



## Intellectual evaluation of children exposed to phthalate-tainted products after the 2011 Taiwan phthalate episode



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### 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  $\mu\text{g/g}$  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 ( $\beta$ ,  $-11.92$ ; SE, 5.33; 95%CI,  $-22.52 \sim -1.33$ ;  $P=0.028$ ) and  $\log_{10}$   $\Sigma$ DBP metabolites ( $\beta$ ,  $-10.95$ ; SE, 4.93; 95%CI,  $-20.74 \sim -1.16$ ;  $P=0.029$ ) 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.

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## 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, 2017).

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., 2015a; Chang et al., 2017). 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; Tellez-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, 2016b, 2016c; 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 ( $\geq 3$  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., 2015a, 2015b) the levels of these metabolites. Briefly, after the urine samples were thawed and sonicated for 10–15 min, a 100- $\mu$ L urine sample was loaded into a 2-mL glass vial containing ammonium acetate (20  $\mu$ L),  $\beta$ -glucuronidase (10  $\mu$ L), and a 10-isotope ( $^{13}\text{C}_4$ ) mixture as the phthalate metabolite standard (100  $\mu$ L). We used HPLC-grade  $\text{H}_2\text{O}$  (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- $\mu$ L 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 $\times$ 4.6 mm; 5  $\mu$ m) was used for extracting and cleaning the samples, whereas the other analytical column (Inertsil Ph-3; 150 mm $\times$ 4.6 mm; 5  $\mu$ m) 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  $\mu$ L/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  $^{13}\text{C}_4$ -labeled internal standard for each phthalate metabolite. The concentration of the blank samples

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