**Supplemental Materials**

**Prenatal Exposure to Organophosphate Esters and** **Growth Trajectory in Early Childhood**

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**Method**

*Study population*

The present study was conducted between 2014 and 2016 at the Wuhan Women and Children Medical Care Center, a major tertiary medical center in Wuhan, China. Pregnant women who received first antenatal care visit before their 16 weeks of pregnancy were invited to participate in this study if they (1) were willing to have prenatal care and give birth at the study hospital; (2) with a singleton pregnancy; (3) agreeing to take in-person interviews, and provide urine samples. A total of 1653 women provided a complete series of urine samples in each of three trimesters and gave birth to live singletons without birth defects between August 2014 and August 2016. Women who without child growth data at the age of 1 or 2 (N = 27) were excluded and 1626 women satisfied the inclusion criteria. Finally, 212 women were randomly selected for OPE measurement in this study. The research protocol was approved by the Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology (No. (2012)07), and the Wuhan Women and Children Medical Care Center (No. 2012003). Written informed consent was provided by all participants.

*Anthropometric measurements*

Obstetric nurses in the study hospital measured birth weight and length. Children were invited to the department of children’s health of the study hospital to measure weight and height at one and two years old by using a standardized measurement. Early-childhood weight and height were normalized to z-scores by applying the World Health Organization child growth standards specified by sex and age (Organization 2006). The z-scores represent the percentiles of birth size or early-childhood growth.

*Urine collection and analysis*

Spot urine samples in the 1st (12.9 ± 0.9 weeks), 2nd (24.2 ± 3.4 weeks), and 3rd trimesters (34.3 ± 3.2 weeks) were collected during the prenatal care visits in the study hospital. All urine samples were stored in polypropylene recipients at -20°C until analyses.

Tris (2-chloroethyl) phosphate (TCEP) and fifteen OPE metabolites were measured, nine compounds were excluded because their detection rates were lower than 20%, finally seven compounds included namely, TCEP, bis (2-butoxyethyl) phosphate (BBOEP), diphenyl phosphate (DPHP), 4-hydroxyphenyl diphenyl phosphate (4-HO-DPHP), bis (1,3-dichloro-2-propyl) phosphate (BDCIPP), 1-hydroxy-2-propyl bis(1-chloro-2-propyl) phosphate (BCIPHIPP), di-ortho-cresyl phosphate (DoCP) and di-para-cresyl phosphate (DpCP) (DoCP & DpCP). The pretreatment of urinary OPEs has been described previously (Hu et al. 2019). In brief, urine was spiked with mixed internal standards, sodium acetate buffer, and β-glucuronidase, then incubated samples overnight at 37 °C. Acetonitrile was added to the mixture; the mixture was vortex-mixed, then centrifuged. After, the mixture was added with methyl tert-butyl ether and vortex oscillation and centrifugation again. Next, removed the organic phase supernatant and concentrated to near dryness and reconstituted with 100 μL of MeOH: H2O (10:90, v: v). Finally, the extract was filtered through a 0.22-μm membrane filter. Target analytes were analyzed by the LC-30A UPLC system coupled to a triple-quadrupole LCMS-8050 (Shimadzu, Japan) in the ESI negative mode. The MS/MS parameters were the same as the previous study (Van den Eede et al. 2015). The recovery for seven compounds in this study was within the range of 72−118%, and the intra-day and inter-day coefficient of variations (CV) were mostly below 20% (Hu et al. 2019). The limits of detections (LODs) for seven compounds ranged from 0.032 to 0.097 ng/ml.

The urinary concentrations of OPE metabolites were standardized with the urinary specific gravity (SG), according to the formula: Pc=P[(SGm−1)/(SG−1)](Duty et al. 2005). SGm is the median of urinary SG of each trimester, and SG is the SG of each urine sample. Concentrations of SG were measured by a hand-held digital refractometer (Atago Co., Ltd., Tokyo, Japan).

*Covariates*

Information on parity, infant sex, pregnancy complications (hypertensive disorders in pregnancy and gestational diabetes mellitus), and birth outcomes were retrieved from medical records. Information on maternal demographic and socioeconomic characteristics, lifestyle factors of mothers and children were obtained in face-to-face interviews using standardized and structured questionnaires. Maternal pre-pregnancy body mass index (BMI) was calculated by pre-pregnancy body weight and height. Self-reported pre-pregnancy body weight was extracted from records of the first prenatal visit, and maternal height was measured by a stadiometer at first prenatal visit. Season at birth was treated as categorical variables [spring (March-May), summer (June-August), fall (September-November), and winter (December-February)].

*Statistical analysis*

Descriptive statistics were provided for subject demographics and maternal concentrations of OPEs. When the concentrations of OPE metabolites below the LOD, a value of LOD/√2 was employed. We calculated the total concentration of OPE metabolites (ΣOPEs) by summing the molar concentrations of the seven metabolites (Liu et al. 2020), and the total concentrations of aromatic OPE metabolites (ΣAr-OPEs: 4-HO-DPHP, DPHP, and DoCP & DpCP) and chlorinated OPE metabolites (ΣCl-OPEs: BDCPP, BCIPHIPP, and TCEP) were calculated in the same way. Due to the right-skewed distributions of OPE metabolites, all concentrations were log2 transformed to obtain normal distributions. We calculated intraclass correlation coefficients (ICCs) using random one-way intercept mixed linear models to estimate between and within-subject variability of log2 transformed concentrations of OPE metabolites over the course of pregnancy. The value of ICC, ranging from 0 to 1, means a good reproducibility when it closes to 1 (Rosner 2000). We excluded TCEP for further analyses because its detection rate was low in samples (< 60%).

Concentrations of OPE metabolites had poor reproducibility throughout the whole pregnancy, which ICCs of all OPE metabolites were < 0.40 (Table SSS). Therefore, to better approximate OPE exposure during pregnancy, we used the average concentrations of OPE metabolites to further analyses.

In order to evaluate the relationships of OPE exposure during pregnancy with growth in early childhood, firstly, we analyzed the growth trajectories of weight and length z-scores using group-based trajectory modeling (GBTM). GBTM is a specialised application of finite mixture modeling and involves a procedure which gathers individuals into meaningful subgroups that show statistically similar trajectories. According to the guide, we first identified the optimal number of trajectory groups (from one to four) by choosing the largest Bayesian information criteria (BIC) score. We found three groups is the Then,

Specifically, we fit models with different numbers (from one to four) of trajectory groups   
using a quadratic form for all trajectories and then generated corresponding BICs. The quadratic function used in this step was to  
help us determine the optimal number of groups and did not represent the final shape of trajectories in this study. The higher BIC  
indicates better model fit, and a difference lower than 2 means  
not enough evidence against the null model and was only used to  
determine the optimal group number. Then, we determined the  
trajectory shapes that best describe the observed trajectories.  
Linear, quadratic, cubic, and quartic functions were all tried.  
Linear and quadratic functions were not enough to capture the  
variations in trajectories. Because we had only five measurements, the quartic function could always perfectly fit the  
observed data, no matter how unrealistic the predictive trajectory  
was, which led to concerns of overfitting. Therefore, as a compromise, the cubic function was finally selected to describe the trajectory shape. After obtaining the optimal number of trajectory  
groups and the proper trajectory shape, we visually presented the  
trajectories for each measure and selected the group that had a  
stable trend near the null z-score as the reference group

We chose two methods to estimate the effect of OPE exposure on growth trajectories. Firstly, we used the mixed model to evaluate associations of log2 transformed concentrations of OPE (log2-OPEs, as a continuous variable) with repeated outcome anthropometric measurements (z-scores of height and weight) measured in early childhood. The estimated β-coefficients were interpreted as a percent change in the averaged weight and height z-scores from birth to 2 years for each doubling increase in concentrations of OPE metabolites. Then, in order to investigate that whether exposure to OPEs affects the shapes of growth trajectories, we classified OPE concentrations into three groups by tertiles (the lowest tertile set as reference) and plotted average weight and height z-scores trajectories by OPE exposure using predicted means at each time point (the LSMEANS function in mixed model), and the difference in predicted means was tested using analysis of variance models specifying a Bonferroni correction for multiple comparison. The mixed model allowed random intercepts and took into account the intrasubject correlation as well as unequal timing of anthropometric measurements (Mervish et al. 2016).

Linear regression models were used to estimate the associations between average concentrations of log2 transformed SG-corrected OPE metabolites and z-scores of children’s weight and height in each age. We used the mixed linear model to estimate associations between average concentrations of maternal urinary OPE metabolites and longitudinal data for weight and height z-scores. We also evaluated an interaction term for concentrations of OPEs and children’s age (birth, one year, and two years old) to assess differences in association over time.

Mixed‐effect polynomial regression models (also known as multi‐level growth curve models) with both fixed and random effects were used to construct the longitudinal growth trajectories of BAZ and HAZ from 3 to 42 months. This method has been widely used to elucidate longitudinal child growth trajectories,19 allowing modelling of nonlinear growth trajectories and assessment of determinants. It mitigates within‐subject correlations and unequal variances over time through use of the covariance structure. Furthermore, it permits modelling of growth using unbalanced longitudinal data and does not exclude participants with missing measurements. Inclusion of random intercepts and slopes allows individual variations in growth trajectory.

All the statistical models were adjusted for potential confounders, including maternal age at recruitment (< 25, 25-29, ≥ 30), maternal pre-pregnancy BMI (categorized using the Chinese standard: < 18.5, 18.5-23.9, ≥ 24.0 kg/m2), education (less than high school, high school or equivalent, college and above), infant sex (boys, girls), and season at birth (spring, summer, fall, and winter). Breastfeeding duration (< 6months, 6-12month, and ≥12months) were additionally included in the model when analyzing associations of OPE exposure with childhood growth at the age of one and two.

A stratified analysis by infant sex was conducted because previous studies reported that OPEs affected the birth outcomes by sex-specific (Hoffman et al. 2018; Luo et al. 2020).Given that preterm children may follow different catch-up growth trajectories (Euser et al. 2008), we conducted a sensitivity analysis by restricting to full-term children (7 infants were preterm birth). Moreover, to investigated whether the critical window of heightened susceptibility of OPE exposure during pregnancy, the trimester-specific associations between concentrations of maternal urinary OPE metabolites and longitudinal data for weight and height z-scores were also estimated by using the mixed linear model.

All statistical analyses were performed using SAS (version 9.4 SAS Institute, Inc., Cary, NC, USA). The statistical significance level was 0.05 for a two-tailed test.

Table 1. The characteristics of study population.

|  |  |
| --- | --- |
| **Characteristics** | **N (%) or mean ± SD** |
| **Maternal characteristics** |  |
| Maternal age (years) | 28.6±3.0 |
| < 25 | 19 (9.0) |
| 26-29 | 118 (55.7) |
| 30-34 | 69 (32.6) |
| ≥ 35 | 6 (2.8) |
| Pre-pregnancy BMI (kg/m2) |  |
| Underweight (< 18.5) | 38 (17.9) |
| Normal (18.5-23.9) | 146 (68.9) |
| Overweight (≥ 24) | 28 (13.2) |
| Parity |  |
| Primiparous | 184 (86.8) |
| Multiparous | 28 (13.2) |
| Maternal education |  |
| Less than high school | 7 (3.3) |
| High school | 33 (15.6) |
| More than high school | 172 (81.1) |
| Hypertension during pregnancy | 4 (1.9) |
| Gestational diabetes mellitus | 15 (7.1) |
| Passive smoking | 74 (34.9) |
| Smoking during pregnancy (Yes) | 0 (0.0) |
| Drinking during pregnancy (Yes) | 0 (0.0) |
| **Children's characteristics** |  |
| Sex |  |
| Female | 98 (46.2) |
| Male | 114 (53.8) |
| Gestational age (weeks) | 39.29±1.28 |
| Weight (kg) |  |
| At birth | 3.31±0.43 |
| 1 year | 10.16±1.14 |
| 2 years | 12.71±1.40 |
| Height or length (cm) |  |
| At birth | 50.26±1.66 |
| 1 year | 76.02±2.39 |
| 2 years | 88.54±3.06 |
| Breastfeeding duration |  |
| < 6 months | 42 (19.8) |
| 6-12 months | 81 (38.2) |
| ≥ 12 months | 82 (38.7) |
| Missing | 7 (3.3) |

Note: BMI, body mass index; SD, standard deviation.

Table 2. The distribution of the OPE metabolites and its reproducibility in pregnancy.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **OPEs (ng/ml or nmol/L)** | **Detection rate (%)** |  | **GM** |  |  |  | **Percentiles** |  |  | **ICC (95% CI) a** |
|  |  | 5th | 25th | 50th | 75th | 95th |
| **Uncorrected** |  |  |  |  |  |  |  |  |  |  |
| BDCIPP | 68.87-70.76 |  | 0.09 |  | < LOD | < LOD | 0.08 | 0.14 | 0.33 | 0.16 (0.09, 0.26) |
| BCIPHIPP | 75.94-77.83 |  | 0.16 |  | < LOD | 0.10 | 0.15 | 0.24 | 0.74 | 0.18 (0.11, 0.28) |
| TCEP | 43.87-50.94 |  | 0.12 |  | < LOD | < LOD | < LOD | 0.19 | 0.57 | 0.40 (0.32, 0.49) |
| DoCP & DpCP | 97.64-98.11 |  | 0.31 |  | 0.04 | 0.12 | 0.30 | 0.79 | 2.35 | 0.26 (0.18, 0.36) |
| DPHP | 98.59-99.10 |  | 0.27 |  | 0.06 | 0.13 | 0.23 | 0.46 | 2.21 | 0.34 (0.26, 0.43) |
| 4-HO-DPHP | 66.51-77.36 |  | 0.09 |  | < LOD | < LOD | 0.07 | 0.16 | 0.58 | 0.23 (0.15, 0.33) |
| BBOEP | 90.57-91.51 |  | 0.11 |  | < LOD | 0.03 | 0.10 | 0.41 | 1.25 | 0.27 (0.19, 0.36) |
| ∑Cl-OPEs (nmol/L) |  |  | 1.47 |  | 0.59 | 0.92 | 1.31 | 2.06 | 4.63 | 0.21 (0.14, 0.31) |
| ∑Ar-OPEs (nmol/L) |  |  | 3.32 |  | 0.71 | 1.56 | 3.22 | 6.19 | 19.35 | 0.27 (0.19, 0.37) |
| ∑OPEs (nmol/L) |  |  | 6.21 |  | 1.69 | 3.50 | 5.98 | 9.81 | 26.26 | 0.24 (0.17, 0.34) |
| **SG-corrected** |  |  |  |  |  |  |  |  |  |  |
| BDCIPP | 68.87-70.76 |  | 0.11 |  | < LOD | < LOD | 0.10 | 0.16 | 0.50 | 0.22 (0.14, 0.32) |
| BCIPHIPP | 75.94-77.83 |  | 0.19 |  | < LOD | 0.11 | 0.17 | 0.28 | 0.77 | 0.16 (0.09, 0.27) |
| TCEP | 43.87-50.94 |  | 0.14 |  | < LOD | < LOD | < LOD | 0.24 | 0.94 | 0.36 (0.28, 0.45) |
| DoCP & DpCP | 97.64-98.11 |  | 0.35 |  | 0.08 | 0.19 | 0.33 | 0.65 | 1.77 | 0.29 (0.21, 0.38) |
| DPHP | 98.59-99.10 |  | 0.31 |  | 0.07 | 0.14 | 0.25 | 0.50 | 3.14 | 0.35 (0.27, 0.44) |
| 4-HO-DPHP | 66.51-77.36 |  | 0.10 |  | < LOD | 0.05 | 0.09 | 0.20 | 0.62 | 0.23 (0.16, 0.33) |
| BBOEP | 90.57-91.51 |  | 0.13 |  | < LOD | 0.04 | 0.14 | 0.41 | 1.32 | 0.31 (0.23, 0.40) |
| ∑Cl-OPEs (nmol/L) |  |  | 1.70 |  | 0.64 | 1.03 | 1.51 | 2.47 | 6.43 | 0.23 (0.15, 0.33) |
| ∑Ar-OPEs (nmol/L) |  |  | 3.83 |  | 1.06 | 2.11 | 3.41 | 6.02 | 19.29 | 0.29 (0.21, 0.38) |
| ∑OPEs (nmol/L) |  |  | 7.17 |  | 2.59 | 4.31 | 6.41 | 10.33 | 26.90 | 0.25 (0.17, 0.35) |

Note: GM, geometric mean; ICC, intraclass correlation coefficient, SG, specific gravity.

a: The point estimates and 95% CIs for ICC were calculated by using each log2-transformed concentrations of urinary analytes to examine temporal variability in the 1st, 2nd and 3rd trimesters.

Duty SM, Ackerman RM, Calafat AM, Russ H. 2005. Personal care product use predicts urinary concentrations of some phthalate monoesters. Environmental health perspectives 113:1530-1535.10.1289/ehp.8083

Euser A, De Wit C, Finken M, Rijken M, Wit J. 2008. Growth of preterm born children. Hormone Research in Paediatrics 70:319-328.doi.org/10.1159/000161862

Hoffman K, Stapleton HM, Lorenzo A, Butt CM, Adair L, Herring AH, et al. 2018. Prenatal exposure to organophosphates and associations with birthweight and gestational length. Environment international 116:248-254.10.1016/j.envint.2018.04.016

Hu L, Tao Y, Luo D, Feng J, Wang L, Yu M, et al. 2019. Simultaneous biomonitoring of 15 organophosphate flame retardants metabolites in urine samples by solvent induced phase transition extraction coupled with ultra-performance liquid chromatography-tandem mass spectrometry. Chemosphere 233:724-732.10.1016/j.chemosphere.2019.05.242

Liu W, Luo D, Xia W, Tao Y, Wang L, Yu M, et al. 2020. Prenatal exposure to halogenated, aryl, and alkyl organophosphate esters and child neurodevelopment at two years of age. J Hazard Mater 408:124856.10.1016/j.jhazmat.2020.124856

Luo D, Liu W, Tao Y, Wang L, Yu M, Hu L, et al. 2020. Prenatal exposure to organophosphate flame retardants and the risk of low birth weight: A nested case-control study in china. Environmental science & technology 54:3375-3385.<https://doi.org/10.1021/acs.est.9b06026>

Mervish NA, Pajak A, Teitelbaum SL, Pinney SM, Windham GC, Kushi LH, et al. 2016. Thyroid antagonists (perchlorate, thiocyanate, and nitrate) and childhood growth in a longitudinal study of u.S. Girls. Environ Health Perspect 124:542-549.10.1289/ehp.1409309

Organization WH. 2006. Who child growth standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and development.

Rosner B. 2000. Fundamentals of biostatistics. . PacificGrove,:CA:Duxbury.

Van den Eede N, Heffernan AL, Aylward LL, Hobson P, Neels H, Mueller JF, et al. 2015. Age as a determinant of phosphate flame retardant exposure of the australian population and identification of novel urinary pfr metabolites. Environment international 74:1-8.10.1016/j.envint.2014.09.005