



# ENVIRONMENTAL HEALTH PERSPECTIVES

<http://www.ehponline.org>

## Prenatal Exposure to Phthalate Esters and Behavioral Syndromes in Children at Eight Years of Age: Taiwan Maternal and Infant Cohort Study

Yin-Ju Lien, Hsiu-Ying Ku, Pen-Hua Su, Suh-Jen Chen,  
Hsiao-Yen Chen, Pao-Chi Liao, Wei-J. Chen, and Shu-Li Wang

<http://dx.doi.org/10.1289/ehp.1307154>

Received: 29 May 2013

Accepted: 2 October 2014

Advance Publication: 3 October 2014

# **Prenatal Exposure to Phthalate Esters and Behavioral Syndromes in Children at Eight Years of Age: Taiwan Maternal and Infant Cohort Study**

Yin-Ju Lien,<sup>1,2\*</sup> Hsiu-Ying Ku,<sup>3,4\*</sup> Pen-Hua Su,<sup>5</sup> Suh-Jen Chen,<sup>6,7</sup> Hsiao-Yen Chen,<sup>4</sup> Pao-Chi Liao,<sup>8</sup> Wei-J. Chen,<sup>2</sup> and Shu-Li Wang<sup>3,4,9</sup>

<sup>1</sup>Department of Health Promotion and Health Education, National Taiwan Normal University, Taipei, Taiwan; <sup>2</sup>Institute of Epidemiology and Preventive Medicine, College of Public Health, National Taiwan University, Taipei, Taiwan; <sup>3</sup>Graduate Institute of Life Science, National Defense Medical Center, Taipei, Taiwan; <sup>4</sup>Division of Environmental Health & Occupational Medicine, National Health Research Institutes, Miaoli, Taiwan; <sup>5</sup>Department of Pediatrics, Chung Shan Medical University, Taichung, Taiwan; <sup>6</sup>Genetic Consultation Center, Chung Shang Medical University Hospital, Taichung, Taiwan; <sup>7</sup>School of Nursing, Chung Shang Medical University, Taichung, Taiwan; <sup>8</sup>Department of Environmental and Occupational Health, College of Medicine, National Cheng Kung University, Tainan, Taiwan; <sup>9</sup>Department of Public Health, National Defense Medical Center, Taipei, Taiwan. \*These authors contributed equally to this work.

**Address correspondence to** Shu-Li Wang, National Health Research Institutes, 35 Keyan Road, Zhunan, Miaoli County 35035, Taiwan. Telephone: +886 37 246166, ext.36509. Fax: +886 37 587 406. E-mail: [slwang@nhri.org.tw](mailto:slwang@nhri.org.tw)

**Running title:** Phthalate exposure and children's behavior

**Acknowledgments:** The authors want to thank to Jein-Wen Chen, Po-Chin Huang, Ya-Tang Liao, Hsin-I Huang, and Yun-Hsiang Liu at the National Health Research Institutes in Taiwan for their assistance of data and specimen collections. The study was funded by the Taiwan Department of National Health Research Institutes (EO-102-PP-05, EO-103-PP-05, EH-102-SP-02, EH-103-SP-02) and National Science Council (102-2325-B-400-008, NSC103-2314-B-400 -006).

**Competing financial interests:** The authors have declared that no competing interests exist.

## Abstract

**Background:** Few studies have shown an association between prenatal phthalate exposure and adverse effects on neurodevelopment and behavior in young children.

**Objectives:** We aimed to assess the relationship between prenatal exposure to phthalate esters and behavior syndromes in children at 8 years of age.

**Methods:** A total of 122 mother-child pairs from the general population in central Taiwan were studied from 2000 to 2009. Mono-methyl phthalate (MMP), mono-ethyl phthalate (MEP), mono-butyl phthalate (MBP), mono-benzyl phthalate (MBzP), and three di- (2-ethylhexyl) phthalate (DEHP) metabolites - mono-2-ethylhexyl, mono-2-ethyl-5-hydroxyhexyl, and mono-2-ethyl-5-oxohexyl phthalates (MEHP, MEHHP and MEOHP) were measured in maternal urine collected during the third trimester of pregnancy using liquid chromatography-electrospray ionization-tandem mass spectrometry. Behavioral syndromes of children at age 8 were evaluated using Child Behavior Checklist (CBCL). Associations between  $\log_{10}$ -transformed creatinine-corrected phthalate concentrations and standardized scores of the CBCL were estimated using linear regression models or multinomial logistic regressions with adjustments for potential confounders.

**Results:** Externalizing problem scores were significantly higher in association with a 1-unit increase in  $\log_{10}$ -transformed creatinine-corrected concentrations of maternal MBP ( $\beta = 4.29$ ; 95% CI 0.59, 7.99), MEOHP ( $\beta = 3.74$  ; 95% CI 1.33, 6.15), and MEHP ( $\beta = 4.28$  ; 95% CI 0.03, 8.26) after adjusting for children's gender, intelligence, and family income. Meanwhile, MBP and MEOHP were significantly associated with Delinquent Behavior and Aggressive Behavior scores. Same pattern was found for borderline and/or clinical ranges.

**Conclusions:** Our findings suggest positive associations between maternal DEHP and dibutyl phthalate (DBP) exposure and externalizing domain behavior problems in eight-year-old children.

## Introduction

Phthalate esters are a family of chemicals which are widely used in daily lives including food packaging, children's toys, and building materials. For instance, di- (2-ethylhexyl) phthalate (DEHP) can be found in food containers, butyl benzyl phthalate (BBzP) in vinyl flooring and wall covering, and di-butyl phthalate (DBP) in personal care products (Duty et al. 2005; Schettler 2006). Due to widespread contamination, these chemicals can enter the human body through daily ingestion and inhalation. In addition, personal care products are likely the predominant source of di-ethyl phthalate (DEP), di-methyl phthalate (DMP) and Di-n-butyl phthalate (DnBP) (Just et al. 2010). Medically related products contaminated with phthalates are another source of exposure (Hernandez-Diaz et al. 2009). Phthalates have been identified in the urine samples of all age groups (Chen et al. 2008).

There is an emerging public health concern that the wide spread use of phthalates may affect brain development and children's behavior. Engel et al. (2010) have reported an association between prenatal low-molecular-weight (LMW) phthalate exposure (the sum of DMP, DEP, DBP, and di-isobutyl phthalate (DiBP)) and poorer scores on aggression, attention problems and depression in children between 4-9 years. Another study using the Child Behavior Checklist reported a positive association between prenatal DnBP and BBzP exposure and clinically withdrawn behavior in 3 year old children (Whyatt et al., 2012). One cross-sectional study revealed that exposure to DEHP might be associated with hyperactivity and attention-deficit/hyperactivity disorder (ADHD) symptoms in school-age children (Kim et al. 2009). These studies showed mixed findings in terms of particular phthalates and internalized (e.g., withdrawal, depression) or externalized behavior problems (e.g., delinquent behavior and aggression). Accordingly, more prospective follow-up studies are needed to clarify whether

exposures to particular phthalates during the fetal period are associated with distinct behavioral profiles later in childhood.

Animal studies have also indicated that phthalate exposures may affect the development of the central nervous system and brain function: Perinatal exposure to DEHP affected the development of coordinated movements in mice offspring during early postnatal development (Tanaka 2002). Perinatal exposure to DBP induced cell apoptosis in hippocampal neurons and impaired the spatial learning and memory in rat pups (Li et al. 2009; Li et al. 2013). These experimental findings suggest that early life exposure to certain phthalates might have adverse effects on behavior, learning, and memory.

Potential long-term effects of phthalate exposure during the fetal period on the behavioral profile of school-age children are uncertain. We conducted a nine-year follow-up study to estimate associations of various prenatal phthalates with distinct behavioral syndromes including internalizing and externalizing problem scores in 8 year-old children in Taiwan.

## **Methods**

### **Study population and recruitment**

This was a birth cohort study examining multiple prenatal and postnatal factors in relation to child health outcomes, part of the nationwide Taiwan Maternal and Infant Cohort Study. (Lin et al. 2011a; Wang et al. 2005). The research profile is depicted in Figure 1. The study participants were pregnant women without clinical complications of eclampsia or preeclampsia, who were between 25 and 34 years of age and delivered their babies in a medical center located in central Taiwan, between December 1, 2000 and November 30, 2001 (n =610). The recruitment and follow-up protocols were both approved by the Institutional Review Board of the National

Health Research Institutes in Taiwan. After receiving detailed explanations of the study, each of pregnant women and their children at 8 years of age provided informed consent for the questionnaire interview, sample collections, and behavioral evaluations. A total of 430 pregnant women in the third trimester were recruited and invited to provide urine samples and information regarding demographic, dietary habits, and reproductive and medical histories. Urine samples were collected during the third trimester (n=388).

Follow-up was done when their children were 8 years old in order to evaluate these children's urinary phthalate metabolite levels as well as their behavioral problems. Of all of those first enrolled in the study center, 251 children were lost to follow-up at 8-9 years mainly because of moving away. An additional 16 caregivers did not complete behavioral evaluations for their children. For a total of 163 eight year-old children, we had completed demographic questionnaires (including parental education levels, parental occupation, family income, children parity) and behavioral evaluations. We also assessed full-scale intelligence quotient (IQ) scores at 8-9 years of age using the Chinese version of the Wechsler Intelligence Scale for Children-version III (WISC-III) (Wechsler 1991). Because urine samples collected from 41 of the pregnant women during the 3rd trimester were not sufficient for phthalate metabolite analyses, we finally had 122 pairs of pregnant women and their children with both urine phthalate metabolite levels and behavioral evaluations.

### **Child Behavior Checklist (CBCL)**

Mothers completed the CBCL at the visit when their children were 8 years old. The mothers' evaluations were based on the status of their children in the preceding 6 months. The CBCL/4-18 (Achenbach 1991) comprised 20 competence items and 118 behavioral/emotional items with a three-point rating scale (0 if not true, 1 if somewhat true or sometimes true, and 2 if very true or

often true). On the basis of cross-informant analysis, eight narrow-band behavioral syndromes (i.e., Withdrawn, Somatic Complaints, Anxious/Depressed, Social Problems, Thought Problems, Attention Problems, Delinquent Behavior, and Aggressive Behavior) were defined according to Achenbach (1991). Some syndromes were further summed to provide scores for broad-band Internalizing Problems (the sum of Withdrawn, Somatic Complaints, and Anxious/Depressed) and Externalizing Problems (the sum of Delinquent Behavior and Aggressive Behavior). The reliability and validity of the Chinese version of the CBCL (CBCL-C) were satisfactory for Taiwanese adolescents (Yang et al. 2000). A recent study further indicated that the CBCL has cross-cultural generalizability in as many as 23 societies, including Taiwan (Ivanova et al. 2007). T scores of the CBCL were derived from well-established normative data in Taiwan (Yang et al. 2000). A T-score of 50 represents the mean of the normative group with the standard deviation of 10. Most of the means (SDs) for the CBCL T scores among the 122 children in this study were similar to the population norm of Taiwanese children, which were 45.36 (6.85) for Withdrawn, 48.26 (8.64) for Somatic Complaints, 49.94 (9.05) for Anxious/Depressed, 51.91 (9.72) for Social Problems, 47.65 (9.83) for Thought Problems, 47.98 (8.55) for Attention Problems, 49.39 (8.25) for Delinquent Behavior, 50.27 (9.85) for Aggressive Behavior, 47.83 (8.24) for Internalizing Problems, and 50.01 (9.33) for Externalizing Problems.

### **Phthalate measurements**

Seven phthalate metabolites: mono-methyl phthalate (MMP), mono-ethyl phthalate (MEP), mono-butyl phthalate (MBP), mono-benzyl phthalate (MBzP), the metabolites of DMP, DEP, DBP and BBzP respectively, and mono-2-ethylhexyl phthalate (MEHP), mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP), the metabolites of DEHP, representing exposures to the five commonly used

phthalates were measured in maternal urine samples collected during the third trimester of pregnancy, and children's urine samples collected at 8–9 years of age. Details of the analysis and, quality assurance and control procedures were described previously (Lin et al. 2011a). In brief, we used  $\beta$ -glucuronidase enzymatic deconjugation of the metabolites from their glucuronidated form, followed by on-line solid-phase extraction (SPE) and quantification using liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS). The intra-day variations were below 10%, and intra-day recoveries were at  $100 \pm 20\%$  at three different concentrations, 25%, 50% and 75%, of the individual substances. The detection limits of MMP, MEP, MBP, MBzP, MEHP, MEHHP, and MEOHP were 3.4, 2.2, 1.6, 0.99, 0.55, 0.23, and 0.26 ng/mL, respectively. When phthalate metabolite levels were below the limit of detection (LOD), we replaced these values with 50% of the LOD values. Urinary creatinine level was measured by the spectrophotometric method, with picric acid as reactive, and read at 520 nm at the central laboratory of Kaohsiung Medical University Chung-Ho Memorial Hospital.

### **Statistical analyses**

Chi-Square tests and independent t tests were used to compare the differences in demographic characteristics between pregnant women with children that we followed and those pregnant women lost to follow-up, while Mann Whitney U tests were used to compare differences in phthalate metabolite levels between these two groups of pregnant women.

The distributions of studied phthalate metabolites were skewed to the right and thus were  $\log_{10}$  transformed to fit the model needs. With 122 maternal and children pairs followed, we could detect correlations of 0.25 or greater between the phthalate metabolites and behavior outcomes with 80% power and 5% type I error. The T scores of the CBCL syndromes were approximately normally distributed. The CBCL scales can be analyzed as continuous scores, or the children can

be classified as the normal, borderline, or clinical range (Achenbach 1991; Yang et al. 2000). According to the percentiles in the normative population in Taiwan (Yang et al. 2000), T scores above the 95<sup>th</sup> percentile represented the clinical range, and T scores ranged from the 90<sup>th</sup> to the 95<sup>th</sup> percentile fall into the borderline range. Multivariable linear regression for continuous outcomes were used to estimate associations between prenatal or children's urine phthalate levels and children's behavioral problem scores at 8-9 years of age. Meanwhile, polytomous logistic regression models were used to estimate ORs comparing exposures among those classified as borderline (90-95<sup>th</sup> percentile) behavior to those with scores <90<sup>th</sup> percentile, and ORs comparing exposures among those classified as clinical (>95<sup>th</sup> percentile) behavior to those with scores <90<sup>th</sup> percentile in the model simultaneously. ORs for borderline/clinical combined were estimated using dichotomous logistic regression. Regarding possible gender difference in the beta estimates, we used independent t tests to assess the difference in CBCL score by child gender, and Wald test to examine the significance level of the cross-product term in the model of  $Y = \alpha + \beta_1 \text{sex} + \beta_2 \log_{10}(\text{creatinine-corrected phthalates}) + \beta_3 \text{sex} \times \log_{10}(\text{creatinine-corrected phthalates})$ . From a pool of covariates which were possibly associated with the phthalate concentrations or CBCL scores in previous studies (Fan et al. 2013; Raadal et al. 1994; Tehrani-Doost et al. 2011), those remaining significantly correlated ( $p < 0.05$ ) with at least one of the behavioral/emotional problems on the CBCL were selected as covariates in regression models. Model covariates for CBCL outcomes were children's gender, IQ, and family income. In this study, children's parental education was not controlled because it was not significantly associated with either phthalate concentration or CBCL scores. Adjusting for children's IQ did not influence coefficients for exposure-outcome associations when models were also adjusted for gender and family income. Analyses were performed with SAS 9.1 for Windows (SAS, Cary,

North Carolina). All tests were two-tailed, and significance was set at  $p < 0.05$  unless otherwise specified.

## Results

### Cohort characteristics

Demographic characteristics of pregnant women with children who were followed ( $n = 122$ ) and those lost to follow-up ( $n = 266$ ) are shown in Table 1. No significant differences in family income or maternal smoking during pregnancy were observed between the groups. Pregnant women who were followed had a greater proportion of higher levels of education (i.e., junior college or university) than those lost to follow-up. The distribution of seven urinary phthalate mono-esters adjusted for creatinine in the two groups is shown in Table 2. Similar concentrations of all phthalate levels were found. We also observed that boys had higher average scores on Attention (mean $\pm$ SD: 49.4 $\pm$ 9.3 for boys vs. 46.3 $\pm$ 7.7 for girls) and Aggressive Behaviors (52.5 $\pm$ 10.5 for boys vs. 48.9 $\pm$ 9.0 for girls), and Externalizing Problems (52.2 $\pm$ 10.2 vs. 48.7 $\pm$ 8.3) compared with girls (see Supplemental Material, Table S1). However, there appeared no significant gender difference in the beta estimates (Supplemental Material, Table S2). We plotted the log<sub>10</sub>-transformed maternal creatinine-corrected MEOHP levels corresponding to externalizing problem scores. The parallel regression lines for boys and girls confirmed the similar pattern of beta estimates between genders (Supplemental Material, Figure S1). Thus the boys and girls combined data were analyzed and presented to produce reasonably precise estimates of association.

## Phthalates and CBCL

Table 3 shows the multivariable linear regression analysis results of  $\log_{10}$  creatinine-corrected concentrations of prenatal phthalate metabolites and children's behavior scores at age 8-9. When adjusted for gender, IQ, and family income, both MBP and MEOHP exhibited average difference in scores associated with a 1-unit increase in  $\log_{10}$ -transformed creatinine-corrected urine concentrations for Delinquent Behavior ( $\beta = 3.57$ , 95% CI: = 0.20, 6.94 for MBP;  $\beta = 3.95$ , 95% CI: 1.81, 6.09 for MEOHP), Aggressive Behavior ( $\beta = 4.21$ , 95% CI: = 0.31, 8.11 for MBP;  $\beta = 3.32$ , 95% CI: = 0.77, 5.87 for MEOHP), and Externalizing Problems (i.e., sum of Delinquent Behavior and Aggressive Behavior) ( $\beta = 4.29$ , 95% CI: = 0.59, 7.59 for MBP). In addition, MEHHP was associated with scores for Delinquent Behavior ( $\beta = 1.87$ , 95% CI: = 0.05, 3.69) while MEHP was associated with scores for Delinquent Behavior ( $\beta = 3.77$ , 95% CI: = 0.16, 7.38) and Externalizing Problems ( $\beta = 4.28$ , 95% CI: = 0.03, 8.26). Other than externalizing domain behavior problem scores, MEOHP was associated with increased scores for Social Problems ( $\beta = 2.98$ , 95% CI: = 0.37, 5.59). Nevertheless, none of the maternal phthalates metabolite concentrations were associated with children's internalizing domain behavior problem scores (Somatic Complaints, Anxious/Depressed, or Internalizing Problem scores) (all p values > 0.05). There were no significant cross-sectional associations between any children's urinary creatinine-corrected phthalates metabolite concentrations measure at 8-yers of age and their behavior problem scores (all p values > 0.05 based on all the 122 children; data not shown).

Higher prenatal phthalate metabolite concentrations were associated with higher odds ratios of borderline or clinical range scores on the CBCL externalizing domain behaviors (Table 4). Given the very small numbers in the borderline or clinical range of the CBCL scores, we thus further merged the borderline group and the clinical one into a single group (borderline/clinical) to

improve statistical power and precision. ORs represent a 1-unit increase on  $\log_{10}$ -transformed creatinine-corrected maternal urine concentrations. Four out of the seven studied phthalate metabolites exhibited increased ORs on the borderline/clinical ranges of Externalizing Problems (14 cases total), MBP (OR = 8.37, 95% CI: 1.61, 43.45), MEOHP (OR = 16.68, 95% CI: 3.08, 90.18), MEHHP (OR = 3.10, 95% CI: 1.09, 8.84), and MEHP (OR = 22.97, 95% CI: 3.34, 158.06). Among these four phthalate metabolites, only MEOHP exhibited increased ORs on the borderline/clinical ranges of Aggressive Behavior (OR = 3.22, 95% CI: 1.12, 9.28 based on 16 cases). Besides, MEP was associated with borderline range, but not clinical range, for Delinquent Behavior (OR = 7.15, 95% CI: 1.26, 40.52 based on 8 cases), and with clinical range on Externalizing Problems (OR = 6.09, 95% CI: 1.01, 36.74 based on 8 cases). With regard to the associations between children's phthalate metabolites and children's behavior problems in terms of borderline/clinical range at age 8-9, only one significant association was implicated (data not shown). There was an association between MMP and scores on the borderline/clinical range of Withdrawn (OR = 9.15, 95% CI: 1.34, 62.64, based on 5 cases). The inclusion or exclusion of child's IQ did not alter the observed patterns of association (data not shown).

## **Discussion**

We estimated positive associations between creatinine-corrected maternal urinary concentrations of DEHP and DBP metabolites and externalizing domain behavior problem scores (i.e., scores for Delinquent and Aggressive Behaviors, and Externalizing Problems) in 8-9-year-old children. Engel et al (2010) reported that externalizing and aggressive problem scores in 4-9 year old boys were associated with maternal urinary MBP and MEP concentrations in the third trimester. In our study population, boys and girls combined, maternal urine MBP concentrations were associated with higher average scores and with scores in the borderline/clinical combined range

for externalizing problems, and maternal MEP concentrations were associated with externalizing problem scores in the clinical range. Whyatt et al. (2012) reported that maternal urine MnBP concentrations were positively associated with CBCL internalizing behavior scores at 3 years of age. We observed a positive but non-significant association between MBP (representing combined MnBP and MiBP concentrations) and internalizing behavior scores at 8–9 years of age in our study population. In addition, we estimated a significant positive association between maternal urine concentrations of MEOHP (a DEHP metabolite) and social problem scores. To our knowledge, this has not been reported previously.

A biological mechanism that might result in negative effects of phthalates on brain development and behavior has not been established. It has been proposed that DEHP might impair fetal brain development through activation of Trim17 protein via PPAR $\gamma$  leading to apoptosis based on an *in vitro* study of neuroblastoma cells (Lin et al. 2011b). Chen et al. proposed that MEHP might disturb neurodevelopment by suppressing cell proliferation and promoting cell differentiation towards a cholinergic phenotype that may relate to hyperactivity based on *in vitro* findings in neuronotypic PC 12 cells (Chen et al. 2011). In rat pups, perinatal exposure to DBP induced cell apoptosis in hippocampal neurons and appeared to impair the spatial learning and memory (Li et al. 2009; Li et al. 2013). In addition, it has been suggested that phthalates might indirectly affect neurodevelopment through effects on essential fatty acids or thyroid function. Xu et al. reported that *in utero* exposure to DEHP influenced the transfer of essential fatty acids across the placenta and was associated with altered lipid content in the rat fetal brain (Xu et al. 2008). In a rat thyroid cell line, DEHP and DBP were appeared to affect iodide uptake activity through modulation of sodium/iodide symporter (Wenzel et al., 2005). In a study of 76 women during the second trimester of pregnancy, urine concentrations of the DBP metabolite MBP were associated

with significantly lower serum free thyroxine (fT4) concentrations (Huang et al. 2007). Furthermore, adverse effects of maternal thyroid dysfunction on neurodevelopment or early postnatal brain development were well known (Haddow et al. 1999; Porterfield and Hendrich 1993). Thus DBP and DEHP might affect growth and development of central nervous system in both direct and indirect modes.

*In utero* exposure assessment for the present study was limited to a single spot urine sample collected in the third trimester of pregnancy. A previous report (Morreale de Escobar et al. 2004) suggested that brain development begins at early conception, which might be the most vulnerable time in neurodevelopment. We assumed that a single urine concentration represented typical exposure, and that sources or exposure patterns were consistent during pregnancy. However, Braun et al. (2012) reported relatively high temporal variability of DEHP metabolites in pregnant women. Non-differential exposure misclassification might attenuate effect estimates leading to underestimated associations between prenatal phthalate and behavior problems.

The attrition rate was high in the current study. We examined differences between the lost and followed groups and found that all studied characteristics and phthalate concentrations were similar, except for higher parental educational level in the followed group. Future larger sample sizes might help the situations and also offer the opportunity of further stratification by sex. In addition, we were not able to adjust for other correlated exposures, such as BPA, in the current study. Lastly we did not distinguish between the two isomers of MnBP and mono-isobutyl phthalate (MiBP). We found that MnBP occupy the majority of MBP for about  $81 \pm 10\%$  in part of our participants (n=99). This is constant with the previous study (Whyatt et al., 2012). Future perspective studies with sufficient sample size will be needed to confirm the sensitive susceptibility to phthalate during pregnancy, and to evaluate gender-specific associations

between phthalates and the risk of behavior problems with consideration of phthalate isomers and co-exposure to other endocrine disruptors in children.

## **Conclusions**

Our data supported positive associations between maternal DBP and DEHP metabolite concentrations measured in the third trimester urine and children's externalizing domain problem scores assessed at ages 8-9 years. The results suggest that prenatal exposure to such chemicals might play a role in the development of behavioral problems later in the children's lives.

## References

- Achenbach TM. 1991. Manual for the child behavior checklist/4-18 and 1991 profile. Burlington, VT:University of Vermont, Department of Psychiatry.
- Braun JM, Smith KW, Williams PL, Calafat AM, Berry K, Ehrlich S, et al. 2012. Variability of urinary phthalate metabolite and bisphenol A concentrations before and during pregnancy. *Environ Health Perspect* 120:739-745.
- Chen ML, Chen JS, Tang CL, Mao IF. 2008. The internal exposure of Taiwanese to phthalate - an evidence of intensive use of plastic materials. *Environ Int* 34:79-85.
- Chen TA, Yang WJ, Li YY, Chen X, Xu SQ. 2011. Mono- (2-ethylhexyl) phthalate impairs neurodevelopment: Inhibition of proliferation and promotion of differentiation in pc12 cells. *Toxicol Lett* 201:34-41.
- Duty SM, Ackerman RM, Calafat AM, Hauser R. 2005. Personal care product use predicts urinary concentrations of some phthalate monoesters. *Environ Health Perspect* 113:1530-1535.
- Engel SM, Miodovnik A, Canfield RL, Zhu CB, Silva MJ, Calafat AM, et al. 2010. Prenatal phthalate exposure is associated with childhood behavior and executive functioning. *Environ Health Persp* 118:565-571.
- Fan RG, Portuguese MW, Nunes ML. 2013. Cognition, behavior and social competence of preterm low birth weight children at school age. *Clinics* 68:915-921.
- Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon J, et al. 1999. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. *The New England Journal of Medicine* 341:549-555.
- Hernandez-Diaz S, Mitchell AA, Kelley KE, Calafat AM, Hauser R. 2009. Medications as a potential source of exposure to phthalates in the U.S. Population. *Environ Health Perspect* 117:185-189.
- Huang PC, Kuo PL, Guo YL, Liao PC, Lee CC. 2007. Associations between urinary phthalate monoesters and thyroid hormones in pregnant women. *Hum Reprod* 22:2715-2722.
- Ivanova MY, Achenbach TM, Dumenci L, Almqvist F, Weintraub S, Bilenberg N, et al. 2007. Testing the 8-syndrome structure of the child behavior checklist in 30 societies. *J Clin Child Adolesc Psychol* 36:405-417.

- Just AC, Adibi JJ, Rundle AG, Calafat AM, Camann DE, Hauser R, et al. 2010. Urinary and air phthalate concentrations and self-reported use of personal care products among minority pregnant women in New York city. *J Expo Sci Environ Epidemiol* 20:625-633.
- Kim BN, Cho SC, Kim Y, Shin MS, Yoo HJ, Kim JW, et al. 2009. Phthalates exposure and Attention-Deficit/Hyperactivity disorder in school-age children. *Biol Psychiatry* 66:958-963.
- Li XJ, Jiang L, Chen L, Chen HS, Li X. 2013. Neurotoxicity of dibutyl phthalate in brain development following perinatal exposure: A study in rats. *Environ Toxicol Pharmacol* 36:392-402.
- Li Y, Zhuang MZ, Li T, Shi N. 2009. Neurobehavioral toxicity study of dibutyl phthalate on rats following *in utero* and lactational exposure. *J Appl Toxicol* 29:603-611.
- Lin LC, Wang SL, Chang YC, Huang PC, Cheng JT, Su PH, et al. 2011a. Associations between maternal phthalate exposure and cord sex hormones in human infants. *Chemosphere* 83:1192-1199.
- Lin CH, Chen TJ, Chen SS, Hsiao PC, Yang RC. 2011b. Activation of trim17 by PPAR-gamma is involved in di (2-ethylhexyl) phthalate (DEHP)-induced apoptosis on neuro-2a cells. *Toxicol Lett* 206:245-251.
- Morreale de Escobar G, Obregon MJ, Escobar del Rey F. 2004. Role of thyroid hormone during early brain development. *Eur J Endocrinol* 151 Suppl 3:U25-37.
- Porterfield SP, Hendrich CE. 1993. The role of thyroid hormones in prenatal and neonatal neurological development—current perspectives. *Endocr Rev* 14 (1):94–106.
- Raadal M, Milgrom P, Cauce AM, Mancl L. 1994. Behavior problems in 5- to 11-year-old children from low-income families. *J Am Acad Child Adolesc Psychiatr* 33:1017-1025.
- Schettler T. 2006. Human exposure to phthalates via consumer products. *Int J Androl* 29:134-139.
- Tanaka T. 2002. Reproductive and neurobehavioural toxicity study of bis (2-ethylhexyl) phthalate (DEHP) administered to mice in the diet. *Food Chem Toxicol* 40:1499-1506.
- Tehrani-Doost M, Shahrivar Z, Pakbaz B, Rezaie A, Ahmadi F. 2011. Normative data and psychometric properties of the child behavior checklist and teacher rating form in an Iranian community sample. *Iran J Pediatr* 21:331-342.

- Wang SL, Su PH, Jong SB, Guo YL, Chou WL, Pöpke O. 2005. In utero exposure to dioxins and polychlorinated biphenyls and its relations to thyroid function and growth hormone in newborns. *Environ Health Perspect* 113:1645-1650.
- Wechsler D. 1991. Wechsler intelligence scale for children-iii. San Antonio: San Antonio, TX: Psychological Corporation.
- Wenzel A, Franz C, Breous E, Loos U. 2005. Modulation of iodide uptake by dialkyl phthalate plasticisers in FRTL-5 rat thyroid follicular cells. *Mol Cell Endocrinol*. 2005 244:63-71.
- Whyatt RM, Liu XH, Rauh VA, Calafat AM, Just AC, Hoepner L, et al. 2012. Maternal prenatal urinary phthalate metabolite concentrations and child mental, psychomotor, and behavioral development at 3 years of age. *Environ Health Persp* 120:290-295.
- Xu Y, Agrawal S, Cook TJ, Knipp GT. 2008. Maternal di- (2-ethylhexyl)-phthalate exposure influences essential fatty acid homeostasis in rat placenta. *Placenta* 29:962-969.
- Yang HJ, Soong WT, Chang CN, Chen WJ. 2000. Competence and behavioral/emotional problems among Taiwanese adolescents as reported by parents and teachers. *J Am Acad Child Adolesc Psychiatr* 39:232-239.

**Table 1.** Demographic characteristics of the pregnant women recruited in 2000-2001 in central Taiwan.

<b>Parental variables</b>	<b>Pregnant women lost to follow-up<sup>a</sup></b>	<b>Pregnant women who were followed</b>	<b>P value</b>
Number	266	122	
Age at delivery (yr)	28.61 ± 4.47	29.28 ± 3.69	0.153
Age at delivery (yr)			0.822
<35	222 (93.3)	115 (94.3)	
≥35	16 (6.7)	7 (5.7)	
Maternal education			0.003
≤High school	132 (53.2)	42 (34.4)	
Junior college	83 (33.5)	57 (46.7)	
≥University	33 (13.3)	23 (18.9)	
Paternal education			0.040
≤High school	119 (49.8)	44 (36.1)	
Junior college	71 (29.7)	49 (40.2)	
≥University	49 (20.5)	29 (23.7)	
Family income per year			0.106
< \$ 20,000	111 (47.2)	43 (35.8)	
\$ 20,000-50,000	120 (51.1)	74 (61.7)	
> \$ 50,000	4 (1.7)	3 (2.5)	
Maternal smoking during pregnancy			0.668
Yes	5 (2.1)	1 (0.8)	
No	235 (97.9)	121 (99.2)	

Data are number (%) or mean±SD. P values for differences between groups using the t test for continuous variables and  $\chi^2$  or Fisher's exact test for categorical variables. <sup>a</sup> One woman gave birth to 2 babies between December 1, 2000 and November 30, 2001.

**Table 2.** Geometric mean (GM) of urinary phthalate metabolite concentrations ( $\mu\text{g/g}$  creatinine) in pregnant women.

Phthalate metabolites	Percent >LOD	Pregnant women lost to follow-up (n=266) <sup>a</sup> mean (SD)	Pregnant women lost to follow-up (n=266) <sup>a</sup> GM (95%CI)	Pregnant women who were followed (n=122) mean (SD)	Pregnant women who were followed (n=122) GM (95%CI)	P-value
MMP	94	80.70 (75.77)	54.13 (48.13-60.88)	87.30 (98.30)	54.53 (44.95-66.14)	0.896
MEP	100	103.2 (158.0)	65.09 (58.59-72.31)	99.87 (134.8)	61.71 (52.45-72.60)	0.535
MBP	100	124.5 (187.0)	74.28 (66.40-83.09)	109.1 (121.6)	66.88 (55.93-79.99)	0.442
MBzP	99	25.06 (22.80)	18.61 (16.98-20.39)	20.20 (16.07)	15.88 (13.87-18.19)	0.103
MEOHP	79	30.48 (72.46)	9.50 (7.71-11.71)	39.18 (99.65)	13.59 (10.27-18.00)	0.104
MEHHP	89	24.93 (51.22)	6.61 (5.25-8.33)	26.77 (57.70)	7.91 (5.69-11.02)	0.538
MEHP	99	34.36 (48.73)	20.02 (17.81-22.51)	30.06 (67.63)	16.93 (14.32-20.02)	0.134

LOD: the limit of detection. *P* for the difference between pregnant women lost to follow up and pregnant women who were followed for analysis was estimated using the Mann-Whitney U test. <sup>a</sup> One woman gave birth to 2 children between December 1, 2000 and November 30, 2001. DEHP metabolites: MEHP, MEHHP, MEOHP.

**Table 3.** Regression coefficients (with 95% confidence intervals) for 1-unit increase in log<sub>10</sub>-transformed creatinine-corrected concentrations of maternal urinary phthalate metabolites on Children Behavior Checklist (CBCL) scores in 8-year-old children (n=122).

Behavior/metabolite (log <sub>10</sub> )	MMP	MEP	MBP	MBzP	MEOHP	MEHHP	MEHP
<b>Without adjusting for covariates</b>							
Withdrawn	-0.42 (-3.07, 2.23)	-2.67 (-5.77, 0.43)	2.72 (-0.08, 5.52)	-1.54 (-5.28, 2.20)	0.94 (-0.86, 2.74)	0.04 (-1.51, 1.59)	1.62 (-1.42, 4.66)
Somatic complaints	-1.52 (-4.81, 1.77)	-1.29 (-5.21, 2.63)	1.53 (-2.02, 5.08)	-1.95 (-6.63, 2.73)	1.86 (-0.39, 4.11)	0.84 (-1.08, 2.76)	1.52 (-2.28, 5.32)
Anxious/depressed	-1.14 (-4.49, 2.21)	-1.12 (-5.12, 2.88)	2.93 (-0.66, 6.52)	-1.24 (-6.02, 3.54)	1.69 (-0.60, 3.98)	1.39 (-0.55, 3.33)	1.13 (-2.73, 4.99)
Social problems	0.10 (-3.57, 3.77)	-3.53 (-7.82, 0.76)	3.87 (-0.01, 7.75)	0.35 (-4.84, 5.54)	3.62 (1.19, 6.05)**	2.36 (0.26, 4.46)*	2.10 (-2.09, 6.29)
Thought problems	-2.75 (-6.49, 0.99)	0.94 (-3.55, 5.43)	0.50 (-3.58, 4.58)	-1.56 (-6.93, 3.81)	-0.09 (-2.29, 2.11)	-0.08 (-2.28, 2.12)	3.56 (-0.75, 7.87)
Attention problems	-0.05 (-3.34, 3.24)	-2.82 (-6.70, 1.06)	1.21 (-2.34, 4.76)	-2.43 (-7.09, 2.23)	1.92 (-0.31, 4.15)	0.51 (-1.41, 2.43)	1.42 (-2.36, 5.20)
Delinquent behavior	-0.22 (-3.40, 2.96)	0.26 (-3.50, 4.02)	3.87 (0.52, 7.22)*	-0.40 (-4.91, 4.11)	4.50 (2.46, 6.54)**	2.57 (0.77, 4.37)**	3.71 (0.10, 7.32)*
Aggressive behavior	0.70 (-2.98, 4.38)	-1.67 (-6.04, 2.70)	4.32 (0.40, 8.24)*	-0.81 (-6.06, 4.44)	3.94 (1.49, 6.39)**	1.98 (-0.14, 4.10)	4.13 (-0.06, 8.32)
Internalizing problems	-1.20 (-4.32, 1.92)	-1.90 (-5.58, 1.78)	2.92 (-0.41, 6.25)	-1.77 (-6.20, 2.66)	1.77 (-0.35, 3.89)	1.77 (-0.35, 3.89)	1.61 (-1.98, 5.20)
Externalizing problems	0.45 (-3.08, 3.98)	-1.17 (-5.36, 3.02)	4.47 (0.75, 8.19)*	-0.74 (-5.78, 4.30)	4.38 (2.07, 6.69)**	2.30 (0.28, 4.32)*	4.28 (0.28, 8.28)*
<b>Adjusting for children's IQ, gender, and family income</b>							
Withdrawn	-0.92 (-3.61, 1.77)	-2.50 (-5.62, 0.62)	2.31 (-0.53, 5.15)	-2.21 (-6.01, 1.59)	0.28 (-1.62, 2.18)	-0.49 (-2.06, 1.08)	1.59 (-1.49, 4.67)
Somatic complaints	-2.00 (-5.41, 1.41)	-1.22 (-5.22, 2.78)	1.02 (-2.65, 4.69)	-2.38 (-7.26, 2.50)	1.46 (-0.95, 3.87)	0.45 (-1.55, 2.45)	1.42 (-2.50, 5.34)
Anxious/depressed	-1.48 (-4.91, 1.95)	-1.00 (-5.02, 3.02)	2.06 (-1.61, 5.73)	-2.67 (-7.53, 2.19)	0.92 (-1.49, 3.33)	0.88 (-1.12, 2.88)	1.32 (-2.60, 5.24)
Social problems	-0.67 (-4.45, 3.11)	-3.37 (-7.74, 1.00)	3.31 (-0.69, 7.31)	-0.76 (-6.13, 4.61)	2.98 (0.37, 5.59)*	1.83 (-0.35, 4.01)	1.90 (-2.41, 6.21)
Thought problems	-3.61 (-7.49, 0.27)	1.42 (-3.17, 6.01)	0.16 (-4.05, 4.37)	-1.73 (-7.34, 3.88)	-0.64 (-3.42, 2.14)	-0.80 (-3.09, 1.49)	3.73 (-0.74, 8.20)
Attention problems	-1.30 (-4.49, 1.89)	-2.00 (-5.70, 1.70)	0.78 (-2.63, 4.19)	-3.46 (-7.97, 1.05)	0.83 (-1.42, 3.08)	-0.50 (-2.36, 1.36)	1.35 (-2.30, 5.00)
Delinquent behavior	-0.99 (-4.18, 2.20)	0.65 (-3.09, 4.39)	3.57 (0.20, 6.94)*	-0.26 (-4.81, 4.29)	3.95 (1.81, 6.09)**	1.87 (0.05, 3.69)*	3.77 (0.16, 7.38)*
Aggressive behavior	-0.49 (-4.21, 3.23)	-0.92 (-5.27, 3.43)	4.21 (0.31, 8.11)*	-1.39 (-6.68, 3.90)	3.32 (0.77, 5.87)*	1.20 (-0.96, 3.36)	4.11 (-0.08, 8.30)
Internalizing problems	-1.70 (-4.86, 1.46)	-1.75 (-5.45, 1.95)	2.17 (-1.20, 5.54)	-2.87 (-7.36, 1.62)	1.02 (-1.21, 3.25)	0.42 (-1.42, 2.26)	1.67 (-1.96, 5.30)
Externalizing problems	-0.68 (-4.23, 2.87)	-0.49 (-4.63, 3.65)	4.29 (0.59, 7.99)*	-1.13 (-6.17, 3.91)	3.74 (1.33, 6.15)**	1.49 (-0.55, 3.53)	4.28 (0.03, 8.26)*

DEHP metabolites: MEHP, MEHHP, MEOHP; \* $P < 0.05$ , \*\* $P < 0.01$ .

**Table 4.** Odds ratios (with 95% confidence intervals) for Children Behavior Checklist (CBCL) scores in the borderline and/ or clinical compared with normal range on the externalizing behaviors for each log<sub>10</sub> unit increase in creatinine-corrected concentrations of maternal urinary phthalate metabolites (n = 122).

<b>Behavior/metabolite (log<sub>10</sub>)</b>	<b>Borderline</b>	<b>Clinical</b>	<b>Borderline/clinical</b>
<b>Delinquent behavior</b>			
No. of observations	N=8	N=6	N=14
MMP	2.07 (0.33, 13.07)	1.14 (0.17, 7.43)	1.55 (0.40, 6.06)
MEP	7.15 (1.26, 40.52)*	1.06 (0.12, 9.75)	3.33 (0.85, 13.00)
MBP	5.34 (0.74, 38.29)	7.28 (0.84, 63.17)	6.17 (1.34, 28.43)*
MBzP	9.99 (0.52, 190.64)	4.83 (0.22, 108.02)	6.94 (0.75, 64.12)
MEOHP	27.31 (3.22, 231.79)**	22.91 (2.32, 226.13)**	24.99 (4.17, 149.65)**
MEHHP	2.62 (0.77, 8.95)	4.21 (0.80, 22.32)	3.11 (1.11, 8.72)*
MEHP	74.04 (4.60, >999.99)**	7.00 (0.65, 75.77)	21.68 (3.41, 137.60)**
<b>Aggressive behavior</b>			
No. of observations	N=7	N=9	N=16
MMP	0.86 (0.16, 4.54)	1.55 (0.25, 9.51)	1.09 (0.31, 3.81)
MEP	0.43 (0.04, 4.18)	4.19 (0.75, 23.51)	1.58 (0.41, 6.16)
MBP	0.25 (0.03, 2.18)	7.18 (1.05, 49.41)*	1.65 (0.44, 6.28)
MBzP	0.90 (0.09, 9.03)	0.94 (0.09, 9.59)	0.89 (0.17, 4.71)
MEOHP	1.95 (0.53, 7.13)	6.88 (1.30, 36.34)*	3.22 (1.12, 9.28)*
MEHHP	0.74 (0.29, 1.92)	4.99 (1.06, 23.54)*	1.49 (0.67, 3.29)
MEHP	0.89 (0.11, 6.96)	9.77 (1.50, 63.70)*	2.92 (0.77, 11.12)
<b>Externalizing problems</b>			
No. of observations	N=6	N=8	N=14
MMP	2.60 (0.30, 22.27)	0.96 (0.18, 5.28)	1.42 (0.35, 5.75)
MEP	0.51 (0.05, 5.64)	6.09 (1.01, 36.74)*	2.20 (0.54, 9.00)
MBP	38.64 (2.31, 646.34)*	3.63 (0.53, 24.81)	8.37 (1.61, 43.45)*
MBzP	0.53 (0.06, 4.82)	6.67 (0.36, 124.50)	1.69 (0.22, 12.81)
MEOHP	10.37 (1.40, 76.54)*	28.49 (3.16, 256.45)**	16.68 (3.08, 90.18)**
MEHHP	1.70 (0.50, 5.80)	7.41 (1.26, 43.38)*	3.10 (1.09, 8.84)*
MEHP	16.42 (1.70, 158.92)*	31.01 (3.01, 319.35)**	22.97 (3.34, 158.06)**

Models controlled for children's gender, IQ and family income; DEHP metabolites: MEHP, MEHHP, MEOHP; T scores of the CBCL were derived from well-established normative data in Taiwan (Yang et al. 2000). Polytomous logistic regression models were used to estimate ORs comparing exposures among those classified as borderline (90-95<sup>th</sup> percentile) behavior to those with scores <90<sup>th</sup> percentile, and ORs comparing exposures among those classified as clinical (>95<sup>th</sup> percentile) behavior to those with scores <90<sup>th</sup> percentile in the model simultaneously. ORs for borderline/clinical combined were estimated using dichotomous logistic regression. \*P<0.05, \*\*P<0.01.

## Figure legend

**Figure 1.** The follow-up diagram of the study population from pregnancy to the age of 8 to 9 years for the children.

Figure 1.

