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Fetal Organophosphate Pesticide Exposure and Child Adiposity Measures at 10 Years of Age in the General Dutch Population

Sophia M. Blaauwendraad,[1,2] Danielle R. Stevens,[3] Michiel A. van den Dries,[1,4] Romy Gaillard,[1,2] Anjoeka Pronk,[5] Suzanne Spaan,[5] Kelly K. Ferguson,[3] and Vincent W.V. Jaddoe[1,2]

[1]Generation R Study Group, Erasmus Medical Center (Erasmus MC), University Medical Center, Rotterdam, the Netherlands
[2]Department of Pediatrics, Erasmus MC, University Medical Center, Rotterdam, the Netherlands
[3]Epidemiology Branch, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Durham, North Carolina, USA
[4]Department of Child and Adolescent Psychiatry, Erasmus MC, University Medical Center, Rotterdam, the Netherlands
[5]Department Risk Analysis for Products in Development, Netherlands Organization for Applied Scientific Research, Utrecht, the Netherlands

Background: Fetal exposure to organophosphate (OP) pesticides might lead to fetal metabolic adaptations, predisposing individuals to adverse metabolic profiles in later life.

Objective: We examined the association of maternal urinary OP pesticide metabolite concentrations in pregnancy with offspring body mass index (BMI) and fat measures at 10 years of age.

Methods: Between 2002 and 2006, we included 642 mother–child pairs from the Generation R Study, a population-based prospective cohort study in Rotterdam, the Netherlands. We measured maternal urinary concentrations of OP pesticide metabolites, namely, dialkyl phosphates, including three dimethyl and three diethyl phosphates in early-, mid- and late-pregnancy. At 10 years of age, child total and regional body fat and lean mass were measured through dual energy X-ray absorptiometry, and abdominal and organ fat through magnetic resonance imaging.

Results: Higher maternal urinary pregnancy-average or trimester-specific dialkyl, dimethyl, or diethyl phosphate concentrations were not associated with childhood BMI and the risk of overweight. In addition, we did not observe any association of dialkyl, dimethyl, or diethyl phosphate concentrations with total and regional body fat, abdominal visceral fat, liver fat, or pericardial fat at child age of 10 y.

Conclusion: We observed no associations of maternal urinary dialkyl concentrations during pregnancy with childhood adiposity measures at 10 years of age. Whether these associations develop at older ages should be further studied. https://doi.org/10.1289/EHP12267

Introduction

Organophosphate (OP) pesticides are a class of insecticides commonly used for agricultural and landscape pest control.[1] The general population is regularly exposed to these pesticides through ingestion of food and, to a lesser extent, through household insecticide use.[2,3] OP pesticide metabolites are able to pass the placental barrier, causing direct fetal exposure, and may interfere with cell differentiation, disrupt thyroid function and disturb the placental transport of nutrients.[4–6] In addition, results from animal studies suggest that these chemicals might affect the cellular metabolism of carbohydrates and lipids, increase insulin resistance, disturb glucose homeostasis, and increase oxidative stress.[7] These fetal developmental adaptations in response to maternal OP pesticides exposure may adversely affect fetal and childhood metabolic development. Among 784 pregnant women within the population-based Generation R Study, higher OP metabolite concentrations in pregnancy were associated with restricted fetal weight and length growth, which are risk factors for higher child body mass index (BMI) in later life.[8,9] Two studies from a Danish prospective cohort that included ∼200 pregnant mother–child pairs reported that maternal occupational exposure to OPs during pregnancy, assessed by questionnaires, was associated with higher offspring BMI and fat percentage at 6–16 years of child age.[10,11] To the contrary, a study among 166 children from the general U.S. population reported no associations of maternal urinary third trimester OP metabolites with offspring BMI at 4–9 years of age.[12] Last, a European meta-analysis among 1,086 mother–child pairs using multi-exposure models reported no associations of maternal urinary OP metabolites measured once in pregnancy with childhood adiposity measures.[13] These inconsistencies might be explained by differences in measurement methods, levels of exposure and variation in sociodemographic, ethnic, and lifestyle characteristics of underlying populations.[14] Moreover, OP pesticide metabolites are highly variable over time, but they were measured only once in pregnancy.[15] In addition, no previous studies included visceral, pericardial, and liver fat outcomes when investigating the effect of maternal OP pesticide exposure on the offspring, even though visceral adipose tissue is more strongly associated with most metabolic risk factors than subcutaneous abdominal adipose tissue.[16]

We hypothesized that fetal exposure to OPs would lead to alterations in fetal metabolic development, causing adverse adiposity profiles in childhood. In a population-based prospective cohort study among 642 mother–child pairs, we assessed the associations of maternal urinary OP metabolite concentrations measured at three time points in pregnancy with child BMI, the risk of overweight or obesity, total and regional body fat, abdominal fat, and organ fat at 10 years of age.

Methods

Study Design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until adulthood in Rotterdam, the Netherlands.[17] Study approval was obtained by the medical ethical committee of the Erasmus Medical Center, University Medical Center, Rotterdam (MEC 198.782/2001/31). Mothers who had a delivery date of from April 2002 to January 2006 were invited to participate through their obstetric health care provider or applied on their own. Mothers were enrolled

Address correspondence to Vincent W.V. Jaddoe, The Generation R Study Group (Na 29-15), Erasmus University Medical Center, 24 P.O. Box 2040, 3000 CA Rotterdam, the Netherlands. E-mail: v.jaddoe@erasmusmc.nl.

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during pregnancy and received no incentives for participation other than the measurements, including fetal ultrasounds, which were performed as part of the study. All participants came from an urban environment given that Rotterdam is the second-largest city in the Netherlands. Written informed consent was obtained from all mothers. From February 2004 to January 2006, 800 women with childhood follow-up data available were selected to determine OP metabolite concentrations in maternal urinary samples, and 778 had urinary OP metabolite measurement at three time points in pregnancy. Of these, 642 mother–child pairs participated in the follow-up study at 10 y (Figure 1).

Urine OP Pesticide Metabolite Analysis

OP exposure was assessed by measuring six dialkyl phosphates (DAP) in maternal urine samples at three time points in pregnancy. DAP metabolites are breakdown products of most OPs, are nonspecific biomarkers of OP pesticides, and provide information on the total exposure to most parent OP pesticides.[18–20] Details of the maternal urine specimen collection have been described elsewhere.[21] Briefly, urine samples were collected in 100-mL polypropylene urine collection containers between 0800 and 2000 hours and were stored for a maximum of 20 h at 4°C before being frozen at −20 °C in 20-mL portions in polypropylene vials. They were kept on −20 °C until analysis and were transported on dry ice by airplane as a priority shipment to the Institut National de Santé Publique in Quebec (INSPQ), Canada. Here, the measurements of the DAP metabolites were performed using gas chromatography coupled with tandem mass spectrometry (GC-MS/MS).[22]

The six DAP metabolites included three dimethyl (DM) metabolites [dimethylphosphate (DMP), dimethylthiophosphate (DMTP), and dimethyldithiophosphate (DMDTP)] and three diethyl (DE) metabolites [diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP)], as previously described.[14] Molar concentrations were used to enable comparison with other studies. The three DM metabolites were summed as total DM, and the three DE metabolites as total DE. The molar sum of the DM and DE metabolite concentrations represent the total urinary DAP concentrations. The molar weights, limits of detection (LODs), percentage of metabolites above the LOD, and descriptive statistics of the metabolites in our study sample are shown in Tables S1 and S2.[14] The LODs were 0.26 μg/L for DMP, 0.40 μg/L for DMTP, 0.09 μg/L for DMDTP, 0.50 μg/L for DEP, 0.12 μg/L for DETP, and 0.06 μg/L for DEDTP. Values below the LOD were imputed with machine-reported values when available.[22] We included measured concentrations below the LOD in the analysis. In our study sample, a small number of concentrations were still missing owing to insufficient sample or machine error. The number of missing values per metabolite was as follows: DMP, 1 missing in the first trimester; DEDTP, 1 missing in the second and 1 missing in the third trimesters; DETP, 12, 19, and 14 missing in the first, second, and third trimesters, respectively; and DEP, 1 missing in the first trimester. To account for urinary dilution, DAP concentrations were expressed in nanomoles per gram creatinine.[14,23,24]

Childhood Measures of Adiposity

We used child adiposity measures at 10 years of age because this was, in our study population, the latest possible time point for assessing long-term effects of fetal pesticide exposure without any pubertal influences on growth or fat distribution. We

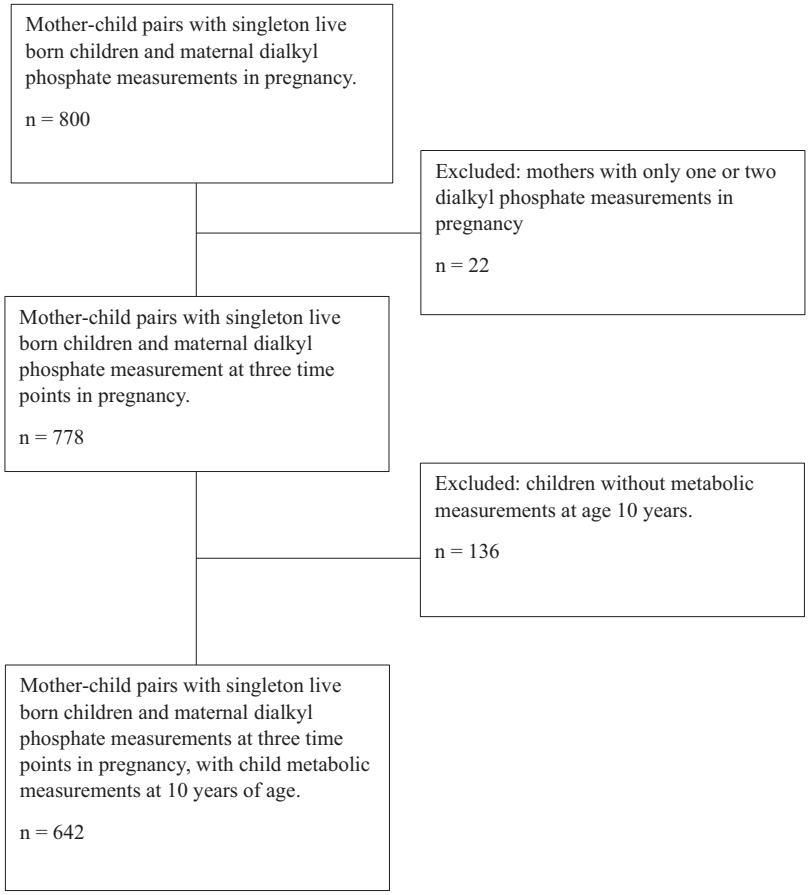

Figure 1. Flowchart of participants included in the study. Generation R Study, Rotterdam, the Netherlands, 2002–2006.

calculated BMI (in kilograms per meter squared) from height and weight, measured without shoes and heavy clothing. Participants were asked to undo themselves from cardigans or jumpers and to empty their pockets. Age- and sex- adjusted standard deviation scores (SDSs) were calculated using a Dutch reference growth chart (Growth Analyzer 4.0; Dutch Growth Research Foundation).[25] Childhood overweight and obesity were defined using the International Obesity Task Force cutoffs.[26] We obtained several measures of visceral adipose tissue because, in adults, excess visceral, pericardial, and liver fat have been linked to various cardiometabolic risk factors and abnormalities, irrespective of BMI.[16,27–30] In addition, in children, an android fat distribution has been associated with cardiovascular risk factors, such as an increased insulin resistance and higher serum cholesterol.[31] Total and regional body fat and lean mass (in kilograms) were measured using dual energy X-ray absorptiometry (DXA) scanning (iDXA; General Electric–Lunar).[32] Children were scanned in the supine position without shoes, heavy clothing, or metal objects. The android-to-gynoid fat mass ratio was calculated and used as a measure of waist-to-hip ratio.[32] Abdominal and organ fat were measured using a 3.0-Tesla magnetic resonance imaging (MRI) scanner (Discovery MR750w; General Electric Healthcare), using standard imaging and positioning protocols, as described previously.[17] Pericardial fat included epicardial and pericardial fat directly attached to the pericardium, ranging from the apex to the left ventricular outflow tract. Total visceral fat volume was generated by summing the volumes of the liver, abdominal and, if necessary, the femoral fat-only scans. Fat masses were obtained by multiplying the total volumes by the specific gravity of adipose tissue, 0.9 g/mL. The liver fat fraction was determined by averaging the mean signal intensities in four regions of interest of at least 4 cm^2 in the central portion of the hepatic volume. To enable comparison between the adiposity outcomes, we computed internal SDSs. To create measures of total fat and lean mass independent of height, we estimated the optimal adjustment by log–log regression analysis and subsequently calculated fat mass index (total fat mass/$height^4$), pericardial fat mass index (pericardial fat mass/$height^3$), visceral fat mass index (total and abdominal visceral fat mass index/$height^3$), and lean mass index (lean mass/$height^2$).[33,34]

Covariates

Information on maternal prepregnancy weight, parity, ethnicity, educational level, and household income was obtained through prenatal questionnaires. For ethnicity, women were categorized as Dutch, European or non-European (including Indonesian, Cape Verdian, Moroccan Surinamese, Turkish, African, American, Asian, and Oceanian).[17] Ethnic background was assessed in accordance with the country of birth of participants themselves and their parents.[17] If both parents were born abroad, the country of birth of the participant's mother determined the ethnic background, in line with the classification from Statistics Netherlands.[35] Nutritional intake in the past 3 months was obtained in early pregnancy using a modified version of a validated semiquantitative food frequency questionnaire.[36,37] A previous study in the same population as the present study showed that maternal early pregnancy fruit and vegetable intake was associated with urinary DAP metabolite concentrations.[14] Therefore, we included daily fruit and vegetable caloric intake, adjusted for the total caloric intake as a covariate. Maternal height was measured without shoes at the time of enrollment, and prepregnancy BMI was calculated.[17] We obtained information about child birthweight, gestational age at birth, and child sex from medical records.

Statistical Analysis

First, we performed a nonresponse analysis. We compared the characteristics of the mother–child pairs with and without metabolic measurements at 10 years of child age, among those with maternal urinary DAP metabolites measurements in pregnancy. We used a Student *t*-test, chi-square test, or Mann-Whitney *U*-test when applicable. Second, because OP metabolites are nonpersistent, rapidly excreted from the body and subsequently highly variable over time, we calculated the average urinary DM, DE, and DAP metabolite concentrations in pregnancy.[15] Pregnancy-average urinary concentrations were calculated by dividing the summed concentrations of the three trimesters by three. This pregnancy-average provides a better estimation of the exposure than any single exposure measurement. Therefore, we considered as main analyses the models including pregnancy-average DAP metabolite concentrations as exposure. Urinary DM, DE, and DAP trimester-specific and pregnancy-average concentrations were $\log_{10}$-transformed to obtain better model fit. Third, we assessed the association of pregnancy-average and trimester-specific maternal urinary DM, DE, and DAP metabolites concentrations with child measures of adiposity using linear regression models. We performed analysis on trimester-specific exposures to determine specific windows of vulnerability to OP pesticide exposure. To the best of our knowledge, the critical exposure periods during pregnancy for child obesity outcomes are not yet known. However, recently, early- and mid-pregnancy have been determined to be windows of higher vulnerability to OP pesticide for the outcome fetal growth.[8] We checked for linearity of all associations by plotting and visually inspecting the residuals vs. the fitted values. Fourth, we examined the associations of maternal urinary DM, DE, and DAP concentrations in pregnancy with the risk of childhood overweight or obesity using logistic regression models. Analysis were adjusted for child sex and age (basic model) and, subsequently, for maternal sociodemographic and lifestyle factors (main model). Potential confounders were selected from the previous literature and defined using a direct acyclic graph (Figure S1). Potential confounders were maternal prepregnancy BMI, ethnicity, parity, pregnancy smoking, educational level, household income, daily fruit and vegetable intake, and child gestational age at birth.[38–40] We included the confounders that changed the effect estimates for >10% for at least one of the outcomes. We added each covariate separately to the basic model. This approach enabled us to assess which covariate most strongly influenced the association of the exposures with the outcomes. Based on this, parity and gestational age at birth were excluded.[41] We checked for multicollinearity of all confounders with the exposures using the variance inflation factor (VIF). The VIF was smaller than 2 for all confounder–exposure combinations, indicating a low possibility of collinearity. We explored potential effect modification by child sex by adding an interaction term of child sex with the exposure to the adjusted model, considering a *p*-value threshold of <0.10. Based on this analysis, we concluded that child sex was not an effect modifier.

As a sensitivity analysis, we first examined whether associations were restricted to women with a relatively high OP exposure within our study population given that associations in previous literature have been mainly observed in highly exposed greenhouse workers. For this, we constructed binary exposure variables for being in the highest 10th exposure percentile and, subsequently, to gain more power, the 25th percentile of our population. We repeated the main analysis of these binary exposure variables with the outcomes. Second, we performed a sensitivity analysis refitting the main models with the exposure expressed in nanomoles per liter and with additional multivariable adjustment for creatinine instead of standardizing by creatinine. We did this

additional analysis to assure that estimates from our main analysis were not driven by nondilution factors related to creatinine excretion.[23] Third, given that a high percentage of values below the LOD might reduce the ability to detect associations owing to low variability, we repeated the main analysis including only individual metabolites in groupings when >50% of women had urinary exposure concentrations above the LOD. Last, to account for a potential within-subject lognormal distribution of the pesticide exposure levels throughout pregnancy, we calculated subject-specific geometric averages of DM, DE, and DAP concentrations (in nanomoles per gram creatinine) and repeated our main analysis.

Missing covariate values were imputed using multiple imputation by the fully conditional specification method.[42] Pooled results from 25 imputed data sets were reported. The percentage of missing values for covariates ranged from 0% to 12.1%; prepregnancy BMI had the highest percentage of missing values at 12.1%. We included as predictors for imputation of covariates the following characteristics: maternal age, prepregnancy BMI, ethnicity, education, household income, parity, smoking, fruit and vegetable intake; paternal age, ethnicity and education; and child age and sex. We additionally included the exposure maternal urinary pregnancy-average DAP and outcome child age- and sex-adjusted BMI. All statistical tests were two-sided. Analyses were performed using R (version 4.1.0; R Development Core Team).

Results

Population Characteristics

The characteristics of the study population are shown in Table 1. Mean maternal age ± SD was 30.1 ± 4.5 y and median prepregnancy BMI was 22.3 kg/m^2 [interquartile range (IQR): 20.6–25.0]. Most mothers were Dutch (61.1%), nulliparous (64.8%), never smoked during pregnancy (78.0%), highly educated (67.6%), and had a household income of >€2,000 per month (72.6%). Children attended the follow-up visit at a mean age of 9.7 ± 0.2 y, and both sexes were equally represented (50.5% male). Median sex- and age-adjusted BMI of children at the follow-up visit was 16.7 kg/m^2 (IQR: 15.5–18.2). As compared with the Generation R participants not included in the study, mothers from our sample were older, higher educated, more often Dutch, and had higher household incomes (Table S3). The median pregnancy-average urinary concentrations of the OP metabolites in our study were 144.7 (105.2, 205.4) nmol/g creatinine for DMP, 128.97 (83.57, 205.79) nmol/g creatinine for DMTP, 8.31 (25th to 75th percentile 2.48, 8.76) nmol/g creatinine for DMDTP, 38.48 (24.34, 59.72) nmol/g creatinine for DEP, 11.31 (6.15, 20.82) nmol/g creatinine for DETP, and 0.14 (0.00, 0.31) nmol/g creatinine for DEDTP (Table S2).

Maternal OP Metabolites and Child Measures of Adiposity

Looking at general adiposity measures, in the basic model, higher maternal pregnancy-average urinary DM, DE, and DAP concentrations were associated with a lower fat mass index: differences of 0.38 [95% confidence interval (CI): −0.67, −0.08], −0.43 (95% CI: −0.67, −0.18), and −0.46 (95% CI: −0.78, −0.15) SDS per 10-fold increase in maternal urinary exposure concentration (Table S4). These associations were mainly present in the second and third trimesters. With additional adjustment for potential confounders, all associations attenuated toward nonsignificant (Table 2). We observed no associations of maternal pregnancy-average urinary DM, DE, or DAP concentrations with BMI, lean mass index or android-to-gynoid fat mass ratio. In the basic model, but not in the main model, higher maternal pregnancy-average urinary DM, DE, and DAP metabolite concentrations were associated with lower odds on obesity or overweight in offspring at 10 years of age [odds ratio = 0.39 (95% CI: 0.16, 0.94), 0.41 (95% CI: 0.20, 0.85), and 0.38 (95% CI: 0.15, 0.95)] (Figure 2; Table S5). Looking at organ fat, in the basic model only higher maternal pregnancy-average urinary DE concentrations were associated with a lower liver fat fraction [difference = −0.31 (95% CI: −0.59, −0.03) SDS], but this association did not remain after adjustment for confounding (Table 3; Table S6). In all models, higher maternal prepregnancy BMI and lower education level had the strongest effect on the change in effect estimates (Table S7).

In our sensitivity analyses, we observed no associations of pregnancy-average maternal urinary DM, DE, and DAP concentrations, with our outcomes comparing the highest 10th exposure percentile to the lowest 90th [cutoff values: DM, 579.3; DE, 120.0; and DAP, 669.2 nmol/g creatinine (Tables S8 and S9)]. Likewise, we observed no associations when comparing the

Table 1. Characteristics of the study population, consisting of 642 mother–child pairs. Mothers were included in pregnancy between 2004 and 2006. Children were measured at 10 years of age. Generation R Study, Rotterdam, the Netherlands, 2002–2006.

	Total sample ($n = 642$)
Maternal characteristics	
Maternal age [y (mean ± SD)]	31.0 ± 4.5
Ethnicity [*n* (%)]	
Dutch	392 (61.1)
European	80 (12.5)
Non-European	170 (26.5)
Parity [*n* (%)]	
Nulliparous	416 (64.8)
Multiparous	223 (34.7)
Prepregnancy BMI (kg/m^2) [median (IQR)]	22.3 (20.6, 25.0)
Smoking [*n* (%)]	
Never smoked during pregnancy	465 (78.0)
Smoked until pregnancy was known	53 (8.9)
Continued smoking in pregnancy	78 (13.1)
Highest education finished [*n* (%)]	
Primary	17 (2.9)
Secondary	176 (29.5)
Higher	403 (67.6)
Household income per month [*n* (%)]	
<€1,200	64 (11.2)
€1,200–€2,000	92 (16.2)
>€2,000	413 (72.6)
Total energy intake per day (kcal) [median (IQR)]	2,071.4 (1,737.8, 2,413.4)
Vegetable intake per day (kcal) [median (IQR)]	140.1 (108.3, 180.0)
Fruit intake per day (kcal) [median (IQR)]	191.8 (120.3, 229.9)
Child characteristics	
Sex [*n* (%)]	
Male	324 (50.5)
Female	318 (49.5)
Gestational age at birth (wk) [median (IQR)]	40.3 (39.4, 41.0)
Birthweight [g (mean ± SD)]	3,461.9 ± 499.0
Child age at visit [y (mean ± SD)]	9.6 ± 0.2
BMI (kg/m^2) [median (IQR)][a]	16.7 (15.5, 18.2)
Overweight or obesity [*n* (%)][b]	106 (16.5)
Total body fat mass (kg) [median (IQR)]	8.2 (6.4, 10.9)
Lean mass [kg (mean ± SD)]	23.7 ± 3.1
Android-to-gynoid fat mass ratio [median (IQR)]	0.2 (0.1)
Total visceral fat mass (g) [median (IQR)]	369.1 (262.4, 495.5)
Pericardial fat mass (g) [median (IQR)]	9.3 (7.4, 13.0)
Liver fat fraction (%) [median (IQR)]	1.9 (1.6, 2.3)

Note: Values presented as mean ± SD, median (IQR), or number of participants (valid percentage). Number of missings per covariate (%): parity, $n = 3$ (0.5); prepregnancy BMI, $n = 78$ (12.1); smoking, $n = 46$ (7.2); education, $n = 46$ (7.2); income, $n = 73$ (11.4); energy intake, $n = 126$ (19.6); total body fat mass, $n = 7$ (1.1); lean mass, $n = 7$ (1.1); android-to-gynoid fat mass ratio, $n = 7$ (1.1); total visceral fat mass, $n = 144$ (38.0), pericardial fat mass, $n = 224$ (34.9), and liver fat fraction, $n = 201$ (31.3). BMI, body mass index; IQR, interquartile range; SD, standard deviation.
[a]According to Dutch reference growth charts, Dutch Growth Research Foundation.
[b]Defined using International Obesity Task Force cutoffs.

Table 2. Associations of average maternal dialkyl metabolite concentrations with measures of general fat mass at 10 years of age, main model. Generation R, Rotterdam, the Netherlands, 2002–2006.

	Measures of adiposity [SDS (95% CI)]							
Period	BMI ($N = 642$)[a]	*p*-Value	Fat mass index ($N = 635$)	*p*-Value	Lean mass index ($N = 635$)	*p*-Value	Android-to-gynoid fat mass ratio ($N = 635$)	*p*-Value
Total DMs[b]								
Average	0.09 (−0.23, 0.41)	0.589	−0.07 (−0.35, 0.21)	0.620	0.13 (−0.17, 0.43)	0.402	0.08 (−0.22, 0.37)	0.598
<18 wk	0.13 (−0.09, 0.35)	0.247	0.09 (−0.11, 0.28)	0.380	0.08 (−0.12, 0.29)	0.426	0.13 (−0.07, 0.33)	0.215
18–25 wk	−0.02 (−0.22, 0.27)	0.866	−0.09 (−0.30, 0.12)	0.401	0.07 (−0.16, 0.30)	0.069	0.01 (−0.21, 0.23)	0.944
>25 wk	−0.01 (−0.24, 0.23)	0.962	−0.11 (−0.31, 0.10)	0.317	0.05 (−0.17, 0.27)	0.686	0.00 (−0.22, 0.21)	0.993
Total DEs[c]								
Average	0.03 (−0.24, 0.30)	0.837	−0.19 (−0.43, 0.04)	0.111	0.11 (−0.14, 0.37)	0.374	−0.03 (−0.28, 0.21)	0.787
<18 wk	0.08 (−0.10, 0.28)	0.377	−0.05 (−0.22, 0.12)	0.568	0.14 (−0.04, 0.31)	0.127	−0.01 (−0.18, 0.16)	0.908
18–25 wk	0.02 (−0.18, 0.22)	0.858	−0.12 (−0.29, 0.06)	0.199	0.00 (−0.19, 0.19)	0.968	−0.06 (−0.25, 0.13)	0.550
>25 wk	−0.09 (−0.28, 0.11)	0.373	−0.14 (−0.31, 0.04)	0.119	−0.05 (−0.23, 0.14)	0.623	−0.04 (−0.22, 0.14)	0.688
Total DAPs[d]								
Average	0.02 (−0.32, 0.36)	0.912	−0.13 (−0.44, 0.17)	0.398	0.11 (−0.21, 0.43)	0.506	0.08 (−0.24, 0.40)	0.634
<18 wk	0.11 (−0.13, 0.34)	0.380	0.05 (−0.16, 0.25)	0.667	0.12 (−0.10, 0.34)	0.298	0.10 (−0.12, 0.32)	0.361
18–25 wk	0.01 (−0.25, 0.28)	0.924	−0.13 (−0.36, 0.09)	0.252	0.05 (−0.19, 0.29)	0.688	0.01 (−0.23, 0.24)	0.956
>25 wk	−0.05 (−0.30, 0.20)	0.699	−0.13 (−0.35, 0.09)	0.230	0.01 (−0.22, 0.25)	0.917	0.01 (−0.22, 0.24)	0.959

Note: Values represent regression coefficients (95% confidence intervals) of the regression models that reflect the difference in SDS of child outcomes for a 10-fold increase in maternal dialkyl urine metabolite concentrations (nmol/g creatinine). Model is corrected for maternal age, ethnicity, prepregnancy BMI, educational level, household income, smoking, daily fruit and vegetable intake, and child sex and age (except for sex- and age-adjusted BMI SDS). *p*-Values are nominal. BMI, body mass index; CI, confidence interval; DAPs, dialkyl phosphates; DEs, diethyl metabolites; DMs, dimethyl metabolites; SDS, standard deviation score.
[a]BMI is sex- and age-adjusted according to Dutch reference growth charts, Dutch Growth Research Foundation.
[b]DMs represent a molar sum of dimethylphosphate, dimethylthiophosphate, and dimethyldithiophosphate.
[c]DEs represent a molar sum of diethylphosphate, diethylthiophosphate, and diethyldithiophosphate.
[d]DAPs represent a molar sum of all of the above.

highest 25th exposure percentile to the lowest 75th [cutoff values: DM, 410.5; DE, 79.5; and DAP 501.7 nmol/g creatinine (Tables S10 and S11)]. Refitting the continuous exposure models with the exposure expressed in nanomoles per liter and with additional multivariate adjustment for creatinine showed similar results to the main analysis (Tables S12 and S13). When we regrouped the exposures including only individual chemicals with >50% of values above the LOD, only the grouping of DE changed, excluding DEDTP (Table S1). Analysis with those exposures did not significantly change the effect estimates as compared with the main analysis (Tables S14 and S15). Last, repeating our analysis using subject-specific geometric averages of DM, DE, and DAP exposure instead of arithmetic pregnancy-averages yielded similar effect estimates as compared with the main analysis (Tables S16 and S17).

Discussion

In this population-based study, we did not observe associations of higher maternal urinary concentrations of average or trimester-specific DM, DE, or DAP metabolites in pregnancy with child BMI, the risk of overweight or obesity, total and regional body fat, abdominal fat, and organ fat at 10 years of child age. Previous studies suggested that *in utero* OP pesticide exposure might adversely affect fetal metabolic development, predisposing children to adverse metabolic profiles in later life. Two studies from the same Danish prospective cohort used questionnaires to determine maternal exposure to 200 different modern nonpersistent pesticides in pregnancy.[10,11] Those mothers were greenhouse workers, and they were classified as occupationally exposed if pesticides were applied in the working area more than once a month and the women were involved in applying these pesticides or handled the treated plants within 1 wk after treatment of the plants. Among them, OPs were the most frequently used insecticides. The first study reported that 184 children of exposed mothers, as compared with 112 children of unexposed mothers, had higher BMIs and fat percentages at pubertal age.[10] The second study reported that 168 children of mothers exposed to modern nonpersistent pesticides in pregnancy had higher absolute weight, but lower weight for gestational age, as compared with 79 controls.[11] To the contrary, 112 children of exposed mothers had a larger increase in BMI *z*-scores and higher body fat percentage at 6–11 years of age, as compared with 65 children of unexposed mothers.[11] A prospective cohort study from the United States among 166 mother–child pairs reported no associations of third trimester urinary maternal DAP metabolite concentrations and BMI *z*-scores at 4, 6, and 7–9 years of age.[12] Last, using multi-exposure models, a European meta-analysis assessed the mixture effects of 77 environmental exposures during pregnancy and 96 exposures in childhood with child BMI, overweight, waist circumference, and fat mass at the average child age of 8 y.[13] That study reported associations of urinary DEP concentrations in childhood with lower risks on adiposity at 8 years of age among ∼1,086 children, but no associations were found for childhood DMP, DMTP, DMDTP, and DETP, nor for any pregnancy exposure.[13]

In the present study, we observed no associations of maternal urinary DAP metabolite concentrations in pregnancy with child adiposity measures at 10 years of age after adjustments for confounders. Discrepancies between our results and the two Danish studies might be due to presumably higher exposure levels and different sources of exposure in the participants of those studies.[10,11] Women included in those studies were workers in greenhouses where pesticides were frequently used, whereas our population lived in urban settings where the main route of exposure is through ingestion of foods.[14] Therefore, exposure to OP pesticides is likely to be lower, even in the highest 10th exposure percentiles in our population. Unfortunately, because the two Danish studies obtained no absolute concentrations of the pesticides exposure, we were not able to compare this with our population. In addition, given that they looked at overall exposure to modern nonpersistent pesticides, they could not determine the specific effect of the OP pesticides. The two other studies that measured maternal concentrations of DAP metabolites in the general population, also through urine samples, neither observed associations of higher DAP concentrations with child BMI, fat mass, and risk of overweight.[12,13] Measurement

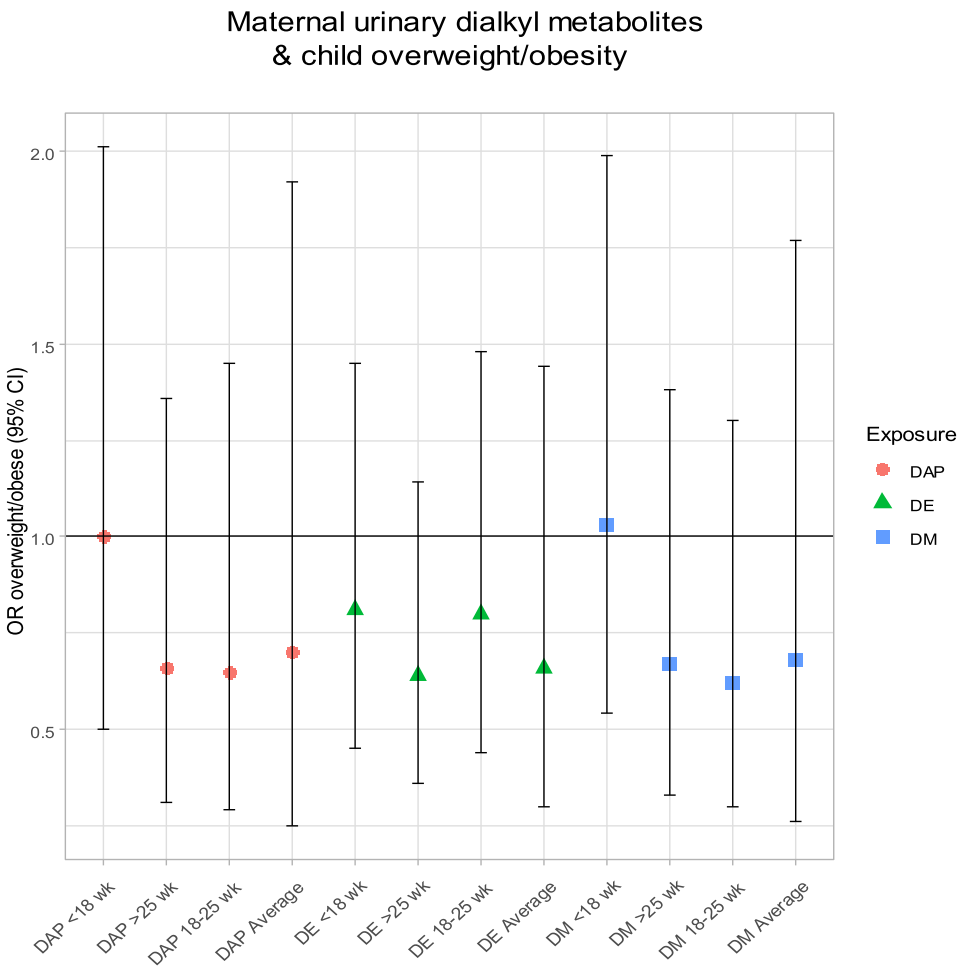

Figure 2. Associations of maternal average dialkyl metabolite urinary concentrations with the risk of overweight/obesity at 10 years of age, main model. Values are ORs (95% CIs) that reflect the risk of overweight/obesity in children with every 10-fold increase in maternal urinary dialkyl metabolite concentration. Model includes maternal age, ethnicity, prepregnancy body mass index, educational level, household income, and smoking. $N = 642$ participants of the Generation R Study, Rotterdam, the Netherlands, 2002–2006. Corresponding data are shown in Table S5. Note: CI, confidence interval; DAPs, dialkyl phosphates (molar sum DM and DE); DEs, diethyl metabolites (molar sum of diethylphosphate, diethylthiophosphate, and diethyldithiophosphate); DMs, dimethyl metabolites (molar sum of dimethylphosphate, dimethylthiophosphate, and dimethyldithiophosphate); OR, odds ratio.

of OP pesticides exposure is more reliable through urine samples as compared with questionnaires, given that the use of questionnaires is prone to recall bias.

We are the first study looking at visceral, pericardial, and liver fat outcomes when investigating the effect of maternal OP pesticide exposure in pregnancy on the offspring. These fat mass outcomes are interesting because visceral adipose tissue is more strongly associated with most metabolic risk factors than subcutaneous abdominal adipose tissue.[16] In addition, MRI is considered the golden standard for the measurement of intra-abdominal and organ fat deposition.[43] We observed no associations of maternal urinary DAP metabolite concentrations in pregnancy with visceral, pericardial, or liver fat outcomes. Still, these outcome measures should be taken forward to future studies, to improve research on fetal OP pesticide exposure and metabolic outcomes, preferably in more highly exposed, agricultural populations and also investigating effects of childhood exposure.

In our study, we observed strong differences between the basic and main models. Adjusting for maternal prepregnancy BMI and education caused the largest changes in effect estimates, attenuating the association of higher maternal urinary DAP metabolite concentrations with lower risks on overweight and lower fat mass index in children at 10 years of age toward the null. Therefore, maternal prepregnancy BMI and socioeconomic status are important factors to consider in future research on OP pesticides. Higher prepregnancy BMI and lower socioeconomic status were associated with lower pesticide exposure in our population, given that those women ate less fruit and vegetables, which are main sources of OP pesticides.[14] Yet, children from lower educated and mothers with higher BMI have higher risks on adverse adiposity profiles.[44] This contrast might be an important source of bias in research on the association of pesticide exposure and fat measures, which we aimed to reduce by adjusting for maternal fruit and vegetable intake.

An important strength of our study is the prospective data collection from early pregnancy onward, allowing repeated measurements of the OP pesticide metabolites throughout pregnancy. A limitation of our study is that DAP metabolites are nonspecific biomarkers of OP pesticide exposure.[45] Measured DAP metabolites are also present in the natural environment.[46,47] Not all DAP metabolites are breakdown products of OP pesticides.[48] In addition, DAP urinary metabolite concentrations give no specific information on the parent OP pesticide they derive from, and they capture only 75% of the OP pesticides in use.[18,20,49] Studies measuring DAP metabolites are unable to determine to which specific OP pesticide the population is exposed. Given that the use of OP pesticides is likely to differ between countries and time, the generalizability of our results and comparability to other

Table 3. Associations of average maternal dialkyl metabolite concentrations with measures of visceral fat at 10 years of age, main model. Generation R, Rotterdam, the Netherlands, 2002–2006.

	Measures of visceral fat [SDS (95% CI)]					
Period	Visceral fat index ($N = 398$)	p-Value	Liver fat fraction ($N = 441$)	p-Value	Pericardial fat mass index ($N = 418$)	p-Value
Total DMs[a]						
Average	−0.16 (−0.52, 0.20)	0.384	−0.05 (−0.41, 0.31)	0.773	−0.09 (−0.46, 0.28)	0.631
<18 wk	−0.02 (−0.28, 0.24)	0.877	0.05 (−0.22, 0.32)	0.725	0.02 (−0.23, 0.27)	0.880
18–25 wk	−0.07 (−0.32, 0.19)	0.621	−0.06 (−0.33, 0.20)	0.634	−0.11 (−0.39, 0.17)	0.442
>25 wk	−0.10 (−0.37, 0.16)	0.435	−0.05 (−0.30, 0.21)	0.726	−0.01 (−0.28, 0.26)	0.948
Total DEs[b]						
Average	−0.04 (−0.34, 0.25)	0.780	−0.10 (−0.39, 0.20)	0.506	0.01 (−0.31, 0.32)	0.972
<18 wk	−0.03 (−0.25, 0.19)	0.791	−0.10 (−0.31, 0.11)	0.336	0.01 (−0.21, 0.23)	0.922
18–25 wk	−0.05 (−0.17, 0.26)	0.659	−0.07 (−0.29, 0.15)	0.521	0.04 (−0.19, 0.27)	0.711
>25 wk	−0.12 (−0.34, 0.10)	0.272	0.01 (−0.19, 0.21)	0.928	−0.04 (−0.26, 0.18)	0.718
Total DAPs[c]						
Average	−0.17 (−0.55, 0.22)	0.397	−0.07 (−0.47, 0.32)	0.721	−0.11 (−0.50, 0.29)	0.599
<18 wk	−0.03 (−0.31, 0.25)	0.820	0.00 (−0.29, 0.29)	0.982	−0.01 (−0.28, 0.26)	0.942
18–25 wk	−0.04 (−0.32, 0.24)	0.769	−0.09 (−0.37, 0.20)	0.545	−0.07 (−0.38, 0.24)	0.646
>25 wk	−0.13 (−0.41, 0.15)	0.356	−0.04 (−0.31, 0.23)	0.764	−0.04 (−0.33, 0.24)	0.763

Note: Values represent regression coefficients (95% confidence intervals) of the regression models that reflect the difference in SDS of child outcomes for a 10-fold increase in maternal dialkyl urine metabolite concentrations (nmol/g creatinine). Model is corrected for maternal age, ethnicity, prepregnancy body mass index, educational level, household income, smoking, daily fruit and vegetable intake, and child sex and age (except for sex- and age-adjusted body mass index SDS). p-Values are nominal. CI, confidence interval; DAPs, dialkyl phosphates; DEs, diethyl metabolites; DMs, dimethyl metabolites; SDS, standard deviation score.
[a]DMs represent a molar sum of dimethylphosphate, dimethylthiophosphate, and dimethyldithiophosphate.
[b]DEs represent a molar sum of diethylphosphate, diethylthiophosphate, and diethyldithiophosphate.
[c]DAPs represent a molar sum of all of the above.

studies should be considered carefully. However, estimation of urinary DAP metabolite concentrations is considered the most appropriate and useful tool to identify and compare levels of OPs pesticides.[1] In addition, DAP metabolites have short biological half-lives, leading to concentrations that are highly variable over time and dependent on diet. In our study, we used three urinary samples, whereas previous studies generally used one maternal spot urine sample.[12,13] The DAP metabolite concentrations between different trimesters in our study sample had correlation coefficients varying from 0.14 to 0.38 (0.30 for total DAP).[15] Achieving better precision of the OP pesticide exposure over pregnancy would require more frequent measurements within each trimester.[50] To our best knowledge, no reference values for absolute visceral, pericardial, and liver fat in childhood exist. Therefore, we could not determine whether the values within our study populations were within the normal clinical ranges. Our study included a relatively healthy and highly educated urban population. This might affect the generalizability of our results toward populations living in more agricultural settings. Our nonresponse analysis showed that several maternal sociodemographic characteristics were different for children that had no outcome data available. However, we did not observe a difference in maternal urinary DAP concentrations during pregnancy between children with and without outcome data. Therefore, it seems unlikely that selection bias owing to loss to follow-up influenced our results. The low pesticide exposure in our population might have reduced the ability to detect associations, and studies among more highly exposed populations are still needed. In addition, residual confounding might have occurred owing to the observational nature of the study. However, a previous study identifying determinants of higher OP pesticides exposure in our study sample has been performed, enabling a targeted selection of covariates in our models.[14] Among others, this previous study reported that common dietary products other than fruit and vegetables, such as grain and dairy, were not related to higher OP pesticide exposure in our population.[14] Last, we adjusted the analyses for fruit and vegetable intake but had no information available for organic food products, which also might play an important role in the exposure.

Conclusion

In the present study, we observed no associations of maternal urinary DAP metabolite concentrations during pregnancy with child adiposity measures at 10 years of child age. Our results are important null observations given that we performed our study in a large prospective cohort among the general population, measured DAP metabolites throughout the whole pregnancy, and used imaging techniques enabling measurement of visceral, pericardial, and liver fat outcomes. The results of our studies should be replicated among more ethnically diverse and more highly exposed populations, such as individuals living and working in areas with agricultural pesticide use. In addition, further studies should assess the associations of OP pesticide exposure in pregnancy with other child cardiometabolic outcomes, such as insulin resistance and vascular health, preferably also among more highly exposed populations.

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