. Scores of WPPSI-R[a] (N = 108), WISC-IV[a] (N = 96), and WAIS-III[a] in participants stratified by gender.

Preschool Child	Boy (N= 67)		Girl (N= 41)		P-value[b]
WPPSI-R Full Scale IQ (FSIQ $_{\geq 3 - <6}$)	109	(76-135)	110	(87-128)	0.718
Performance IQ (PIQ $_{\geq 3 - <6}$)	105	(54-133)	111	(91-131)	0.051[#]
Verbal IQ (VIQ $_{\geq 3 - <6}$)	109	(66-135)	107	(79-128)	0.300
WAIS-III Maternal FSIQ	110	(83-137)	110	(85-140)	0.869
School-aged Children	Boy (N= 56)		Girl (N= 40)		P-value[b]
WISC-IV Full Scale IQ (FSIQ $_{\geq 6 - <12}$)	110	(67-138)	114	(85-144)	0.237
Verbal Comprehension Index (VCI $_{\geq 6 - <12}$)	113	(77-144)	111	(85-151)	0.378
Perceptual Reasoning Index (PRI $_{\geq 6 - <12}$)	110	(61-139)	111	(85-142)	0.967
Working Memory Index (WMI $_{\geq 6 - <12}$)	107	(72-138)	114	(81-142)	**0.021**[*]
Process Speed Index (PSI $_{\geq 6 - <12}$)	98	(53-136)	105	(78-132)	**0.024**[*]
WAIS-III Maternal FSIQ	112	(89-140)	111	(81-132)	0.508

[a] Data are presented as mean (range); WPPSI-R: Wechsler Preschool and Primary Scale of Intelligence-Revised; WISC-IV: Wechsler Intelligence Scale for Children-IV; WAIS-III: Wechsler Adult Intelligence Scale-III;

[b] Mann–Whitney *U* test; *: $P < .05$; #: $P < .10$.

Table 4. Multiple regression results for DEHP and DBP exposure regarding the WPPSI-R scores for preschool children aged ≥3–<6 y (N = 108).

WPPSI-R	Model 1[a] (N= 108)					Model 2[b] (N= 108)				
	Beta	SE	95%CI		P-value	Beta	SE	95%CI		P-value
FSIQ ≥3 - <6										
Log MEHP	-0.91	1.83	-4.53	2.72	0.622	1.37	1.96	-2.53	5.26	0.488
Log MEOHP	-1.23	2.16	-5.53	3.06	0.570	1.38	2.60	-3.78	6.54	0.597
Log MEHHP	-1.00	3.26	-7.47	5.46	0.759	4.35	3.97	-3.53	12.22	0.276
Log MnBP	-1.35	3.43	-8.15	5.44	0.694	2.68	3.64	-4.54	9.90	0.464
Log MiBP	1.50	3.38	-5.20	8.21	0.658	4.48	3.46	-2.39	11.35	0.198
Log ΣDEHP	-1.63	3.28	-8.13	4.86	0.619	3.60	4.01	-4.35	11.55	0.371
Log ΣDBP	-0.43	3.62	-7.62	6.75	0.905	3.89	3.81	-3.68	11.46	0.310
PIQ ≥3 - <6										
Log MEHP	-2.34	2.13	-6.56	1.88	0.273	-0.24	2.32	-4.85	4.38	0.919
Log MEOHP	0.69	2.53	-4.33	5.71	0.785	1.87	3.07	-4.22	7.96	0.544
Log MEHHP	0.89	3.81	-6.66	8.45	0.815	3.29	4.70	-6.04	12.62	0.486
Log MnBP	2.86	4.00	-5.06	10.78	0.475	4.84	4.28	-3.65	13.34	0.260
Log MiBP	2.99	3.94	-4.83	10.81	0.450	4.65	4.10	-3.48	12.78	0.259
Log ΣDEHP	0.09	3.83	-7.51	7.68	0.982	2.76	4.74	-6.65	12.17	0.562
Log ΣDBP	3.37	4.22	-5.00	11.74	0.427	5.71	4.49	-3.20	14.62	0.206
VIQ ≥3 - <6										
Log MEHP	1.22	1.85	-2.45	4.89	0.511	2.89	1.99	-1.06	6.85	0.149
Log MEOHP	-2.15	2.18	-6.47	2.18	0.328	1.59	2.66	-3.68	6.86	0.550
Log MEHHP	-1.18	3.30	-7.72	5.36	0.721	5.64	4.04	-2.38	13.65	0.166
Log MnBP	-3.09	3.46	-9.95	3.76	0.373	1.44	3.73	-5.95	8.84	0.699
Log MiBP	1.96	3.42	-4.82	8.74	0.567	5.66	3.52	-1.33	12.65	0.111
Log ΣDEHP	-1.24	3.32	-7.81	5.34	0.709	5.56	4.07	-2.52	13.65	0.175
Log ΣDBP	-1.50	3.66	-8.77	5.76	0.683	3.40	3.90	-4.35	11.14	0.386

Abbreviations are listed in the footnote of Table 2 and Table 3.

[a]Model 1: multiple regression for crude model

[b]Model 2: multiple regression after adjustment for age, gender, estimate DEHP $_{ADD}$, maternal IQ (WAIS-III) and education, passive smoking, maternal active smoking (during pregnancy) and passive smoking (during pregnancy).

Table 5. Multiple regression results for DEHP and DBP exposure regarding the WISC-IV scores for school-aged children aged ≥6–<12 y (N = 96).

WISC-IV	Model 1[a] (N= 96)					Model 2[b] (N= 96)				
	Beta	SE	95%CI		P-value	Beta	SE	95%CI		P-value
FSIQ ≥6 - <12										
Log MEHP	-0.69	3.22	-7.09	5.70	0.830	-2.13	3.35	-8.78	4.53	0.527
Log MEOHP	-7.40	4.10	-15.55	0.75	0.074	-6.20	5.04	-16.21	3.82	0.222
Log MEHHP	-7.40	4.99	-17.31	2.51	0.142	-6.76	6.07	-18.82	5.31	0.269
Log MnBP	-7.10	4.79	-16.61	2.42	0.142	-6.11	5.01	-16.08	3.85	0.226
Log MiBP	-5.84	3.83	-13.44	1.76	0.130	-5.97	3.83	-13.58	1.65	0.123
Log ΣDEHP	-7.40	5.15	-17.63	2.84	0.155	-7.19	6.24	-19.58	5.21	0.252
Log ΣDBP	-6.52	4.55	-15.56	2.52	0.156	-6.07	4.65	-15.31	3.17	0.195
VCI ≥6 - <12										
Log MEHP	-1.10	3.45	-7.95	5.75	0.751	-3.82	3.60	-10.97	3.32	0.290
Log MEOHP	-10.74	4.33	-19.34	-2.13	**0.015**	-11.92	5.33	-22.52	-1.33	**0.028**
Log MEHHP	-7.86	5.35	-18.49	2.76	0.145	-8.25	6.53	-21.23	4.74	0.210
Log MnBP	-11.77	5.05	-21.79	-1.74	**0.022**	-11.32	5.31	-21.89	-0.76	**0.036**
Log MiBP	-10.08	4.02	-18.06	-2.10	**0.014**	-8.85	4.08	-16.96	-0.74	**0.033**
Log ΣDEHP	-8.80	5.51	-19.74	2.14	0.114	-10.16	6.69	-23.45	3.14	0.133
Log ΣDBP	-11.73	4.78	-21.22	-2.24	**0.016**	-10.95	4.93	-20.74	-1.16	**0.029**
PRI ≥6 - <12										
Log MEHP	-1.13	3.61	-8.30	6.04	0.755	-2.03	3.85	-9.68	5.62	0.599
Log MEOHP	-4.70	4.66	-13.94	4.54	0.315	1.04	5.84	-10.56	12.64	0.859
Log MEHHP	-7.00	5.62	-18.15	4.15	0.216	-2.09	7.01	-16.03	11.85	0.766
Log MnBP	-5.53	5.40	-16.26	5.20	0.309	-4.18	5.79	-15.69	7.33	0.472
Log MiBP	-4.74	4.32	-13.31	3.83	0.275	-3.86	4.44	-12.69	4.97	0.388
Log ΣDEHP	-6.66	5.80	-18.18	4.86	0.254	-1.96	7.21	-16.29	12.38	0.787
Log ΣDBP	-5.24	5.13	-15.43	4.95	0.310	-4.18	5.37	-14.86	6.50	0.439
WMI ≥6 - <12										
Log MEHP	0.47	3.29	-6.06	6.99	0.888	-0.88	3.39	-7.62	5.85	0.795
Log MEOHP	-6.29	4.21	-14.65	2.07	0.138	-9.27	5.04	-19.28	0.75	0.069
Log MEHHP	-5.71	5.12	-15.88	4.45	0.267	-9.24	6.09	-21.35	2.87	0.133
Log MnBP	-5.73	4.91	-15.47	4.02	0.246	-4.85	5.08	-14.95	5.25	0.343
Log MiBP	-2.98	3.94	-10.80	4.84	0.451	-4.18	3.90	-11.93	3.57	0.286
Log ΣDEHP	-5.79	5.28	-16.27	4.70	0.276	-9.84	6.26	-22.28	2.60	0.120
Log ΣDBP	-4.86	4.67	-14.13	4.41	0.301	-4.94	4.71	-14.31	4.43	0.297
PSI ≥6 - <12										
Log MEHP	-0.17	3.97	-8.05	7.71	0.966	1.38	4.23	-7.03	9.79	0.744
Log MEOHP	-0.78	5.14	-0.78	5.14	0.880	1.28	6.41	-11.47	14.03	0.842
Log MEHHP	-4.22	6.21	-16.54	8.10	0.498	-4.39	7.70	-19.69	10.91	0.570
Log MnBP	4.67	5.95	-7.14	16.48	0.434	4.44	6.36	-8.20	17.09	0.487
Log MiBP	1.73	4.77	-7.74	11.19	0.718	-0.58	4.90	-10.32	9.17	0.906
Log ΣDEHP	-3.04	6.41	-15.76	9.69	0.637	-2.44	7.92	-18.19	13.31	0.759
Log ΣDBP	5.00	5.64	-6.20	16.21	0.378	3.79	5.91	-7.96	15.55	0.523

Abbreviations are listed in the footnote below Table 2 and Table 3.

[a]Model 1: Multiple regression for crude model; [b]Model 2: Multiple regression after adjustment for age, gender, estimate DEHP $_{ADD}$, maternal IQ (WAIS-III) and education, passive smoking, maternal active smoking (during pregnancy) and passive smoking (during pregnancy).

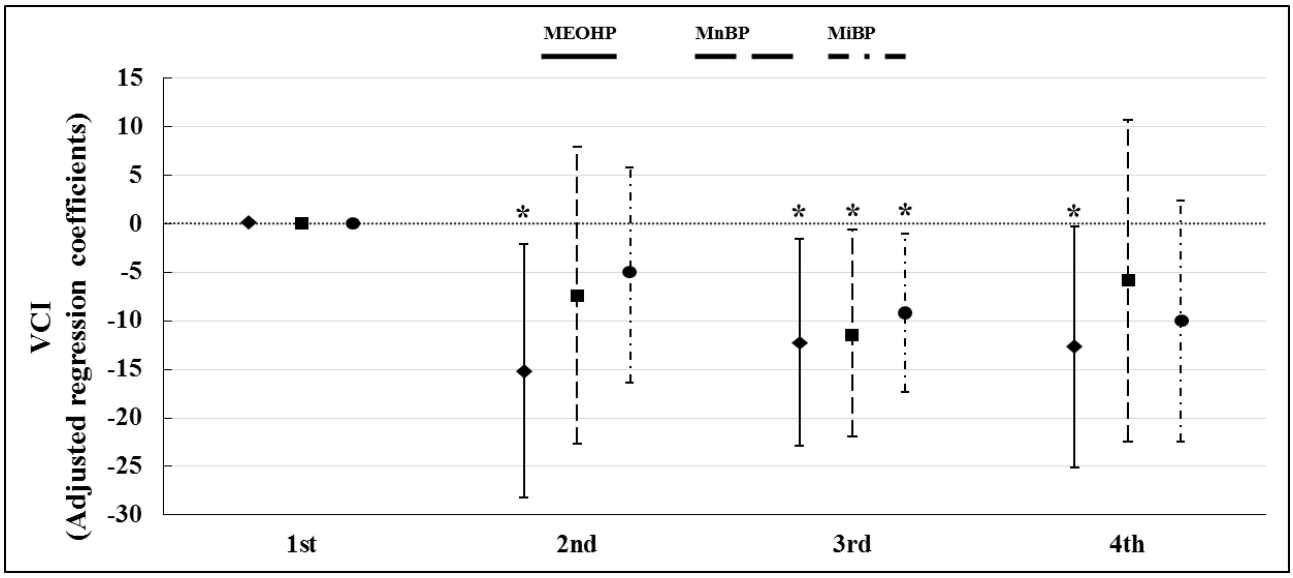

Figure 1. Regression coefficients (95% confidence intervals) for change in $VCI_{\geq 6-<12}$ scores associated with quartiles of urinary levels of MEOHP, MnBP, and MiBP. All models were adjusted for age, gender, estimate DEHP $_{ADD}$, maternal IQ (WAIS-III) and education, passive smoking, maternal active and passive smoking (during pregnancy).