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**Trimester-Specific Effects of Maternal Exposure to Organophosphate Flame Retardants
on Offspring Size at Birth: A Prospective Cohort Study in China**

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ABSTRACT: Organophosphate flame retardants (OPFRs) are substantially applied as flame retardants and plasticizers in consumer products. Although the embryonic developmental toxicity of OPFRs has been reported, human data are limited and the critical windows of susceptibility to OPFRs exposure need to be identified. Here, we have investigated the trimester-specific associations between OPFRs exposure during pregnancy and birth size in a prospective cohort from Wuhan, China. The concentrations of 15 OPFR metabolites and tris(2-chloroethyl) phosphate were repeatedly determined in urine samples collected from 213 pregnant women in the first, second, and third trimesters, and anthropometric data were retrieved from medical records. In adjusted multiple informant models, urinary concentrations of bis(1,3-dichloro-2-propyl) phosphate (BDCIPP) and bis(2-butoxyethyl) phosphate (BBOEP) in the third trimester, 4-hydroxyphenyl-diphenyl phosphate (4-HO-DPHP) in the second trimester, and diphenyl phosphate (DPHP) in the first trimester were significantly negatively associated with birth weight, among which a significant difference in exposure-effect relationships across the three trimesters was observed for BDCIPP. BBOEP concentrations in the third trimester were negatively correlated to birth length with significant varying exposure effects. Our results suggest that prenatal exposure to certain OPFRs may impair fetal growth, and the fetus is vulnerable to the developmental toxicity of BDCIPP and BBOEP in the third trimester.

TOC/Abstract Art

INTRODUCTION

With the progressive phasing out of brominated flame retardants (BFRs) from the global flame retardants market, organophosphate flame retardants (OPFRs) are being substantially produced and applied as the most suitable substitute for BFRs.^{1, 2} OPFRs, used as flame retardants, plasticizers, and other additive agents, have been extensively applied in numerous industrial applications and commercial products, such as furniture, textiles, mattresses, electronics, vehicles, building materials, petroleum, hydraulic fluids, and lubricants.³ As high-production-volume chemicals, OPFRs were reported to have a worldwide annual consumption of 200,000 tons in 2004 and 500,000 tons in 2011, and it was evaluated to more than 680,000 tons in 2015, indicating an increasing trend for the usage of OPFRs over time.⁴ OPFRs are frequently added to consumer products by physical addition rather than chemical bonding with polymers, implying they easily escape and are leak into the peripheral environment.⁴ Hence, there has been a growing concern regarding their environmental occurrence in recent years. A large number of literatures has well documented that OPFRs could be found in various environmental media, such as dust, air, soil, water, sediment, biota samples, and foodstuffs.⁵⁻¹⁰

It is reported that the ubiquitous OPFRs in the environment lead to human being exposed via inhalation, ingestion, and skin contact.^{11, 12} Unlike typical persistent organic pollutants, as most OPFRs have short half-lives, they are rapidly metabolized by the liver as hydrophilic or oxidized metabolites after being ingested and are then excreted through urine.¹ Recently, numerous studies from different countries or regions have revealed the widespread exposure of different populations, such as the general population, pregnant women, and children, to OPFRs.¹³⁻¹⁵

As the organs and tissues of the fetus are not yet fully developed, the fetus is a highly sensitive and vulnerable population affected by environmental pollutants.¹⁶ Exposure to toxic environmental pollutants may cause immense damage to fetal development.¹⁷ OPFRs have been proven to have the ability to pass through the placental barrier into the fetal environment, and thus may harm fetal health.¹⁸⁻²⁰ Currently, an increasing body of toxicological evidence suggests the reproductive and developmental toxicity of OPFRs in different organisms. After

85 exposure to triphenyl phosphate (TPHP) and tris(1,3-dichloro-2-propyl) phosphate (TDCIPP)
86 at the environmental concentrations, the fecundity of *Daphnia magna* was suppressed, and the
87 body length of the parental generation and offspring were evidently reduced.^{21, 22} In zebrafish
88 models, TDCIPP exposure led to the inhibition of offspring development, including reduced
89 survival, body length, and heart rate, in which the downregulation of genes in the growth
90 hormone/insulin-like growth factor (GH/IGF) axis and the imbalance of the hypothalamic-
91 pituitary-thyroid (HPT) axis might partially explain this phenomenon.^{23, 24} In addition, tris(2-
92 butoxyethyl) phosphate (TBOEP) exposure of zebrafish has also been linked with a decrease
93 in weight and length.²⁵

94 Although the developmental toxicity induced by OPFRs exposure has been well-studied
95 in animal models, the available epidemiological evidence is limited with inconsistent findings.
96 Furthermore, the susceptibility of the fetus to environmental exposure varies greatly during
97 different gestational periods, which may cause varying degrees of damage to fetal health.
98 Hence, the critical windows of fetal susceptibility, being interpreted as certain special periods
99 of our lives in which exposure may cause more serious health hazards, urgently need to be
100 identified.²⁶ There are four epidemiological studies from the USA and China investigating the
101 relationship of prenatal OPFRs exposure with birth outcomes.²⁷⁻³⁰ Hoffman et al. identified an
102 inverse trend between urinary isopropyl-phenyl phenyl phosphate (ip-PPP) levels and birth
103 weight among female infants.²⁷ Our previous work found that maternal urinary diphenyl
104 phosphate (DPHP) levels were positively associated with the risk of low birth weight.²⁸
105 However, Feng et al. and Kuiper et al. found non-significant associations of OPFRs exposure
106 during pregnancy with birth weight and length.^{29, 30} To our knowledge, there is no study
107 exploring the trimester-specific associations of prenatal OPFRs exposure with birth size.

108 In a prospective study from China, we investigated the profile of OPFRs exposure
109 throughout pregnancy in 213 pregnant women with repeated urine samples collected at
110 different stages of pregnancy, estimated the trimester-specific effects of maternal exposure to
111 OPFRs on offspring size at birth, and identified the critical windows of heightened
112 susceptibility to OPFRs exposure.

MATERIALS AND METHODS

Study Population

The pregnant women in this research were enrolled from the ongoing birth cohort project conducted in the Wuhan Maternal and Child Healthcare Hospital that is focusing on the effects of environmental exposure on maternal and infant health. Pregnant women with gestational age <16 weeks were recruited from 2014 to 2016 at their first prenatal visit to the designated hospital, according to the following criteria: a) resided in Wuhan city with the ability to comprehend the Chinese language; b) had a singleton gestation; c) was willing to provide their biological samples during pregnancy tests; and d) planned to deliver at the designated hospital. Considering the availability of urine samples and the measurement costs, we only chose 213 participants who had spot urine collected in the first (12.9 ± 0.9 weeks), second (23.9 ± 2.9 weeks), and third trimesters (34.4 ± 3.2 weeks) during pregnancy to determine OPFR metabolite concentrations.

All pregnant women included in our study understood this project prior to signing informed consent forms. The research protocol was authorized by the ethics committee of Tongji Medical College, Huazhong University of Science and Technology, and the Wuhan Maternal and Child Healthcare Hospital.

Assessment of Offspring Size at Birth and Covariates

Anthropometric measurements of the newborns, including birth weight (in grams) and birth length (in centimeters), were retrieved from the electronic medical records of the study hospital. According to the standardized procedures of anthropometric measurements, experienced obstetric nurses measured the birth weight and length of newborns at the time of delivery. The information regarding gestational age at birth, sex of the newborn, parity, delivery mode, mother's weight at delivery, and medical history were retrieved from medical records. All pregnant women were encouraged to complete the standardized face-to-face interview conducted by well-trained nurses to collect information, such as demographic characteristics (age, weight, height, etc.), socioeconomic status (education, occupation, annual household

income, etc.), and lifestyle habits during pregnancy (smoking, alcohol use, passive smoking, etc.). Maternal prepregnancy body mass index (BMI) was calculated based on self-reported weight and height. Pregnancy weight gain was assigned to the difference between prepregnancy weight and weight at delivery. Gestational age was assessed based on the date of the last menstrual period (LMP) or ultrasound examination, in which the ultrasound-based data were adopted when the LMP of participants was unclear or the difference between the self-reported LMP and ultrasound examination was over 7 days. Live birth with birth weight less than 2,500 g was defined as low birth weight.

Urine Collection and Analysis of OPFR Metabolites

Spot urine was obtained when participants came to the study hospital for antenatal examination in different trimesters. These samples were separated into aliquots and stored in 5-mL polyethylene containers at -20°C before further use. Fifteen OPFR metabolites and tris(2-chloroethyl) phosphate (TCEP) in urine were measured in the present study; however, here we only list the detectable metabolites and TCEP: bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), 1-hydroxy-2-propyl bis(1-chloro-2-propyl) phosphate (BCIPHIPP), TCEP, dibutyl phosphate (DBP), 4-hydroxyphenyl-diphenyl phosphate (4-HO-DPHP), DPHP, di-o-cresyl phosphate (DoCP), di-p-cresyl phosphate (DpCP), and bis(2-butoxyethyl) phosphate (BBOEP).

The solvent-induced phase transition extraction linked with ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) was used to determine urinary OPFR metabolite levels as reported in our previous study.^{28, 31} In short, urine was first spiked with mixed internal standards, sodium acetate buffer, and β -glucuronidase; the mixture was then digested at 37°C for 12 h. Acetonitrile was used to extract target compounds from the mixture and methyl tert-butyl ether was subsequently added to successively separate the organic phase and water phase. The supernatants were collected, evaporated, and reconstituted for further instrumental analysis. The levels of OPFR metabolites were analyzed using a UPLC-MS/MS system (Shimadzu, Kyoto, Japan) equipped with Agilent Zorbax Eclipse Plus C18 column (2.1×50 mm, $1.8 \mu\text{m}$).

The identification and quantification of OPFR metabolites were completed using an

isotope-labeled internal standard method. To improve the acceptability of data, two quality control samples (spiked with 2 ng/mL and 20 ng/mL), three procedural blanks, and one reagent blank were run within a batch of 30 real samples. Urinary levels of OPFR metabolites were corrected by subtracting the average value of procedural blanks. The method limits of detection (LOD) of target analytes were obtained by calculating three times the standard deviation of blank samples spiked with the lowest concentration of calibration curve, and ranged from 0.012 to 0.25 ng/mL. The recovery tests exhibited an acceptable accuracy for OPFR metabolites within the range of 72–118%. As the separate quantification of the two metabolites of tricresyl phosphate (TCrP) was difficult, the total concentration of DoCP and DpCP was presented as sum of DoCP & DpCP. Moreover, the specific gravity (SG) of the urine sample was determined using a hand-held refractometer to correct for urine dilution. The formula is as follows: $P_c = P \times [(SG_m - 1)/(SG - 1)]$, where P_c is the SG-adjusted concentration (ng/mL), P represents the measured concentration (ng/mL), and SG_m represents the median SG of all urine samples.

Statistical Analysis

Descriptive statistics for demographic characteristics and distribution profiles of SG-adjusted urinary OPFR metabolite concentrations of the participants were firstly summarized. The non-detectable target analytes ($<LOD$) were replaced by $LOD/\sqrt{2}$. The total concentrations of OPFR metabolites ($\Sigma mOPFRs$) were equal to the molar sum of the eight detectable target analytes. Due to the right-skewed distributions of urinary OPFR metabolite concentrations as verified by the Shapiro-Wilk normality test, natural logarithm ($\ln$) transformations of OPFR metabolite concentrations were performed to better achieve normal distributions. Only the predominant OPFR metabolites detected in $>60\%$ of urine samples were considered for main statistical analysis.

Pearson correlation coefficients (r_s) for correlations among frequently detected OPFR metabolites were computed in different trimesters. We also calculated intraclass correlation coefficients (ICCs) using a linear mixed effects model to assess the temporal variability of unadjusted and SG-adjusted OPFR metabolite levels throughout the whole pregnancy. The ICCs used as indicators of reproducibility of exposure biomarkers over time were defined as

the ratio of inter-subject variation to the total variation with the range of 0 (poor reproducibility) to 1 (perfect reproducibility).

As urinary OPFR metabolites were repeatedly measured during the pregnancy, we utilized a multiple informant method to jointly evaluate the trimester-specific relationships between OPFR metabolite levels in urine and offspring size at birth. In the multiple informant model, the exposure windows (different trimesters) were treated as informants and the effect estimation of individual OPFR exposure in different trimesters related with offspring size could be performed using generalized estimating equations with a linear function. The multiple informant model allowed us to reveal the critical windows of susceptibility to environmental toxicants throughout pregnancy. Simultaneously, these models could construct a score type test to analyze differences in relationships between OPFR metabolite concentrations in each trimester and offspring size at birth, with the null hypothesis that effect estimates were equal across all three trimesters. In addition to the method mentioned above, we also analyzed the association between tertiles of OPFR metabolite concentrations and offspring size, with the first tertile as the reference. We performed trend tests by assigning the median of each tertile of OPFR metabolites in the model to clarify the potential linear relationships.

Adjustments of confounders were performed in the multiple informant models based on biologic plausibility and statistical considerations. The change-in-effect estimate method was adopted, in which some covariates changed the main effect estimates by 10% and more were introduced into these models.³² The following potential confounders were included in the final linear regression model: maternal age (years), education level (less than high school/high school or equivalent/college or above), prepregnancy BMI ($<18.5/18.5-23.9/\geq 24.0$ kg/m²), passive smoking during pregnancy (yes/no), delivery mode (vaginal/cesarean), parity (nulliparous/multiparous), pregnancy weight gain (kg), gestational age (weeks), and newborn sex (male/female). Maternal active smoking and alcohol were not adjusted in the final model because no pregnant women reported smoking or alcohol use.

In consideration of the estrogenic-like activity of some OPFRs,³³ we assessed the potential modification effect by newborn sex. The stratified analysis was conducted by stratifying the

models by newborn sex and additionally inserting an interaction term between OPFR metabolites, trimester, and newborn sex into the full regression models. Furthermore, to test the robustness of these findings, we implemented several sensitivity analyses by additionally adjusting for hypertensive disorders of pregnancy and gestational diabetes mellitus in the multivariate models, and excluding newborns with gestational age at birth less than 37 weeks.

All data analyses were executed using SAS version 9.4 (SAS Institute, Inc., Cary, NC). The two-tailed p-value was set to be statistically significant at 0.05.

RESULTS

Characteristics of the Mother-Newborn Pairs

Baseline characteristics of the participants enrolled in this research are summarized in Table 1. The maternal age was 28.6 ± 3.0 years old on average at enrollment. Of the 213 mothers, 68.5% of them had a prepregnancy BMI within the normal range, and the average weight gain during pregnancy was 16.3 ± 4.6 kg. The majority of mothers were highly educated (college or above level, 81.2%) and nearly half of mothers had an annual household income of >100,000 Yuan (45.5%). Most mothers were employed during pregnancy (65.3%) and were nulliparous (86.9%), and approximately 52.1% women gave birth by cesarean section. In total, 34.7% of mothers were exposed to second-hand smoke during pregnancy. Moreover, 1.9% and 7.0% of mothers had hypertensive disorders of pregnancy and gestational diabetes mellitus, respectively. Among the newborns, boys and girls accounted for 54.0% and 46.0%, respectively. The average gestational age, birth weight, and birth length of newborns were 39.3 ± 1.3 weeks, 3315.4 ± 434.5 g, and 50.3 ± 1.7 cm, respectively.

Distributions and Variability of Urinary OPFR Metabolite Concentrations

The detection frequency and distribution profile of SG-adjusted urinary OPFR metabolite concentrations in urine of our study population are given in Table S1 and Figure 1, except for seven non-detectable targeted analytes. Among these targeted analytes, BDCIPP, BCIPHPP, 4-HO-DPHP, DPHP, DoCP & DpCP, and BBOEP were frequently detected in 69.8–98.8% of all urine samples, indicating that these analytes were the predominant OPFR metabolites in

Chinese pregnant women. Of the six analytes, the geometric mean concentrations of DoCP & DpCP ranked in the top (0.34 ng/mL) throughout the whole pregnancy (639 urine samples), followed by DPHP (0.30 ng/mL), BCIPHIPP (0.19 ng/mL), BBOEP (0.13 ng/mL), 4-HO-DPHP (0.10 ng/mL), and BDCIPP (0.10 ng/mL). At the same time, the detection frequency and distribution concentrations of most OPFR metabolites showed similarities across the three trimesters.

For six major OPFR metabolites, correlations were assessed using the Pearson correlation test (Table S2). There were positive and weak correlations between BDCIPP, BCIPHIPP, 4-HO-DPHP, DPHP, DoCP & DpCP, and BBOEP, with r_s of 0.15–0.47 in different trimesters. Furthermore, we estimated the temporal variability of urinary OPFR metabolite concentrations in urine across the three trimesters. The ICC values for SG-adjusted target compounds were relatively low, with the range of 0.17–0.36, which indicated poor reproducibility across the whole pregnancy (Table S3). The unadjusted results of ICC were similar to the SG-adjusted values mentioned above.

Maternal Exposure to OPFRs and Birth Size

The associations of trimester-specific maternal exposure to OPFRs with birth weight and birth length are shown in Table 2 and Table 3, respectively, and some statistically significant results were observed in different trimesters. After adjusting for the main confounders, each ln-transformed unit increase in third trimester BDCIPP concentrations was significantly related to a 85.12-g reduction in birth weight (95% CI: –148.98, –21.27), with a significant difference in exposure effects across the three trimesters ($p_{\text{tri-int}} = 0.04$). After stratification by newborn sex, the inverse relationship between the third trimester BDCIPP concentrations and birth weight was only found in male newborns; nevertheless, the effect modification of sex was not significant ($p_{\text{sex-int}} = 0.57$). For each ln-transformed unit increase in second trimester 4-HO-DPHP and first trimester DPHP concentrations, birth weight significantly decreased by 48.46 g (95% CI: –94.50, –2.42) and 43.34 g (95% CI: –81.35, –5.33), respectively. The varying associations over the time of exposure (different trimesters) of 4-HO-DPHP and DPHP did not reach a statistically significant level ($p_{\text{tri-int}} = 0.89$ and 0.80). The sex-based results only

revealed a significant association between the first trimester DPHP concentrations and birth weight in female newborns with non-significant sex modification ($p_{\text{sex-int}} = 0.23$). In the third trimester, there were significant reductions in birth weight per one ln-transformed unit increment of BBOEP concentrations ($\beta = -35.48$, 95% CI: $-68.23, -2.73$); the relationship was not different across the three trimesters ($p_{\text{tri-int}} = 0.22$). Following the stratification by sex, the negative association between BBOEP levels and birth weight remained among male newborns, but this association was not modified by sex ($p_{\text{sex-int}} = 0.98$). In addition, the second trimester ΣmOPFRs levels had negative correlation with birth weight, with β value of -82.83 (95% CI: $-148.46, -17.20$). No significant relationships between most target analytes and birth length were found except that third trimester BBOEP concentrations were negatively correlated with birth length ($\beta = -0.19$, 95% CI: $-0.32, -0.06$) with a significant varying exposure effect ($p_{\text{tri-int}} = 0.02$). After stratifying based on sex, the above association was only found in male newborns, and the effect modification of sex was not significant ($p_{\text{sex-int}} = 0.56$).

Associations between tertiles of OPFR metabolite concentrations and offspring size at birth were also estimated (Figure 2). Similar to the findings shown above, some significant linear associations between OPFR metabolite levels and birth size were confirmed by the trend test. Urinary BDCIPP levels in the third trimester had significantly negative linear relationships with birth weight identified by the trend test (p for trend = 0.02). Second trimester 4-HO-DPHP and first trimester DPHP concentrations trended to be in inverse relation to birth weight (p for trend = 0.03 and 0.01, respectively). The negative dose-response relationships between the levels of ΣmOPFRs in the second and third trimester and birth weight were statistically significant (p for trend = 0.04 and 0.02, respectively). Additionally, DoCP & DpCP, BBOEP, and ΣmOPFRs levels in the third trimester were inversely related to birth length with significant linear trends (all p for trend <0.05).

When performing the sensitivity analyses by additionally adjusting for hypertensive disorders of pregnancy and gestational diabetes mellitus or restricting to term newborns, the values of effect estimates did not change significantly compared with those in the main results (Table S4-S5).

DISCUSSION

In the present study of 213 mother-newborn pairs who were recruited in China, we systematically evaluated the internal exposure level of OPFRs in pregnant women throughout pregnancy, and examined the trimester-specific associations between maternal exposure to OPFRs and offspring size at birth for the first time. We observed that pregnant women were widely exposed to OPFRs throughout pregnancy, and the urinary concentrations of certain OPFR metabolites in some trimesters showed significantly inverse associations with birth weight and birth length, among which the associations of BDCIPP and BBOEP levels with birth size were significantly different across the three trimesters with a critical window of susceptibility in the third trimester. However, no significant effect modifications by newborn sex were observed, even though some associations between OPFR metabolites and birth size were only found in male or female newborns.

Many studies have reported the exposure level of OPFRs in different populations as summarized in Table S6. We compared the urinary concentrations of OPFR metabolites in this work with that of pregnant women from different regions in the USA. BDCIPP and DPHP, the most common OPFR metabolites, had significantly lower concentrations in our Chinese cohort than those from California,³⁴ North Carolina,²⁷ and Massachusetts.¹⁴ We also observed generally lower concentrations of BDCIPP, DPHP, DoCP & DpCP, and BBOEP compared to pregnant women from Canada.³⁵ Moreover, BDCIPP levels in our research were lower than those in pregnant women from Shenzhen, China, whereas DPHP, DoCP & DpCP, and BBOEP concentrations were higher than those reported in Shenzhen, China.³⁶ Similarly, compared with the general population in the USA,³⁷ Australia,³⁸ and Canada,³⁹ BDCIPP and DPHP concentrations in our study were still at relatively low levels. BCIPHIPP levels in our samples were one order of magnitude lower than those in the general population in Australia.³⁸ DoCP & DpCP concentrations in this research were slightly lower than those in the general population in Canada, whereas BBOEP concentrations were comparable to those in Canada.³⁹ The concentrations of BDCIPP and DPHP from a national survey of 13 cities in China were

comparable to those detected in our samples, whereas DoCP & DpCP and BBOEP were lower than those in our population.¹³ The discrepancies in OPFR metabolite concentrations between different countries or regions can be explained by the consumption pattern of flame retardants.³ Dietary intake habits may also play a role in different OPFRs exposure levels between these countries or regions.⁴⁰

We observed poor reproducibility of urinary OPFR metabolite concentrations throughout pregnancy, with the SG-adjusted ICC values of 0.17–0.36. In the present study, the ICC of 0.23 for BDCIPP was lower than that reported in most previous studies among pregnant women, with ICCs ranging from 0.36 to 0.60,^{30, 41–43} but comparable to that of a birth cohort from Northern Puerto Rico (ICC = 0.23).⁴⁴ DPHP concentrations throughout pregnancy in the present study were less variable (ICC = 0.36) than those in other studies with ICCs of 0.16–0.27,^{30, 43, 44} and it showed similar temporal variability to that in a study conducted in North Carolina (ICC = 0.40).⁴¹ The demographic characteristics, environmental exposure level, dietary and lifestyle habits during pregnancy, and sample size varied between different studies, which might have contributed to the differences in the variability of OPFR metabolite concentrations during pregnancy among these studies. The reason for the poor reproducibility of target compounds throughout pregnancy in our study may be the short half-lives of OPFRs and the physiological changes that occur during pregnancy.^{1, 45} Our findings regarding temporal variability suggest that multiple measurements of OPFR metabolites during pregnancy in the future research are essential in future studies to accurately evaluate OPFRs exposure among pregnant women.

There are limited human data on the association between maternal exposure to OPFRs and offspring growth. As far as we know, only four epidemiological studies have examined the effect of OPFRs exposure during pregnancy on offspring size at birth, with conflicting results.^{27–30} Inconsistent with our findings, the study conducted by Hoffman et al. in North Carolina reported that maternal urinary ip-PPP concentrations (gestational week 24–30) were negatively associated with birth weight among 157 female infants, while no significant association between other target compounds (i.e., BDCIPP, DPHP, and BCIPHIPP) and birth

size was observed.²⁷ Kuiper et al. found non-significant associations of the repeated measurements of urinary levels of OPFR metabolites in 76 pregnant women enrolled in Maryland with birth weight and length, which was contrary to our results.³⁰ Similarly, a small sample study with 23 pregnant women from Shanghai, China, evaluated the relationships of urinary BDCIPP and DPHP levels with birth outcomes and observed no significant association.²⁹ However, in our previous nested case-control study of 339 participants, the increased risk of low birth weight was related to high urinary DPHP concentrations in pregnant women,²⁸ which was in line with the current results to some extent. These discrepant findings on the effects of OPFRs exposure during pregnancy on birth size may be due to differences in OPFRs exposure levels, exposure windows, study population and design, confounders, and sample size between the above studies. It is worth noting that no prior study has investigated the relationship of maternal urinary BBOEP and 4-HO-DPHP levels with birth size. Given the extensive exposure to OPFRs and the low number of studies, further research is warranted to confirm the observed associations in this present study.

The toxic effects of exposure to OPFRs on embryonic development have been investigated *in vivo* experiments. A study on zebrafish reported that TDCIPP exposure was related to a decrease in body weight, length, survival rate, and heart rate in the offspring.^{23,46} Similar results of TDCIPP exposure were obtained for *Daphnia magna*.²² Long-term exposure of *Daphnia magna* to TPHP at a concentration of 500 µg/L could lead to a great reduction in body length of parent and offspring.²¹ An embryo toxicity test in Japanese medaka pointed out that exposure to TPHP, TBOEP, TCEP, and tri-n-butyl phosphate could decrease hatchability, delay time to hatching, body length, and heart rate, and increase the occurrence of malformation.⁴⁷ In addition, TBOEP has been found to induce malformations and growth delay, and reduce heart rate, body weight, and length.^{25, 48, 49}

While the biological mechanisms of the developmental toxicity of OPFRs are not clearly understood, a growing body of studies may provide clues to explain the potential mechanisms. According to previous reports, OPFRs had diverse properties of human nuclear receptors, such as estrogen receptor activation, androgen receptor antagonism, glucocorticoid receptor

antagonism, and pregnane X receptor activation, which could directly interfere with the signal transduction process of different nuclear receptor pathways to change various hormone levels in the body.³³ Evidence supported that exposure to OPFRs could affect the normal physiological function of endogenous hormones by altering the regulatory circuits of the endocrine axis, which might eventually result in inhibition of the growth and development of the body.^{50, 51} For example, exposure of zebrafish to TBEOP could disturb the balance of sex hormones by regulating the gene transcription of the hypothalamic-pituitary-gonadal axis.⁵⁰ TDCIPP and TPHP exposure in early life were found to alter the levels of thyroid hormones via interference with the transcription of genes (e.g., *crh* and *tsh*) in the HPT axis in a sex-dependent manner in the zebrafish model.⁵¹ It was reported that both hyper- and hypothyroidism during pregnancy could increase the risk of preterm birth and low birth weight.⁵² In addition, the growth inhibition of zebrafish caused by TDCIPP and TBOEP exposure might be attributed to the downregulation of expression of GH/IGF axis-related genes.^{25, 46} Following exposure to some OPFRs, oxidative stress is enhanced and has also been proposed to have link with adverse pregnancy outcomes.^{53, 54}

The fetus can be more affected by exposure to environmental pollutants than adults. It is proposed that the prenatal exposure to pollutants in specific stages of pregnancy might cause greater health damage to the fetus than exposure during other periods of pregnancy.²⁶ Thus, it is extremely necessary to identify the critical windows of fetal susceptibility to environmental compound exposure throughout pregnancy to improve infants' health. In our study, using multiple informant models, we observed that associations of BDCIPP and BBOEP levels with birth size significantly varied over the exposure windows. The third trimester was found to be the critical window of susceptibility for BDCIPP levels to affect birth weight and BBOEP levels to affect birth length in our study. To our knowledge, no epidemiological studies have considered the timing of OPFRs exposure during pregnancy and how this affects fetal growth. The third trimester is the peak period of fetal growth and development, with the fastest increase in fetal weight and has been considered to be the stage when the fetus is more susceptible to environmental exposure.⁵⁵ Several investigations have suggested significant and strong

relationships between exposure to environmental pollutants (e.g., bisphenols, arsenic) in the third trimester and birth outcomes, while null or weaker associations were observed in other trimesters.^{56, 57} More studies should be done to validate our results.

The major strength of the current study relies on its prospective cohort design and the repeated measurements of OPFR metabolites in urine collected in the first, second, and third trimesters. This allowed to feasibly investigate the trimester-specific effects of OPFRs exposure on fetal growth and identify the critical windows of fetal susceptibility to OPFRs exposure in early life. Additionally, information regarding confounders was collected from face-to-face standardized questionnaires or medical records to control for potential biases.

Nevertheless, there are several limitations in our work. First, we only captured urinary OPFR metabolite concentrations at a single time point in different trimesters, which due to the non-persistent character of OPFRs might lead to the misclassification of exposure estimation during a single pregnancy. Second, the sample size of our study is relatively small; thus, the statistical power to detect subtle effects and obtain reliable conclusions might be insufficient. Third, although major confounders have been controlled for as far as possible in our analyses, we could not completely eliminate the potential effect of other unavailable confounders on the reliability of our findings, such as maternal dietary habits, nutritional supplements, and daily activities. Finally, people are usually exposed to a large number of environmental pollutants (e.g., phthalates, bisphenols, metals, and others) that may simultaneously affect fetal growth, so the individual effects of OPFRs exposure on fetal size at birth are difficult to identify. Our findings should be interpreted cautiously and additional longitudinal cohort studies considering these limitations should be carried out to replicate these results.

Taken together, in this prospective cohort study, we provided epidemiological evidence that several OPFR metabolite concentrations in urine in some trimesters were in inverse relation to birth weight and length. Also, the associations of BDCIPP and BBOEP levels with birth size significantly varied by trimester with a critical window of susceptibility in the third trimester. These results improve the current understanding of toxic effects of OPFRs exposure on embryonic development. Further research with large sample sizes is warranted to obtain

more reliable findings.

ASSOCIATED CONTENT

Supporting Information

Distribution of SG-adjusted urinary OPFR metabolite concentrations (ng/mL) of the study population (Table S1); Pearson correlation coefficients of ln-transformed SG-adjusted concentrations of urinary OPFR metabolites in different trimesters (Table S2); Intraclass correlation coefficients (ICCs) for maternal urinary OPFR metabolite concentrations across three trimesters (Table S3); Adjusted trimester-specific associations between ln-transformed SG-adjusted urinary concentrations of OPFR metabolites and offspring size with additional adjustment for hypertensive disorders of pregnancy and gestational diabetes mellitus (Table S4); Adjusted trimester-specific associations between ln-transformed SG-adjusted urinary concentrations of OPFR metabolites and offspring size among term infants (Table S5); Comparisons of urinary geometric concentrations of OPFR metabolites in different countries or regions (Table S6) (PDF).

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Notes

The authors declare no competing financial interest.

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658

Table 1. Demographic characteristics of the mother-newborn pairs in this study^a

Characteristics	n	Mean $\pm$ SD or percent (%)
Mothers		
Maternal age (years)	213	28.6 $\pm$ 3.0
Prepregnancy BMI (kg/m ²)		
Underweight (<18.5)	38	17.8%
Normal (18.5–23.9)	146	68.5%
Overweight ($\geq$ 24.0)	29	13.6%
Pregnancy weight gain (kg)	213	16.3 $\pm$ 4.6
Maternal education level		
Less than high school	7	3.3%
High school or equivalent	33	15.5%
College or above	173	81.2%
Annual household income (RMB, Yuan)		
<50,000	42	19.7%
50,000–100,000	74	34.7%
>100,000	97	45.5%
Employment during pregnancy		
Unemployed	73	34.3%
Employed	139	65.3%
Missing	1	0.5%
Parity		
Nulliparous	185	86.9%
Multiparous	28	13.1%
Delivery mode		
Vaginal	102	47.9%
Cesarean	111	52.1%
Passive smoking during pregnancy		
Yes	74	34.7%
No	139	65.3%
Hypertensive disorders of pregnancy		
Yes	4	1.9%
No	209	98.1%
Gestational diabetes mellitus		
Yes	15	7.0%
No	198	93.0%
Newborns		
Sex		
Male	115	54.0%

Female	98	46.0%
Gestational age (weeks)	213	39.3 ± 1.3
Birth weight (g)	213	3315.4 ± 434.5
Birth length (cm)	213	50.3 ± 1.7

^aAbbreviations: SD, standard deviation; BMI, body mass index.

Table 2. Adjusted trimester-specific associations between ln-transformed SG-adjusted urinary concentrations of OPFR metabolites and birth weight^{a,b}

Analytes	All (n = 213) ^c		Male (n =115) ^d		Female (n = 98) ^d		<i>p</i> _{sex-int} ^e
	β (95% CI)	<i>p</i> -Value	β (95% CI)	<i>p</i> -Value	β (95% CI)	<i>p</i> -Value	
BDCIPP							
1st trimester	−32.46 (−83.54, 18.63)	0.21	−50.08 (−112.94, 12.78)	0.12	−45.23 (−122.43, 31.96)	0.25	0.65
2nd trimester	2.39 (−63.21, 67.99)	0.94	28.86 (−59.64, 117.35)	0.52	−37.05 (−129.30, 55.20)	0.43	0.55
3rd trimester	−85.12 (−148.98, −21.27)	0.01	−89.62 (−165.04, −14.21)	0.02	−86.40 (−187.72, 14.93)	0.09	0.57
<i>p</i> _{tri-int} ^f	0.04		0.07		0.60		
BCIPHIPP							
1st trimester	−50.60 (−109.56, 8.36)	0.09	−66.62 (−134.40, 1.17)	0.05	−31.70 (−130.56, 67.16)	0.53	0.94
2nd trimester	−53.70 (−127.09, 19.70)	0.15	−30.17 (−124.90, 64.56)	0.53	−54.86 (−160.21, 50.48)	0.31	0.82
3rd trimester	18.79 (−34.15, 71.73)	0.49	−11.83 (−91.18, 67.53)	0.77	29.92 (−40.00, 99.83)	0.40	0.93
<i>p</i> _{tri-int} ^f	0.06		0.45		0.17		
4-HO-DPHP							
1st trimester	−36.06 (−80.26, 8.13)	0.11	−61.96 (−116.24, −7.67)	0.03	−14.78 (−81.52, 51.97)	0.66	0.27
2nd trimester	−48.46 (−94.50, −2.42)	0.04	−45.14 (−98.32, 8.04)	0.10	−42.84 (−118.85, 33.17)	0.27	0.46
3rd trimester	−34.26 (−78.97, 10.44)	0.13	−42.38 (−95.10, 10.33)	0.12	−21.83 (−93.16, 49.50)	0.55	0.37
<i>p</i> _{tri-int} ^f	0.89		0.83		0.73		
DPHP							
1st trimester	−43.34 (−81.35, −5.33)	0.03	−27.87 (−75.82, 20.08)	0.25	−59.09 (−114.96, −3.23)	0.04	0.23
2nd trimester	−34.79 (−76.22, 6.64)	0.10	−27.48 (−84.22, 29.26)	0.34	−30.07 (−86.39, 26.24)	0.30	0.69
3rd trimester	−27.17 (−67.27, 12.93)	0.18	−47.90 (−102.04, 6.24)	0.08	1.82 (−52.86, 56.50)	0.95	0.83
<i>p</i> _{tri-int} ^f	0.80		0.83		0.12		
DoCP & DpCP							
1st trimester	19.22 (−31.50, 69.94)	0.46	−3.92 (−66.02, 58.19)	0.90	48.64 (−28.60, 125.87)	0.22	0.30

2nd trimester	-51.59 (-104.48, 1.30)	0.06	-40.51 (-115.09, 34.07)	0.29	-20.35 (-93.62, 52.93)	0.59	0.61
3rd trimester	-39.90 (-89.36, 9.55)	0.11	-46.84 (-112.13, 18.45)	0.16	-17.01 (-87.79, 53.76)	0.64	0.85
$p_{\text{tri-int}}^f$	0.04		0.38		0.18		
BBOEP							
1st trimester	0.02 (-29.57, 29.61)	1.00	17.27 (-19.15, 53.70)	0.35	-19.69 (-64.03, 24.65)	0.38	0.81
2nd trimester	-22.54 (-54.30, 9.23)	0.16	-4.90 (-44.96, 35.16)	0.81	-44.17 (-93.03, 4.69)	0.08	0.40
3rd trimester	-35.48 (-68.23, -2.73)	0.03	-48.11 (-90.20, -6.01)	0.03	-1.48 (-49.57, 46.61)	0.95	0.98
$p_{\text{tri-int}}^f$	0.22		0.04		0.21		
Σ mOPFRs							
1st trimester	-42.00 (-101.35, 17.35)	0.17	-47.69 (-120.47, 25.08)	0.20	-27.58 (-116.81, 61.66)	0.54	0.97
2nd trimester	-82.83 (-148.46, -17.20)	0.01	-57.78 (-142.80, 27.23)	0.18	-70.50 (-167.27, 26.26)	0.15	0.88
3rd trimester	-43.02 (-103.84, 17.80)	0.17	-89.83 (-178.38, -1.28)	0.05	2.26 (-78.29, 82.81)	0.96	0.83
$p_{\text{tri-int}}^f$	0.41		0.80		0.25		

^aAbbreviations: SG, specific gravity; β , regression coefficient; CI, confidence interval.

^bThe bold indicated statistical significance.

^cAdjusted for maternal age, education, prepregnancy BMI, passive smoking during pregnancy, delivery mode, parity, pregnancy weight gain, gestational age, and infant sex.

^dAdjusted for maternal age, education, prepregnancy BMI, passive smoking during pregnancy, delivery mode, parity, pregnancy weight gain, and gestational age.

^e p values for interaction between OPFR metabolite concentrations and infant sex.

^fScore test of homogeneity of effect estimates across the three trimesters.

Table 3. Adjusted trimester-specific associations between ln-transformed SG-adjusted urinary concentrations of OPFR metabolites and birth length^{a,b}

Analytes	All (n = 213) ^c		Male (n = 115) ^d		Female (n = 98) ^d		<i>p</i> _{sex-int} ^e
	β (95% CI)	<i>p</i> -Value	β (95% CI)	<i>p</i> -Value	β (95% CI)	<i>p</i> -Value	
BDCIPP							
1st trimester	−0.09 (−0.29, 0.12)	0.42	−0.05 (−0.32, 0.23)	0.73	−0.11 (−0.41, 0.19)	0.46	0.34
2nd trimester	0.14 (−0.13, 0.41)	0.30	0.24 (−0.15, 0.62)	0.23	−0.04 (−0.40, 0.31)	0.81	0.36
3rd trimester	−0.25 (−0.51, 0.01)	0.06	−0.25 (−0.58, 0.08)	0.13	−0.21 (−0.60, 0.19)	0.30	0.35
<i>p</i> _{tri-int} ^f	0.03		0.04		0.69		
BCIPHIPP							
1st trimester	−0.23 (−0.47, 0.01)	0.06	−0.19 (−0.48, 0.11)	0.21	−0.22 (−0.61, 0.16)	0.25	0.70
2nd trimester	−0.13 (−0.42, 0.17)	0.41	−0.17 (−0.58, 0.24)	0.42	−0.07 (−0.48, 0.34)	0.74	0.58
3rd trimester	0.04 (−0.18, 0.25)	0.74	−0.15 (−0.49, 0.19)	0.39	0.08 (−0.19, 0.35)	0.55	0.45
<i>p</i> _{tri-int} ^f	0.23		0.96		0.34		
4-HO-DPHP							
1st trimester	−0.08 (−0.26, 0.10)	0.36	−0.13 (−0.36, 0.11)	0.30	−0.04 (−0.30, 0.21)	0.74	0.61
2nd trimester	−0.06 (−0.25, 0.12)	0.50	−0.15 (−0.38, 0.08)	0.20	0.04 (−0.26, 0.33)	0.81	0.55
3rd trimester	−0.16 (−0.34, 0.02)	0.09	−0.20 (−0.43, 0.03)	0.09	−0.08 (−0.36, 0.19)	0.56	0.57
<i>p</i> _{tri-int} ^f	0.62		0.81		0.73		
DPHP							
1st trimester	−0.12 (−0.28, 0.03)	0.12	0.00 (−0.20, 0.21)	0.97	−0.24 (−0.45, −0.02)	0.03	0.09
2nd trimester	−0.08 (−0.25, 0.09)	0.36	−0.07 (−0.31, 0.18)	0.60	−0.10 (−0.31, 0.12)	0.39	0.65
3rd trimester	−0.14 (−0.30, 0.02)	0.09	−0.22 (−0.45, 0.02)	0.07	−0.08 (−0.29, 0.13)	0.48	0.93
<i>p</i> _{tri-int} ^f	0.75		0.21		0.09		
DoCP & DpCP							
1st trimester	0.01 (−0.20, 0.21)	0.94	0.00 (−0.27, 0.27)	0.99	−0.08 (−0.38, 0.21)	0.58	0.68

2nd trimester	-0.10 (-0.32, 0.11)	0.34	-0.03 (-0.36, 0.29)	0.84	-0.06 (-0.35, 0.22)	0.67	0.63
3rd trimester	-0.16 (-0.36, 0.04)	0.12	-0.05 (-0.34, 0.23)	0.71	-0.20 (-0.47, 0.07)	0.15	0.20
$p_{\text{tri-int}}^f$	0.31		0.89		0.55		
BBOEP							
1st trimester	0.00 (-0.12, 0.12)	0.97	0.05 (-0.11, 0.20)	0.58	-0.06 (-0.23, 0.11)	0.51	0.58
2nd trimester	-0.08 (-0.21, 0.05)	0.24	0.01 (-0.16, 0.19)	0.87	-0.16 (-0.35, 0.03)	0.09	0.26
3rd trimester	-0.19 (-0.32, -0.06)	0.01	-0.19 (-0.37, -0.01)	0.04	-0.11 (-0.29, 0.08)	0.26	0.56
$p_{\text{tri-int}}^f$	0.02		0.01		0.46		
Σ mOPFRs							
1st trimester	-0.20 (-0.44, 0.04)	0.11	-0.10 (-0.41, 0.22)	0.55	-0.26 (-0.60, 0.09)	0.14	0.27
2nd trimester	-0.23 (-0.50, 0.03)	0.09	-0.13 (-0.50, 0.24)	0.48	-0.27 (-0.64, 0.11)	0.16	0.42
3rd trimester	-0.24 (-0.48, 0.01)	0.06	-0.34 (-0.72, 0.05)	0.08	-0.18 (-0.49, 0.13)	0.26	0.47
$p_{\text{tri-int}}^f$	0.96		0.42		0.81		

^aAbbreviations: SG, specific gravity; β , regression coefficient; CI, confidence interval.

^bThe bold indicated statistical significance.

^cAdjusted for maternal age, education, prepregnancy BMI, passive smoking during pregnancy, delivery mode, parity, pregnancy weight gain, gestational age, and infant sex.

^dAdjusted for maternal age, education, prepregnancy BMI, passive smoking during pregnancy, delivery mode, parity, pregnancy weight gain, and gestational age.

^e p values for interaction between OPFR metabolite concentrations and infant sex.

^fScore test of homogeneity of effect estimates across the three trimesters.

Figure Legends

Figure 1. Distributions of specific gravity (SG)-adjusted urinary OPFR metabolite concentrations in different trimesters. The bottom bar, the horizontal line, and the top bar of the box indicates the 25th, 50th and 75th percentiles, respectively. The box whiskers present the 5th and 95th percentiles. ^aThe unit is nmol/L.

Figure 2. Adjusted regression coefficients (β s) and 95% confidence intervals (CIs) for trimester-specific relationships of tertiles of OPFR metabolite concentrations with birth weight (A) and birth length (B). All models were adjusted for maternal age, education, prepregnancy BMI, passive smoking during pregnancy, delivery mode, parity, pregnancy weight gain, gestational age, and infant sex. T1, the first tertile; T2, the second tertile; T3, the third tertile. *P* for trend presents the linear trend by assigning the median of each tertile of OPFR metabolite concentrations.

Figure 1

Figure 2