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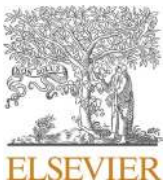

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# Association of prenatal co-exposure to OPEs and PAEs with ASD symptom trajectories in preschool children: the modifying role of vitamin D

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ABSTRACT

*Introduction:* Few studies have examined prenatal co-exposure to organophosphate esters (OPEs) and phthalic acid esters (PAEs) in relation to childhood autism spectrum disorder (ASD) symptoms, and whether vitamin D modifies these associations.

*Methods:* Included 2400 mother–child dyads from the Ma'anshan Birth Cohort and examined levels of maternal OPEs, PAEs, and vitamin D across the three trimesters. Used the Clancy Autism Behavior Scale to assess preschoolers' ASD symptoms at ages 3, 5, and 6, then used group-based trajectory modeling to fit ASD symptom trajectories.

*Results:* ASD symptom scores fit 3 trajectories: low, moderate, and high. Average-MEOHP, average-ΣHMWP, first-trimester-ΣHMWP, third-trimester-DPHP, and third-trimester-BCIPP were associated with increased risk in the moderate group ($p < 0.05$). Average-MMP, average-ΣHMWP, second-trimester-BEHP, and third-trimester-ΣHMWP were associated with increased risk in the high-score group ($p < 0.05$). By contrast, first-trimester-BCIPP was negatively correlated with the moderate group. DPHP, DBP, and Σdi-OPEs showed sex-specific effects ($p_{sex\text{-}int} < 0.05$). Maternal vitamin D levels modified these associations, with women with vitamin D deficiency showing significantly higher risks. OPEs and PAEs showed mixed effects.

*Conclusion:* Prenatal exposure to OPEs/PAEs exhibited heterogeneous associations with preschoolers' ASD symptom trajectories, and maternal vitamin D deficiency exacerbates the effects of OPEs/PAEs individual exposure on symptom trajectories.

## 1. Introduction

Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders characterized by impairments in social communication and interaction, as well as restricted, repetitive patterns of behavior and narrow interests, typically emerging in early childhood (Szatmari et al., 2016). ASD symptoms usually become apparent before age 3, though in some cases they may not become evident until school age or even later.

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Some studies suggest that initial signs may appear between 6 and 18 months (Matrone & Ferretti, 2023; Szatmari et al., 2016). The latest data indicate that in 2021, approximately 1 in 127 individuals worldwide were living with ASD (GBD 2021 Autism Spectrum Disorder Collaborators, 2025), and the disease burden associated with ASD ranks among the top ten neurological disorders (GBD 2021 Diseases and Injuries Collaborators, 2024). A systematic review encompassing 71 global studies reported an ASD prevalence rate of 1.19% (ranging from 0.11‰ to 4.36%) (Zeidan et al., 2022). This upward trend is consistently observed across diverse countries and regions, yet the exact drivers of this rapid growth remain incompletely understood (Hansen et al., 2015). Notably, subclinical functional impairments or partial manifestations (ASD symptoms) are far more common than clinically diagnosed mental disorders (Sayal et al., 2018). Consequently, early identification and intervention during childhood are critical for improving long-term health outcomes. However, the etiology of ASD remains poorly understood. Identifying modifiable environmental factors associated with ASD—such as endocrine disruptors—and implementing preventive measures are crucial to promoting healthy child development and well-being. Organophosphate esters (OPEs) and phthalic acid esters (PAEs) are two prevalent classes of emerging environmental contaminants. They share overlapping applications as plasticizers, similar exposure pathways, and frequent co-detection in human biospecimens, suggesting potential similarities in adverse health effects (Gan et al., 2025). Consequently, co-exposure to OPEs and PAEs warrants serious consideration, particularly during the critical prenatal window of fetal neurodevelopment.

Animal studies have shown that prenatal exposure to di (2-ethylhexyl) phthalate (DEHP) leads to ASD characteristics and ASD-like behaviors in offspring (Li et al., 2022; Zhang et al., 2021). Limited human studies have indicated that early-life exposure to PAEs is positively correlated with the risk of ASD in children (Jeddi et al., 2016). Since OPEs have chemical structures highly similar to those of organophosphorus pesticides, they may be neurotoxic. Although it is believed that OPEs have a weak inhibitory effect on acetylcholinesterase due to minor differences in molecular structure, multi-level evidence from human, animal, and cellular studies suggests that OPEs may affect the neurodevelopment and behavior of offspring by interfering with glutamate, γ-aminobutyric acid, and endocrine functions (Patisaul et al., 2021). Currently, there are relatively few studies on the relationship between prenatal OPE exposure and ASD.

Interestingly, the research found that among pregnant women who took prenatal vitamins, exposure to PAEs was associated with a reduced risk of ASD in children (Shin et al., 2018). Previously, our team's research also found that taking vitamin D during pregnancy could reduce the risk of ADHD symptoms in offspring caused by prenatal OPEs exposure (Li et al., 2024). Vitamin D is an important fat-soluble steroid derivative that not only supports bone health and normal nerve and muscle function but also regulates neurotransmitters and activates signal transduction pathways (Murthi et al., 2017; Saidi et al., 2023). However, vitamin D deficiency and insufficiency are particularly common among pregnant and postpartum women in many regions, including the Americas, Europe, the Eastern Mediterranean, Southeast Asia, and the Western Pacific (Saraf et al., 2016). A Meta-analysis of prospective studies showed that children with vitamin D deficiency or insufficiency during pregnancy or after birth have a 54% higher risk of ASD (Wang et al., 2020). Based on the above background, it is crucial to understand whether prenatal vitamin D supplementation can reduce the risk of ASD caused by OPEs and PAEs exposure.

Based on the above research background, several important knowledge gaps remain. First, most previous studies assessed ASD symptoms at a single time point, with limited attention to longitudinal symptom trajectories. Second, few studies investigate sex-specific effects, despite growing evidence that prenatal OPEs and PAEs exposure has different effects on the development of fetuses of different sexes. Third, because the sensitive period for infant neural development varies across trimesters and the half-lives of OPEs and PAEs are relatively short, repeated exposure assessment across trimesters is essential for identifying sensitive windows. Finally, the effects of co-exposure to OPEs and PAEs on ASD symptom trajectories have not been examined. Therefore, relying on the Ma'anshan Birth Cohort (MABC) with repeated maternal urine collection during pregnancy and repeated assessment of children's ASD symptoms, we aimed to (1) investigate associations of prenatal exposure to individual and mixed OPEs and PAEs with preschoolers' ASD symptom trajectories; (2) determine the sensitive exposure window and the sex-specific associations; (3) explore effect modification by prenatal vitamin D levels on the association.

## 2. Methods

### 2.1. Study population

Based on MABC, the cohort was recruited from May 2013 to September 2017 at Ma'anshan Maternal and Child Health Hospital from pregnant women in the first trimester. Previous studies have reported detailed information on MABC (Huang et al., 2024). The Ethics Committee of Anhui Medical University approved the study (No. 20131195). All research subjects were informed of the study's purpose and nature and signed the informed consent form. Among the 3474 pregnant women included in MABC, 39 cases of twins, 75 cases of fetal arrest, 45 cases of spontaneous abortion, 30 cases of therapeutic induction of labor, 10 cases of stillbirth, and 2 cases of ectopic pregnancy were further excluded. A total of 3273 singleton live-born infants remained. We then excluded 47 pregnant women who did not provide urine samples in the three trimesters. Among the children, we excluded 826 individuals with fewer than two ASD symptom questionnaire evaluations across the 3-, 5-, and 6-year intervals. Finally, we included 2400 mother–child dyads: 2301 in the first trimester, 2320 in the second, and 2247 in the third. The specific inclusion and exclusion process is shown in **Fig. S1**.

### 2.2. Sample collection and OPE/PAEs/25 (OH) D measurement

Maternal morning urine samples were collected during the first trimester (10.41 weeks), second trimester (25.98 weeks), and third trimester (34.37 weeks) using 50 mL polypropylene urine cups. On-site laboratory technicians transferred the urine into 5 mL polypropylene cryovials and stored them at –80 °C until measurement. During follow-up visits in the three trimesters, clinical laboratory personnel drew blood samples before 10:00 a.m. after overnight fasting. Stored at –80 °C after centrifugation until measurement.

Building on preliminary method development and a comprehensive literature review, we quantified six OPEs in maternal urine: tris(2-chloroethyl) phosphate (TCEP), diphenyl phosphate (DPHP), bis(1-chloro-2-propyl) phosphate (BCIPP), bis(2-butoxyethyl) phosphate (BBOEP), bis(2-ethylhexyl) phosphate (BEHP), and dibutyl phosphate (DBP). These targets were selected due to their prevalence and high detection rates in human biomonitoring studies (Wang et al., 2025). Urinary concentrations were determined using solvent-induced phase change extraction (SIPTE) coupled with ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) on a Waters Xevo TQ-S system. Chromatographic separation was achieved on an ACQUITY® C18 BEH column (50 × 2.1 mm, 1.7 μm). Quantification was performed via isotope dilution, supported by rigorous quality assurance and control (QA/QC) procedures to ensure data accuracy (Gan et al., 2025). Detailed methodological parameters have been reported previously (Gan et al., 2025). The detection rate of OPE metabolites ranged from 65.3% to 97.3% (Table S1).

Six major PAEs were quantified in urine: monomethyl phthalate (MMP), mono-ethyl phthalate (MEP), mono-butyl phthalate (MBP), mono-2-ethylhexyl phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), and mono-(2-ethyl-5-oxohexyl) phthalate

(MEOHP). We quantified PAE concentrations in urine samples using an isotopic internal standard method: solid-phase extraction followed by high-performance liquid chromatography–tandem mass spectrometry (6410 LC–MS, Agilent Technologies, Santa Clara, CA, USA). We implemented stringent QA/QC procedures throughout the analytical process to ensure reliable quantification of urinary PAE (Gan et al., 2025). The detection rate of PAEs was ≥ 97% across all samples (Table S1).

Urinary creatinine (CR) concentrations were quantified using a commercial assay kit (Jiancheng Bioengineering Institute, Nanjing, China) based on the picric acid (Jaffe) reaction to adjust for inter-individual variation in urine dilution.

The serum 25(OH)D concentration was measured by radioimmunoassay. Based on the vitamin D level limits published by the European Food Safety Authority, vitamin D nutritional status was classified as deficiency (< 20 ng/mL) or non-deficiency (≥ 20 ng/mL) (Li et al., 2024). The mean value across the three trimesters was calculated to represent the average level throughout pregnancy.

### 2.3. Collection of information on children's ASD symptoms

Using the Clancy Autism Behavior Scale (CABS), children's ASD symptoms were screened at the 3–6-year follow-up. This scale has clear, concise items, is time-efficient, and is widely used in pediatric neurobehavioral cognitive assessments and in maternal and child health care institutions. The scale consists of 14 items and was revised in 1983, changing the original dichotomy to three response intensities: "never 0 points," "occasionally 1 point," and "often 2 points." The revised scale uses a total score ≥ 14 points as the initial criterion for screening for ASD symptoms. This scale is one of the most widely used ASD screening and diagnostic tools at home and abroad, with high diagnostic sensitivity and the ability to effectively reflect common behavioral problems. In Chinese children, the sensitivity, specificity, and positive predictive value were 58%, 84%, and 65%, respectively (Gao et al., 2024; Qin et al., 2022). The Cronbach α coefficients for the 3-, 5-, and 6-year periods in this study were 0.800, 0.798, and 0.851, respectively, demonstrating good reliability and validity.

### 2.4. Covariates

Based on previous studies (Hoffman et al., 2025; Lu et al., 2024) and the analysis results, this research also used directed acyclic graphs to select potential confounding factors. Finally, the following covariates were included in this study: maternal age at inclusion (continuous variable), pre-pregnancy BMI (continuous variable), maternal intelligence quotient (IQ) (continuous variable), gestational weight gain (continuous variable), parity (categorical variable: primiparous or multiparous), maternal educational level (categorical variable: junior high school or below; high school; college; bachelor's degree or above), per capita monthly family income (categorical variable: < 2500 yuan; 2500 ~ 4000 yuan; > 4000 yuan), breastfeeding pattern in the first 6 months (categorical variable: breastfeeding; artificial feeding; mixed feeding), delivery mode (categorical variable: vaginal delivery or cesarean section), and child sex (categorical variable: boy or girl). As shown in **Fig. S2**.

### 2.5. Data processing and statistical analysis

If the concentrations of OPEs and PAEs were below the limit of detection (LOD), they were replaced with LOD/$\sqrt{2}$ (Hornung & Reed, 1990). To correct for individual urine dilution, we corrected OPEs and PAEs in urine using creatinine. The formula is as follows:

$$corrected\ concentration\ of\ OPEs/PAEs\ in\ urine\ (\mu g/g) = \frac{mass\ fraction\ of\ OPEs/PAEs\ by\ volume\ (ng/mL)}{urinary\ creatinine\ concentration\ (mol/L) \times 113.12(g/mol)}$$

Based on the chemical structures and functions of OPEs, this study classified six OPEs into chlorinated-alkyl OPEs (cl-OPEs) and di-esters of OPEs (di-OPEs). The cl-OPEs included TCEP and BCIPP, while the di-OPEs comprised DPHP, BBOEP, BEHP, and DBP. The total molar concentrations were calculated separately as ∑cl-OPEs, ∑di-OPEs, and ∑OPEs (Liu et al., 2021). Similarly, PAEs were divided into low-molecular-weight phthalates (LMWP) and high-molecular-weight phthalates (HMWP) based on molecular weight, with LMWP including MMP, MEP, and MBP, and HMWP including MEHP, MEOHP, and MEHHP (Gao et al., 2023). We also calculated total molar concentrations separately for ∑LMWP, ∑HMWP, and ∑PAEs.

Description of the basic demographic characteristics of the study population: Continuous variables were reported as mean ± standard deviation, and categorical variables as frequencies and proportions. Comparisons of general demographic characteristics between included and excluded mother–child dyads were conducted using independent-samples *t*-tests and chi-square tests. Because OPEs and PAEs concentrations were generally skewed, their distributions before and after CR-adjustment were summarized using geometric means (*GM*) and 25th and 75th percentiles ($P_{25}$, $P_{75}$). Intraclass correlation coefficients (*ICCs*) were calculated for OPEs and PAEs before and after CR-adjustment to assess reproducibility. Results showed poor reproducibility of OPEs and PAEs across all three trimesters (all *ICCs* ≤ 0.29), so we computed the *GM* across the three trimesters to better represent average exposure levels throughout pregnancy. Among the 2400 participants, 2124 provided samples in all three trimesters, 220 in two trimesters, and 56 in only one trimester. If one trimester value was missing, we used the GM of the remaining two trimesters to calculate the maternal average concentration; if two trimester values were missing, we used the single available trimester value as the representative maternal concentration (Li et al., 2024). Because OPEs and PAEs were highly skewed, we applied a natural logarithmic (ln) transformation to the CR-adjusted concentrations to reduce the influence of extreme values and achieve normality for subsequent analyses. We used Spearman's rank correlation analysis to examine pairwise correlations between OPE and PAE concentrations across the entire pregnancy and within individual trimesters. Missing values in covariates were imputed using random forest imputation. Group comparisons were performed using one-way *ANOVA* for continuous variables with three groups, independent-samples *t*-tests for two groups, and chi-square tests for categorical variables.

Using group-based trajectory modeling (GBTM), ASD symptom scale scores for preschool children at ages 3, 5, and 6 were modeled as trajectories. This model uses maximum likelihood estimation to handle missing data and assigns individuals with similar measurement patterns over time to the same group. This method can integrate all the information in high-resolution longitudinal data (Nagin & Tremblay, 2001). To determine the optimal number of stable trajectories to include in the model and to test the statistical hypotheses, estimates were first conducted for each dimension separately. The model started with a single category and gradually increased the number of categories until the model fit indicators no longer met the recommended standards or could not distinguish meaningful trajectories. The fit indicators evaluated were: (1) the Bayesian information criterion (BIC); the closer the BIC value is to 0, the better the model fits; (2) higher entropy indicates greater distinction between trajectory category classifications across groups; (3) the average posterior probability (AvePP) is greater than 0.7; (4) the proportion of participants in each trajectory is greater than 5%. However, the optimal trajectories also need to be selected in combination with clinical significance and actual circumstances, with the latter being more important (Nguena Nguefack et al., 2020). Subsequently, data visualization methods were used to evaluate data separation and model fit. The results showed that the three-trajectory model in this study fit the data relatively well, so we used the low-score trajectory group as a reference for subsequent analysis.

We carried out the analysis in four steps. First, using restricted cubic

splines (RCS), we explored whether there was a nonlinear association between prenatal OPEs and PAEs and trajectories of children's ASD symptom scale scores. Second, we used logistic regression to analyze the association between OPE and PAE concentrations throughout pregnancy and trajectory groups, adjusting for potential confounders. Additionally, we conducted stratified logistic regression analyses by vitamin D status during pregnancy (deficient vs. non-deficient) and sex. Third, using the quantile-based g-computation (QGC) and Bayesian kernel machine regression (BKMR) models, we analyzed the association between the mixed exposure to OPEs and PAEs during pregnancy and the trajectory groups with moderate and high scores. Because individual components of a mixture may show associations in opposite directions, we first characterized the mixture's directional heterogeneity using component-specific coefficients estimated from the QGC model and partitioned OPEs and PAEs into positive- and negative-direction exposure subsets. We then conducted exploratory, direction-specific BKMR analyses to reduce potential masking from opposing associations within the overall mixture, further characterize joint exposure–response patterns within each directional subset, and evaluate the relative importance of individual analytes within each subset. Fourth, through the following two steps, we analyzed the relationship between the exposure of OPEs and PAEs and the ASD symptoms of the offspring: First, we used generalized linear models (GLMs) or logistic regression to separately analyze the association between the ASD scores or symptoms of offspring at different ages and the exposure; then, we used generalized estimating equations (GEEs) to comprehensively evaluate the longitudinal association between OPEs, PAEs and the above indicators. To reduce the false positive rate (Type I error) due to multiple comparisons, we adjusted *p*-values using the Benjamini–Hochberg method at a false discovery rate (*FDR*) of 0.05(Wang et al., 2025).

The data analysis was performed using SPSS 23.0 (SPSS Inc, Chicago, IL), Stata 14.0 (StataCorp, TX, USA), and R (4.1.2). Graphs were drawn using GraphPad Prism 9.0 (GraphPad Inc, California, USA). The significance level was 0.05.

### 2.6. Sensitivity analysis

To assess the robustness of the main results, we conducted several sensitivity analyses. (1) Premature infants (Crump et al., 2021) were excluded to evaluate whether their inclusion would affect the results; (2) Given that maternal smoking/drinking before or during pregnancy, as well as folic acid supplementation, might influence children's neurodevelopment (Bölte et al., 2019), in addition to the original adjustment for confounding factors, the maternal smoking/drinking before or during pregnancy were further adjusted, and folic acid supplementation was then controlled (Hoxha et al., 2021), to explore whether the results would change. Because the entropy of the final trajectory in our study is relatively low (0.644), classification errors may occur. Therefore, we derive posterior probabilities and construct inverse probability weights (IPW = 1/posterior probability). Using these IPW weights, we conduct multivariate logistic regression. By varying the exposure quantile settings (q = 3, 4, and 5) and removing the quantile transformation used to standardize exposure levels by IQR, we compare the consistency of effect directions for each chemical across model settings.

## 3. Results

### 3.1. Demographic characteristics description

Table 1 summarizes the demographic characteristics of the included, excluded, and total populations in this study, as well as the comparison between the included and excluded groups. Among the 2400 mother–child dyads included in the study, the average maternal age was 26.71 years, the average pre-pregnancy BMI was 20.84 kg/m$^2$, and 1936 mothers had a high school education or higher. Half of the children were delivered vaginally (50.6%), 1230 were boys, and the proportion of

**Table 1**
Basic characteristics of included and excluded participants [Mean ± SD /n(%)].

| Variables | All ($N$ = 3474) | Included ($N$ = 2400) | Excluded ($N$ = 1074) | *p*-value |
|---|---|---|---|---|
| Maternal characteristics | | | | |
| Age at enrollment (years) | 26.67 ± 3.69 | 26.71 ± 3.63 | 26.57 ± 3.81 | 0.282 |
| ≤ 24 | 1 008 (29.0) | 684 (28.5) | 324 (30.2) | 0.584 |
| 25–29 | 1 829 (52.7) | 1 270 (52.9) | 559 (52.0) | |
| ≥ 30 | 637 (18.3) | 446 (18.6) | 191 (17.8) | |
| Pre-pregnancy BMI (kg/m$^2$) | 20.90 ± 2.84 | 20.84 ± 2.84 | 21.04 ± 2.82 | 0.062 |
| < 18.5 | 637 (18.3) | 464 (19.3) | 173 (16.1) | **0.043** |
| 18.5–23.9 | 2 407 (69.3) | 1 652 (68.9) | 755 (70.3) | |
| ≥ 24 | 430 (12.4) | 284 (11.8) | 146 (13.6) | |
| Educational level | | | | |
| Junior high school and below | 719 (20.7) | 464 (19.3) | 255 (23.7) | **0.002** |
| High school/technical | 786 (22.6) | 527 (22.0) | 259 (24.1) | |
| Secondary school college | 1 059 (30.5) | 752 (31.3) | 307 (28.6) | |
| Bachelor's degree or above | 910 (26.2) | 657 (27.4) | 253 (23.6) | |
| Per capita monthly family income (yuan) | | | | |
| < 2500 | 918 (26.4) | 662 (27.6) | 256 (23.8) | 0.067 |
| 2500–4000 | 1 493 (43.0) | 1 013 (42.2) | 480 (44.7) | |
| > 4000 | 1 063 (30.6) | 725 (30.2) | 338 (31.5) | |
| Maternal smoking before or during pregnancy | | | | |
| Yes | 150 (4.3) | 94 (3.9) | 56 (5.2) | 0.082 |
| No | 3 324 (95.7) | 2 306 (96.1) | 1018 (94.8) | |
| Maternal alcohol consumption before or during pregnancy | | | | |
| Yes | 276 (7.9) | 190 (7.9) | 86 (8.0) | 0.927 |
| No | 3 198 (92.1) | 2 210 (92.1) | 988 (92.0) | |
| Parity | | | | |
| Primiparous | 3 063 (88.2) | 2 125 (88.5) | 938 (87.3) | 0.310 |
| Multiparous | 411 (11.8) | 275 (11.5) | 136 (12.7) | |
| Gestational diabetes mellitus* | | | | |
| Yes | 429 (12.9) | 289 (12.0) | 140 (15.2) | **0.015** |
| No | 2 892 (87.1) | 2 111 (88.0) | 781 (84.8) | |
| Hypertensive disorder of pregnancy* | | | | 0.753 |
| Yes | 200 (6.1) | 145 (6.0) | 55 (6.3) | |
| No | 3 066 (93.9) | 2 254 (94.0) | 812 (93.7) | |
| Gestational weight gain (kg)* | 17.83 ± 5.09 | 17.76 ± 5.00 | 17.80 ± 5.35 | 0.248 |
| IQ | 95.75 ± 10.56 | 96.08 ± 10.64 | 95.03 ± 10.34 | **0.007** |
| Delivery mode* | | | | **0.037** |
| Vaginal | 1 619 (49.5) | 1 214 (50.6) | 405 (46.5) | |
| Cesarean | 1 650 (50.5) | 1 184 (49.4) | 466 (53.5) | |
| Vitamin D status during pregnancy(ng/mL)* | | | | **0.027** |
| < 20 | 1 867 (54.4) | 1 288 (53.7) | 579 (56.1) | |
| ≥ 20 | 1 563 (45.6) | 1 110 (46.3) | 453 (43.9) | |
| Children's characteristics | | | | |
| Sex* | | | | 0.778 |
| Boy | 1 670 (51.1) | 1 230 (51.2) | 440 (50.7) | |
| Girl | 1 598 (48.9) | 1 170 (48.8) | 428 (49.3) | |
| Feeding mode at 5 months of age* | | | | **0.008** |

(*continued on next page*)

**Table 1** (*continued*)

| Variables | All ($N = 3474$) | Included ($N = 2400$) | Excluded ($N = 1074$) | *p*-value |
|---|---|---|---|---|
| Breastfeeding | 1 072 (34.0) | 813 (34.6) | 259 (32.3) | |
| Mixed feeding | 1 221 (38.8) | 930 (39.6) | 291 (36.3) | |
| Artificial feeding | 857 (27.2) | 605 (25.8) | 252 (31.4) | |

Note: * Some values are missing and the effective proportion is reported. Gestational diabetes mellitus in 153cases, hypertensive disorder of pregnancy in 208 cases, gestational weight gain in 255 cases, delivery mode in 205 cases, vitamin D status deficiency in 44 cases, Sex in 206 cases, and feeding mode at 5 months of age in 324 case.

breastfeeding was 34.6%. The included and excluded groups differed significantly in education level, gestational diabetes, mother's IQ, mode of delivery, vitamin D supplementation during pregnancy, and breastfeeding patterns. However, the two groups did not differ significantly in other variables.

### 3.2. Distribution characteristics of OPEs and PAEs concentrations

As shown in **Table S1**, the detection rates of DPHP, DBP, BCIPP, MMP, MEP, MBP, MEHP, MEOHP, and MEHHP were all ≥ 90%. Among the OPEs, BBOEP had the lowest concentration; and among the PAEs, MBP had the highest concentration. For both unadjusted and CR-adjusted concentrations, the *ICCs* for the 6 OPE metabolites were ≤ 0.29, indicating poor reproducibility and high temporal variability. The concentration distributions of OPEs and PAEs after ln-transformation and CR-adjustment were normal, as shown in **Fig. S3**. There were no statistically significant differences in ln-transformed and CR-adjusted concentrations of OPEs and PAEs across trimesters or between boys and girls ($p > 0.05$). Detailed information is provided in **Fig. S4** and **Table S2**. **Fig. S5** shows the results of the Spearman rank correlation analysis after ln-transformation and CR-adjustment for OPEs and PAEs: OPE metabolites showed weak but significant correlations throughout pregnancy and in each trimester ($0.05 \leq r \leq 0.38$), and PAEs showed significant, meaningful correlation coefficients ranging from 0.11 to 0.85. The correlations between most OPEs and PAEs were statistically significant ($p < 0.05$).

### 3.3. Symptoms of ASD in preschool children

**Table S3 shows** that the detection rate of ASD symptoms gradually decreases with age. The detection rates at ages 3, 5, and 6 are 8.8%, 5.1%, and 4.2%, respectively. ASD symptom scale scores are consistently higher for boys than for girls. Based on combined model analysis and practical significance, although the BIC and Akaike information criterion (AIC) values of the 4-category model are closer to zero, its entropy is lower, and the practical significance of the 4-category groups is limited. Therefore, this study determines that the typical trajectory of ASD symptom scale scores can be divided into 3 categories. **Table S4** summarizes the BIC and AIC indicators used for GBTM model selection, as well as the posterior probability of the selected model. Fig. 1 shows the 3 categories: low-score trajectory (50.0%), moderate-score trajectory (46.4%), and high-score trajectory (3.6%).

### 3.4. Association between individual exposure to OPEs/PAEs and children's ASD symptom trajectory

Using the RCS model and after further adjustment for confounding factors, the results showed that MBP in the second trimester and BCIPP in the third trimester had a non-linear association with the moderate-score group; MEOHP in the first trimester, DPHP in the third trimester, and MEHP throughout the pregnancy had a non-linear association with the high-score group, whereas no non-linear association was observed for other metabolites with the trajectory of children's ASD symptom scores. Specific details are shown in **Figs. S6- S13**.

As shown in Table 2, after adjusting for confounding factors, it was found that compared with the low-score group of ASD symptom scores, in the whole pregnancy, the levels of MEOHP (*OR* = 1.34, 95%*CI*: 1.03, 1.73) and ∑HMWP (*OR* = 1.19, 95%*CI*: 1.02, 1.39) were positively correlated with the moderate-score group, and the concentrations of MMP (*OR* = 1.86, 95%*CI*: 1.07, 3.24) and ∑HMWP (*OR* = 1.63, 95%*CI*: 1.20, 3.11) were positively correlated with the high-score group; BCIPP in the first trimester (*OR* = 0.89, 95%*CI*: 0.81, 0.99) was negatively correlated with the moderate-score group, while ∑HMWP (*OR* = 1.13, 95%*CI*: 1.00, 1.28) was positively correlated with the moderate-score group; for BEHP in the second trimester, for every increase of one ln concentration, the risk of the high-score group of children's ASD symptom scores increased by 1.22 (95%*CI*: 1.00, 1.49); in the third trimester, DPHP (*OR* = 1.09, 95%*CI*: 1.00, 1.198) and BCIPP (*OR* = 1.10, 95%*CI*: 1.00, 1.20) would increase the risk of the moderate-score group, while ∑HMWP would increase the risk of the high-score group (*OR* = 1.67,

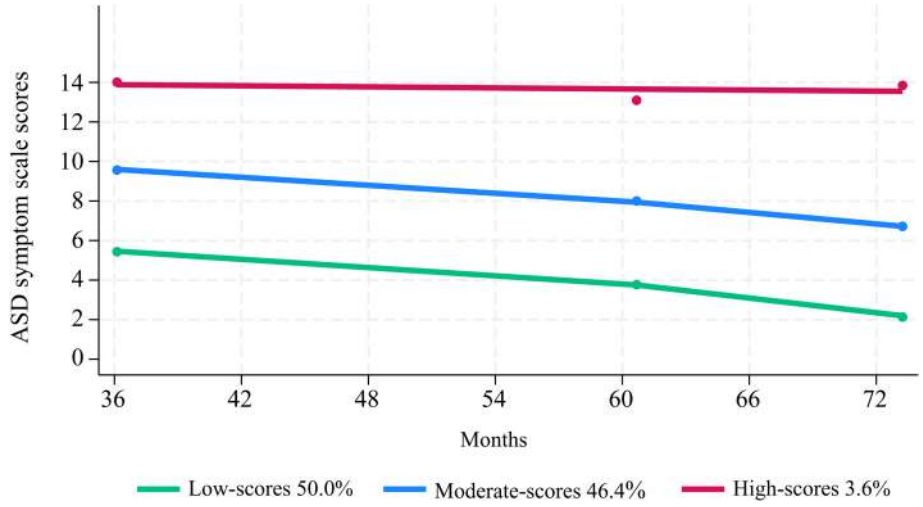


**Fig. 1.** Trajectories of preschoolers' ASD symptom scale scores derived from group-based trajectory models. The groups are named based on their initial scores and subsequent trends. The green line indicates the low-score trajectory, the blue line indicates the moderate-score trajectory, and the red line indicates the high-score trajectory. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Table 2**
Association between OPEs and PAEs concentration (ln-transformed, CR-adjusted) in whole pregnancy and ASD symptom trajectories.

| Chemical | Across pregnancy | | | First trimester | | | Second trimester | | | Third trimester | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *OR* (95%*CI*) | *p*-value | *q*-value | *OR* (95%*CI*) | *p*-value | *q*-value | *OR* (95%*CI*) | *p*-value | *q*-value | *OR* (95%*CI*) | *p*-value | *q*-value |
| Moderate-scores vs. Low-scores | | | | | | | | | | | | |
| DPHP | 1.10 (0.98, 1.23) | 0.098 | 0.351 | 1.05 (0.96, 1.15) | 0.316 | 0.500 | 1.05 (0.97, 1.14) | 0.200 | 0.783 | **1.09 (1.00, 1.19)** | **0.040** | 0.405 |
| BBOEP | 0.94 (0.87, 1.02) | 0.156 | 0.401 | 0.95 (0.89, 1.01) | 0.131 | 0.390 | 0.97 (0.92, 1.03) | 0.348 | 0.783 | 0.99 (0.92, 1.05) | 0.670 | 0.779 |
| BEHP | 1.01 (0.92, 1.11) | 0.781 | 0.908 | 0.98 (0.91, 1.05) | 0.561 | 0.651 | 1.04 (0.98, 1.11) | 0.238 | 0.783 | 0.97 (0.91, 1.04) | 0.452 | 0.779 |
| DBP | 0.89 (0.80, 1.00) | 0.050 | 0.300 | 0.95 (0.87, 1.03) | 0.193 | 0.390 | 0.93 (0.86, 1.00) | 0.057 | 0.783 | 0.97 (0.89, 1.06) | 0.570 | 0.779 |
| TCEP | 1.08 (0.98, 1.18) | 0.117 | 0.351 | 1.05 (0.98, 1.14) | 0.178 | 0.390 | 1.03 (0.96, 1.10) | 0.403 | 0.806 | 1.01 (0.94, 1.09) | 0.775 | 0.779 |
| BCIPP | 0.98 (0.87, 1.11) | 0.805 | 0.908 | **0.89 (0.81, 0.99)** | **0.025** | 0.387 | 0.99 (0.91, 1.08) | 0.813 | 0.879 | **1.10 (1.00, 1.20)** | **0.045** | 0.405 |
| ∑cl-OPEs | 1.04 (0.93, 1.16) | 0.510 | 0.835 | 0.94 (0.86, 1.04) | 0.226 | 0.407 | 1.04 (0.96, 1.13) | 0.317 | 0.783 | 1.06 (0.97, 1.16) | 0.172 | 0.779 |
| ∑di-OPEs | 0.98 (0.87, 1.12) | 0.807 | 0.908 | 0.95 (0.85, 1.06) | 0.361 | 0.500 | 1.00 (0.92, 1.08) | 0.914 | 0.914 | 1.04 (0.94, 1.15) | 0.415 | 0.779 |
| ∑OPEs | 0.99 (0.87, 1.14) | 0.909 | 0.909 | 0.90 (0.80, 1.02) | 0.092 | 0.390 | 1.01 (0.93, 1.11) | 0.790 | 0.879 | 1.07 (0.97, 1.20) | 0.182 | 0.779 |
| MMP | 0.99 (0.89, 1.10) | 0.869 | 0.909 | 0.99 (0.89, 1.10) | 0.836 | 0.836 | 0.99 (0.94, 1.05) | 0.830 | 0.879 | 1.03 (0.95, 1.12) | 0.418 | 0.779 |
| MEP | 0.97 (0.89, 1.07) | 0.591 | 0.887 | 1.02 (0.94, 1.11) | 0.579 | 0.651 | 0.95 (0.89, 1.03) | 0.198 | 0.783 | 0.99 (0.92, 1.06) | 0.727 | 0.779 |
| MBP | 1.06 (0.93, 1.21) | 0.401 | 0.722 | 1.05 (0.95, 1.16) | 0.359 | 0.500 | 1.05 (0.95, 1.15) | 0.329 | 0.783 | 0.99 (0.89, 1.09) | 0.779 | 0.779 |
| MEHP | 1.03 (0.89, 1.19) | 0.725 | 0.908 | 1.04 (0.94, 1.16) | 0.419 | 0.539 | 1.02 (0.89, 1.17) | 0.787 | 0.879 | 0.95 (0.85, 1.06) | 0.325 | 0.779 |
| MEOHP | **1.34 (1.03, 1.73)** | **0.028** | 0.252 | 1.11 (0.95, 1.29) | 0.195 | 0.390 | 1.06 (0.89, 1.27) | 0.493 | 0.879 | 1.16 (0.89, 1.51) | 0.268 | 0.779 |
| MEHHP | 0.86 (0.67, 1.10) | 0.219 | 0.438 | 0.97 (0.83, 1.14) | 0.740 | 0.784 | 0.96 (0.83, 1.12) | 0.627 | 0.879 | 0.91 (0.71, 1.16) | 0.444 | 0.779 |
| ∑HMWP | **1.19 (1.02, 1.39)** | **0.026** | 0.252 | **1.13 (1.00, 1.28)** | **0.043** | 0.387 | 1.06 (0.97, 1.15) | 0.185 | 0.783 | 1.03 (0.91, 1.17) | 0.620 | 0.779 |
| ∑LMWP | 1.09 (0.96, 1.24) | 0.192 | 0.432 | 1.08 (0.98, 1.20) | 0.123 | 0.390 | 1.01 (0.94, 1.08) | 0.808 | 0.879 | 1.02 (0.93, 1.13) | 0.650 | 0.779 |
| ∑PAEs | 1.13 (0.98, 1.30) | 0.094 | 0.351 | 1.10 (0.99, 1.23) | 0.085 | 0.390 | 1.02 (0.95, 1.10) | 0.628 | 0.879 | 1.04 (0.93, 1.16) | 0.487 | 0.779 |
| High-scores vs. Low-scores | | | | | | | | | | | | |
| DPHP | 1.19 (0.82, 1.71) | 0.365 | 0.666 | 1.03 (0.76, 1.41) | 0.833 | 0.937 | 1.29 (1.00, 1.67) | 0.054 | 0.486 | 1.05 (0.79, 1.40) | 0.724 | 0.832 |
| BBOEP | 0.95 (0.72, 1.24) | 0.684 | 0.770 | 0.98 (0.79, 1.20) | 0.817 | 0.937 | 0.95 (0.79, 1.14) | 0.563 | 0.817 | 0.97 (0.78, 1.20) | 0.749 | 0.832 |
| BEHP | 1.32 (0.99, 1.77) | 0.061 | 0.366 | 1.06 (0.85, 1.33) | 0.594 | 0.937 | **1.22 (1.00, 1.49)** | **0.047** | 0.486 | 1.11 (0.89, 1.37) | 0.362 | 0.724 |
| DBP | 0.78 (0.55, 1.12) | 0.181 | 0.407 | 1.07 (0.82, 1.38) | 0.628 | 0.937 | 0.83 (0.65, 1.06) | 0.129 | 0.774 | 0.78 (0.59, 1.04) | 0.090 | 0.536 |
| TCEP | 0.99 (0.74, 1.33) | 0.949 | 0.949 | 1.24 (0.98, 1.56) | 0.071 | 0.426 | 1.02 (0.82, 1.26) | 0.887 | 0.888 | 0.87 (0.68, 1.11) | 0.255 | 0.574 |
| BCIPP | 0.74 (0.50, 1.09) | 0.125 | 0.407 | 0.78 (0.57, 1.07) | 0.120 | 0.511 | 0.86 (0.65, 1.13) | 0.280 | 0.817 | 1.04 (0.78, 1.39) | 0.786 | 0.832 |
| ∑cl-OPEs | 1.30 (0.93, 1.83) | 0.126 | 0.407 | 1.10 (0.83, 1.46) | 0.520 | 0.936 | 0.93 (0.71, 1.22) | 0.590 | 0.817 | 1.02 (0.77, 1.35) | 0.910 | 0.910 |
| ∑di-OPEs | 0.79 (0.57, 1.09) | 0.144 | 0.407 | 1.16 (0.84, 1.61) | 0.357 | 0.803 | 1.18 (0.92, 1.51) | 0.192 | 0.817 | 1.08 (0.78, 1.48) | 0.654 | 0.832 |
| ∑OPEs | 0.88 (0.56, 1.36) | 0.561 | 0.770 | 1.14 (0.81, 1.62) | 0.457 | 0.914 | 1.08 (0.81, 1.44) | 0.583 | 0.817 | 1.06 (0.75, 1.49) | 0.735 | 0.832 |
| MMP | **1.86 (1.07, 3.24)** | **0.028** | 0.252 | 0.81 (0.57, 1.15) | 0.232 | 0.696 | 1.10 (0.90, 1.35) | 0.362 | 0.817 | 1.16 (0.90, 1.50) | 0.246 | 0.574 |
| MEP | 0.76 (0.31, 1.84) | 0.540 | 0.770 | 0.99 (0.76, 1.29) | 0.938 | 0.980 | 0.89 (0.70, 1.13) | 0.350 | 0.817 | 0.82 (0.64, 1.06) | 0.126 | 0.536 |
| MBP | 1.19 (0.51, 2.79) | 0.682 | 0.770 | 1.04 (0.78, 1.40) | 0.783 | 0.937 | 0.98 (0.71, 1.34) | 0.888 | 0.888 | 0.88 (0.63, 1.23) | 0.470 | 0.808 |
| MEHP | 0.92 (0.64, 1.33) | 0.652 | 0.770 | 1.39 (1.00, 1.93) | 0.051 | 0.426 | 0.87 (0.57, 1.33) | 0.520 | 0.817 | 1.33 (0.83, 2.14) | 0.242 | 0.574 |
| MEOHP | 1.19 (0.79, 1.78) | 0.407 | 0.666 | 0.68 (0.45, 1.03) | 0.069 | 0.426 | 1.17 (0.67, 2.05) | 0.585 | 0.817 | 2.06 (0.87, 4.87) | 0.102 | 0.536 |
| MEHHP | 1.02 (0.66, 1.58) | 0.932 | 0.949 | 1.25 (0.80, 1.96) | 0.320 | 0.803 | 0.95 (0.58, 1.56) | 0.853 | 0.888 | 0.80 (0.35, 1.80) | 0.582 | 0.832 |
| ∑HMWP | **1.93 (1.20, 3.11)** | **0.007** | 0.126 | 1.32 (0.91, 1.90) | 0.142 | 0.511 | 1.09 (0.84, 1.42) | 0.522 | 0.817 | **1.67 (1.19, 2.34)** | **0.003** | 0.054 |
| ∑LMWP | 1.19 (0.79, 1.77) | 0.407 | 0.666 | 0.94 (0.67, 1.32) | 0.716 | 0.937 | 1.03 (0.82, 1.30) | 0.775 | 0.888 | 1.12 (0.81, 1.53) | 0.494 | 0.808 |

(*continued on next page*)

**Table 2** (*continued*)

| Chemical | Across pregnancy | | | First trimester | | | Second trimester | | | Third trimester | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *OR* (95%*CI*) | *p*-value | *q*-value | *OR* (95%*CI*) | *p*-value | *q*-value | *OR* (95%*CI*) | *p*-value | *q*-value | *OR* (95%*CI*) | *p*-value | *q*-value |
| ∑PAEs | 1.35 (0.88, 2.07) | 0.167 | 0.407 | 1.00 (0.69, 1.44) | 0.980 | 0.980 | 1.03 (0.81, 1.32) | 0.782 | 0.888 | 1.28 (0.92, 1.79) | 0.149 | 0.536 |

Note: Model was adjusted for maternal age, pre-pregnancy BMI, gestational weight gain, per capita monthly family income, maternal educational level, delivery mode, parity, maternal IQ, infant sex, and breastfeeding mode; The whole pregnancy is defined as geometric mean concentration for three trimesters; q-values were corrected by Benjamini-Hochberg method.

95%*CI*: 1.19, 2.34). After *FDR* multiple corrections, the association was not statistically significant.

### 3.5. Stratified analysis by child sex and maternal vitamin D levels: associations between prenatal OPEs and PAEs exposure and children's ASD symptom trajectory

After adjusting for confounding factors, among boys, DBP concentrations throughout pregnancy were negatively associated with both the moderate- and high-score groups ($p_{sex\text{-}int} < 0.05$). BCIPP was negatively associated with the high-score group, while TCEP and MMP were positively associated with the high-score group, and ∑HMWP was positively associated with the moderate-score group. Among girls, only DPHP showed a positive association with the moderate-score group ($p_{sex\text{-}int} < 0.05$); TCEP was negatively associated with the high-score group, whereas ∑di-OPEs ($p_{sex\text{-}int} < 0.05$), MEHP, and ∑HMWP were all positively associated with the high-score group. In the first trimester, MEOHP and ∑HMWP were positively associated with the moderate-score group in boys, whereas ∑LMWP showed a negative association; TCEP was positively associated with the high-score group. In girls, DPHP and ∑LMWP were positively associated with the moderate-score group, and DBP ($p_{sex\text{-}int} < 0.05$) and MEHP were positively associated with the high-score group. In the second trimester, DBP and BCIPP in boys were negatively associated with the high-score group ($p_{sex\text{-}int} < 0.05$); in girls, DPHP was positively associated with the moderate-score group, and BEHP, ∑di-OPEs ($p_{sex\text{-}int} < 0.05$), and ∑OPEs ($p_{sex\text{-}int} < 0.05$) were positively associated with the high-score group. In the third trimester, BCIPP in boys was positively associated with the moderate-score group, DBP was negatively associated with the high-score group, and ∑HMWP was positively associated with the high-score group; in girls, TCEP was negatively associated with the high-score group, whereas ∑HMWP was positively associated with the high-score group. Detailed results are presented in Tables S5-S8.

Logistic regression models revealed that among mothers with vitamin D deficiency during pregnancy, prenatal exposure to MEOHP, ∑HMWP, ∑LMWP, ∑PAEs, and MEHP was positively associated with ASD symptom trajectories in all children, while DBP showed a negative association. However, among mothers without vitamin D deficiency during pregnancy, only TCEP showed a positive association, and no other compounds were found to be associated with ASD symptom trajectories. Among boys, prenatal exposure to BCIPP, MBP, MEOHP, and ∑HMWP was positively associated with ASD symptom trajectories in children born to mothers with vitamin D deficiency; however, among mothers without vitamin D deficiency, only BCIPP showed a negative association. Among girls, prenatal exposure to DPHP and MEHP was positively associated with ASD symptom trajectories in children born to mothers with vitamin D deficiency; among mothers without vitamin D deficiency, DPHP and MBP were positively associated with the moderate-score group. Detailed findings are shown in Fig. 2. The QGC model indicates that in the group without vitamin D deficiency during pregnancy, for every additional quartile increase in the mixture concentration of OPEs and PAEs throughout pregnancy, the risk of ASD symptoms decreased by 0.94 times (95%*CI*: 0.89, 0.99) in the moderate-score group (**Table S9**).

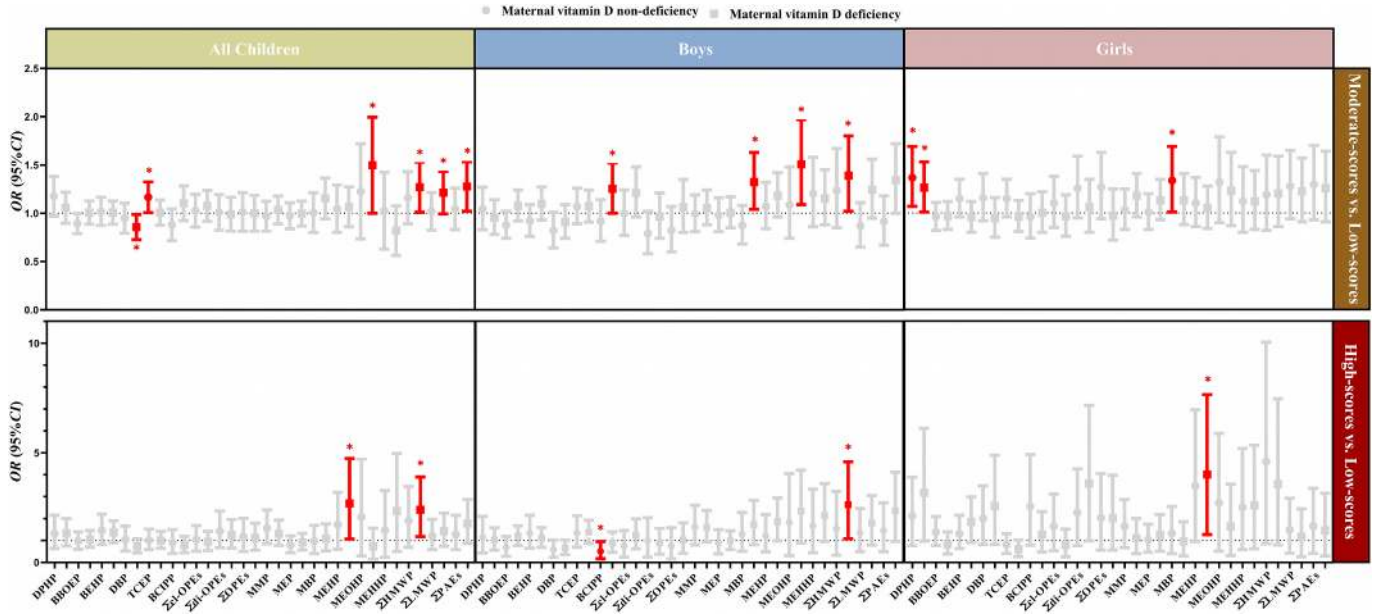


**Fig. 2.** Association between maternal OPEs and PAEs in the whole pregnancy with ASD symptom trajectories after maternal vitamin D status. Model was adjusted for maternal age, pre-pregnancy BMI, gestational weight gain, per capita monthly family income, maternal educational level, delivery mode, parity, maternal IQ, infant sex, and breastfeeding mode; The whole pregnancy is defined as geometric mean concentration for three trimesters; *$p < 0.05$, **$p < 0.01$, #*p-FDR* $< 0.05$。.

### 3.6. Association between co-exposure to OPEs and PAEs and children's ASD symptom trajectory

We employed QGC and BKMR models to analyze the association between co-exposure to OPEs and PAEs during pregnancy and preschoolers' ASD symptom trajectory, adjusting for relevant confounding factors. The QGC model revealed that for each interquartile increase in the mixture concentration of OPEs and PAEs throughout pregnancy, the risk of the moderate-score group increased by 1.07-fold (95% *CI*: 1.05, 1.10), with MEOHP having the highest positive weight (0.326). Furthermore, higher concentrations of co-exposure to OPEs and PAEs were positively associated with the high-score group, although this association was not statistically significant in the QGC model (*OR* = 1.32, 95% *CI*: 0.99, 1.75), with MEHP showing the highest positive weight (0.516). Fig. 3 shows detailed results**.**

The BKMR model of the overall mixture showed that mixed exposure to OPEs and PAE metabolites was not significantly associated with the moderate-score and high-score groups of ASD symptom trajectories in preschool children, consistent with the QGC results (**Fig. S14**). Based on QGC findings, prenatal exposure to OPEs and PAEs was divided into two groups—positive and negative effects—for further BKMR analysis. **Table S10** presents the group posterior inclusion probabilities (groupPIPs) and conditional posterior inclusion probabilities (condPIPs) derived from the BKMR model for ASD symptom trajectory groups. The BKMR results indicated a positive association between co-exposure to OPEs and PAEs and the high-score group, with a significant positive correlation when concentrations exceeded the 60th percentile; MEOHP showed the strongest significance (condPIP = 0.609). A positive association was also observed between co-exposure and the moderate-score group, though it was not statistically significant (Fig. 4). **Fig. S15** illustrates the potential nonlinear exposure–response relationships for individual OPEs and PAEs, with concentrations of the other 11 chemicals fixed at the 50th percentile. Throughout pregnancy, MEHP showed a nonlinear association with the high-score group, consistent with previous RCS results; in the first trimester, BCIPP was nonlinearly associated with the moderate-score group, while MEOHP and MEHP showed such associations with the high-score group. In the second DBP and MBP were nonlinearly related to the moderate-score group, and TCEP showed a nonlinear association with the high-score group. In the third trimester, DPHP was nonlinearly associated with the moderate-score group, whereas TCEP and MEOHP were nonlinearly associated with the high-score group. When concentrations of the other 11 OPEs and PAEs were fixed at the 25th, 50th, and 75th percentiles, BCIPP at the 25th and 50th percentiles in early pregnancy showed negative associations with the moderate-score group, while DPHP at the 25th and 50th percentiles in the third trimester showed positive associations with the moderate-score group. Additionally, MEHP throughout pregnancy and MEOHP in the third trimester were positively associated with the high-score group (**Fig. S16**).

### 3.7. Association between OPEs and PAEs exposure during pregnancy and children's ASD symptom scores and their symptoms

Regarding ASD symptom scores (continuous variable), GLM results showed that, after adjusting for confounding factors, throughout pregnancy, BEHP, MEOHP ($q$-value < 0.05), ∑HMWP ($q$-value < 0.05), and ∑PAEs were significantly positively associated with ASD symptom scores in children at age 3; BEHP was positively associated with ASD symptom scores at age 6, whereas BCIPP showed a negative association. GEE models indicated that BEHP, MEOHP, ∑HMWP, and ∑PAEs were positively associated with children's ASD symptom scores, whereas DBP was negatively associated. In the first trimester, TCEP was negatively associated with ASD symptom scores in 6-year-old children ($q$-value < 0.05), whereas MEHHP and ∑LMWP were positively associated. GEE results showed that ∑HMWP was positively associated with children's ASD symptom scores, whereas BCIPP was negatively associated. In the second trimester, MEOHP ($q$-value < 0.05), ∑HMWP ($q$-value < 0.05), and ∑PAEs were positively associated with ASD symptom scores in 3-year-olds; DPHP and BEHP were positively associated with ASD scores in 5- and 6-year-olds, respectively, whereas DBP (in 3-year-olds), BCIPP (in 6-year-olds), and MEP (in 3-year-olds) ($q$-value < 0.05) were negatively associated. GEE results indicated that BEHP and MEOHP were positively associated with children's ASD symptom scores, whereas DBP and MEP were negatively associated. In the third trimester, BEHP (in 3-year-olds), ∑HMWP (in 3-year-olds), and MEOHP (in 5-year-olds) were positively associated with ASD symptom scores. GEE results showed that MEOHP was positively associated with children's ASD symptom scores. Specific details are provided in Tables S11-S14.

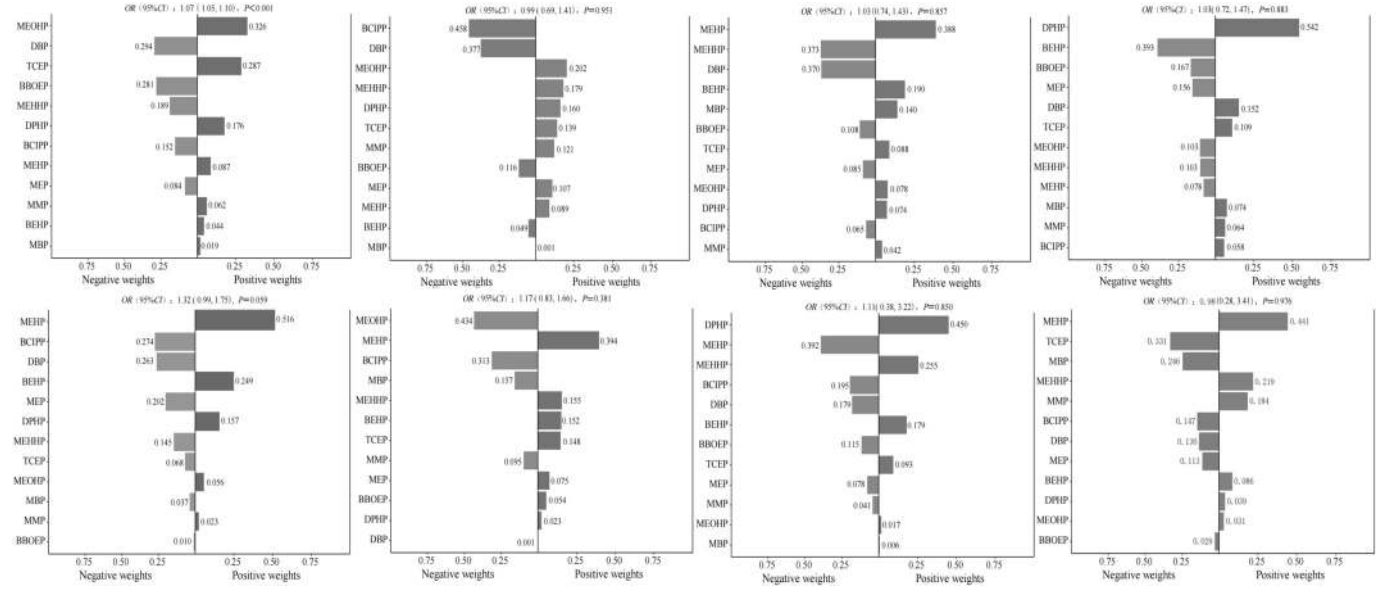


**Fig. 3.** The impact of mixed exposure to OPEs and PAEs on the ASD symptom trajectory of preschoolers by quantile-based g-computation model. Model was adjusted for maternal age, pre-pregnancy BMI, gestational weight gain, per capita monthly family income, maternal educational level, delivery mode, parity, maternal IQ, infant sex, and breastfeeding mode; The whole pregnancy is defined as geometric mean concentration for three trimesters; The top row represents the group with moderate-scores in the children's ASD symptom trajectory, and the bottom row represents the high-score group.

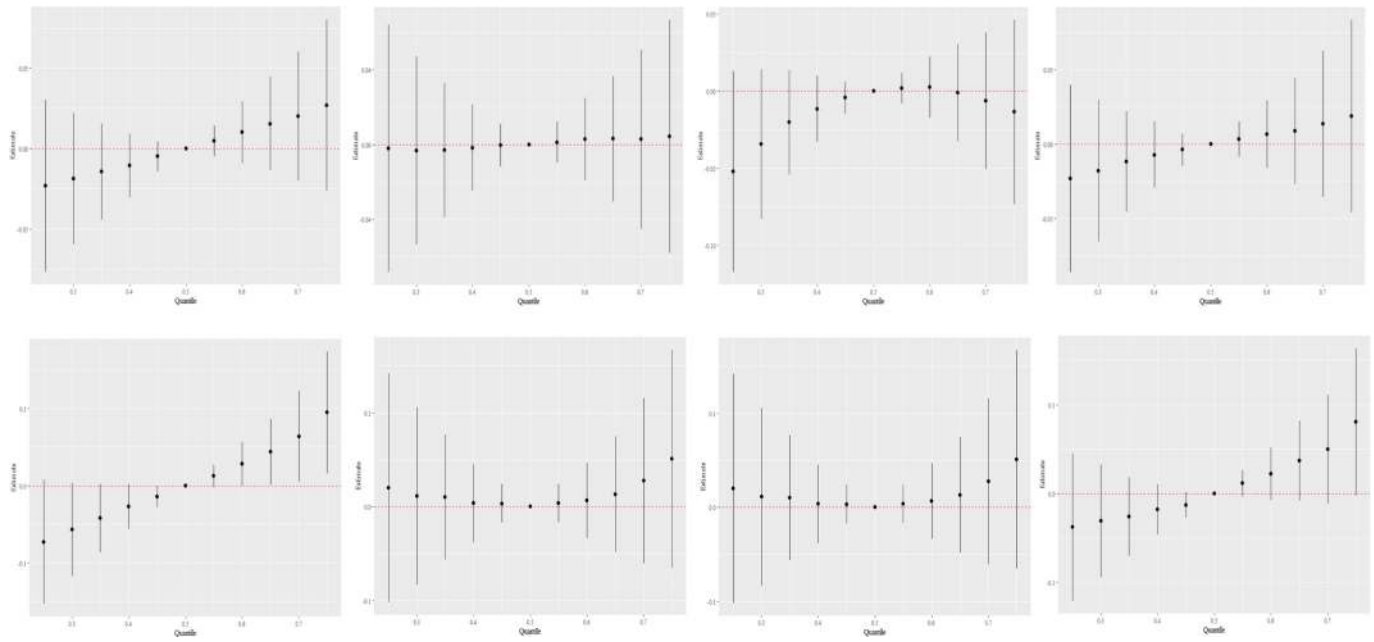

**Fig. 4.** The impact of mixed exposure to OPEs and PAEs on the ASD symptom trajectory of preschool by directional stratified Bayesian kernel machine regression model. Model was adjusted for maternal age, pre-pregnancy BMI, gestational weight gain, per capita monthly family income, maternal educational level, delivery mode, parity, maternal IQ, infant sex, and breastfeeding mode; The whole pregnancy is defined as geometric mean concentration for three trimesters; The top row represents the group with moderate-scores in the children's ASD symptom trajectory, and the bottom row represents the high-score group.

Regarding ASD symptoms (binary variable), throughout the pregnancy, GLM results indicated that BEHP ($q$-value $< 0.05$), ∑cl-OPEs, ∑diOPEs, ∑OPEs, and ∑HMWP ($q$-value $< 0.05$) may increase the risk of ASD symptoms in 3-year-old children; MEHP may increase the risk in 5-year-olds; MMP and ∑PAEs may increase the risk in 6-year-olds, while BCIPP and ∑cl-OPEs showed negative associations. GEE models suggested that BEHP and ∑HMWP may increase the risk of ASD symptoms in children, whereas MEP showed a negative association. In the first trimester, ∑cl-OPEs, ∑diOPEs, and ∑OPEs ($q$-value $< 0.05$) may increase the risk of ASD symptoms in 3-year-olds; ∑HMWP may increase the risk in 6-year-olds, while BCIPP and ∑cl-OPEs were negatively associated. GEE results indicated that BEHP and ∑diOPEs may increase the risk of ASD symptoms in children. In the second trimester, MEOHP may increase the risk in 3-year-olds, while DBP and MEP were negatively associated; DPHP and MMP may increase the risk in 6-year-olds, while BCIPP was negatively associated. GEE results showed that BEHP may increase the risk of ASD symptoms in children, while DBP and MEP were negatively associated. In the third trimester, MEOHP and ∑HMWP ($q$-value $< 0.05$) may increase the risk of ASD symptoms in 3-year-olds; ∑OPEs may increase the risk in 6-year-olds, while BEHP showed a negative association. GEE results indicated that ∑HMWP may increase the risk of ASD symptoms in children. Specific details are provided in **Table S15-S18**.

### 3.8. Sensitivity analysis

After excluding premature infants, the only significant finding was a positive correlation between MEHP and the high-score group. No other results showed significant differences. In addition to the existing confounding factors, this study also included maternal smoking/drinking before or during pregnancy and, on this basis, folic acid supplementation during pregnancy. The results remained stable. Sensitivity analyses incorporating IPW weights (derived as 1/posterior probability) into a multinomial logistic regression in SPSS yielded effect estimates that were directionally consistent with the primary results, mitigating concerns about trajectory misclassification. For details, see **Table S19**. Overall, the directional results from QGC in the medium-score group were highly stable. In the high-score group, some analytes were more sensitive to quantile settings, but the continuous exposure model was generally consistent with the main analysis results, indicating that the directional structure identified by QGC is generally robust (**Table S20**).

## 4. Discussion

We found that MEOHP and ΣHMWP throughout pregnancy increased the risk of the moderate-score group in preschool children's ASD symptom scores, whereas MMP and ΣHMWP increased the risk of the high-score group. First trimester: ΣHMWP was positively correlated with the moderate-score group, whereas BCIPP was negatively correlated. Second trimester: BEHP concentration was positively correlated with the high-score group. Third trimester: DPHP and BCIPP concentrations were positively correlated with the moderate-score group, and ΣHMWP was positively correlated with the high-score group. Among boys, ΣHMWP throughout pregnancy was positively associated with the moderate-score group (DBP: negative), and TCEP and MMP were positively associated with the high-score group (DBP and BCIPP: negative). In girls, DPHP throughout pregnancy was positively correlated with the moderate-score group; MEHP and Σdi-OPEs were positively correlated with the high-score group, whereas TCEP showed a negative association. Maternal vitamin D levels modified these associations, with women with vitamin D deficiency showing significantly higher risks. Mixture models indicated that QGC identified increased risk for the moderate-score group with co-exposure to OPEs and PAEs, whereas BKMR showed increased risk for the high-score group when mixture concentrations exceeded the 60th percentile. GEE analyses demonstrated positive associations of BEHP and ΣHMWP with both ASD scores and symptoms, MEOHP and ΣPAEs with scores, and negative associations for DBP (scores) and MEP (symptoms).

In our study, the detection rates of ASD symptoms were 8.8%, 5.1%, and 4.2%, respectively, which were significantly higher than the prevalence rates reported in other studies. A systematic review of 71 global studies showed that the incidence of ASD was 1.19% (Zeidan et al., 2022), and the latest research also indicated that the prevalence rate was approximately 1090.2 per 100,000 people, with the highest

prevalence rate in Japan (1559.5 per 100,000 people) and the lowest in Bangladesh (588.2 per 100,000 people) (Zeidan et al., 2022). The reason for the higher rate in this study is that it was a screening-based ASD rather than a diagnostic one. Although the scale does not have the high sensitivity and specificity of clinical diagnostic results, we cannot deny that, in large-scale epidemiological surveys, initial screening with the scale can improve efficiency and provide preliminary identification of the disease (Gao et al., 2024). The differences between our results and those of other studies are also partly due to age. Previous studies have shown that the prevalence rate varies among different age groups of children (Jia et al., 2025), and the reason is that ASD usually presents early manifestations such as language delay and social withdrawal, which usually appear at the age of 2 to 3 years, and the diagnosis rate is higher in children under 5 years old (Zwaigenbaum et al., 2015). At the same time, this study found that the detection rate of ASD gradually decreases with age, consistent with previous studies (Zwaigenbaum et al., 2015). Finally, it must be acknowledged that the results of this study are based on parents' reports, which will inevitably be influenced by the parents' educational level and cultural background. In birth cohort studies, repeated measurements at multiple time points of children's ASD changes trajectories to better observe the trend of ASD symptoms in children and adolescents with age. Compared with single-time-point measurements, the symptom change trajectory can comprehensively capture information such as timing and rate (Li et al., 2024; Wang et al., 2025).

Currently, there are 2 studies on the association between prenatal OPEs and childhood ASD. Choi et al.'s study did not find a statistically significant association between the two (Choi et al., 2024). However, the latest study, which included 4159 subjects in a birth cohort, found that prenatal exposure to OPEs would increase the risk of ASD in the offspring (Ames et al., 2025). Currently, there are 11 studies on the association between prenatal PAEs exposure and childhood ASD (Al-Saleh et al., 2025; Braun et al., 2014; Day et al., 2021; Gao et al., 2024; Haggerty et al., 2021; Miodovnik et al., 2011; Oulhote et al., 2020; Shin et al., 2018; Tsai et al., 2023; Uldbjerg et al., 2024; van den Dries et al., 2021). Among them, 6 studies showed that prenatal exposure to PAEs would increase the occurrence of ASD in the offspring, while the remaining 5 studies did not find a statistical association between the two. Two studies from Canada (Oulhote et al., 2020) and China (Gao et al., 2024) showed that prenatal supplementation with nutrients (folic acid and vitamin D) could reduce PAE-induced neurotoxicity, with a notable sex difference. One study from the United States (Day et al., 2021) and two studies from China (Gao et al., 2024; Tsai et al., 2023) reported a cumulative, mixed effect across the compounds. This study also found a cumulative, mixed effect, suggesting that we should pay attention to multiple compound exposures in the real world rather than fall into the “cocktail effect”. This also highlights the public health significance, namely, the need to conduct large-scale population screening and further diagnose individuals who test positive. However, currently, the screening questionnaires for ASD are often limited, which suggests that future research can further focus on the development of ASD screening questionnaires to improve the efficiency of ASD screening in the population. Of course, we are well aware that this study only used a scale to measure ASD, which is often insufficient for determining the results. This suggests that future studies should use more professional clinical diagnostic methods to further verify the results of this study. Currently, there are few studies that combine OPEs and PAEs exposure. Our previous team's research confirmed that combined prenatal exposure to OPEs and PAEs would impair offspring cognition (Lu et al., 2024). This study also found that the combined exposure of the two substances would increase the risk of ASD symptoms in preschool children.

This study revealed significant differences in vitamin D levels across the three trimesters of pregnancy: the concentration was highest in the second trimester (17.85 ± 8.23 ng/ml), followed by the third trimester (22.84 ± 10.45 ng/ml), and lowest in the first trimester (20.20.43 ± 10.09 ng/ml). The vitamin D deficiency rates in the first, second, and third trimesters were 68.2%, 46.0%, and 57.2%, respectively (**Table S21**). These rates were close to the 54% reported in a *meta*-analysis covering over 54,000 pregnant women, and the study also found that the prevalence was higher in South Asia, the Middle East, and parts of Africa (Saraf et al., 2016). These data indicate that vitamin deficiency during pregnancy is widespread, and recent studies have consistently shown that the serum vitamin D concentration of pregnant women is the main determinant of the vitamin D status of newborns and infants, as cord blood and early postpartum levels are closely related to the maternal circulating concentration at delivery; that is, vitamin deficiency during pregnancy can lead to vitamin deficiency in infants (Płudowski et al., 2023) In our study, vitamin D levels during the third trimester and throughout the entire pregnancy were negatively correlated with 3-year ASD symptoms (**Table S22**), and stratified results for vitamin D levels throughout the entire pregnancy showed that the vitamin D concentration during pregnancy would modify the effects of OPEs and PAEs exposure on the trajectory of ASD symptom scores, which is consistent with previous research results (Bandini et al., 2026; Guetterman et al., 2026). The positive role of vitamin D in fetal brain development has a biological rationale, and the related mechanisms may include: regulating neuronal proliferation and migration; regulating immunity and inflammation (by reducing the levels of pro-inflammatory cytokines in the mother's intestine and the fetal brain); and synthesizing and balancing dopaminergic, GABAergic, and serotonergic neurotransmitters and neurotrophic factors (Bandini et al., 2026; Testa et al., 2012). Because vitamin D deficiency may be confounded by factors such as poverty, diet, and indoor plastic pollution that are not measured, we compared compound concentrations between the deficient and adequate groups. For most compounds, there were no statistically significant differences between the two groups (**Table S23**). Although we have corrected for per capita monthly family income to control for socioeconomic confounding, the results of this study should be interpreted with caution. Future studies in representative populations are needed to conduct large-scale intervention studies to clarify the actual benefits of prenatal vitamin D supplementation for maternal and infant health.

The mechanisms by which prenatal exposure to OPEs/PAEs affects the occurrence of ASD in offspring remain unclear. However, current studies indicate three potential mechanisms: endocrine disruption, oxidative stress, and placental effects. Animal experiments have shown that PAEs can induce hypothyroidism, thereby leading to ASD (Miodovnik et al., 2014). At the same time, population studies have found that prenatal exposure to OPEs/PAEs can affect the thyroid function of both the mother and the offspring (Li et al., 2025; Yin et al., 2024). Thyroid hormone in the mother plays an important role in the normal brain development of the offspring, and some studies have found that a lack of thyroid hormone in the pregnant mother is associated with an increased risk of ASD in children (Levie et al., 2018). Meanwhile, OPEs can alter methylation pathways, including methionine synthase activity, and oxidative stress is associated with methionine synthase activity, thereby influencing the occurrence of ASD (Choi et al., 2024). Some epidemiological studies have found that prenatal exposure to OPEs may increase oxidative stress, which may in turn mediate the association between prenatal OPEs and fetal thyroid hormone (Yao et al., 2021). The placenta is not only the hub for material exchange between mother and fetus, but also an endocrine and neuroactive organ that directly affects fetal brain development by secreting hormones and neurotransmitters. This “placenta-brain” axis regulates the biological dialogue between mother and fetus (Kratimenos & Penn, 2019), and its dysfunction can alter neurogenesis, neuronal migration, and synaptic maturation (Vacher et al., 2021). Moreover, the placenta acts as a biological recorder of maternal environmental exposure, converting stress, diet, infection, and circadian rhythm disorders into epigenetic and metabolic imprints. These imprints may persist after birth and affect the risk of individual mental disorders. Studies have shown that placental

DNA methylation, gene expression, and metabolomics characteristics are influenced by genetic variations, are sensitive to environmental changes, and have quantifiable associations with neurodevelopmental outcomes such as ASD diagnosis, social behavior, and cognitive ability (Guglielmo et al., 2025). Current research has found that prenatal exposure to OPEs/PAEs causes a series of genomic changes in the placenta, including changes in gene expression, DNA methylation, transcriptomics, and metabolism (Lapehn et al., 2025; Lu et al., 2023; Luan et al., 2025).

The placenta plays a mediating role in the neurotoxicity of OPEs, with higher accumulation of OPEs in the male placenta, leading to decreased serotonin in the anterior brain, inflammation, and endocrine dysfunction (Hernandez-Castro et al., 2023). PAEs exhibit complex interactions with sex hormones, which may be the main reason for the observed sex-specific responses, and their effects vary with the exposure time window. For example, early-life exposure to endocrine disruptors is closely related to an increase in male behavioral defects, while females may be more sensitive to stress in later life (Al-Saleh et al., 2025). Under the stress of the intrauterine environment, the placenta exhibits significant sex-differential functional adaptations. Compared with the placentas of female fetuses, those of male fetuses exhibit lower adaptability and plasticity (Miller et al., 2020). Environmental factors can alter placental epigenetics and gene expression, thereby affecting placental development and function (Liang et al., 2010). Therefore, sex-specific disparity in gene expression in placental tissue may also be a key mechanism underlying sex-specific adverse maternal and infant outcomes caused by environmental exposure (Rosenfeld, 2015). The specific mechanism remains unclear, and the sex-specific disparity findings should be interpreted cautiously, indicating that future research should explore this relationship in greater depth.

The advantages of this study: First, this study was based on the MABC prospective birth cohort, which ensured the temporal sequence between prenatal exposure and childhood outcomes and thus strengthened the evidence supporting a potential causal association; Second, this study repeatedly measured urine from pregnant mothers for OPEs and PAEs at multiple time points during pregnancy, which can further explore sensitivity across pregnancy periods, and the assessment of ASD symptoms in the offspring is also multi-time point and fitted trajectories, allowing for not only the association at a single age point but also the changes in ASD symptoms during the preschool period; Third, this study discusses OPEs and PAEs together, for the first time clarifying the association between mixed exposure to OPEs and PAEs during pregnancy and the trajectory of ASD symptoms in preschool children; Finally, this study explores sex differences in the association and the effect-modification role of prenatal vitamin D in the association through stratified analysis.

However, this study still has some limitations: First, although this study included a series of confounders, residual confounding factors may remain, such as exposure to OPEs and PAEs during childhood, physical activity, and sleep patterns. Second, this study uses parent-completed questionnaires to assess children's ASD. Although these scales are suitable for large-scale epidemiological surveys, their sensitivity and specificity are limited. Moreover, among the 2400 mother–-child dyads included in our study, response rates for ASD symptom assessment at ages 3, 5, and 6 were 97.2%, 75.5%, and 86.2%, respectively, which may introduce bias when interpreting model results by age stratum. Therefore, exercise caution when comparing models across age strata. Third, this study detected only 6 OPEs and 6 PAEs, the tip of the iceberg of numerous compounds, leading to the neglect of more complex effects. Fourth, we corrected urinary dilution using conventional creatinine adjustment. Because urinary creatinine is influenced by factors beyond hydration status, this approach may introduce bias, and residual bias cannot be excluded. Future studies should consider covariate-adjusted standardization to assess the robustness of these findings (O'Brien et al., 2016). Fifth, because geographic location, lifestyle habits, and the level of environmental pollutant control vary across regions, exposure levels of OPEs and PAEs in the population also vary. Our study is limited to Ma'anshan, which further limits the generalizability of the results. Sixth, our study is based on a birth cohort, and each round of follow-up will have cases of loss to follow-up. Although we compared the basic demographic characteristics of the included and excluded mother–child pairs, this may still affect the reliability of the study results. Thus, these findings offer preliminary epidemiological evidence for a potential link between prenatal OPE/PAE exposure and ASD symptom trajectories in preschoolers, warranting cautious interpretation. Prospective cohorts and animal studies are needed to confirm causality and elucidate sex-specific mechanisms.

## 5. Conclusion

In summary, based on MABC, our results indicate that exposure to OPEs and PAEs during pregnancy may increase the risk of high scores in offspring with ASD, and the association is trimester- and sex-specific. Moreover, this increased risk is more pronounced in pregnant women with vitamin D deficiency. The combined effect of these two substances is also significant. There are relatively few studies on the association between prenatal exposure to OPEs/PAEs and offspring ASD, and the biological mechanism of this association is not yet clear. Nevertheless, this study also suggests certain public health significance. That is, “plastic reduction” actions in daily life are necessary, especially during pregnancy. Reducing exposure to these two types of substances during pregnancy has important implications for better reproductive health and can promote the neural development of the offspring.

## CRediT authorship contribution statement

**Xing Wang:** Conceptualization, Formal analysis, Software, Writing – original draft. **Juan Tong:** Conceptualization, Formal analysis, Software editing. **Ling Luo:** Formal analysis, Software editing. **Ping Lv:** Data curation, Formal analysis. **Qizheng Huang:** Data curation, Software. **Yimin Zheng:** Data curation, Software. **Shuaishuai Zhou:** Data curation, Software. **Hong Gan:** Data curation, Investigation. **Menglong Geng:** Data curation, Investigation. **Shuman Tao:** Project administration, Supervision. **Xingyong Tao:** Project administration, Supervision. **Guopeng Gao:** Project administration, Supervision. **Shuangqin Yan:** Project administration, Supervision. **Xiaoyan Wu:** Conceptualization, Project administration, Supervision. **Kun Huang:** Conceptualization, Project administration, Supervision. **Yunxia Cao:** Conceptualization, Project administration, Supervision. **Mengjuan Lu:** Data curation, Formal analysis, Funding acquisition. **Hui Gao:** Data curation, Formal analysis, Funding acquisition. **Fangbiao Tao:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

## Ethics declaration

Written informed consent to take part in the study and to publish the article has been obtained from all participants or their legal representatives. The privacy rights of participants have been observed.

This study included organ or tissue donors.

This study includes human biological material and consent was obtained by donors, or their next of kin or legal representatives, for use in this study and for publication of the article. The samples used in this research were not sourced from executed prisoners or prisoners of conscience.

## Studies in human

This study was performed in compliance with relevant laws, regulatory frameworks and guidelines where the research took place.

This study was approved by the The Ethics Committee of Anhui Medical University.

(Approval No. 20131195)

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2026.110510.

## Data availability

The authors do not have permission to share data.

Data from all participants included in this study were anonymized and were available only to authorized members of the study team.

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