Supplemental Materials

Prenatal Exposure to Organophosphate Esters and Growth Trajectory in Early Childhood

Context

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: $P_c=P[(SG_m-1)/(SG-1)]$(Duty et al. 2005). SG_m 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.

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 is close 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. Therefore, to better approximate OPE exposure during pregnancy, we used the average concentrations of OPE metabolites to further analyses.

To evaluate the relationships of OPE exposure during pregnancy with growth in early childhood, we analyzed the growth trajectories of weight and length z-scores using group-based trajectory modeling (GBTM). GBTM is a specialized application of finite mixture modeling and involves a procedure that gathers individuals into meaningful subgroups that show statistically similar trajectories. According to the guide (Jones et al. 2001; Nagin 2005), first, we identified the optimal number of trajectory groups (from one to four) by choosing the largest Bayesian information criteria (BIC) score and appropriate 2ΔBIC (>2 means positive evidence against the null model). Meanwhile, trajectory size was also greater than or equal to 5% of the sample. Next, we tried linear, quadratic, and cubic functions to determine the trajectory shapes. An appropriate smoothness was selected using the BIC, and posterior probabilities were used to determine which trajectory a child was most likely to be a member of. The overall uncertainty of trajectory membership was assessed using entropy (defined from 0 to 1), where values approaching zero indicate an equal posterior probability of membership among trajectories and values approaching one indicate a strong posterior probability of belonging to

a single trajectory. Last, we identified three trajectory groups with cubic functions (order 3 3 3). Table S1 shows the BIC used for model selection and posterior probability for model goodness of fit.

After obtaining the proper trajectory groups, we investigate the association between OPEs and growth trajectories using logistic regression. We visually presented the trajectories for each measure and selected the group with a stable trend near the null z-score as the reference group. Odds ratios (ORs) and 95% confidence interval (95%CI) represented increases in odds of different growth trajectories per doubling in prenatal OPE concentrations in comparison with the reference group. Based on the directed acyclic graph (DAG), we select maternal sociodemographic, nutritional, prenatal, postnatal, and environmental factors as covariates in our analysis (Figure S1). All the statistical models were adjusted for maternal age, pre-pregnancy BMI, maternal education, and infant sex.

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.

Reference

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

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

Jones BL, Nagin DS, Roeder K. 2001. A sas procedure based on mixture models for estimating developmental trajectories. Sociological Methods & Research 29:374 - 393

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

Nagin DS. 2005. Group-based modeling of development.

Word Health Organization. 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. Environ Int 74:1-8.10.1016/j.envint.2014.09.005

Table S1. BIC and 2ΔBIC used for model selection and posterior probability for model goodness of fit

Number of groups	BIC (N=212)	Null model	2ΔBIC	Average posterior probability
Weight-for-age z-score (WAZ)				0.90
1	-1126.84			
2	-1040.28	1	173.12	
3	-1009.91	2	60.74	
4	-1012.33	3	-4.84	
Height-for-age z-score (WAZ)				0.90
1	-1091.53			
2	-1015.64	1	151.78	
3	-989.38	2	52.52	
4	-990.58	3	-2.4	

BIC = Bayesian information criterion

2ΔBIC: between the current model and the null model. A value lower than 2 means no enough evidence against the null model.

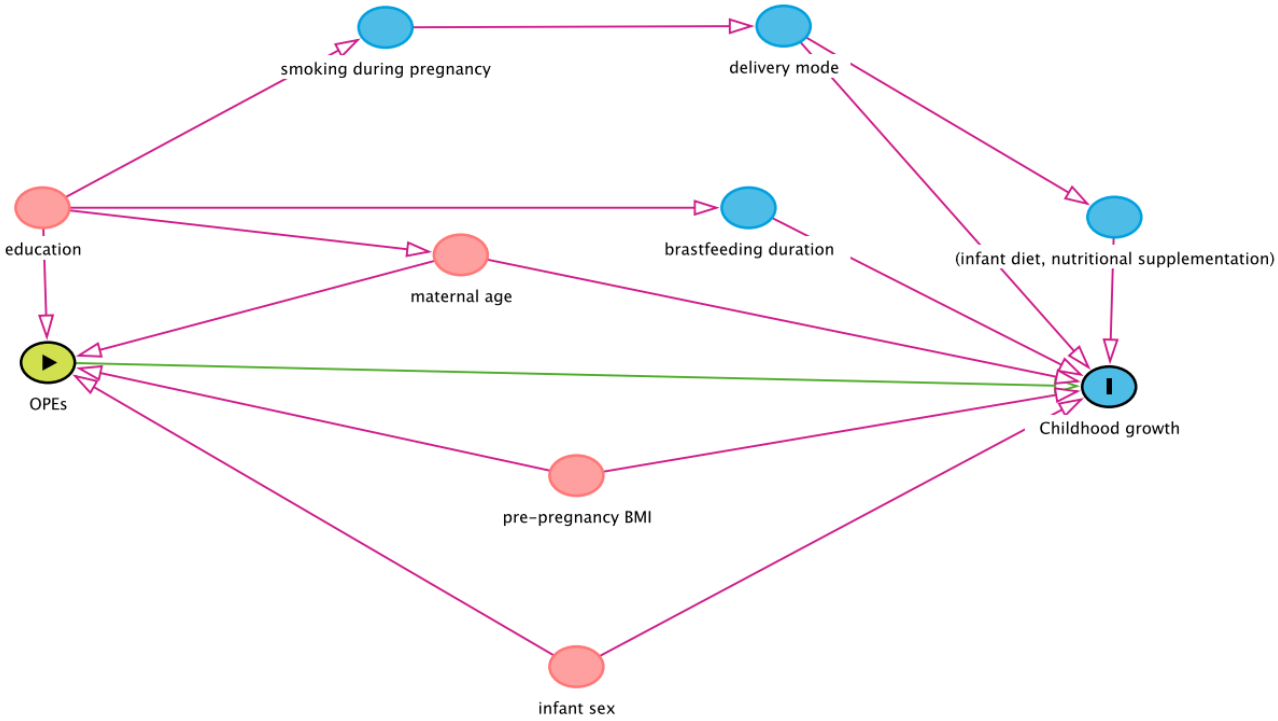

Figure S1. The directed acyclic graph for covariates considered in this study.
The variables with parenthesis indicate possible unmeasured/uncontrolled confounders that should be evaluated for their potential impacts in future studies. We collected other variables with bule or pink in this study. Variables with pink were included in the adjusted models.

Table S2. 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/m^2)	
Underweight (< 18.5)	38 (17.9)
Normal (18.5-23.9)	146 (68.9)
Overweight (≥ 24)	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)
Smoking during pregnancy (Yes)	0 (0.0)
Drinking during pregnancy (Yes)	0 (0.0)
Children's characteristics	
Gestational age (weeks)	39.29±1.28
Sex	
Female	98 (46.2)
Male	114 (53.8)
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 S3. 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.

Table S4. Odds ratios for associations of OPEs with children's weight growth trajectories.

	OR (95% CI)	
	Persistently LOW development vs. Persistently MODERATE development	Persistently HIGH development vs. Persistently MODERATE development
BDCIPP	0.95 (0.56, 1.59)	0.89 (0.60, 1.32)
BCIPHIPP	1.26 (0.74, 2.15)	0.91 (0.58, 1.43)
∑Cl-OPEs	1.03 (0.59, 1.79)	0.95 (0.61, 1.49)
DoCP & DpCP	1.57 (1.01, 2.43)	1.26 (0.91, 1.74)
DPHP	1.17 (0.87, 1.58)	0.89 (0.69, 1.15)
4-HO-DPHP	1.27 (0.88, 1.85)	1.08 (0.81, 1.46)
∑Ar-OPEs	1.68 (1.10, 2.56)	1.06 (0.76, 1.47)
BBOEP	1.49 (1.12, 1.98)	1.19 (0.98, 1.45)
∑OPEs	1.84 (1.10, 3.08)	1.12 (0.74, 1.68)

Models were adjusted for maternal age, pre-pregnancy BMI, maternal education, and infant sex.

Table S5. Odds ratios for associations of OPEs with children's height growth trajectories.

	OR (95% CI)	
	Persistently LOW development vs. Persistently MODERATE development	Persistently HIGH development vs. Persistently MODERATE development
BDCIPP	0.70 (0.39, 1.26)	0.72 (0.47, 1.10)
BCIPHIPP	1.70 (0.98, 2.94)	0.69 (0.43, 1.10)
∑Cl-OPEs	1.14 (0.64, 2.06)	0.68 (0.42, 1.09)
DoCP & DpCP	0.86 (0.53, 1.39)	0.99 (0.72, 1.38)
DPHP	1.08 (0.76, 1.53)	0.75 (0.57, 0.99)
4-HO-DPHP	1.09 (0.73, 1.62)	0.98 (0.72, 1.32)
∑Ar-OPEs	0.99 (0.62, 1.60)	0.75 (0.53, 1.07)
BBOEP	1.01 (0.76, 1.35)	0.90 (0.73, 1.10)
∑OPEs	0.94 (0.53, 1.68)	0.60 (0.38, 0.95)

Models were adjusted for maternal age, pre-pregnancy BMI, maternal education, and infant sex.